

## COMPOSITIONS FOR MITOPHAGY INDUCTION AND USES THEREOF

### 1. CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the priority benefit of U.S. provisional application no. 63/426,424, filed November 18, 2022, the contents of which are incorporated herein in their entireties by reference thereto.

### 2. SEQUENCE LISTING

**[0002]** The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML Sequence Listing, created on November 8, 2023, is named RMG-004WO\_SL.xml and is 42,441 bytes in size.

### 1. BACKGROUND

**[0003]** Mitochondrial dysfunction is caused by a variety of factors, including mutations in genes encoding mitochondrial proteins, tRNA, and rRNA, accumulation of mutations in the mitochondrial genome, mismanagement of mitochondrial proteins, mismanagement of intracellular organelles such as the endoplasmic reticulum and lysosome, and mitochondrial protein quality control defects. Mitochondrial protein quality control defects can be caused by a variety of factors, such as mismanagement of intracellular organelles such as the endoplasmic reticulum and the lysosome. Among these, quality control by mitochondrial biogenesis and autophagy (mitophagy) and dynamics by fusion/fission is where various mitochondrial dysfunctions converge. Mitochondrial quality control defects associated with various diseases continue to be intensively studied as targets for disease therapy. Target diseases include not only mitochondrial diseases, but also neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and ALS, heart failure, diabetes, and immunodeficiency for cancer, infectious diseases, and autoimmune diseases. Small molecule compounds have been developed to enhance biogenesis, to induce mitophagy, and to suppress excessive fission, *etc.* For example, coenzyme Q10, idebenone, and metformin have been shown to induce mitophagy.

**[0004]** However, no compound with a clear mode of action has yet reached the clinic. Accordingly, there remains a need for compositions and methods for ameliorating mitochondrial dysfunction.

### 2. SUMMARY

**[0005]** The disclosure provides polypeptides capable of inducing double strand breaks (DSBs) in mitochondria to enable a temporary and partial reduction in the number of mitochondria in a cell. Compared to the nucleus, mitochondria have poor gene repair mechanisms. In response

to the stress of DSBs, mitochondria strongly transmit signals to the nucleus to promote replication of the mitochondrial genome and increase production of mitochondrial component proteins. Without being bound by theory, it is believed that introducing DSBs in mitochondrial DNA can be used to efficiently promote mitochondrial turnover, thereby ameliorating mitochondrial dysfunction through the generation of new mitochondria. DSBs can be introduced into mitochondrial DNA through use of a polypeptide comprising a mitochondrial targeting sequence (MTS) fused to an endonuclease. However, one potential problem with this approach is excessive endonuclease activity. To provide sensitive ON/OFF control of the endonuclease, the present disclosure provides polypeptides having a destabilization domain in addition to a mitochondrial targeting sequence and endonuclease sequence. When stabilized by a stabilizing agent, the destabilization domain permits the polypeptide to retain structure and endonuclease activity; in absence of the stabilizing agent, the destabilization domain loses stability leading to degradation of the polypeptide by the proteasome.

**[0006]** Accordingly, in one aspect, the disclosure provides polypeptides comprising a mitochondrial targeting sequence (MTS), an endonuclease sequence, for example XbaIR, and a destabilizing domain sequence. Inclusion of an MTS is useful for directing the polypeptide to the desired site of endonuclease activity, namely the mitochondrial genome. Inclusion of a destabilizing domain sequence, which is stabilized by a stabilizing agent, enables sensitive ON/OFF control of the endonuclease. For example, a cell can be contacted with the polypeptide in the presence of the stabilizing agent for a period of time (during which the polypeptide is active) and, subsequently, the stabilizing agent can be removed, thereby leading to destabilization and degradation of the polypeptide.

**[0007]** Exemplary features of polypeptides of the disclosure are described in Sections 4.2 and specific embodiments 1 to 84, *infra*.

**[0008]** In another aspect, the disclosure provides nucleic acids encoding a polypeptide of the disclosure, particles comprising the nucleic acids, such as viral particles, and host cells comprising a nucleic acid of the disclosure. Exemplary nucleic acids include vectors such as viral (e.g., retroviral) genomes, plasmids, and mRNA molecules. Exemplary particles include viral particles (e.g., retroviral particles). Further exemplary features of nucleic acids, particles and host cells of the disclosure are described in Section 4.3 and specific embodiments 85 to 96, *infra*.

**[0009]** In another aspect, the disclosure provides methods of (a) inducing mitophagy in a cell, and/or (b) increasing mitochondrial turnover in a cell, and/or (c) increasing mitochondrial mass, and/or (d) inducing double strand breaks in mitochondrial DNA and/or (e) inducing epigenomic modifications in a cell by contacting the cell with a polypeptide, nucleic acid, or particle of the disclosure and a stabilizing agent. For example, a cell can be transfected with a nucleic acid

encoding a polypeptide, transduced with a viral particle containing a nucleic acid encoding the polypeptide, or injected with the polypeptide, and cultured in the presence of the stabilizing agent. Following a period of time (*e.g.*, 6 hours to 5 days), the stabilizing agent can be removed to allow destabilization and degradation of the polypeptide.

**[0010]** In another aspect, the disclosure provides cells and populations of cells obtained or obtainable by the methods of (a) inducing mitophagy in a cell, and/or (b) increasing mitochondrial turnover in a cell, and/or (c) increasing mitochondrial mass, and/or (d) inducing double strand breaks in mitochondrial DNA and/or (e) inducing epigenomic modifications in a cell described herein. Unless required otherwise by context, reference herein to a “cell” encompasses single cells as well as populations of cells.

**[0011]** In another aspect, the disclosure provides methods of treating subjects with cells and populations of cells of the disclosure. For example, the subject can be a subject having an age-related disease, a mitochondrial disease or disorder, a neurodegenerative disease, an eye disease (*e.g.*, a retinal disease), diabetes, a hearing disorder, a genetic disease, heart failure, an immunodeficiency, cancer, or an infectious disease.

**[0012]** Further exemplary features of methods and cells of the disclosure are described in Section 4.4 and specific embodiments 97 to 195, *infra*.

**[0013]** In further aspects, the disclosure provides pharmaceutical compositions comprising a polypeptide, nucleic acid, particle or cell (including populations of cells) of the disclosure. Such pharmaceutical compositions can be used, for example, in the methods of treatment described herein.

**[0014]** In yet another aspect, the disclosure provides a kit comprising a polypeptide, nucleic acid, or particle of the disclosure and a stabilizing agent. Kits can be used, for example, in the methods of the disclosure.

**[0015]** Further exemplary features of pharmaceutical compositions and kits are described in Section 4.5 and specific embodiments 196 to 200, *infra*.

### **3. BRIEF DESCRIPTION OF THE FIGURES**

**[0016]** FIG. 1 shows a retroviral vector map with an MTS-XbaIR-DHFR polypeptide (Example 1).

**[0017]** FIG. 2 shows a molecular model of the MTS-XbaIR-DHFR polypeptide of Example 1.

**[0018]** FIG. 3 shows a retroviral vector map with an EGFR-DHFR polypeptide (Example 1).

**[0019]** FIG. 4 shows a molecular model of the EGFR-DHFR polypeptide of Example 1.

**[0020]** FIG. 5 shows fluorescence microscopy images of HeLa cells transduced with an EGFP-DHFR retroviral vector (Example 1).

**[0021]** FIG. 6 shows FACS data for HeLa cells transduced with an EGFP-DHFR retroviral vector, showing EGFP expression when cultured in the presence of TMP (Example 1).

**[0022]** FIG. 7 shows relative EGFP mRNA expression levels in HeLa cells transduced with an EGFP-DHFR retroviral vector cultured with TMP (Example 1).

**[0023]** FIGS. 8A-8D show fluorescence intensity of HeLa cells transduced with an EGFP-DHFR retroviral vector by fluorescence microscopy and FACS after culturing in media with TMP for two days followed by washout of TMP at 0, 1, 2, 4, 6, 8, 24, and 48 hours post-washout (Example 1). FIG. 8A: Time course; FIG. 8B: Fluorescence images; FIG. 8C: FACS analysis, FIG. 8D: Mean fluorescence intensity (MFI) in FACS analysis over time.

**[0024]** FIG. 9A-9D show fluorescence intensity of HeLa cells transduced with an EGFP-DHFR retroviral vector by fluorescence microscopy and FACS after culturing in media with TMP for several durations (0, 1, 2, 4, 6, 8, 24, and 48 hours). FIG. 9A: Time course; FIG. 9B: Fluorescence images; FIG. 9C: FACS analysis; FIG. 9D: MFI in FACS analysis over time

**[0025]** FIG. 10A-10C show XbaI expression (FIG. 10A) and mtDNA copy number (CN) (FIG. 10B) for HeLa cells transduced with a retroviral vector encoding an MTS-XbaIR-DHFR polypeptide (HeLa MXD sc20) and cultured over time in the absence or presence of 0.5  $\mu$ M TMP for two days followed by TMP washout (FIG. 10C) (Example 1).

**[0026]** FIG. 11A-11D show MFIs of Mitogreen staining that is an indicator for mitochondrial mass (mtMass) (FIG. 11A), MFIs of TMRM staining that is an indicator for global mitochondrial membrane potential (mtMP) (FIG. 11B), and relative TMRM/Mitogreen ratios that is an indicator for mtMP per mass unit (FIG. 11C) in HeLa MXD sc20 incubated with TMP for 3 different durations (16, 20, and 48 hours) following cell culture over time (FIG. 11D)

**[0027]** FIGS. 12A-12C show cell number (FIG. 12A) and cell viability (FIG. 12B) for HeLa MXD sc20 cells cultured in the absence or presence of 0.5  $\mu$ M TMP for two days followed by TMP washout (FIG. 12C). (Example 1).

**[0028]** FIGS. 13A-13B show mitophagy index (FIG. 13A) by FACS analysis and mtDNA CN estimated by qPCR (FIG. 13B) for HeLa MDX sc20 cells transduced with a retroviral vector encoding mtKeimaRed and PARK2 and cultured without or with TMP for several durations. CCCP is used for a positive control of mitophagy at 10  $\mu$ M. (Example 1).

**[0029]** FIGS. 14A-14D show changes in mitochondrial biogenesis in HeLa\_GiM cells stably expressing a genetically induced mitophagy (GiM) unit, in the presence and absence of TMP. Mitochondrial ROS (mtROS) (FIG. 14A), PGC1 $\alpha$  (FIG. 14B), NRF1 (FIG. 14C), and TFAM (FIG. 14D) were assessed over time using FACS. (Example 2).

**[0030] FIGS. 15A-15F** show the expression of several mitochondrial proteins over time in HeLa\_GiM cells in the presence and absence of TMP. FIG. 15A shows western blot images of mitochondrial proteins on Days 2, 4, 6, and 8. FIGS. 15B-15F show the quantified levels of the same proteins. (Example 2).

**[0031] FIGS. 16A-16B** show phase contrast (PhC) and fluorescence microscopy images of mtKeimaRed-expressing HeLa\_GiM cells in the presence and absence of TMP (FIG. 16A) and the percentage of cells that are mitophagy positive (FIG. 16B). (Example 2).

**[0032] FIGS. 17A-17B** show colocalization of the autophagosomal membrane marker LC3, mitochondrial marker TOM20, and the nuclear stain DAPI in HeLa\_GiM cells in the presence or absence of TMP and BafA1 (FIG. 17A) and quantified values for the areas that correspond to autophagosomes (FIG. 17B) (Example 3).

**[0033] FIGS. 18A-18B** show LC3-II expression levels in HeLa\_GiM cells in the absence or presence TMP or TMP + BafA1. FIG. 18A shows the western blot images and FIG. 18B is a graphical representation of the triplicate quantification. (Example 3).

**[0034] FIGS. 19A-19C** show results of respirometry, displaying OXPHOS and glycolysis changes over time (FIG. 19A), two-dimensional expansion of OXPHOS and Glycolysis relation (FIG. 19B), and changes in ATP production, basal respiration, proton leak, and spare capacity over time (FIG. 19C). (Example 4).

**[0035] FIGS. 20A-20G** show the assay setup used in Example 5 (FIG. 20A), FACS results in untreated Alzheimer's Disease (AD) fibroblasts and control NHDF cells (FIG. 20B) and in AD fibroblasts at 7, 14, and 21 days after genetically induced mitophagy (GiM) (FIG. 20C), mtMass and mtMP levels in untreated (FIGS. 20D and 20E, respectively) and in AD fibroblasts at 7 and 14 days after GiM, relative to controls (FIGS. 20F and 20G, respectively), which are described further in Example 5.

#### **4. DETAILED DESCRIPTION**

**[0036]** The disclosure provides polypeptides capable of inducing double strand breaks (DSBs) in mitochondria to enable a temporary and partial reduction in the number of mitochondria in a cell.

**[0037]** The mitochondrial genome encodes respiratory chain proteins that are under a strict regulation balanced with the translation of nuclear-coded respiratory proteins. Therefore, the partial loss of the mitochondrial genome directly links to insufficient proton uptake, resulting in depolarization of mitochondrial membrane potential. The mitochondrial membrane potential is depolarized in dysfunctional mitochondrial compartments, and a key regulator for mitophagy. Without being bound by theory, it is believed that the more depolarized portions can be preferentially subject to mitophagy following introduction of DSBs.

**[0038]** In response to the stress of DSBs, mitochondria strongly transmit signals to the nucleus (e.g., as part of the mitochondrial unfolded protein response (UPRmt)) to promote replication of the mitochondrial genome and increase production of mitochondrial component proteins. In addition to signals of the UPRmt, the metabolic alterations resulting from mitochondrial genome reduction can affect the epigenomic status of the cell. For example, the reduction can decrease some intermediates of TCA cycle, which are utilized for acetylation and methylation in the nuclear genome and histone. Without being bound by theory, it is believed that introducing DSBs in mitochondrial DNA can be used to efficiently promote mitochondrial turnover, thereby ameliorating mitochondrial dysfunction through the generation of new mitochondria. Mitochondrial DNA may possess some modifications, such as 8-oxo-7,8-dihydroguanine (8-OXOG), which is an oxidized form of guanine. With aging, damaging modifications accumulate. Since newly generated mitochondrial genomes lack these modifications, mitochondrialogenesis can promote a regain of healthy mitochondrial function.

**[0039]** DSBs can be introduced into mitochondrial DNA through use a polypeptide comprising a mitochondrial targeting sequence (MTS) fused to an endonuclease. However, one potential problem with this approach is excessive endonuclease activity. To provide sensitive ON/OFF control of the endonuclease, the present disclosure provides polypeptides having a destabilization domain in addition to a mitochondrial targeting sequence and endonuclease sequence. When stabilized by a stabilizing agent, the destabilization domain permits the polypeptide to retain structure and endonuclease activity; in absence of the stabilizing agent, the destabilization domain loses stability leading to degradation of the polypeptide by the proteasome.

#### **4.1. Definitions**

**[0040]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. The following definitions are provided for the full understanding of terms used in this specification.

**[0041]** As used herein, the following terms are intended to have the following meanings:

**[0042] A, An, The:** As used herein, the term "a", "an", "the" and similar terms used in the context of the present disclosure (especially in the context of the claims) are to be construed to cover both the singular and plural unless otherwise indicated herein or clearly contradicted by the context. As such, the terms "a" (or "an"), "one or more", and "at least one" can be used interchangeably herein.

**[0043] And/or:** The term "and/or" means that each one or both or all the components or features of a list are possible variants, especially two or more thereof in an alternative or cumulative way.

**[0044] Destabilization Domain (DD):** The term destabilization domain refers to a polypeptide domain which when fused to a second polypeptide domain such as an endonuclease causes the polypeptide to be degraded in the absence of a stabilizing agent which prevents or inhibits the degradation otherwise elicited by the destabilization domain. Exemplary destabilization domains include dihydrofolate reductase (DHFR) destabilization domains (which can be stabilized by the exemplary stabilizing agent trimethoprim), FK506-binding protein (FKBP) destabilization domains (which can be stabilized by the exemplary stabilizing agents Shield-1 (Shld1), rapamycin and FK506) and PDE5 destabilization domains (which can be stabilized by the exemplary stabilizing agents sildenafil, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil, benzamidenafil, dasantafil, and beminafil). Exemplary DHFR destabilization domains are described in Iwamoto *et al.*, 2010, Chem Biol. 17(9):981-8, Liu *et al.*, 2014 Int. J. Parasitol. 44(10):729-735, and US 9,487,787; exemplary FKBP destabilization domains are described in Banaszynski *et al.*, 2006, Cell 126(5):995-1104 and US 9,487,787; and exemplary PDE5 destabilization domains are described in WO 2018/237323, the contents of each of which are incorporated herein by reference in their entireties.

**[0045] Effective amount:** The term "effective amount" or "therapeutically effective amount" means the amount or quantity of an agent or composition that is sufficient to elicit the required or desired response, or in other words, the amount that is sufficient to elicit an appreciable biological response when administered to a subject. Said amount preferably relates to an amount that is therapeutically or in a broader sense also prophylactically effective against the progression of a disease or disorder as disclosed herein. It is understood that an "effective amount" or a "therapeutically effective amount" can vary from subject to subject, due to variation in metabolism of an agent, age, weight, general condition of the subject, the condition being treated, the severity of the condition being treated, and the judgment of the prescribing physician.

**[0046] Endonuclease:** The term "endonuclease" refers to an enzyme that cleaves a polynucleotide chain by separating nucleotides other than nucleotides at a 5' or 3' end. Endonucleases differ from exonucleases, which cleave nucleotides from a 5' or 3' ends of a polynucleotide chain. Exemplary endonucleases include restriction endonucleases capable of cleaving double-stranded DNA at or near specific recognition sites to form double strand breaks (DSB) in the DNA. Exemplary restriction endonucleases include XbaI, EcoRI, SmaI, AflII, BamHI, BclI, EcoRI, HaeIII, HindII, HindIII, NdeI, PvuII, PstI, and SpeI. Exemplary endonuclease amino acid sequences are described in publicly available databases such as

UniProt. For example, an exemplary XbaI amino acid sequence has UniProt accession number O68567; an exemplary EcoRI amino acid sequence has UniProt accession number P00642; an exemplary SmaI amino acid sequence has UniProt accession number P14229; an exemplary AflII amino acid sequence has UniProt accession number E3VX87; an exemplary BamHI amino acid sequence has UniProt accession number P23940; an exemplary BclI amino acid sequence has UniProt accession number E5LGB8; an exemplary HaeIII amino acid sequence has UniProt accession number O68584; an exemplary HindII amino acid sequence has UniProt accession number P44413; an exemplary HindIII amino acid sequence has UniProt accession number P43870; an exemplary PvuII amino acid sequence has UniProt accession number A0A4R7BM34; an exemplary PstI amino acid sequence has UniProt accession number P00640; and an exemplary SpeI amino acid sequence has UniProt accession number F1KM35.

**[0047] Mitochondrial Targeting Sequence (MTS):** The term “mitochondrial targeting sequence” refers to an amino acid sequence capable of directing the transport of a polypeptide containing the sequence to mitochondria. An MTS is typically 10-70 amino acids in length. An MTS frequently comprises an alternating pattern of hydrophobic and positively charged amino acids to form an amphipathic helix.

**[0048] Or:** Unless indicated otherwise, an “or” conjunction is intended to be used in its correct sense as a Boolean logical operator, encompassing both the selection of features in the alternative (A or B, where the selection of A is mutually exclusive from B) and the selection of features in conjunction (A and B, where both A and B are selected). In some places in the text, the term “and/or” is used for the same purpose, which shall not be construed to imply that “or” is used with reference to mutually exclusive alternatives.

**[0049] Peptide, protein, and polypeptide:** The terms peptide, protein, and polypeptide are used interchangeably to refer to a natural or synthetic molecule comprising two or more amino acids linked by the carboxyl group of one amino acid to the alpha amino group of another. The amino acids may be natural or synthetic, and can contain chemical modifications such as disulfide bridges, substitution of radioisotopes, phosphorylation, substrate chelation (*e.g.*, chelation of iron or copper atoms), glycosylation, acetylation, formylation, amidation, biotinylation, and a wide range of other modifications. There is expressly no requirement that a polypeptide must contain an intended function; a polypeptide can be functional, non-functional, function for unexpected/unintended purposes, or have unknown function. A polypeptide is comprised of approximately twenty standard naturally occurring amino acids, although natural and synthetic amino acids which are not members of the standard twenty amino acids may also be used. The standard twenty amino acids include alanine (Ala, A), arginine (Arg, R), asparagine (Asn, N), aspartic acid (Asp, D), cysteine (Cys, C), glutamine (Gln, Q), glutamic acid (Glu, E), glycine (Gly, G), histidine (His, H), isoleucine (Ile, I), leucine (Leu, L), lysine (Lys, K),

methionine (Met, M), phenylalanine (Phe, F), proline (Pro, P), serine (Ser, S), threonine (Thr, T), tryptophan (Trp, W), tyrosine (Tyr, Y), and valine (Val, V). The terms "polypeptide sequence" or "amino acid sequence" are an alphabetical representation of a polypeptide molecule.

**[0050] Percentage identity:** Percentage identity between two amino acid sequences is calculated by multiplying the number of matches between a pair of aligned sequences by 100, and dividing by the length of the aligned region. Identity scoring only counts perfect matches and does not consider the degree of similarity of amino acids to one another, nor does it consider substitutions or deletions as matches. Alignment for purposes of determining percent sequence identity can be achieved in various ways that are within the skill in the art, for instance, by manual alignment or using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for achieving maximum alignment.

**[0051] Subject:** As used herein, the term "subject" means a human.

**[0052] Treat, treating, treatment:** As used herein, the term "treat", "treating" or "treatment" of any disease or disorder refers in one embodiment to ameliorating the disease or disorder (e.g., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms or pathological features thereof). In another embodiment "treat", "treating" or "treatment" refers to alleviating or ameliorating at least one physical parameter or pathological features of the disease, e.g., including those, which may not be discernible by the subject. In yet another embodiment, "treat", "treating" or "treatment" refers to modulating the disease or disorder, either physically, (e.g., stabilization of at least one discernible or non-discernible symptom), physiologically (e.g., stabilization of a physical parameter) or both. In yet another embodiment, "treat", "treating" or "treatment" refers to preventing or delaying the onset or development or progression of the disease or disorder, or of at least one symptoms or pathological features associated thereof. In yet another embodiment, "treat", "treating" or "treatment" refers to preventing or delaying progression of the disease to a more advanced stage or a more serious condition. The benefit to a patient to be treated is either statistically significant or at least perceptible to the patient or to the physician. However, it will be appreciated that when a medicament is administered to a patient to treat a disease, the outcome may not always be an effective treatment.

#### **4.2. Polypeptides**

**[0053]** In one aspect, the disclosure provides polypeptides comprising a mitochondrial targeting sequence (MTS), an endonuclease sequence, and a destabilizing domain (DD) sequence. Exemplary features of mitochondrial targeting sequences, endonuclease and destabilizing domains that can be included in polypeptides of the disclosure are described in Sections 4.2.1, 4.2.2, and 4.2.3, respectively.

**[0054]** The MTS, endonuclease sequence, and DD can be positioned in any suitable N-terminal to C-terminal order. For example, the MTS can be positioned at the N-terminal or C-terminal end of the polypeptide. In some embodiments, the MTS is positioned at the N-terminal end of the polypeptide. The endonuclease sequence can be positioned N-terminal to the DD, or C-terminal to the DD. In some embodiments, the polypeptide comprises, in N-terminal to C-terminal order, the MTS, the endonuclease sequence, and the DD sequence. The MTS, endonuclease sequence and the DD sequence can be directly linked, or can be separated by a spacer sequence, for example, a short amino acid sequence, for example of one, two, three, four or more amino acids.

#### **4.2.1. Mitochondrial Targeting Sequences**

**[0055]** Mitochondria have about 1500 proteins encoded by the nuclear genome. They are translated in the cytosol, and imported to the mitochondrial inner or outer membrane, intermembrane space, or matrix, depending upon the MTS. Polypeptides of the disclosure can include a full length MTS of a mitochondrial protein, or a variant of a wild-type MTS (e.g., a truncated version of a full-length MTS and/or an MTS with one or more amino acid substitutions compared to a wild-type sequence, for example one or more conservative amino acid substitutions).

**[0056]** Polypeptides of the disclosure can include a human MTS or a non-human MTS (e.g., rodent such as mouse or rat or non-human primate such as cynomolgus monkey). For example, an MTS of a polypeptide of the disclosure can comprise an MTS of a TCA cycle-related enzyme, a chaperone protein, a mitochondrial genome replication protein, a protease, an mRNA processing protein, a mitochondrial RNA degradation protein, a deoxynucleotide triphosphate synthesis-related protein, a mitochondrial protein, a phospholipid metabolism-related protein, a protein involved in metabolism of toxic compounds, a disulfide relay system-related protein, an iron-sulfur protein assembly protein, a tRNA modification protein, an aminoacyl-tRNA synthetase, a release factor, or an elongation factor.

**[0057]** In some embodiments, the MTS comprises an MTS of a cytochrome c oxidase subunit (for example, either a full-length MTS or a truncated version thereof which retains mitochondrial targeting activity), for example cytochrome c oxidase subunit VIII (COX8), cytochrome c oxidase subunit X (COX10), or cytochrome c oxidase subunit IV (COX4).

**[0058]** In some embodiments, the MTS comprises an MTS of a frataxin (FXN) protein.

**[0059]** In some embodiments, the MTS comprises an MTS of a TCA cycle-related enzyme, for example, Pyruvate dehydrogenase, Citrate synthase, Aconitase, Isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, Succinyl-CoA synthetase, Succinic dehydrogenase, Fumarase, Malate dehydrogenase, or Pyruvate carboxylase.

- [0060] In other embodiments, the MTS comprises an MTS of a chaperone protein, for example, mtHSP10, mtHSP60, mtHSP70, or mtHSP90.
- [0061] In other embodiments, the MTS comprises an MTS of a mitochondrial genome replication protein, for example, TFAM, Twinkle, PolG, TFB2M, TEFM, or MTERF1.
- [0062] In other embodiments, the MTS comprises an MTS of a protease, for example, MPP, CLPXP, LON ATPase, or PreP.
- [0063] In other embodiments, the MTS comprises an MTS of an mRNA processing protein, for example, LRPPRC, TACO1, ELAC2, PNPT1, HSD17B10, MTPAP, or PTC1.
- [0064] In other embodiments, the MTS comprises an MTS of a mitochondrial RNA degradation protein, for example, PNPase, REX02, or SUV3.
- [0065] In other embodiments, the MTS comprises an MTS of a deoxynucleotide triphosphate synthesis-related protein, for example, DGUOK, TK2, TYMP, MGME1, SUCLG1, SUCLA2, RNASEH1, or C10orf2.
- [0066] In other embodiments, the MTS comprises an MTS of a mitochondrial protein, for example, MRPS16, MRPS22, MRPL3, MRPL12, or MRPL44.
- [0067] In other embodiments, the MTS comprises an MTS of a phospholipid metabolism-related protein, for example, AGK, SERAC1, or TAZ.
- [0068] In other embodiments, the MTS comprises an MTS of a protein involved in metabolism of toxic compounds, for example, HIBCH, ECHS1, ETHE1 or MPV17.
- [0069] In other embodiments, the MTS comprises an MTS of a disulfide relay system-related protein, for example, GFER.
- [0070] In other embodiments, the MTS comprises an MTS of an iron-sulfur protein assembly protein, for example, ISCU, BOLA3, NFU1, or IBA57.
- [0071] In other embodiments, the MTS comprises an MTS of a tRNA modification protein, for example, MTO1, GTP3BP, TRMU, PUS1, MTFMT, TRIT1, TRNT1 or TRMT5.
- [0072] In other embodiments, the MTS comprises an MTS of an aminoacyl-tRNA synthetase, for example, AARS2, DARS2, EARS2, RARS2, YARS2, FARS2, HARS2, LARS2, VARS2, TARS2, IARS2, CARS2, PARS2, NARS2, KARS, GARS, SARS2 or MARS2.
- [0073] In other embodiments, the MTS comprises an MTS of an elongation factor, for example, TUFM, TSFM, or GFM1.
- [0074] Exemplary mitochondrial targeting sequences are set forth in Table 1.

|                                                |
|------------------------------------------------|
| <p><b>TABLE 1</b><br/><b>MTS Sequences</b></p> |
|------------------------------------------------|

| Description               | Sequence                              | SEQ ID NO |
|---------------------------|---------------------------------------|-----------|
| COX8 MTS<br>(human short) | MSVLTPLLLRGLTGSARR                    | 1         |
| COX8 MTS<br>(human long)  | MSVLTPLLLRGLTGSARRLPVPRAKIHSL         | 2         |
| COX8 MTS<br>(mouse)       | MSVLTPLLLRSLTGSARRLMVPRA              | 3         |
| COX10 MTS                 | MAASPHLTSSRLTGCVGGSVWYLERRT           | 4         |
| FXN MTS                   | MWTLGRRVAGLLASPSAQ                    | 5         |
| ACO2 MTS                  | MAPYSLLVTRLQKALG                      | 6         |
| CS MTS                    | MALLTAAARLLGTKNASCLVLAARHASA          | 7         |
| TXN MTS                   | MVKQIESKTAFQEALDAAGDKLVVDFSATWC       | 8         |
| GSS MTS                   | MATNWGSLLQDKQQLEELARQAVDRALAEGVLLRTSQ | 9         |
| TFAM MTS                  | MAFLRSMWGVLSALGRSGA                   | 10        |
| TWINK MTS                 | MWVLLRSGYPLRILLPLRG                   | 11        |
| POLG MTS                  | MSRLLWRKVAGATVGPGVPAPG                | 12        |
| PMPCA<br>MTS              | MKRNTLVELLTFWKNWHFRLL                 | 13        |
| HSPA9 MTS                 | MISASRAAAARLVGAAASRGPTAA              | 14        |
| DNM1L MTS                 | MEALIPVINKLQDVFNTVGA                  | 15        |

**[0075]** A polypeptide of the disclosure can include an MTS identified in Table 1 or a variant thereof (for example, an MTS having one or more conservative amino acid substitutions and/or a truncation). A truncation can be a truncation of the C-terminal sequence (for example, an MTS can correspond to a sequence set forth in Table 1, but with a C-terminal truncation of one or more amino acids, e.g., one, two, three, four, five or more than five amino acids). In some embodiments, an MTS comprises at least 15 N-terminal amino acids of a MTS sequence set forth in Table 1. A variant MTS can include, for example, an MTS that is at least 80%, at least 95%, at least 90%, or at least 95% identical to an MTS listed in Table 1.

**[0076]** Those of skill in the art will appreciate that additional mitochondrial targeting sequences beyond those identified in this section can be used. Various tools for predicting MTS can be used to identify additional mitochondrial targeting sequences, including SignalP (Bendtsen *et al.*, 2004, J. Mol. Biol. 340:783-795; Teufel *et al.*, 2022 Nat Biotechnol. doi.org/10.1038/s41587-021-01156-3), MitoFates (Fukasawa *et al.*, 2015 Mol Cell Proteomics 14(4):1113-1126), and MitoProt (Claros, 1995, Comput Apl Biosci. 11(4):441-7).

#### 4.2.2. Endonuclease

**[0077]** Various endonucleases can be used in the polypeptides of the disclosure. For example, the endonuclease can be a restriction endonuclease, an RNA-guided endonuclease (e.g., Cas9 or Cas12), a zinc finger nuclease, or a transcription activator-like effector nuclease (TALEN). The endonuclease can comprise a catalytic domain (for example, from a wild-type or

engineered endonuclease) and, optionally one or more additional domains, for example all domains present in a full-length wild-type or engineered endonuclease.

**[0078]** Endonucleases can be bacterial in origin. Many restriction enzymes are known in the art and include, for example, XbaIR, EcoRI, SmaI, AflII, BamHI, BclI, HaeIII, HindII, HindIII, NdeI, PvuII, PstI, and SpeI.

**[0079]** In some embodiments, the endonuclease is XbaIR. An exemplary XbaIR sequence is set forth in SEQ ID NO:16:

MTTLEKIKLLADGYADRLKLAIDGRVLEMQGDDVSHYLIYRVLGVAQEEGRLIDVYQNKG RFLY  
KYAGSFLEAATKLCFKEAFDPSASLRPNLTQGQRPTVEIDCLVGNDAL EIKWKDATTDGDHIT  
KEHTRIKVISDAGYKPIRIMFYYPHRTQAIRIQETLETLYNGVHGEYHYGEAAWDYVLQRTSVNL  
KVALEQIADSR TNEAA (SEQ ID NO:16).

**[0080]** In some embodiments, the endonuclease comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, more than 95%, or 100% identity with SEQ ID NO:16.

**[0081]** In some embodiments, the endonuclease is EcoRI.

**[0082]** In some embodiments, the endonuclease is SmaI.

**[0083]** In some embodiments, the endonuclease is AflII.

**[0084]** In some embodiments, the endonuclease is BamHI.

**[0085]** In some embodiments, the endonuclease is BclI.

**[0086]** In some embodiments, the endonuclease is HaeIII.

**[0087]** In some embodiments, the endonuclease is HindII.

**[0088]** In some embodiments, the endonuclease is HindIII.

**[0089]** In some embodiments, the endonuclease is NdeI.

**[0090]** In some embodiments, the endonuclease is PvuII.

**[0091]** In some embodiments, the endonuclease is PstI.

**[0092]** In some embodiments, the endonuclease is SpeI.

**[0093]** In some embodiments, the endonuclease sequence is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to the amino acid sequence of: UniProt accession number O68567, UniProt accession number P00642, UniProt accession number P14229, UniProt accession number E3VX87, UniProt accession number P23940, UniProt accession number E5LGB8, UniProt accession number O68584, UniProt accession number P44413,

UniProt accession number P43870, UniProt accession number A0A4R7BM34, UniProt accession number P00640, or UniProt accession number F1KM35.

**[0094]** Exemplary RNA-guided endonucleases such as Cas9 and Cas12 are described in US 11,001,863 B2, WO 2014/093661 and WO 2019/233990, the contents of which are incorporated herein by reference in their entireties. In some embodiments, the endonuclease is SaCas9 or SpCas9. When an RNA-guided endonuclease is used, the polypeptide can be used in combination with one or more guide RNA molecules targeting mitochondrial DNA.

**[0095]** Exemplary Zinc finger nucleases are described in WO 2001/025255, and WO 2003/066828, the contents of which are incorporated herein by reference in their entireties.

**[0096]** Exemplary TALEN nucleases are described in WO 2014/134412, WO 2015/013583, and WO 2013/163628, the contents of which are incorporated herein by reference in their entireties.

#### 4.2.3. Destabilizing Domain

**[0097]** The polypeptides of the disclosure include a destabilization domain (DD), which allows for ON/OFF control of the endonuclease. Exemplary DDs include DHFR, FKBP, and PDE5 DDs.

**[0098]** Exemplary DHFR DDs are described in US 9,487,787, the contents of which are incorporated herein in their entirety. An amino acid sequence of wild-type *E coli* DHFR is as follows:

MISLIAALAVDGHVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNIILSS  
QPSTDDRVTWKSVDIAACGDVPEIMVIGGGRVYEQLPKAQKLYLTHIDAEVEGDTHFPD  
YEPDDWESVFSEFHDADAQNSHSYCFEILERR (SEQ ID NO:17)

**[0099]** A DHFR DD can comprise a wild-type DHFR sequence or can comprise one or more amino acid substitutions and/or truncations at the N and/or C terminal end. For example, a DHFR DD sequence can be at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:17. Exemplary amino acid substitutions and combinations that can be included in a DHFR DD include Y100I, G121V, N18T/A19V, F103L, H12Y/Y100I, H12L/Y100I, R98H/F103S, M42T/H114R, I61F/T68S. Combinations of the foregoing substitutions can also be used. In some embodiments, the DHFR comprises an amino acid sequence which is identical to SEQ ID NO:17 except for a Y100I, G121V, N18T/A19V, F103L, H12Y/Y100I, H12L/Y100I, R98H/F103S, M42T/H114R, or I61F/T68S substitution(s), or a combination thereof. In some embodiments, a DHFR DD lacks an N-terminal methionine. For example, in some embodiments, the DHFR comprises an amino acid sequence which is identical to SEQ ID NO:17 except for a Y100I, G121V, N18T/A19V, F103L, H12Y/Y100I, H12L/Y100I,

R98H/F103S, M42T/H114R, or I61F/T68S substitution(s), or a combination thereof, and lack of the N-terminal methionine.

**[0100]** In some embodiments, a DHFR DD has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to

ISLIAALAVDHHVIGMETVMPWNLPAFLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNILSSQP  
STDDRVTWVKSVDEAIAACGDVPEIMVIGGGRVYEQLPKAQKLYLTHIDAEVEGDTHFPDYEP  
DDWESVFSEFHDADAQNSHSYCFEILERR (SEQ ID NO:18). An exemplary nucleotide  
sequence encoding SEQ ID NO:18 is the following:

atcagtctgattgcggcgtagcggtagatcacgttatcgcatggaaaccgtcatgccgtggaacctgcctgccgatctgcctggtt  
aaacgcaacaccttaataaaccgtgattatggccgccatacctgggaatcaatcggtcgtccgttgccaggacgcaaaaatatt  
atcctcagcagtaaccgagtagcgatcggtacgtgggtgaagtcggtggaagccatcgccggtgtggtgacgtacc  
agaaatcatggtattggcggcggtcggttatgaacagttctgccaaaagcgcaaaaactgtatctgacgcatacgacgcagaa  
gtggaaggcgacacccattcccgattacgagccggtgactgggaatcggtattcagcgaattccacgatgctgatgacgcagaa  
ctctcacagctattgcttgagattctggagcgcgataa (SEQ ID NO:19).

**[0101]** An exemplary stabilizing agent for a DHFR DD is trimethoprim.

**[0102]** Exemplary FKBP DDs are described in US 9,487,787, the contents of which are incorporated herein in their entirety. An amino acid sequence of an exemplary FKBP DD (having a F36V substitution compared to the wild-type sequence) is as follows:

GVQVETISPGDGRTPFKRGQTCVVHYTGMLDGKKVDSSRDRNKPFFMLGKQEVIRGWEE  
GVAQMSVGRRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO:20)

**[0103]** A FKBP DD can comprise a wild-type FKBP sequence or can comprise one or more amino acid substitutions. For example, a FKBP DD sequence can be at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to SEQ ID NO:20. Exemplary amino acid substitutions that can be included in a FKBP DD include F15S, V24A, H25R, E60G, L106P, D100G, M66T, R71G, D100N, E102G, and K105I. Combinations of the foregoing substitutions can also be used. In some embodiments, the DD comprises an amino acid sequence which is identical to SEQ ID NO:20 except for a F15S, V24A, H25R, E60G, L106P, D100G, M66T, R71G, D100N, E102G, or K105I substitution, or a combination thereof.

**[0104]** Exemplary FKBP DD stabilizing agents include Shield-1 (Shld1), rapamycin and FK506.

**[0105]** Exemplary PDE5 DDs are described in WO 2018/237323, the contents of which are incorporated herein in their entirety. PDE5 DDs may be derived from PDE5A, Isoform 1 (SEQ ID NO:21); PDE5A Isoform 2 (SEQ ID NO:22) and/or PDE5A Isoform 3 (SEQ ID NO:23). These isoforms differ at their N terminal regions and have unique first exons followed by a common sequence of 823 amino acids.

[0106] All PDE5A isoforms contain a catalytic domain that is located near the C terminus of the protein and is relatively selective for cGMP as a substrate at physiological levels. The substrate binding site is also the binding site for several known PDE5 inhibitors such as sildenafil, which have been utilized to treat cardiovascular diseases and erectile dysfunction. Towards the N terminus, two homologous GAF domains are located. One of the GAF domains, GAF-A contains a high affinity binding site for cGMP. Occupancy of this domain by cGMP is known to cause activation of the catalytic domain. Moreover, the affinity of this site for cGMP is increased by cGMP-dependent protein kinase-mediated phosphorylation of serine 92. In another embodiment, a PDE5A DD can comprise the catalytic domain of PDE5A, spanning from amino acid position 535 to position 860 of UniProt ID: O76074 (SEQ ID NO:21), as represented in SEQ ID NO:24. In addition to the catalytic domain, PDE5A DDs may also comprise one or more GAF domains and/or the C terminal portion that extends beyond the catalytic domain. In one embodiment, the PDE5A derived DD comprises amino acids from position 535 to position 875 of SEQ ID NO:21. In another embodiment, the PDE5 DD comprises amino acids from position 466 to 875 or position 420 to 875 of SEQ ID NO:21. Exemplary PDE5 DD sequences are set forth in Table 2.

| TABLE 2         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |            |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| PDE5 DD         | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | SEQ ID NO: |
| PDE5A Isoform 1 | MERAGPSFGQQRQQQPPQQKQQQRDQDSVEAWLDDHWDFTFSYFVRKATREM<br>VNAWFAERVHTIPVCKEGIRGHTESSCPLQQSPRADNSAPGTPTRKISASEFDRPL<br>RPIVVKDSEGTVSFLSDSEKKEQMPLTPPRFDHDEGDQCSRLLELVKDISHLDVTAL<br>CHKIFLHIHGLISADRYSLFLVCESSNDKFLISRLFDVAEGSTLEEVSNNCIRLEWNK<br>GIVGHVAALGEPLNIKDAYEDPRFNAEVDQITGYKTSILCMPIKNHREEVVGVQAQAIN<br>KKSGNGGTFTEKDEKDFAAAYLAFCGIVLHNAQLYETSLLENKRNQVLLDLASLIFEEQ<br>QSLEVILKKIAATISFMQVQKCTIFIVDEDCSDSFSSVFHMECEELEKSSDTLTREHDA<br>NKINYMYAQYVKNTMEPLNIPDVSKDKRFPWTENTGNVNQQCIRSLCTPIKNGKK<br>NKVIGVCQLVNKMEENTGKVKPFNRNDEQFLEAFVIFCGLGIQNTQMYEAVERAMAK<br>QMVLTLEVLSYHASAAEEETRELQSLAAAVVPSAQLTKITDFSFSDFELSDLETALCTIR<br>MFTDLNLVQNFQMKHEVLCRWILSVKKNYRKNVAYHNWRHAFNTAQCMFAALKAG<br>KIQNKLTDLAILALLIALSHDLDRGVNNSYIQRSEHPLAQLYCHSIMEHHHFDQCLM<br>ILNSPGNQILSGLSIEEYKTTLKIQQAILATDLALYIKRRGEFFELIRKNQFNLEDPHQKE<br>LFLAMLMTACDLSAITKPWPIQQRIAEVATEFFDQGDREKELNIEPTDLMNREKKN<br>KIPSMQVGFIDAICLQLYEALTHVSEDCFPLLDGCRKNRQKWQALAEQKEKMLINGE<br>SGQAKRN | 21         |
| PDE5A Isoform 2 | MLPFGDKTREMVNAWFAERVHTIPVCKEGIRGHTESSCPLQQSPRADNSAPGTPT<br>RKISASEFDRPLRPIVVKDSEGTVSFLSDSEKKEQMPLTPPRFDHDEGDQCSRLLELV<br>KDISHLDVTALCHKIFLHIHGLISADRYSLFLVCESSNDKFLISRLFDVAEGSTLEEV<br>SNNCIRLEWNKGIVGHVAALGEPLNIKDAYEDPRFNAEVDQITGYKTSILCMPIKNH                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 22         |

| TABLE 2                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |            |
|---------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| PDE5 DD                                                                                                                         | Amino Acid Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | SEQ ID NO: |
|                                                                                                                                 | REEVVGVAQAINKKSGNGGTFTEKDEKDFAAYLAFCGIVLHNAQLYETSLENKRNQ<br>VLLDLASLIFEQQSLEVLKKIAATIISFMQVQKCTIFIVDEDCSDSFSSVFHMECEELE<br>KSSDTLTREHDANKINYMYAQYVKNTMEPLNIPDVSKDKRFPWTTENTGNVNQQCIR<br>SLLCTPIKNGKKNKVIGVCQLVNKMEENTGKVKPFNRNDEQFLEAFVIFCGLGIQNTQ<br>MYEAVERAMAKQMVLTLEVLSYHASAAEEETRELQSLAAAVVPSAQT KITDFSFSDF<br>ELSDLETALCTIRMFTDLNLVQNFQMKHEVLCRWILSVKKNYRKNVAYHNWRHAFNT<br>AQCMFAALKAGKIQNKLTDEILALLIAALSHDLDRGVNNSYIQRSEHPLAQLYCHSI<br>MEHHHFDQCLMILNSPGNQILSGLSIEEYKTTLKIIKQAILATDLALYIKRRGEFFELIRK<br>NQFNLEDPHQKELFLAMLTACDLSAITKPWPIQQRIAELVATEFFDQGDREKELNIEPT<br>DLMNREKKNKIPSMQVGFIDAICLQLYEALTHVSEDCFPLLDGCRKNRQKWQALAEQQ<br>EKMILINGESGQAKRN                                                                                                                                                                                                                                               |            |
| PDE5A<br>Isoform 3                                                                                                              | MVNAWFAERVHTIPVCKEGIRGHTESCSCPLQQSPRADNSAPGTPTRKISASEFDRP<br>LRPIVVKDSEGTVSFLSDSEKKEQMPLTPPRFDHDEGDQCSRLELVKDISSHLDVTA<br>LCHKIFLHHGLISADRYSLFLVCEDSSNDKFLISRLFDVAEGSTLEEVSNNCIRLEWVK<br>GIVGHVAALGEPLNIKDAYEDPRFNAEVDQITGYKTSILCMTPIKNHREEVVGVAQAIN<br>KKSGNGGTFTEKDEKDFAAYLAFCGIVLHNAQLYETSLENKRNQVLLDLASLIFEQQ<br>QSLEVLKKIAATIISFMQVQKCTIFIVDEDCSDSFSSVFHMECEELEKSSDTLTREHDA<br>NKINYMYAQYVKNTMEPLNIPDVSKDKRFPWTTENTGNVNQQCIR SLLCTPIKNGKK<br>NKVIGVCQLVNKMEENTGKVKPFNRNDEQFLEAFVIFCGLGIQNTQMYEAVERAMAK<br>QMVLTLEVLSYHASAAEEETRELQSLAAAVVPSAQT KITDFSFSDFELSDLETALCTIR<br>MFTDLNLVQNFQMKHEVLCRWILSVKKNYRKNVAYHNWRHAFNTAQCMFAALKAG<br>KIQNKLTDEILALLIAALSHDLDRGVNNSYIQRSEHPLAQLYCHSIMEHHHFDQCLM<br>ILNSPGNQILSGLSIEEYKTTLKIIKQAILATDLALYIKRRGEFFELIRKNQFNLEDPHQKE<br>LFLAMLTACDLSAITKPWPIQQRIAELVATEFFDQGDREKELNIEPTDLMNREKKN<br>KIPSMQVGFIDAICLQLYEALTHVSEDCFPLLDGCRKNRQKWQALAEQQEKMILINGE<br>SGQAKRN | 23         |
| Catalytic<br>domain of<br>PDE5A<br>(position<br>535 to<br>position<br>860 of<br>UniProt<br>ID:<br>O76074<br>(SEQ ID<br>NO. 21)) | EETRELQSLAAAVVPSAQT KITDFSFSDFELSDLETALCTIRMFTDLNLVQNFQMKH<br>EVLCDRWILSVKKNYRKNVAYHNWRHAFNTAQCMFAALKAGKIQNKLTDEILALLIAA<br>LSHDLDRGVNNSYIQRSEHPLAQLYCHSIMEHHHFDQCLMILNSPGNQILSGLSIEE<br>YKTTLKIIKQAILATDLALYIKRRGEFFELIRKNQFNLEDPHQKELFLAMLTACDLSAIT<br>KPWPIQQRIAELVATEFFDQGDREKELNIEPTDLMNREKKNKIPSMQVGFIDAICLQ<br>LYEALTHVSEDCFPLLDGCRKNRQKWQALAEQQ                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 24         |

**[0107]** Exemplary amino acid substitutions that can be included in PDE5 DDs include one or more amino acid substitutions selected from E535D, E536G, Q541R, K555R, F559L S560G, F561L, F564L, F564S, V585A, N587S, K591E, I599V, K604E, K608E, N609H, K630R, K633E, N636S, I648V, N661S, S663P, L675P, Y676D, Y676N, C677R, H678R, D687A, T711A, T712S, D724N, L738H, N742S, F744L, L746S, F755L, A762S, D764V, D764N, D764G, S766F, K795E, L797F, I799T, L804P, T802P, S815C, M816A, M816T, I824T, C839S, F840S, and K852E. The PDE5 DDs can also contain additional substitutions such as Q589R. In some embodiments, a PDE5 DD sequence comprises a sequence selected from the group of amino acid sequences identified by SEQ ID NOs. 19-35 of WO 2018/237323 and SEQ ID NOs.66-69 of WO 2018/237323.

**[0108]** Exemplary stabilizing agents for PDE5 DDs include sildenafil, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil, benzamidenafil, dasantafil, and beminafil.

#### **4.3. Nucleic acids, particles and host cells**

**[0109]** In another aspect, the disclosure provides nucleic acids encoding a polypeptide of the disclosure, *e.g.*, as described in Section 4.2. The nucleic acid can be, for example, a vector such as a viral genome or a plasmid, or an mRNA molecule.

**[0110]** Exemplary vectors include viral expression vectors (*e.g.*, viral vectors based on vaccinia virus; poliovirus; adenovirus (see, *e.g.*, Li *et al.*, 1994, Invest Ophthalmol Vis Sci 35:2543-2549; Borrás *et al.*, 1999, Gene Ther 6:515-524; Li and Davidson, 1995, PNAS 92:7700-7704; Sakamoto *et al.*, 1999, Hum Gene Ther 5:1088-1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (AAV) (see, *e.g.*, Ali *et al.*, 1998, Hum Gene Ther 9:81-86; Flannery *et al.*, 1997, PNAS 94:6916-6921; Bennett *et al.*, 1997, Invest Ophthalmol Vis Sci 38:2857-2863; Jomary *et al.*, 1997, Gene Ther 4:683-690; Rolling *et al.*, 1999, Hum Gene Ther 10:641-648; Ali *et al.*, 1996, Hum Mol Genet 5:591-594; WO 93/09239); SV40; herpes simplex virus; human immunodeficiency virus (see, *e.g.*, Miyoshi *et al.*, 1997, PNAS 94:10319-23; Takahashi *et al.*, 1999, J Virol 73:7812-7816); a retroviral vector (*e.g.*, Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like. In some cases, a recombinant expression vector of the present disclosure is a recombinant lentivirus vector. In some cases, a recombinant expression vector of the present disclosure is a recombinant retroviral vector.

[0111] In some embodiments, the vector comprises a retroviral genome. Nucleic acids, such as retroviral genomes, can be provided in the form of a particle, for example a viral particle (*e.g.*, retroviral particle).

[0112] Nucleic acids encoding a polypeptide of the disclosure can further include one or more regulatory sequences, for example a promoter, for example a SV40, CMV, or CAG promoter. An exemplary SV40 promoter sequence is as follows:

GTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAG  
CATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAG  
AAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCC  
ATCCCGCCCCCTAACTCCGCCCAGTTCGCCCCATTCTCCGCCCCATGGCTGACTAATTTTTT  
TTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAG  
GCTTTTTTGGAGGCCTAGGCTTTTGCAA (SEQ ID NO:25).

[0113] In another aspect, the disclosure provides host cells comprising a nucleic acid of the disclosure. Host cells can be prokaryotic (*e.g.*, bacterial such as *E. coli*) or eukaryotic (*e.g.*, a human cell line such as HEK293 or 293T). Host cells can be used, for example, to propagate a nucleic acid such as a retroviral genome or plasmid, or to propagate and package a particle, for example a retroviral particle.

#### 4.4. Methods of inducing mitophagy and methods of treatment

[0114] In further aspects, the disclosure provides methods of inducing mitophagy in a cell, and/or increasing mitochondrial turnover in a cell, and/or increasing mitochondrial mass, and/or inducing double strand breaks in mitochondrial DNA and/or (e) inducing epigenomic modifications in a cell using the polypeptides, nucleic acids, and particles of the disclosure, *e.g.*, polypeptides, nucleic acids, and particles as described in Sections 4.2 and 4.3.

[0115] The methods typically comprise contacting a cell with a polypeptide, nucleic acid, or particle and a stabilizing agent capable of stabilizing the DD. A polypeptide can be introduced to the cell by electroporation, injection, or a carrier (*e.g.*, lipid-based carrier such as a liposome), or any other means known in the art for delivering polypeptides to cells. A nucleic acid can be introduced to a cell by transfection, electroporation, injection, a carrier, or any other means known in the art for delivering nucleic acids to cells. A viral particle can be introduced to a cell by transduction.

[0116] The cell can be contacted with a stabilizing agent, for example, by culturing the cell in a medium comprising the stabilizing agent. The cell can be cultured in the medium with the stabilizing agent for a period of time to allow the endonuclease to introduce DSBs in mitochondrial DNA. In some embodiments, the cell is cultured in a medium with the stabilizing agent for at least 8 hours (*e.g.*, at least 12 hours, at least 1 day, at least 2 days, or more) and/or

up to 5 days (e.g., up to 4 days, up to 3 days, or up to 2 days). Subsequently, the stabilizing agent can be removed, for example by culturing the cell in a culture medium without the stabilizing agent. Once the stabilizing agent is removed, the polypeptide will be destabilized, leading to degradation of the polypeptide.

**[0117]** Following removal of the stabilizing agent, the cell can be cultured for a period of time without the stabilizing agent, during which the cell can produce new mitochondria. In some embodiments, the cell is cultured for at least 6 hours in a medium that does not include the stabilizing agent (e.g., at least 12 hours, at least 1 day, at least 2 days, at least 3 days, at least 4 days) and/or up to 10 days (e.g., up to 8 days, up to 6 days, or up to 4 days).

**[0118]** In some embodiments, the methods of the disclosure result in the induction of mitophagy in the cell. In some embodiments, the methods of the disclosure result in an increase in mitochondrial turnover in the cell. In some embodiments, the methods of the disclosure result in an increase in mitochondrial mass in the cell. In some embodiments, the methods result in an induction of DSBs in mitochondrial DNA in the cell. In some embodiments, the methods result in epigenomic modifications in the cell, e.g., induced by mitochondrial depletion. It has been previously reported that rho0 cells, which are completely depleted of mitochondrial genome, show significant levels of epigenomic changes (see, e.g., Hertzog Santos, 2021 Free Radic Biol Med. 170:69-69). Thus, it is believed that compositions of the disclosure can be used to induce epigenomic modifications. In some embodiments, the methods of the disclosure result in one, two, three, four, or all five of the following in the cell: (a) induction of mitophagy, (b) increased mitochondrial turnover, (c) increased mitochondrial mass, (d) DSBs in mitochondrial DNA, and (e) epigenomic modifications.

**[0119]** Exemplary cells that can be used in the methods include mammalian cells, preferably human cells, more preferably human somatic cells. Cell types that can be used include bone marrow cells, stem cells such as hematopoietic stem cells (HSCs) or a mesenchymal stem cells (MSCs), immune cells such as T cells, phagocytes, microglial cells, and macrophages. In some embodiment, the cell is a T cell such as a CD4+ and/or CD8+ T cell. Primary cells obtained from a subject, as well as progeny thereof can be used.

**[0120]** The cells be normal cells (e.g., from a healthy donor) or have dysfunctional mitochondria (e.g., from a subject having a disease or disorder). For example, cells can be from a subject having an age-related disease or disorder, such as an autoimmune disease, a metabolic disease, a genetic disease, cancer, a neurodegenerative disease, or immunosenescence.

**[0121]** As another example, cells can be from a subject having a mitochondrial disease or disorder such as chronic progressive external ophthalmoplegia (CPEO), Pearson syndrome, Kearns-Sayre syndrome (KSS), diabetes and deafness (DAD), mitochondrial diabetes, Leber hereditary optic neuropathy (LHON), LHON-plus, neuropathy, ataxia, retinitis pigmentosa

syndrome (NARP), maternally inherited Leigh syndrome (MILS), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged-red fiber disease (MERRF), familial bilateral striatal necrosis/striatonigral degeneration (FBSN), Luft disease, aminoglycoside-induced Deafness (AID), or multiple deletions of mitochondrial DNA syndrome. Additional mitochondrial diseases and disorders include Mitochondrial DNA depletion syndrome-4A, mitochondrial recessive ataxia syndrome (MIRAS), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), mitochondrial DNA depletion syndrome (MTDPS), DNA polymerase gamma (POLG)-related disorders, sensory ataxia neuropathy dysarthria ophthalmoplegia (SANDO), leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), co-enzyme Q10 deficiency, Leigh syndrome, mitochondrial complex abnormalities, fumarase deficiency,  $\alpha$ -ketoglutarate dehydrogenase complex (KGDHC) deficiency, succinyl-CoA ligase deficiency, pyruvate dehydrogenase complex deficiency (PDHC), pyruvate carboxylase deficiency (PCD), carnitine palmitoyltransferase I (CPT I) deficiency, carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acyl-carnitine (CACT) deficiency, autosomal dominant-/ autosomal recessive-progressive external ophthalmoplegia (ad-/ar-PEO), infantile onset spinal cerebellar atrophy (IOSCA), mitochondrial myopathy (MM) spinal muscular atrophy (SMA), growth retardation, aminoaciduria, cholestasis, iron overload, early death (GRACILE), and Charcot-Marie-Tooth disease type 2 A (CMT2A).

**[0122]** As another example, cells can be from a subject having a neurodegenerative disease such as amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, Charcot Marie Tooth disease or leukodystrophy. In some embodiments, cells are from a subject having Alzheimer's disease, for example a subject having an APOE4 allele, e.g., an E3/E4 or E4/E4 genotype.

**[0123]** As yet another example, cells can be from a subject having an eye disease (e.g., a retinal disease) such as age-related macular degeneration, macular edema or glaucoma.

**[0124]** In yet further examples, cells can be from a subject having diabetes, a hearing disorder, a genetic disease (such as Hutchinson-Gilford Progeria Syndrome, Werner Syndrome, or Huntington's disease), heart failure, immunodeficiency, cancer, or an infectious disease.

**[0125]** Cells obtained or obtainable by the methods described herein can be administered to a subject, for example, administered to the subject from which the cells originate or in the case of cells from a healthy donor, administered to a different subject.

**[0126]** Accordingly, in another aspect, the disclosure provides a method of treating a subject having an age-related disease, mitochondrial disease or disorder, neurodegenerative disease, retinal disease, diabetes, hearing disorder, genetic disease, heart failure, immunodeficiency, cancer, or infectious disease by administering a therapeutically effective amount of cells

obtained or obtainable by the methods described herein. For example, the subject can have a disease or disorder described in this Section.

#### **4.5. Pharmaceutical compositions and kits**

**[0100]** In another aspect, the disclosure provides a pharmaceutical composition comprising a polypeptide of the disclosure (e.g., as described in Section 4.2), a nucleic acid of the disclosure, (e.g., as described in Section 4.3), a particle of the disclosure (e.g., as described in Section 4.3, or a cell of the disclosure (e.g., a obtained by a method described in 4.4) and a pharmaceutically acceptable excipient. For example, pharmaceutical compositions can be prepared by mixing a polypeptide, nucleic acid, particle, or cell with one or more physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., aqueous solutions or suspensions (see, e.g., Hardman *et al.*, 2001, Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro, 2000, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y.; Weiner and Kotkoskie, 2000, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.).

**[0127]** In another aspect, the disclosure provides kits comprising a polypeptide of the disclosure (e.g., as described in Section 4.2), a nucleic acid of the disclosure, (e.g., as described in Section 4.3), or a particle of the disclosure and a stabilizing agent. For example, the kit can include trimethoprim (TMP) when the DD sequence of the polypeptide is a DHFR DD sequence, Shield-1, rapamycin, or FK506 when the DD sequence of the polypeptide is a FKBP DD sequence, or sildenafil, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil, benzamidenafil, dasantafil, or beminafil when the DD sequence of the polypeptide is a PDE5 DD sequence.

### **5. EXAMPLES**

#### **5.1. Example 1: Polypeptides for inducing mitophagy, biogenesis, and acceleration of mitochondria turnover**

This Example describes compositions and methods for simultaneously activating not only mitophagy, but also biogenesis, and for accelerating mitochondria turnover by eliminating dysfunctional mitochondria and generating new mitochondria.

##### **5.1.1. Design of Transgenes**

**[0128]** XbaIR was selected to induce DSBs in the mitochondrial genome. XbaIR has five cleavage sites in the mitochondrial genome consensus sequence. To direct the XbaIR endonuclease to the mitochondria, a Cox8a post-mitochondrial signal was placed on the N-terminal side of XbaIR. Inducing DSBs in the mitochondrial genome leads to a strong temporary energy depletion and, to control endonuclease activity, a DHFR destabilization domain (Liu *et al.*, 2014 Int. J. Parasitol. 44(10):729-735) was fused to the C-terminal end of XbaIR to allow

sensitive ON/OFF control of the endonuclease. The DHFR destabilization domain is stabilized by the antibiotic trimethoprim (TMP).

[0129] A retroviral vector having the MTS-XbaIR-DHFR coding sequences was constructed (FIG. 1). The nucleotide sequence of the vector is shown in Table 3.

| TABLE 3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pMXs-puro-MTS-XbaI-ecDHFR vector nucleotide sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Nucleotide sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| CCCGAAAAGTGCCACCTGCATAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCA<br>TTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAAAAGTTCAGATCAAGGTCAGGAACAGATGGAA<br>CAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCGGCTCAGGGCCAAAGAACAG<br>ATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCGGCTCAGGGCCAA<br>GAACAGATGGTCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGT<br>GCCCCAAGGACCTGAAATGACCCGTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTCTG<br>CGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCCAACCCCTCACTCGGCGCGCCAGTCCCTCCGATT<br>GACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGACGTTGCATCCGACTTGTGGTCTCGCT<br>GTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTGACGGGGGGTCTTTCATTTGGGGGCTCGT<br>CCGGGATCGGGAGACCCCTGCCAGGGACCCGACCCACCACGGGAGGTAAGCTGGCCAGCAACTT<br>ATCTGTGTCTGTCCGATTGTCTAGTGTCTATGACTGATTTTATGCGCCTGCGTCCGTACTAGTTAGCTAAC<br>TAGCTCTGTATCTGGCGGACCCGTGGTGAAGTACGAGTTCGGAACACCCGGCCGCAACCCCTGGGAG<br>ACGTCCCAGGGACTTCGGGGGCGGTTTTGTGGCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGAC<br>TCTTTGGTGACCCCCCTAATAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACAGTTCCC<br>GCCTCCGTCTGAATTTTGTCTTGGTTTGGGACCGAAGCCGCGCCGCGCTTGTCTGCTGCAGCAT<br>CGTTCTGTGTTGCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATTAGGGCCAGACTGTTACCACTC<br>CCTTAAGTTTGACCTTAGGTCACTGGAAGATGTGAGCGGATCGCTCACAACCAAGTCGGTAGATGTCAA<br>GAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTAACGTCGGATGGCCGCGAGACGG<br>CACCTTAACCGAGACCTCATCCCCAGGTTAAGATCAAGGTCTTTTACCTGGCCCGCATGGACACCCA<br>GACCAGTCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTACCCCCCTCCCTGGGTCAAGCCCTTT<br>GTACACCCTAAGCCTCCGCTCCTCTTCCCTCATCCGCCCGTCTCTCCCCCTGAACCTCCTCGTTTCA<br>CCCCGCTCGATCCTCCCTTATCCAGCCCTCACTCCTTCTAGGCGCCCCCATATGGCCATATGAGAT<br>CTTATATGGGGCACCCCGCCCCCTGTAAACTTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCC<br>TCTCTCCAAGCTCACTTACAGGCTCTCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGGCAGCCT<br>ACCAAGAACAAGTGGACCGACCGGTGGTACCTACCCCTACCGAGTCGGCGACACAGTGTGGGTCCGCC<br>GACACCAGACTAAGAACCTAGAACCTCGCTGGAAAGGACCTTACACAGTCTGCTGACCACCCCAACG<br>CCCTCAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAAGGCTGCCGACCCCGGGGGT<br>GGACCATCCTCTAGACTGCCGGATCTAGCTAGTTAATTAAGGATCCgcccaccatgctcgtcctgacgcccgtgctgctg<br>gggcttgacaggctcgcccgccgctccagtgccgcccgaagatccattcgttggttaacatgacaacctagaaaagatcaaattgctggcagat<br>gggtacgcggatcgctaaagctgctattgatggcgcttttagagatgcaaggatgacgtgtccactacatgctaccgtgttctcgagtcgcaca<br>agaagaaggctgctgattgacgtctatcaaaacaaggccgattcctctcaaatatgcgggatcattctggaggccgcaaaaagctgctcaaaag<br>aggctttccgattccgcatcgctaagggtaccgaacacgcagggtcaacgcccgcgaacagtcgagatcgattgcttgtaggcaacgacgcttggaa<br>attaagtggaaagatgccagcactgatggcgacatatacaaaagagcacactcgatcaaaagtcatttcagacgcccgtacaagcctattcgaataa<br>tgttactactccccatagaacgcaggcaattcggatatacaaaaactctgagaccctgtacaatggagttcatggtagtatcactacggcgaagcagcct<br>gggactacgtcttgcaacgcacctctgtaaacctcaaatagcactggaacaaattgctgattctcggaactaagcgaagctgcagcaatcagctgattgctg |

cgttagcggtagatcacgttatcgcatggaacccgtcatgccgtggaacctgctgccgatctcgctgglttaacgcaacaccttaataaaccctga  
ttatgggcccatacctgggaatcaatcggtcgtccgttgccaggacgcataatattatcctcagcagcaaccgagtagcgacgatcgctaaccgtg  
gtgaagtcggtgtagaagccatcgccggtgtggtgacgtaccagaaatcatggttatggcggcggtcggtttatgaacagttcttgcctaaagcgca  
aaaactgtatcgaacatcgacgcagaaagtggaaggcgacacccatttcccgattacgagccgatgactgggaatcggtattcagcgaattccac  
gatgctgatgacgagaactcagacgtattgctttgagattctggagcggcgataaCTCGAGCGGCCGCCAGCACAGTGGTCGACC  
TGTGGAATGTGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCAAAGCA  
TGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGAAAGTATGCAAA  
GCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCGCC  
CAGTTCCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCG  
GCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTGGAGGCCTAGGCTTTTGAAAAAGCTTACCA  
TGACCAGTACAAGCCACGGTGCCTCGCCACCCGCGACGACGTCCCCAGGGCCGTACGCACCCCTC  
GCCGCCGCGTTCGCCGACTACCCGCCACGCGCCACACCGTCGATCCGGACCGCCACATCGAGCGGGT  
CACCGAGCTGCAAGAACTTTCCTCACGCGCGTCGGGCTCGACATCGGCAAGGTGTGGGTGCGCGACG  
ACGGCGCCGCGGTGGCGGTCTGGAACACGCCGAGAGCGTCGAAGCGGGGGCGGTGTTGCGCGAGAT  
CGGCCCGCGCATGGCCGAGTTGAGCGGTTCCCGGCTGGCCGCGCAGCAACAGATGGAAGGCCTCCTG  
GCGCCGACCCGGCCCAAGGAGCCCGCTGTTCTGCGCCACCGTCGGCGTCTCGCCCGACCCAGG  
GCAAGGGTCTGGGACGCGCGTCTGCTCCCCGGAGTGGAGGCGGCGGAGCGCGCGGGGTGCCCCG  
CCTTCTGAGACCTCCGCGCCCCGCAACCTCCCCCTTCTACGAGCGGCTCGGCTTACCGTACCGCCG  
ACGTCGAGTGCCCGAAGGACCGCGGACCTGGTGCATGACCCGCAAGCCCGGTGCCTGACGCCCCG  
CCACGACCCGCGAGCCCCGACCGAAAGGAGCGCACGACCCATCGTCGACGATAAAATAAAAGATTTTAT  
TTAGTCTCCAGAAAAAGGGGGAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCC  
ATTTTGCAAGGCATGGAATAACATAACTGAGAATAGAGAAGTTGAGATCAAGGTCAGGAACAGATGGA  
ACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACA  
GATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCA  
AGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGT  
GCCCCAAGGACCTGAAATGACCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTG  
CGCGCTTCTGCTCCCCGAGCTCAATAAAGAGCCCAACCCCTCACTCGGGGCGCCAGTCCCTCGATT  
GACTGAGTCGCCGGGTACCCGTGTATCCAATAAACCCCTTTGCAATTGCATCCGACTTGTTGGTCTCGCT  
GTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTCAGCGGGGGTCTTTCACATGCAGCATGTAT  
CAAAATTAATTTGGTTTTTTTTCTTAAGTATTTACATTAATAGGCCATAGTTGCATTAATGAATCGGCCAACG  
CGCGGGGAGAGGCGGTTTGGCTATTGGCGCTCTTCCGCTTCTCGCTCACTGACTCGCTGCGCTCGGT  
CGTTCCGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAAATCAGGGGA  
TAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT  
GGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCG  
AAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCG  
ACCCTGCCGCTTACCGGATACCTGTCCGCTTTTCTCCCTTCGGGAAGCGTGCGCTTTTCTCATAGCTCAC  
GCTGTAGGTATCTCAGTTCCGTTGAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTT  
AGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGTAGTCCAACCCGGTAAGACACGACTTATCGCC  
ACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAA  
GTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACC  
TTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCCGCTGGTAGCGGTGGTTTTTTGTTT  
GCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGA  
CGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTACCTAGA  
TCCTTTTGGCGCCGGCCGCAATCAATCTAAAGTATATGAGTAACTTGGTCTGACAGTTACCAATGCT  
TAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCATAGTTGCCTGACTCCCCGCTG

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| TAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGC<br>TCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA<br>ACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCAGTTAATAG<br>TTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATTC<br>AGCTCCGGTTCCTCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCT<br>TCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAAGTTATCACTCATGGTTATGGCAGCACTGCA<br>TAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCT<br>GAGAAAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATA<br>GCAGAACTTTAAAGTGCTCATCATTGAAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCT<br>GTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTACCAGCG<br>TTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTT<br>GAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA<br>TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTT (SEQ ID NO:26) |
| Nucleotide positions of components:<br><br>LTR = 22-276; MMLV Ψ = 678-932; gag (truncated) = 1094-1348; pol region = 1520-1774; Kozak<br>sequence = 1923-1928; MTS (COX8A) = 1929-2015; XbaIR = 2022-2648; ecDHFR = 2652-3128;<br>SV40 promoter = 3167-3496; Puromycin resistance gene = 3506-4105; LTR = 4188-4796; AmpR =<br>5849-6709; AmpR promoter = 6711-6814                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |

[0130] Molecular simulation was used to confirm that the three functional domains of the polypeptide construct (MTS, XbaI, and DHFR) adopt a three-dimensional structure without interfering with each other's structure (FIG. 2). In addition, a retroviral vector with EGFP-DHFR as the transgene was created to evaluate the responsiveness of the DHFR/TMP system (FIG. 3 and FIG. 4). The nucleotide sequence of the vector is shown in Table 4.

| TABLE 4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pMSx-puro-EGFP_ecDHFR vector nucleotide sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Nucleotide sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| CCCGAAAAGTGCCACCTGCATAATGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCA<br>TTTTGCAAGGCATGGAAAAATACATAACTGAGAAAGTTCAGATCAAGGTCAGGAACAGATGGAA<br>CAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAG<br>ATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAA<br>GAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGT<br>GCCCCAAGGACCTGAAATGACCCTGTGCCTATTTGAACTAACCAATCAGTTGCTTCTCGCTTCTGTTCCG<br>CGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCACAAACCCCTCACTCGGCGCGCCAGTCCCTCCGATT<br>GACTGAGTCGCCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGCAAGTTGCATCCGACTTGTGGTCTCGCT<br>GTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTGAGCGGGGGTCTTTCAATTTGGGGGCTCGT<br>CCGGGATCGGGAGACCCCTGCCAGGGACCACCGACCCACCACCGGGAGGTAAGCTGGCCAGCAACTT<br>ATCTGTGTCTGTCGATTGTCTAGTGTCTATGACTGATTTTATGCGCCTGCGTCGGTACTAGTTAGCTAAC<br>TAGCTCTGTATCTGGCGGACCCGTGGTGGAAGTACGAGTTGGAACACCCGGCCGCAACCCCTGGGAG<br>ACGTCACAGGGACTTCGGGGGCCGTTTTGTGGCCCGACCTGAGTCCAAAAATCCCGATCGTTTTGGAC |

TCTTTGGTGCACCCCCCTAATAGGAGGGATATGTGGTTCTGGTAGGAGACGAGAACCTAAAACAGTTCCC  
GCCTCCGTCTGAATTTTTGCTTTCGGTTTGGGACCGAAGCCGCGCCGCGCTTGTCTGCTGCAGCAT  
CGTTCTGTGTTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATTAGGGCCAGACTGTTACCACTC  
CCTTAAGTTTGACCTTAGGTCACCTGGAAGATGTCGAGCGGATCGCTCACAACCAGTCGGTAGATGTCAA  
GAAGAGACGTTGGGTTACCTTCTGCTCTGCAGAATGGCCAACCTTTAACGTCGGATGGCCGCGAGACGG  
CACCTTTAACCGAGACCTCATCACCCAGGTTAAGATCAAGGTCTTTTACCTGGCCCGCATGGACACCCA  
GACCAGGTCCCCTACATCGTGACCTGGGAAGCCTTGGCTTTTGACCCCCCTCCCTGGGTCAAGCCCTTT  
GTACACCCTAAGCCTCCGCTCCTCTTCCCTCCATCCGCCCGCTCTCTCCCCCTTGAACCTCCTCGTTTCA  
CCCCGCCTCGATCCTCCCTTTATCCAGCCCTCACTCCTTCTCTAGGCGCCCCCATATGGCCATATGAGAT  
CTTATATGGGGCACCCCCGCCCCCTTGTAACCTCCCTGACCCTGACATGACAAGAGTTACTAACAGCCCC  
TCTCTCCAAGCTCACTTACAGGCTCTCTACTTAGTCCAGCACGAAGTCTGGAGACCTCTGGCGCGACGCT  
ACCAAGAACAACCTGGACCGACCGGTGGTACCTCACCCCTTACCGAGTCGGCGACACAGTGTGGGTCCGCC  
GACACCAGACTAAGAACCTAGAACCTCGCTGGAAGGACCTTACACAGTCCTGCTGACCACCCCCACCG  
CCCTCAAAGTAGACGGCATCGCAGCTTGGATACACGCCGCCACGTGAAGGCTGCCGACCCCCGGGGGT  
GGACCATCCTCTAGACTGCCGATCTAGCTAGTTAATTAAGGATCCgcccaccATGGTGAGCAAGGGCGAGG  
AGCTGTTACCCGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGC  
GTGTCGGCGGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGG  
CAAGCTGCCCCGTGCCCTGGCCACCCCTCGTGACCACCCCTGACCTACGGCGTGACGTGCTTCAGCCGCTA  
CCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCAC  
CATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGT  
GAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGT  
ACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTCAA  
GATCCGCCACAACATCGAGGACGGCAGCGTGACGCTCGCCGACCACTACCAGCAGAACACCCCCATCG  
GCGACGGCCCCGTGCTGCTGCCCCACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCC  
AACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGA  
CGAGCTGTACAagatcagtcgattgcggttagcggtatcacgttatcggtatggaaccgatgcggtggaacctgctgctgctgctg  
tggttaaacgcaacacctaataaaccggtgattatgggcccataccdggaatcaatcggtgctcggttgcaggacgcaaaatattatcctcagc  
agtaaccgagtagcgagatcggttaacggtggtgaagtcggtggtgaagccatcgcggtggtgacgtaccagaaatcatggttattgctg  
ggtcggttattgaacagttcttgcaaaagcgcaaaactgtatcgacgcatatcgacgagagtggaaggcgacacccatttccggattacgagcc  
ggatgactgggaatcggtattcagcgaattccacgatgctgacgagacgagaaactcacagctattgctttagattcggagcggtgataaCTCAGGC  
GGCCGCCAGCACAGTGGTCGACCTGTGGAATGTGTGTGCTAGTTAGGGTGTGGAAGTCCCCAGGCTCCC  
CAGCAGGCAGAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCT  
CCCCAGCAGGCAGAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCC  
GCCCATCCCGCCCCCTAACTCCGCCCAAGTTCGCCCAATTCTCCGCCCATGGCTGACTAATTTTTTTTATTT  
ATGCAGAGGCCGAGGCCGCTCGGCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCC  
TAGGCTTTTGCAAAAAGCTTACCATGACCGAGTACAAGCCACGGTGCGCCTCGCCACCCGCGACGACG  
TCCCCAGGGCCGTACGCACCCCTCGCCGCCGCGTTCCGCCGACTACCCGCCACGCGCCACACCGTCGAT  
CCGGACCGCCACATCGAGCGGGTCACCGAGCTGCAAGAACTTCTCTACGCGCGTCCGGCTCGACAT  
CGGCAAGGTGTGGGTGCGGACGACGGCGCCGCGGTGGCGGTCTGGACCACGCCGAGAGCGTCGAA  
GCGGGGGCGGTGTTCCGCCGAGATCGCCCCGCGCATGGCCGAGTTGAGCGGTTCCCGGCTGGCCGCGC  
AGCAACAGATGGAAGGCCTCCTGGCGCCGACCCGGCCCAAGGAGCCCCGCTGGTTCCTGGCCACCGTC  
GGCGTCTGCCCCGACCACAGGGCAAGGTCTGGGACGCGCGTCTGTCTCCCCGGAGTGAGGGCGG  
CCGAGCGCGCGGGGTGCCCGCCTTCTGGAGACCTCCGCGCCCCGCAACCTCCCCTTCTACGAGCGG  
CTCGGCTTACCGTCAACGCCGACGTGAGTGCCCGAAGGACCGCGCGACCTGGTGCATGACCCGCAA  
GCCCGGTGCCTGACGCCCGCCCCACGACCCGACGCGCCGACCGAAAGGAGCGCACGACCCATCGTC

GACGATAAAATAAAAGATTTTATTTAGTCTCCAGAAAAAGGGGGAATGAAAGACCCACCTGTAGGTTTG  
GCAAGCTAGCTTAAGTAACGCCATTTTGCAAGGCATGGAAAAATACATAACTGAGAATAGAGAAGTTCAGA  
TCAAGGTCAGGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCC  
CCGGCTCAGGGCCAAGAACAGATGGAACAGCTGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC  
CCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCAGCCCTCAGCAGTTTCTAGAGA  
ACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCA  
GTTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCAATAAAAGAGCCACAACCCCTCACT  
CGGGGCGCCAGTCTCCGATTGACTGAGTCGCCCGGGTACCCGTGTATCCAATAAACCCCTCTTGACGTT  
GCATCCGACTTGTGGTCTCGCTGTTCTTGGGAGGGTCTCCTCTGAGTGATTGACTACCCGTACGCGGG  
GGTCTTTTACATGCAGCATGTATCAAAATTAATTTGGTTTTTTTCTTAAGTATTACATTAATGGCCATAG  
TTGCATTAATGAATCGGCCAACGCGCGGGGAGAGCGGTTTTCGTATTGGGCGCTCTTCCGCTTCCTCG  
CTCACTGACTCGCTGCGCTCGGTCTCGGTCTCGGCTCGGCGGAGCGGTATCAGCTCACTCAAAGCGGTAATA  
CGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGG  
AACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATC  
GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCT  
CCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAA  
GCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTTCGCTCCAAGCTGG  
GCTGTGTGCACGAACCCCGCTTACGCCCAGCCGCTGCGCTTATCCGGTAACATATCGTCTTGAGTCCA  
ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATG  
TAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT  
CTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACC  
GCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATC  
CTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAACTCACGTTAAGGGATTTTGGTCATGAG  
ATTATCAAAAAGGATCTTACCTAGATCCTTTTTCGGCCGCGCCGCAAACTCAATCTAAAGTATATATGAGTA  
AACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTAT  
CCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTG  
CTGCAATGATACCGCGAGACCCACGCTCACC GGCTCCAGATTTATCAGCAATAAACCCAGCCAGCCGGA  
GGGCCGAGCGCAGAAGTGGTCTGCAACTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAG  
CTAGAGTAAGTAGTTCCGAGTTAATAGTTTGCACAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTG  
ACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCTAACGATCAAGGCGAGTTACATGATCCCC  
ATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAAGTAAGTTGGCCGCAAGTGT  
TATCACTCATGGTTATGGCAGCACTGCATAATCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTG  
ACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGT  
CAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAACGTTCTTCGGG  
GCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTGACCCAACTGA  
TCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAA  
GGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTTATCA  
GGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCA  
CATTTT (SEQ ID NO:27)

Nucleotide positions of components:

LTR = 22-276; MMLV  $\Psi$  = 678-932; gag (truncated) = 1094-1348; pol region = 1520-1774; Kozak  
sequence = 1923-1928; EGFP = 1929-2639; ecDHFR = 2646-3122; SV40 promoter = 3161-3490;

Puromycin resistance gene = 3500-4099; LTR = 4182-4790; AmpR = 5843-6703; AmpR promoter = 6704-6808

### 5.1.2. Responsiveness of DHFR/TMP system

[0131] The EGFP-DHFR retroviral vector was transduced into Hela cells. Infection efficiency was high, and TMP exposure was performed for two days at various concentrations without enriching infected cells, followed by fluorescent expression of EGFP using fluorescence microscopy and FACS (FIG. 5 and FIG. 6). EGFP mRNA was observed to be transcribed and present (FIG. 7), but the protein was not observed in the absence of TMP (FIG. 5 and FIG. 6).

[0132] To confirm the OFF control of the construct, medium washout was performed after two days of TMP exposure, and the fluorescence intensity over time was checked by fluorescence microscopy and FACS (FIG. 8A). The EGFP fluorescence abruptly declined even one hour after removing TMP in the culture medium, then four hours later, the fluorescence dissipated (FIG. 8B and FIG. 8C). Additionally, thereafter expression of the transgene product was not observed to leak out in the 48 hours following TMP OFF (FIG. 8D).

[0133] To confirm the ON control of the construct, addition of TMP was performed, and the fluorescence intensity over time was checked by fluorescence microscopy and FACS (FIG. 9A). The EGFP fluorescence abruptly turned on even one hour after adding TMP in the culture medium, then six hours later, the fluorescence reached to over 80% of the maximum intensity (FIG. 9B and FIG. 9C). From eight hours later, the intensity plateaued until the 48 hour time point (FIG. 9D).

### 5.1.3. Mitochondria Characteristics following Genetically Induced Mitophagy

[0134] Hela cells were transfected with the MTS-XbaIR-DHFR vector and exposed to 0.5  $\mu$ M TMP for two days followed by washout. XbaRI RNA expression was measured following TMP exposure. The transcript level of the transgene was not significantly changed (FIG. 10A). On Day 2, CN decreased to less than half of the initial value (FIG. 10B).

[0135] Hela transfectants with MTS-XbaIR-DHFR were cloned by limiting dilution, named as Hela MXD sc20. Hela MXD sc20 was subject to 0.5 $\mu$ M TMP exposure for several time durations (16, 20, and 48 hours) to examine mitochondrial mass (mtMass), which was measured by Mito Green staining, global mitochondrial membrane potential (mtMP), which was measured by TMRM staining, and corrected mtMP with mtMass, which was calculated as the ratio of mtMP to mtMass (FIGS. 11A-11C). Regardless of the duration of TMP exposure, the mtMass temporally increased about to two-fold compared to the resting state, then returned to the initial volume at 10 hours following TMP ON, suggesting that mitochondrial biogenesis was temporarily and strongly activated (FIG. 11A). Both the global and corrected mtMP

demonstrated a sharp decline and then increase, indicating poor pumping up of hydrogen ions through either respiratory chain complex I, III, and IV or counter clock-wise rotated complex V (FIG. 11B and FIG. 11C).

**[0136]** The introduced transgene appears to have caused DSB in the mitochondrial genome and decreased CN and, in addition, MM increased very responsively to this stress despite the need for protein from the nucleus. Based on these two factors, the density of the respiratory chain complex is thought to decrease, and MMP is thought to decrease as a phenotype. The mitochondrial genome appeared to return to normal by Day 6, with MM increased slightly. Without being bound by theory, it is believed that this indicates that mitochondrial biogenesis was enhanced in the MTS-XbaIR-DHFR/TMP system and that mitochondrial capacity as an abundance of mitochondria was increased.

**[0137]** Next, it was investigated whether changes in cell proliferation and viability occurred in this system and affected the above-described changes. No significant difference in cell number or viability in this system with or without TMP was observed (FIGS. 12A-12B). Thus, the main benefit of this system was determined to be the intervention on mitochondria by the MTS-XbaIR-DHFR polypeptide.

#### **5.1.4. Effects on Mitophagy following MTS-XbaIR-DHFR/TMP**

**[0138]** mtKeima-Red-transfected Hela MXD sc20 cells with PARK2 overexpression were generated in order to quantify mitophagy in a more sophisticated fashion. Various durations of TMP exposure to the transfectants were examined to measure mitophagy (16, 20, 24, 40, 44, and 48 hours). Mitophagy inducer carbonyl cyanide 3-chlorophenylhydrazone (CCCP) was used as a positive control. More mitophagy was observed with increasing duration of TMP exposure (FIG. 13A). Mitophagy reached a plateau at 40 hours of TMP exposure (FIG. 13A). Simultaneously, measured mtDNA CN was observed to return to the initial value regardless of the duration of TMP exposure (FIG. 13B). In total, the results indicate that mitochondrial turnover was accelerated by MTS-XbaIR-DHFR/TMP.

### **5.2. Example 2: Changes in mitochondrial functioning and biogenesis due to genetically induced mitophagy**

**[0139]** This Example describes mitochondrial biogenesis and functional changes associated with genetically induced mitophagy (GiM).

#### **5.2.1. Materials and Methods**

**[0140]** MTS-XbaI-ecDHFR was cloned into Hela cells after gene transfer using a retrovirus, and stable transfectant that constitutively expresses the GiM unit (Hela\_GiM) was generated. The endonuclease, XbaI, was transiently present in the mitochondrial matrix during treatment of Hela\_GiM cells with trimethoprim (TMP) for 2 days. Since TMP was dissolved in DMSO, the control group was treated with the same amount of DMSO.

**[0141]** The reactive oxygen species (ROS) over time were assessed by staining cells with mitoSox and measuring fluorescence intensity with FACS, setting a threshold line compared to stress-free cells, and measuring the percentage of positive cells. The mitochondrial biogenesis-related peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$  (PGC1 $\alpha$ ), nuclear factor receptor 1 (NRF1), which is involved in mitochondriogenesis, and mitochondrial transcription factor A (TFAM), which forms nucleoids with mtDNA and is deeply involved in mtDNA transcription, replication, and maintenance, were assessed with qPCR. Expression levels of several nuclear-encoded and mtDNA-encoded proteins were quantified via Western Blotting.

### 5.2.2. Results

**[0142]** The level of ROS increased initially in the TMP-treated group but was essentially unchanged in the control group throughout the assessments. On Day 2, the percentage of mitoSox-positive cells in the TMP-treated group was higher than in the control group. On Day 4 and onward, the percentage of mitoSox-positive cells were comparable for the two groups (FIG. 14A). Mitochondriogenesis-promoting transcription factors PGC1 $\alpha$  and NRF1 were also found to be elevated in the TMP-treated group relative to the control group at all observation points (FIGS. 14B and 14C) and TFAM was largely maintained on Days 2, 4, and 6 in the TMP-treated group (FIG. 14D).

**[0143]** Without being bound by theory, it is believed that these results indicate that ROS production is temporarily enhanced by transient mitochondrial genome reduction, whereby mitochondrial biogenesis is brought about by amplification of transcription factors such as PGC1 $\alpha$  and NRF1. It is further believed, again without being bound by theory, that the maintenance of the elevated transcript levels of TFAM, a major constituent protein of nucleoids, indicates that mitochondrial biogenesis continues for some time after mitochondrial genome reduction has been triggered.

**[0144]** Next, the effect of GiM on the expression of nuclear-encoded and mtDNA-encoded proteins was assessed. The expression level of ATP5A, a respiratory chain complex encoded in the nucleus, was essentially unchanged by mitochondrial genome reduction (FIGS. 15A and 15B). The expression of translocase of the outer membrane (TOM20), also encoded in the nucleus, was reduced immediately after mitochondrial genome reduction but returned to a level comparable to that observed in the DMSO-treated group by Day 6 (FIGS. 15A and 15C). On the other hand, ATP6, which is encoded by mtDNA, was greatly reduced immediately after mitochondrial genome reduction in the TMP-treated group, then increased on Day 4, and later returned to a comparable level as the control group (FIGS. 15A and 15D). This trend was also observed with the ATP6/ATP5A ratio (FIGS. 15A and 15E). These results indicate that GiM

causes a transient surge of mitochondrial proteins derived from the mitochondrial genome, but not from the nuclear genome.

**[0145]** Changes in the level of the activated form of AMPK, p-AMPK, were used to estimate changes in mitochondrial energy production after mitochondrial genome reduction. Mitochondrial energy production was confirmed by an increase in p-AMPK on Day 4 (FIGS. 15A and 15F). Transient mitochondrial genome reduction was associated with a reduced competence of the respiratory chain complex on Day 2, and the effect was directly linked to cellular energy depletion, leading to a marked increase in p-AMPK on Day 4.

### **5.3. Example 3: Autophagy associated with GiM-induced transient mitochondrial genome reduction**

**[0146]** Mitophagy occurs when mitochondria are incorporated into the phagophore and become autolysosomes by fusion with lysosomes. This Example describes compositions and methods for detecting autolysosome formation and autophagy following GiM-induced transient mitochondrial genome reduction.

#### **5.3.1. Materials and Methods**

**[0147]** Autolysosomes have lower pH values than mitochondria that are not fused with lysosomes. Using a pH-sensitive mitochondrial reporter, mtKeimaRed, autolysosome formation was assessed in cells after GiM-induced transient mitochondrial genome reduction. mtKeimaRed emits fluorescence with a peak at 440 nm (green) at pH > 6 and a peak at 620 nm (red) at pH < 5 and has a transport signal that allows its transport into mitochondria. Cells that stably express mKeimaRed emit red light when the environmental pH is below 5. To quantify autolysosomes upon GiM-induced transient mitochondrial genome reduction, Hela\_GiM cells described in Section 5.2.1 were retrovirally engineered with a sequence encoding mKeimaRed. The percentage of cells undergoing mitophagy was quantified every two days for two weeks using fluorescence and phase contrast microscopy.

**[0148]** The final step of autophagy flux depends on lysosomal V-ATPase activity. Therefore, the final step of flux was suppressed after GiM-induced transient mitochondrial genome reduction using a lysosomal V-ATPase inhibitor, bafilomycinA1 (BafA1), to assess the mitochondria-targeted autophagy flux. Antibody staining for LC3 (MAP1LC3: Microtubule-associated protein 1 light chain 3), a representative marker for autophagosome formation, along with TOM20 staining as a mitochondrial membrane marker, was used to quantify autophagosomes. Given that LC3 is present on autophagosome membranes as LC3-II with PE, LC3-II was quantified by western blotting in Hela\_GiM cells treated with BafA1, wherein protein was extracted from the cells on Day 8 after 48 h exposure to TMP.

### 5.3.2. Results

**[0149]** The peak of mtKeimaRed signal associated with GiM-induced autolysosome formation was on Day 8 (FIG. 16A), with mitophagy occurring in about 20% of cells (FIG. 16B). The time course analysis suggested that kinetics of mitophagy increased on Day 6 and decreased to control levels by Day 14, with only a few percent of cells undergoing mitophagy (FIG. 16B). This transient surge in induced mitophagy was not associated with cell death or a decrease in viability. Without being bound by theory, these results suggest that the adverse reactions of excess mitophagy, such as mitophagy-induced cell death, can be suppressed by controlling GiM.

**[0150]** Autophagosomes formed upon fusion of mitochondria and lysosomes were detected as spots where LC3 staining and TOM20 staining overlap (FIG. 17A). In the absence of TMP, the spots with overlapping LC3 and TOM20 staining were about 80  $\mu\text{m}^2$  in size and were not significantly affected by the use of BafA1. On the other hand, in the TMP-exposed group, the area of spots with overlapping LC3 and TOM20 staining significantly increased to about 120  $\mu\text{m}^2$  with the addition of BafA1 (FIG. 17B). Although LC3-II was found to be significantly increased by GiM, the increase was more pronounced and significant when BafA1 was used (FIGS. 18A and 18B). Taken together, these results suggest that GiM significantly promotes autophagy.

### 5.4. Example 4: Metabolic Effects of Mitochondrial Genome Reduction

**[0151]** Mitochondrial genome reduction was performed using Hela\_GiM cells described in Section 5.2.1 with 2 days of TMP exposure. Respirometry using SeaHorse over time was performed to evaluate oxidative phosphorylation (OXPHOS) and glycolysis. OXPHOS (FIG. 19A, left panel) decreased until day 4 and gradually increased from day 6 until approaching the starting level on day 10. Glycolysis, on the other hand, increased until day 8 and then decreased by day 10 (FIG. 19A, right panel). The two-dimensional plot depicting OXPHOS and Glycolysis relationship, showed a circular change, indicating that the metabolic change was transient (FIG. 19B). Individual assessments of ATP production, basal respiration, proton leak, and spare capacity further supported the transient nature of GiM-associated metabolic changes (FIG. 19C). Even in terms of oxygen consumption, the metabolic effects of GiM indicate that the temporary mitochondrial genome reduction is a reversible change.

### 5.5. Example 5: Genetically Induced Mitophagy in AD Fibroblasts

**[0152]** Alzheimer's Disease (AD) is associated with mitochondrial dysfunction. This example describes how transient mitochondrial genome reduction by gene transfer can transform the cellular phenotype by enhancing mitochondrial turnover in fibroblasts obtained from a patient with AD.

### 5.5.1. Materials and Methods

**[0153]** Normal Human Dermal Fibroblasts (NHDF) and fibroblasts obtained from forearm skin samples of a patient with Alzheimer's disease whose APOE genotype is E3/E45 (AD fibroblasts) were utilized as target cells. Transient mitochondrial genome reduction was performed by transferring the plasmid carrying the gene encoding endonuclease XbaIR downstream of the mitochondrial transfer signal derived from human Cox8, and expressing the puromycin resistance as a selection marker under a different promoter (pCAGGS-MTS-XbaIR). Electroporation was used as the gene transfer method, and transfected target cells were enriched by exposure to puromycin at a concentration of 3 µg/mL for 24 hours on day 2 after electroporation. This condition was set by the criteria of 70~80% GFP expression and viability of more than 90%, using a plasmid with recombinant GFP gene instead of XbaIR. After gene transfer, the copy number of the mitochondrial genome was assessed on days 7, 14, and 21 to confirm genome reduction and subsequent biogenesis (FIG. 20A). In addition, mitochondrial phenotype was evaluated by measuring mitochondrial volume (mtMass) and mitochondrial membrane potential (mtMP) using MitoTracker Green and TMRM, respectively.

### 5.5.2. Results

**[0154]** In untreated AD fibroblasts mtMP was significantly decreased relative to untreated NHDF (FIGS. 20B and 20E). Similarly, mtMass was decreased in untreated AD fibroblasts relative to untreated NHDF (FIG. 20B and 20D). The two fluorescent signals were expanded in two dimensions, and a quadrant analysis was performed using NHDFs as a positive control to set a threshold line. The fraction ratio is used as a biomarker of aging in lymphocytes for detecting mitochondrial dysfunction. This double positive rate was found to be about half of that of NHDF. The percentage of double positives did not change significantly on Day 7 after genetically induced mitophagy (GiM), but increased to 60.3% on Day 14 and furthermore to 87.3% on Day 21, comparable to the control NHDF (FIG. 20C).

**[0155]** Both mtMass and mtMP were quantified using mean fluorescent intensity (MFI) as an indicator of fluorescence intensity. Both mtMass and mtMP were lower in untreated AD fibroblast cells relative to NHDF cells. In AD fibroblasts, mtMass and mtMP levels were even lower on day 7 after GiM induction, but increased to a level comparable to NHDF on Day 14 (FIGS. 20F-20G), suggesting that both the mitochondrial volume and mitochondrial membrane potential in AD fibroblasts were restored to the same levels as those of healthy NHDF cells by GiM.

**[0156]** These results indicate that the promotion of mitochondrial turnover by GiM is qualitatively favorable for nascent generated mitochondria. Given that accumulation of dysfunctional mitochondria is a common phenomenon in various neurodegenerative diseases and

in aging, GiM can be employed as a therapeutic strategy for the treatment of neurodegenerative diseases and also for the reacquisition of normal function in aging cells.

## 6. SPECIFIC EMBODIMENTS

**[0157]** The present disclosure is exemplified by the specific embodiments below.

1. A polypeptide comprising:
  - (a) a mitochondrial targeting sequence (MTS);
  - (b) an endonuclease sequence; and
  - (c) a destabilizing domain sequence.
2. The polypeptide of embodiment 1, wherein the MTS comprises a human MTS.
3. The polypeptide of embodiment 1, wherein the MTS comprises a non-human MTS.
4. The polypeptide of any one of embodiments 1 to 3, wherein the MTS comprises an MTS of a mitochondrial protein.
5. The polypeptide of any one of embodiments 1 to 4, wherein the MTS comprises an MTS of a TCA cycle-related enzyme, a chaperone protein, a mitochondrial genome replication protein, a protease, an mRNA processing protein, a mitochondrial RNA degradation protein, a deoxynucleotide triphosphate synthesis-related protein, a mitoribosomal protein, a phospholipid metabolism-related protein, a protein involved in metabolism of toxic compounds, a disulfide relay system-related protein, an iron-sulfur protein assembly protein, a tRNA modification protein, an aminoacyl-tRNA synthetase, a release factor, or an elongation factor.
6. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a cytochrome c oxidase subunit.
7. The polypeptide of embodiment 6, wherein the MTS comprises an MTS of a cytochrome c oxidase subunit VIII (COX8).
8. The polypeptide of embodiment 6, wherein the MTS comprises an MTS of a cytochrome c oxidase subunit X (COX10).
9. The polypeptide of embodiment 6, wherein the MTS comprises an MTS of a cytochrome c oxidase subunit IV (COX4).
10. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a frataxin (FXN) protein.

11. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a TCA cycle-related enzyme, optionally which is Pyruvate dehydrogenase, Citrate synthase, Aconitase, Isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, Succinyl-CoA synthetase, Succinic dehydrogenase, Fumarase, Malate dehydrogenase, or Pyruvate carboxylase.
12. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a chaperone protein, optionally which is mtHSP10, mtHSP60, mtHSP70, or mtHSP90.
13. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a mitochondrial genome replication protein, optionally which is TFAM, Twinkle, PolG, TFB2M, TEFM, or MTERF1.
14. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a protease, optionally which is MPP, CLXP, LON ATPase, or PreP.
15. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of an mRNA processing protein, optionally which is LRPPRC, TACO1, ELAC2, PNPT1, HSD17B10, MTPAP, or PTCD1.
16. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a mitochondrial RNA degradation protein, optionally which is PNPase, REX02, or SUV3.
17. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a deoxynucleotide triphosphate synthesis-related protein, optionally which is DGUOK, TK2, TYMP, MGME1, SUCLG1, SUCLA2, RNASEH1, or C10orf2.
18. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a mitochondrial protein, optionally which is MRPS16, MRPS22, MRPL3, MRP12, or MRPL44.
19. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a phospholipid metabolism-related protein, optionally which is AGK, SERAC1, or TAZ.
20. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a protein involved in metabolism of toxic compounds, optionally which is HIBCH, ECHS1, ETHE1 or MPV17.

21. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a disulfide relay system-related protein, optionally which is GFER.

22. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of an iron-sulfur protein assembly protein, optionally which is ISCU, BOLA3, NFU1, or IBA57.

23. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a tRNA modification protein, optionally which is MTO1, GTP3BP, TRMU, PUS1, MTFMT, TRIT1, TRNT1 or TRMT5.

24. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of an aminoacyl-tRNA synthetase, optionally which is AARS2, DARS2, EARS2, RARS2, YARS2, FARS2, HARS2, LARS2, VARS2, TARS2, IARS2, CARS2, PARS2, NARS2, KARS, GARS, SARS2 or MARS2.

25. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of a release factor, optionally which is a C12orf65.

26. The polypeptide of any one of embodiments 1 to 5, wherein the MTS comprises an MTS of an elongation factor, optionally which is TUFM, TSFM, or GFM1.

27. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80% identical to MSVLTPLLLRGLTGSARR (SEQ ID NO:1).

28. The polypeptide of embodiment 27, wherein the MTS comprises a sequence that is at least 85% identical to MSVLTPLLLRGLTGSARR (SEQ ID NO:1).

29. The polypeptide of embodiment 27, wherein the MTS comprises a sequence that is at least 90% identical to MSVLTPLLLRGLTGSARR (SEQ ID NO:1).

30. The polypeptide of embodiment 27, wherein the MTS comprises a sequence that is at least 95% identical to MSVLTPLLLRGLTGSARR (SEQ ID NO:1).

31. The polypeptide of embodiment 27, wherein the MTS comprises a sequence that is 100% identical to MSVLTPLLLRGLTGSARR (SEQ ID NO:1).

32. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80% identical to MSVLTPLLLRGLTGSARRLPVPRAKIHSL (SEQ ID NO:2).

33. The polypeptide of embodiment 32, wherein the MTS comprises a sequence that is at least 85% identical to MSVLTPLLLRGLTGSARRLPVPRAKIHSL (SEQ ID NO:2).

34. The polypeptide of embodiment 32, wherein the MTS comprises a sequence that is at least 90% identical to MSVLTPLLLRGLTGSARRLPVPRAKIHSL (SEQ ID NO:2).

35. The polypeptide of embodiment 32, wherein the MTS comprises a sequence that is at least 95% identical to MSVLTPLLLRGLTGSARRLPVPRAKIHSL (SEQ ID NO:2).

36. The polypeptide of embodiment 32, wherein the MTS comprises a sequence that is 100% identical to MSVLTPLLLRGLTGSARRLPVPRAKIHSL (SEQ ID NO:2)

37. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80% identical to MSVLTPLLLRSLTGSARRLMVPRA (SEQ ID NO:3).

38. The polypeptide of embodiment 37 wherein the MTS comprises a sequence that is at least 85% identical to MSVLTPLLLRSLTGSARRLMVPRA (SEQ ID NO:3).

39. The polypeptide of embodiment 37 wherein the MTS comprises a sequence that is at least 90% identical to MSVLTPLLLRSLTGSARRLMVPRA (SEQ ID NO:3).

40. The polypeptide of embodiment 37 wherein the MTS comprises a sequence that is at least 95% identical to MSVLTPLLLRSLTGSARRLMVPRA (SEQ ID NO:3).

41. The polypeptide of embodiment 37 wherein the MTS comprises a sequence that is 100% identical to MSVLTPLLLRSLTGSARRLMVPRA (SEQ ID NO:3).

42. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80% identical to MAASPHLTSSRLTGCVGGSVWYLERRT (SEQ ID NO:4).

43. The polypeptide of embodiment 42, wherein the MTS comprises a sequence that is at least 85% identical to MAASPHLTSSRLTGCVGGSVWYLERRT (SEQ ID NO:4).

44. The polypeptide of embodiment 42, wherein the MTS comprises a sequence that is at least 90% identical to MAASPHLTSSRLTGCVGGSVWYLERRT (SEQ ID NO:4).

45. The polypeptide of embodiment 42, wherein the MTS comprises a sequence that is at least 95% identical to MAASPHLTSSRLTGCVGGSVWYLERRT (SEQ ID NO:4).

46. The polypeptide of embodiment 42, wherein the MTS comprises a sequence that is 100% identical to MAASPHLTSSRLTGCVGGSVWYLERRT (SEQ ID NO:4).

47. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80% identical to MWTLGRRVAGLLASPSAQ (SEQ ID NO:5).

48. The polypeptide of embodiment 47, wherein the MTS comprises a sequence that is at least 85% identical to MWTLGRRVAGLLASPSAQ (SEQ ID NO:5).

49. The polypeptide of embodiment 47, wherein the MTS comprises a sequence that is at least 90% identical to MWTLGRRVAGLLASPSAQ (SEQ ID NO:5).

50. The polypeptide of embodiment 47, wherein the MTS comprises a sequence that is at least 95% identical to MWTLGRRVAGLLASPSAQ (SEQ ID NO:5).

51. The polypeptide of embodiment 47, wherein the MTS comprises a sequence that is 100% identical to MWTLGRRVAGLLASPSAQ (SEQ ID NO:5).

52. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MAPYSLLVTRLQKALG (SEQ ID NO:6).

53. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MALLTAAARLLGTKNASCLVLAARHASA (SEQ ID NO:7).

54. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MVKQIESKTAFQEALDAAGDKLVVVDTSATWC (SEQ ID NO:8).

55. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MATNWGSLQDKQLEELARQAVDRALAEGLLRSTSQ (SEQ ID NO:9).

56. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MAFLRSMWGVLSALGRSGA (SEQ ID NO:10).

57. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MWVLLRSGYPLRILLPLRG (SEQ ID NO:11).

58. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MSRLLRWKVAGATVGPVPVAPG (SEQ ID NO:12).

59. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MKRNTLVELLTFWKNWHFRL (SEQ ID NO:13).

60. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MISASRAAAARLVGAAASRGPTAA (SEQ ID NO:14).

61. The polypeptide of embodiment 1, wherein the MTS comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to MEALIPVINKLQDVFNTVGA (SEQ ID NO:15).

62. The polypeptide of any one of embodiments 1 to 61, wherein the endonuclease is a restriction endonuclease, an RNA-guided endonuclease (e.g., Cas9 or Cas12), a zinc finger nuclease, or a transcription activator-like effector nuclease (TALEN).

63. The polypeptide of embodiment 62, wherein the endonuclease is a restriction endonuclease.

64. The polypeptide of embodiment 63, wherein the restriction endonuclease is an XbaIR, EcoRI, SmaI, AflII, BamHI, BclI, HaeIII, HindII, HindIII, NdeI, PvuII, PstI, or SpeI endonuclease.

65. The polypeptide of embodiment 64, wherein the restriction endonuclease is an XbaIR endonuclease.

66. The polypeptide of any one of embodiments 1 to 63, wherein the endonuclease comprises a sequence that is at least 80% identical to MTTLEKIKLLADGYADRLKLAIDGRVLEMQGDDVSHYLIYRVLGVAQEEGRIDVYQNKGRFLY KYAGSFLEAATKLCFKEAFDPSASLRPLNTQGQRPRTVEIDCLVGNDALAIKWKDATTDGDHIT KEHTRIKVISDAGYKPIRMFYYPHRTQAIQETLETLYNGVHGHEYHYGEAAWDYVLQRTSVNL KVALEQIADSRTNEAA (SEQ ID NO:16).

67. The polypeptide of embodiment 66, wherein the endonuclease sequence comprises a sequence that is at least 85% identical to MTTLEKIKLLADGYADRLKLAIDGRVLEMQGDDVSHYLIYRVLGVAQEEGRIDVYQNKGRFLY KYAGSFLEAATKLCFKEAFDPSASLRPLNTQGQRPRTVEIDCLVGNDALAIKWKDATTDGDHIT KEHTRIKVISDAGYKPIRMFYYPHRTQAIQETLETLYNGVHGHEYHYGEAAWDYVLQRTSVNL KVALEQIADSRTNEAA (SEQ ID NO:16).

68. The polypeptide of embodiment 66, wherein the endonuclease sequence comprises a sequence that is at least 90% identical to  
MTTLEKIKLLADGYADRLKLAIDGRVLEMQGDDVSHYLIYRVLGVAQEEGRLLIDVYQNKGRFLY  
KYAGSFLEAATKLCFKEAFPDSASLRPNTQGQRPRTVEIDCLVGNDALAIKWKDATTGDGHIT  
KEHTRIKVISDAGYKPIRIMFYYPHRTQAIRIQETLETLYNGVHGHEYHYGEAAWDYVLQRTSVNL  
KVALEQIADSRTNEAA (SEQ ID NO:16).

69. The polypeptide of embodiment 66, wherein the endonuclease sequence comprises a sequence that is at least 95% identical to  
MTTLEKIKLLADGYADRLKLAIDGRVLEMQGDDVSHYLIYRVLGVAQEEGRLLIDVYQNKGRFLY  
KYAGSFLEAATKLCFKEAFPDSASLRPNTQGQRPRTVEIDCLVGNDALAIKWKDATTGDGHIT  
KEHTRIKVISDAGYKPIRIMFYYPHRTQAIRIQETLETLYNGVHGHEYHYGEAAWDYVLQRTSVNL  
KVALEQIADSRTNEAA (SEQ ID NO:16).

70. The polypeptide of embodiment 66, wherein the endonuclease sequence comprises a sequence that is 100% identical to  
MTTLEKIKLLADGYADRLKLAIDGRVLEMQGDDVSHYLIYRVLGVAQEEGRLLIDVYQNKGRFLY  
KYAGSFLEAATKLCFKEAFPDSASLRPNTQGQRPRTVEIDCLVGNDALAIKWKDATTGDGHIT  
KEHTRIKVISDAGYKPIRIMFYYPHRTQAIRIQETLETLYNGVHGHEYHYGEAAWDYVLQRTSVNL  
KVALEQIADSRTNEAA (SEQ ID NO:16).

71. The polypeptide of any one of embodiments 1 to 70, wherein the destabilizing domain sequence is a DHFR, FKBP, or PDE5 destabilization domain sequence.

72. The polypeptide of embodiment 71, wherein the destabilization domain sequence is a DHFR destabilization domain sequence.

73. The polypeptide of embodiment 72, wherein the destabilization domain sequence is an *E. coli* DHFR (ecDHFR) destabilization domain sequence.

74. The polypeptide of any one of embodiments 1 to 73, wherein the destabilization domain sequence comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to  
MISLIAALVDHVIGMENAMPWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNIILSS  
QPSTDDRVTWKSVDIAIACGDVPEIMVIGGGRVYEQLPKAQKLYLTHIDAEVEGDTHFPD  
YEPDDWESVFSEFHDADAQNSHSYCFEILERR (SEQ ID NO:17).

75. The polypeptide of any one of embodiments 1 to 73, wherein the destabilization domain sequence comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to

ISLIAALAVDHHVIGMETVMPWNLPA DLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNILSSQP  
STDDRVTWVKSVDEAIAACGDVPEIMVIGGGRVYEQLPKAQKLYLTHIDAEVEGDTHFPDYEP  
DDWESVFSEFHDADAQNSHSCFEILERR (SEQ ID NO:18).

76. The polypeptide of embodiment 74, wherein the destabilization domain sequence has one or more of the following amino acid substitutions: N18T/A19V, F103L, Y100I, G121V, H12Y/Y100I, H12L/Y100I, R98H/F103S, M42T/H114R, and I61F/T68S.

77. The polypeptide of any one of embodiments 1 to 71, wherein the destabilization domain sequence comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to  
GVQVETISPGDGRTPKRGQTCVVHYTGMLDGKKVDSSRDRNKPFK FMLGKQEVIRGWEE  
GVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO:20).

78. The polypeptide of embodiment 77, wherein the destabilization domain sequence has one or more of the following amino acid substitutions: F15S, V24A, H25R, E60G, L106P, D100G, M66T, R71G, D100N, E102G, and K105I.

79. The polypeptide of any one of embodiments 1 to 71, wherein the destabilization domain sequence comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to any one of SEQ ID NOs: 19-35 and 66-69 of WO 2018/237323.

80. The polypeptide of any one of embodiments 1 to 79, wherein the MTS is positioned N-terminal to the endonuclease sequence and the destabilizing domain sequence.

81. The polypeptide of any one of embodiments 1 to 79, wherein the MTS is positioned C-terminal to the endonuclease sequence and the destabilizing domain sequence.

82. The polypeptide of any one of embodiments 1 to 81, wherein the endonuclease sequence is positioned N-terminal to the destabilization domain sequence.

83. The polypeptide of any one of embodiments 1 to 81, wherein the endonuclease sequence is positioned C-terminal to the destabilization domain sequence.

84. The polypeptide of any one of embodiments 1 to 79, wherein the MTS is positioned N-terminal to the endonuclease sequence and the endonuclease sequence is positioned N-terminal to the destabilizing domain sequence.

85. A nucleic acid encoding the polypeptide of any one of embodiments 1 to 84.

86. The nucleic acid of embodiment 85, which comprises a promoter operably linked to the nucleotide sequence encoding the polypeptide.

87. The nucleic acid of embodiment 86, wherein the promoter is a SV40 promoter, a CMV promoter or a CAG promoter.

88. The nucleic acid of embodiment 87, wherein the promoter is a SV40 promoter.

89. The nucleic acid of embodiment 88, wherein the SV40 promoter comprises a nucleotide sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO:25.

90. The nucleic acid of any one of embodiments 85 to 89, which is a vector.

91. The nucleic acid of embodiment 90, wherein the vector is a retroviral genome.

92. The nucleic acid of embodiment 91, wherein the vector is a murine retroviral genome.

93. The nucleic acid of embodiment 90, wherein the vector is a plasmid.

94. The nucleic acid of embodiment 85, which is an mRNA molecule.

95. A particle comprising the nucleic acid of any one of embodiments 85 to 94, optionally wherein the particle is a retroviral particle.

96. A host cell comprising the nucleic acid of any one of embodiments 85 to 94.

97. A method of (a) inducing mitophagy in a cell, and/or (b) increasing mitochondrial turnover in a cell, and/or (c) increasing mitochondrial mass, and/or (d) inducing double strand breaks in mitochondrial DNA and/or (e) inducing epigenomic modifications in a cell, the method comprising contacting the cell with (i) the polypeptide of any one of embodiments 1 to 84, the nucleic acid of any one of embodiments 85 to 94, or the particle of embodiment 95 and (ii) a stabilizing agent.

98. The method of embodiment 97, wherein the stabilizing agent is trimethoprim (TMP) when the destabilizing domain sequence is a DHFR destabilization domain sequence.

99. The method of embodiment 97, wherein the stabilizing agent is Shield-1, rapamycin, or FK506 when the destabilizing domain sequence is a FKBP destabilization domain sequence.

100. The method of any one of embodiments 97 to 99, wherein the contacting the cell with the stabilizing agent comprises culturing the cell in a medium comprising the stabilizing agent.

101. The method of embodiment 100, which comprises culturing the cell for at least 8 hours in a medium comprising the stabilizing agent.

102. The method of embodiment 100, which comprises culturing the cell for at least 12 hours in a medium comprising the stabilizing agent.

103. The method of embodiment 100, which comprises culturing the cell for at least 1 day in a medium comprising the stabilizing agent.

104. The method of embodiment 100, which comprises culturing the cell for at least 2 days in a medium comprising the stabilizing agent.

105. The method of any one of embodiments 100 to 104, which comprises culturing the cell for up to 5 days in a medium comprising the stabilizing agent.

106. The method of any one of embodiments 100 to 104, which comprises culturing the cell for up to 4 days in a medium comprising the stabilizing agent.

107. The method of any one of embodiments 100 to 104, which comprises culturing the cell for up to 3 days in a medium comprising the stabilizing agent.

108. The method of any one of embodiments 100 to 104, which comprises culturing the cell for up to 2 days in a medium comprising the stabilizing agent.

109. The method of any one of embodiments 97 to 108, which further comprises, subsequent to contacting the cell with the stabilizing agent, removing the stabilizing agent from the cell.

110. The method of embodiment 109, wherein removing the stabilizing agent from the cell comprises culturing the cell in a medium that does not include the stabilizing agent.

111. The method of embodiment 110, which comprises culturing the cell for at 3 hours in a medium that does not include the stabilizing agent.

112. The method of embodiment 110, which comprises culturing the cell for at least 6 hours in a medium that does not include the stabilizing agent.

113. The method of embodiment 110, which comprises culturing the cell for at least 12 hours in a medium that does not include the stabilizing agent.

114. The method of embodiment 110, which comprises culturing the cell for at least 1 day in a medium that does not include the stabilizing agent.

115. The method of embodiment 110, which comprises culturing the cell for at least 2 days in a medium that does not include the stabilizing agent.

116. The method of embodiment 110, which comprises culturing the cell for at least 3 days in a medium that does not include the stabilizing agent.

117. The method of embodiment 110, which comprises culturing the cell for at least 4 days in a medium that does not include the stabilizing agent.

118. The method of any one of embodiments 110 to 117, which comprises culturing the cell for up to 10 days in a medium that does not include the stabilizing agent.

119. The method of any one of embodiments 100 to 117, which comprises culturing the cell for up to 8 days in a medium that does not include the stabilizing agent.

120. The method of any one of embodiments 100 to 117, which comprises culturing the cell for up to 6 days in a medium that does not include the stabilizing agent.

121. The method of any one of embodiments 100 to 117, which comprises culturing the cell for up to 4 days in a medium that does not include the stabilizing agent.

122. The method of any one of embodiments 97 to 121, which comprises contacting the cell with the polypeptide of any one of embodiments 1 to 84.

123. The method of embodiment 122, wherein the contacting comprises introducing the polypeptide to the cell via electroporation, injection, or a carrier.

124. The method of any one of embodiments 97 to 121, which comprises contacting the cell with the nucleic acid of any one of embodiments 85 to 94.

125. The method of embodiment 124, wherein the contacting comprises introducing the nucleic acid to the cell via transfection, electroporation, injection, or a carrier.

126. The method of embodiment 124 or embodiment 125, wherein the polypeptide is transiently expressed in the cell.

127. The method of any one of embodiments 97 to 126, further comprising administering the cell to a subject.
128. The method of any one of embodiments 97 to 127, which induces mitophagy in the cell.
129. The method of any one of embodiments 97 to 128, which increases mitochondrial turnover in the cell.
130. The method of any one of embodiments 97 to 129, which increases mitochondrial mass.
131. The method of any one of embodiments 97 to 130, which induces double strand breaks in mitochondrial DNA.
132. The method of any one of embodiments 97 to 131, which induces epigenomic modifications in the cell.
133. A cell obtained or obtainable by the method of any one of embodiments 97 to 132.
134. A cell comprising the polypeptide of any one of embodiments 1 to 84, the nucleic acid of any one of embodiments 85 to 94, or particle of embodiment 95.
135. The cell of embodiment 134, further comprising a stabilizing agent.
136. The cell of embodiment 135, wherein the stabilizing agent is trimethoprim (TMP) when the destabilizing domain sequence is a DHFR destabilization domain sequence.
137. The cell of embodiment 135, wherein the stabilizing agent is Shield-1, rapamycin, or FK506 when the destabilizing domain sequence is a FKBP destabilization domain sequence.
138. The method of any one of embodiments 97 to 132 or cell of any one of embodiments 133 to 137, wherein the cell is a mammalian cell.
139. The method or cell of embodiment 138, wherein the cell is a human cell.
140. The method or cell of any one of embodiments 138 to 139, wherein the cell is a somatic cell.

141. The method or cell of any one of embodiments 138 to 140, wherein the cell is a bone marrow cell.

142. The method or cell of any one of embodiments 138 to 141, wherein the cell is a hematopoietic stem cell (HSC) or a mesenchymal stem cell (MSC).

143. The method or cell of any one of embodiments 138 to 140, wherein the cell is an immune cell.

144. The method or cell of embodiment 143, wherein the cell is a T cell, a phagocyte, a microglial cell, or a macrophage.

145. The method or cell of embodiment 144, wherein the cell is CD4+ T cell.

146. The method or cell of embodiment 144 or embodiment 145, wherein the cell is CD8+ T cell.

147. The method or cell of any one of embodiments 138 to 146, wherein the cell is a primary cell.

148. The method or cell of any one of embodiments 138 to 146, wherein the cell is a progeny of a primary cell.

149. The method or cell of any one of embodiments 138 to 148, wherein the cell has dysfunctional mitochondria.

150. The method or cell of any one of embodiments 138 to 149, wherein the cell is from a subject having an age-related disease.

151. The method or cell of embodiment 150, wherein the age-related disease is an autoimmune disease, a metabolic disease, a genetic disease, cancer, a neurodegenerative disease, or immunosenescence.

152. The method or cell of any one of embodiments 138 to 151, wherein the cell is from a subject having a mitochondrial disease or disorder.

153. The method or cell of embodiment 152, wherein the mitochondrial disease or disorder is caused by mitochondrial DNA abnormalities, nuclear DNA abnormalities, or both.

154. The method or cell of embodiment 152 or embodiment 153, wherein the mitochondrial disease or disorder is chronic progressive external ophthalmoplegia (CPEO), Pearson syndrome, Kearns-Sayre syndrome (KSS), diabetes and deafness (DAD),

mitochondrial diabetes, Leber hereditary optic neuropathy (LHON), LHON-plus, neuropathy, ataxia, retinitis pigmentosa syndrome (NARP), maternally inherited Leigh syndrome (MILS), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged-red fiber disease (MERRF), familial bilateral striatal necrosis/striatonigral degeneration (FBSN), Luft disease, aminoglycoside-induced Deafness (AID), or multiple deletions of mitochondrial DNA syndrome.

155. The method or cell of embodiment 152 or embodiment 153, wherein the mitochondrial disease or disorder is Mitochondrial DNA depletion syndrome-4A, mitochondrial recessive ataxia syndrome (MIRAS), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), mitochondrial DNA depletion syndrome (MTDPS), DNA polymerase gamma (POLG)-related disorders, sensory ataxia neuropathy dysarthria ophthalmoplegia (SANDO), leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), co-enzyme Q10 deficiency, Leigh syndrome, mitochondrial complex abnormalities, fumarase deficiency,  $\alpha$ -ketoglutarate dehydrogenase complex (KGDHC) deficiency, succinyl-CoA ligase deficiency, pyruvate dehydrogenase complex deficiency (PDHC), pyruvate carboxylase deficiency (PCD), carnitine palmitoyltransferase I (CPT I) deficiency, carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acyl-carnitine (CACT) deficiency, autosomal dominant-/ autosomal recessive- progressive external ophthalmoplegia (ad-/ar-PEO), infantile onset spinal cerebellar atrophy (IOSCA), mitochondrial myopathy (MM) spinal muscular atrophy (SMA), growth retardation, aminoaciduria, cholestasis, iron overload, early death (GRACILE), or Charcot-Marie-Tooth disease type 2 A (CMT2A).

156. The method or cell of any one of embodiments 138 to 155, wherein the cell is from a subject having a neurodegenerative disease.

157. The method or cell of embodiment 156, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, Charcot Marie Tooth disease or leukodystrophy.

158. The method or cell of embodiment 156, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS).

159. The method or cell of embodiment 156, wherein the neurodegenerative disease is Huntington's disease.

160. The method or cell of embodiment 156, wherein the neurodegenerative disease is Alzheimer's disease, optionally wherein the cell is from a subject having an APOE4 allele, for example an E3/E4 or E4/E4 genotype.

161. The method or cell of embodiment 156, wherein the neurodegenerative disease is Parkinson's disease.

162. The method or cell of embodiment 156, wherein the neurodegenerative disease is Friedreich's ataxia. Charcot Marie Tooth disease.

163. The method or cell of embodiment 156, wherein the neurodegenerative disease is Leukodystrophy.

164. The method or cell of any one of embodiments 138 to 163, wherein the cell is from a subject having a retinal disease.

165. The method or cell of embodiment 164, wherein the retinal disease is age-related macular degeneration, macular edema or glaucoma.

166. The method or cell of any one of embodiments 138 to 165, wherein the cell is from a subject having diabetes.

167. The method or cell of any one of embodiments 138 to 166, wherein the cell is from a subject having a hearing disorder.

168. The method or cell of any one of embodiments 138 to 167, wherein the cell is from a subject having a genetic disease.

169. The method or cell of embodiment 168, wherein the genetic disease is Hutchinson-Gilford Progeria Syndrome, Werner Syndrome, or Huntington's disease.

170. The method or cell of any one of embodiments 138 to 169, wherein the cell is from a subject having heart failure.

171. The method or cell of any one of embodiments 138 to 170, wherein the cell is from a subject having immunodeficiency.

172. The method or cell of any one of embodiments 138 to 171, wherein the cell is from a subject having cancer.

173. The method or cell of any one of embodiments 138 to 172, wherein the cell is from a subject having an infectious disease.

174. The method or cell of any one of embodiments 138 to 148, wherein the cell is from a healthy donor.

175. The method or cell of any one of embodiments 138 to 174, which is an *ex vivo* cell.

176. The method of embodiment 175, further comprising administering the cell to a subject, optionally wherein the subject is the same subject from which the cell originated.

177. A method of treating a subject having an age-related disease, mitochondrial disease or disorder, neurodegenerative disease, retinal disease, diabetes, hearing disorder, genetic disease, heart failure, immunodeficiency, cancer, or infectious disease, the method comprising administering a therapeutically effective amount of cells according to any one of embodiments 133 to 175 to the subject.

178. The method of embodiment 177, wherein the subject has an age-related disease.

179. The method of embodiment 178, wherein the age-related disease is an autoimmune disease, a metabolic disease, a genetic disease, cancer, a neurodegenerative disease, or immunosenescence.

180. The method of embodiment 177, wherein the subject has a mitochondrial disease or disorder.

181. The method of embodiment 180, wherein the mitochondrial disease or disorder is caused by mitochondrial DNA abnormalities, nuclear DNA abnormalities, or both.

182. The method of embodiment 180 or embodiment 181, wherein the mitochondrial disease or disorder is chronic progressive external ophthalmoplegia (CPEO), Pearson syndrome, Kearns-Sayre syndrome (KSS), diabetes and deafness (DAD), mitochondrial diabetes, Leber hereditary optic neuropathy (LHON), LHON-plus, neuropathy, ataxia, and retinitis pigmentosa syndrome (NARP), maternally inherited Leigh syndrome (MILS), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged-red fiber disease (MERRF), familial bilateral striatal necrosis/striatonigral degeneration (FBSN), Luft disease, aminoglycoside-induced Deafness (AID), and multiple deletions of mitochondrial DNA syndrome.

183. The method of embodiment 180 or embodiment 181, wherein the mitochondrial disease or disorder is Mitochondrial DNA depletion syndrome-4A, mitochondrial recessive ataxia syndrome (MIRAS), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), mitochondrial DNA depletion syndrome (MTDPS), DNA polymerase gamma (POLG)-related disorders, sensory ataxia neuropathy dysarthria ophthalmoplegia (SANDO),

leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), co-enzyme Q10 deficiency, Leigh syndrome, mitochondrial complex abnormalities, fumarase deficiency,  $\alpha$ -ketoglutarate dehydrogenase complex (KGDHC) deficiency, succinyl-CoA ligase deficiency, pyruvate dehydrogenase complex deficiency (PDHC), pyruvate carboxylase deficiency (PCD), carnitine palmitoyltransferase I (CPT I) deficiency, carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acyl-carnitine (CACT) deficiency, autosomal dominant-/ autosomal recessive- progressive external ophthalmoplegia (ad-/ar-PEO), infantile onset spinal cerebellar atrophy (IOSCA), mitochondrial myopathy (MM) spinal muscular atrophy (SMA), growth retardation, aminoaciduria, cholestasis, iron overload, early death (GRACILE), or Charcot-Marie-Tooth disease type 2 A (CMT2A).

184. The method of embodiment 177, wherein the subject has a neurodegenerative disease.

185. The method of embodiment 184, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, Charcot Marie Tooth disease or leukodystrophy.

186. The method of embodiment 177, wherein the subject has a retinal disease.

187. The method of embodiment 186, wherein the retinal disease is age-related macular degeneration, macular edema or glaucoma.

188. The method of embodiment 177, wherein the subject has diabetes.

189. The method of embodiment 177, wherein the subject has a hearing disorder.

190. The method of embodiment 177, wherein the subject has a genetic disease.

191. The method of embodiment 190, wherein the genetic disease is Hutchinson-Gilford Progeria Syndrome, Werner Syndrome, or Huntington's disease.

192. The method of embodiment 177, wherein the subject has heart failure.

193. The method of embodiment 177, wherein the subject has immunodeficiency.

194. The method of embodiment 177, wherein the subject has cancer.

195. The method of embodiment 177, wherein the subject has an infectious disease.

196. A pharmaceutical composition comprising the polypeptide of any one of embodiments 1 to 84, the nucleic acid of any one of embodiments 85 to 94, the particle of

embodiment 95, or the cell of any one of embodiments 133 to 175 and a pharmaceutically acceptable excipient.

197. A kit comprising (a) the polypeptide of any one of embodiments 1 to 84, the nucleic acid of any one of embodiments 85 to 94, or the particle of embodiment 95 and (b) a stabilizing agent.

198. The kit of embodiment 197, wherein the stabilizing agent is trimethoprim (TMP) when the destabilizing domain sequence is a DHFR destabilization domain sequence.

199. The kit of embodiment 197, wherein the stabilizing agent is Shield-1, rapamycin, or FK506 when the destabilizing domain sequence is a FKBP destabilization domain sequence.

200. The kit of embodiment 197, wherein the stabilizing agent is sildenafil, vardenafil, tadalafil, avanafil, lodenafil, mirodenafil, udenafil, benzamidenafil, dasantafil, or beminafil when the destabilizing domain sequence is a PDE5 destabilization domain sequence.

## 7. CITATION OF REFERENCES

**[0158]** All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. In the event that there is an inconsistency between the teachings of one or more of the references incorporated herein and the present disclosure, the teachings of the present specification are intended

## WHAT IS CLAIMED IS:

1. A polypeptide comprising:
  - (a) a mitochondrial targeting sequence (MTS);
  - (b) an endonuclease sequence; and
  - (c) a destabilizing domain sequence.
2. The polypeptide of claim 1, wherein the MTS comprises a human MTS.
3. The polypeptide of claim 1, wherein the MTS comprises a non-human MTS.
4. The polypeptide of any one of claims 1 to 3, wherein the MTS comprises an MTS of a mitochondrial protein.
5. The polypeptide of any one of claims 1 to 4, wherein the MTS comprises an MTS of a TCA cycle-related enzyme, a chaperone protein, a mitochondrial genome replication protein, a protease, an mRNA processing protein, a mitochondrial RNA degradation protein, a deoxynucleotide triphosphate synthesis-related protein, a mitoribosomal protein, a phospholipid metabolism-related protein, a protein involved in metabolism of toxic compounds, a disulfide relay system-related protein, an iron-sulfur protein assembly protein, a tRNA modification protein, an aminoacyl-tRNA synthetase, a release factor, or an elongation factor.
6. The polypeptide of any one of claims 1 to 5, wherein the MTS comprises an MTS of a cytochrome c oxidase subunit.
7. The polypeptide of claim 6, wherein the MTS comprises an MTS of a cytochrome c oxidase subunit VIII (COX8), cytochrome c oxidase subunit X (COX10), cytochrome c oxidase subunit IV (COX4).
8. The polypeptide of any one of claims 1 to 5, wherein the MTS comprises:
  - (a) an MTS of a frataxin (FXN) protein;
  - (b) an MTS of a TCA cycle-related enzyme, optionally which is Pyruvate dehydrogenase, Citrate synthase, Aconitase, Isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, Succinyl-CoA synthetase, Succinic dehydrogenase, Fumarase, Malate dehydrogenase, or Pyruvate carboxylase;
  - (c) an MTS of a chaperone protein, optionally which is mtHSP10, mtHSP60, mtHSP70, or mtHSP90;

- (d) an MTS of a mitochondrial genome replication protein, optionally which is TFAM, Twinkle, PolG, TFB2M, TEFM, or MTERF1;
- (e) an MTS of a protease, optionally which is MPP, CLPXP, LON ATPase, or PreP;
- (f) an MTS of an mRNA processing protein, optionally which is LRPPRC, TACO1, ELAC2, PNPT1, HSD17B10, MTPAP, or PTCD1;
- (g) an MTS of a mitochondrial RNA degradation protein, optionally which is PNPase, REX02, or SUV3;
- (h) an MTS of a deoxynucleotide triphosphate synthesis-related protein, optionally which is DGUOK, TK2, TYMP, MGME1, SUCLG1, SUCLA2, RNASEH1, or C10orf2;
- (i) an MTS of a mitochondrial protein, optionally which is MRPS16, MRPS22, MRPL3, MRP12, or MRPL44;
- (j) an MTS of a phospholipid metabolism-related protein, optionally which is AGK, SERAC1, or TAZ;
- (k) an MTS of a protein involved in metabolism of toxic compounds, optionally which is HIBCH, ECHS1, ETHE1 or MPV17;
- (l) an MTS of a disulfide relay system-related protein, optionally which is GFER;
- (m) an MTS of an iron-sulfur protein assembly protein, optionally which is ISCU, BOLA3, NFU1, or IBA57;
- (n) an MTS of a tRNA modification protein, optionally which is MTO1, GTP3BP, TRMU, PUS1, MTFMT, TRIT1, TRNT1 or TRMT5;
- (o) an MTS of an aminoacyl-tRNA synthetase, optionally which is AARS2, DARS2, EARS2, RARS2, YARS2, FARS2, HARS2, LARS2, VARS2, TARS2, IARS2, CARS2, PARS2, NARS2, KARS, GARS, SARS2 or MARS2;
- (p) an MTS of a release factor, optionally which is a C12orf65; or
- (q) an MTS of an elongation factor, optionally which is TUFM, TSFM, or GFM1.

9. The polypeptide of claim 1, wherein the MTS comprises a sequence that is at least 80% identical to any one of SEQ ID NOs. 1-15.

10. The polypeptide of claim 9, wherein the MTS comprises a sequence that is 100% identical to SEQ ID NO:1.

11. The polypeptide of claim 9, wherein the MTS comprises a sequence that is 100% identical to SEQ ID NO:2.
12. The polypeptide of any one of claims 1 to 11, wherein the endonuclease is a restriction endonuclease, an RNA-guided endonuclease (e.g., Cas9 or Cas12), a zinc finger nuclease, or a transcription activator-like effector nuclease (TALEN).
13. The polypeptide of claim 12, wherein the endonuclease is a restriction endonuclease.
14. The polypeptide of claim 13, wherein the restriction endonuclease is an XbaIR, EcoRI, SmaI, AflII, BamHI, BclI, HaeIII, HindII, HindIII, NdeI, PvuII, PstI, or SpeI endonuclease.
15. The polypeptide of claim 14, wherein the restriction endonuclease is an XbaIR endonuclease.
16. The polypeptide of any one of claims 1 to 13, wherein the endonuclease comprises a sequence that is at least 80% identical to SEQ ID NO:16.
17. The polypeptide of claim 16, wherein the endonuclease sequence comprises a sequence that is 100% identical to SEQ ID NO:16.
18. The polypeptide of any one of claims 1 to 17, wherein the destabilizing domain sequence is a DHFR, FKBP, or PDE5 destabilization domain sequence.
19. The polypeptide of claim 18, wherein the destabilization domain sequence is a DHFR destabilization domain sequence.
20. The polypeptide of claim 19, wherein the destabilization domain sequence is an *E. coli* DHFR (ecDHFR) destabilization domain sequence.
21. The polypeptide of any one of claims 1 to 20, wherein the destabilization domain sequence comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO:18.
22. The polypeptide of any one of claims 1 to 18, wherein the destabilization domain sequence comprises a sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or 100% identical to SEQ ID NO:20.
23. The polypeptide of any one of claims 1 to 22, wherein the MTS is positioned N-terminal to the endonuclease sequence and the destabilizing domain sequence.

24. The polypeptide of any one of claims 1 to 22, wherein the MTS is positioned C-terminal to the endonuclease sequence and the destabilizing domain sequence.
25. The polypeptide of any one of claims 1 to 24, wherein the endonuclease sequence is positioned N-terminal to the destabilization domain sequence.
26. The polypeptide of any one of claims 1 to 24, wherein the endonuclease sequence is positioned C-terminal to the destabilization domain sequence.
27. The polypeptide of any one of claims 1 to 22, wherein the MTS is positioned N-terminal to the endonuclease sequence and the endonuclease sequence is positioned N-terminal to the destabilizing domain sequence.
28. A nucleic acid encoding the polypeptide of any one of claims 1 to 27.
29. A particle comprising the nucleic acid of claim 28, optionally wherein the particle is a retroviral particle.
30. A host cell comprising the nucleic acid of claim 28.
31. A method of (a) inducing mitophagy in a cell, and/or (b) increasing mitochondrial turnover in a cell, and/or (c) increasing mitochondrial mass, and/or (d) inducing double strand breaks in mitochondrial DNA and/or (e) inducing epigenomic modifications in a cell, the method comprising contacting the cell with (i) the polypeptide of any one of claims 1 to 27, the nucleic acid of claim 28, or the particle of claim 29 and (ii) a stabilizing agent.
32. The method of claim 31, wherein the stabilizing agent is trimethoprim (TMP) when the destabilizing domain sequence is a DHFR destabilization domain sequence.
33. The method of claim 31, wherein the stabilizing agent is Shield-1, rapamycin, or FK506 when the destabilizing domain sequence is a FKBP destabilization domain sequence.
34. The method of any one of claims 31 to 33, which further comprises, subsequent to contacting the cell with the stabilizing agent, removing the stabilizing agent from the cell.
35. A cell obtained or obtainable by the method of any one of claims 31 to 34.
36. A cell comprising the polypeptide of any one of claims 1 to 27, the nucleic acid of claim 28, or particle of claim 29.

37. The method of any one of claims 31 to 34 or cell of claim 35 or claim 36, wherein the cell is a mammalian cell.

38. The method or cell of claim 37, wherein the cell is a human cell.

39. The method or cell of claim 37 or claim 38, wherein the cell is a somatic cell, bone marrow cell, hematopoietic stem cell (HSC) or a mesenchymal stem cell (MSC), or an immune cell.

40. The method or cell of any one of claims 37 to 39, wherein the cell is from a subject having an age-related disease.

41. The method or cell of claim 40, wherein the age-related disease is an autoimmune disease, a metabolic disease, a genetic disease, cancer, a neurodegenerative disease, or immunosenescence.

42. The method or cell of any one of claims 37 to 41, wherein the cell is from a subject having a mitochondrial disease or disorder.

43. The method or cell of claim 42, wherein the mitochondrial disease or disorder is chronic progressive external ophthalmoplegia (CPEO), Pearson syndrome, Kearns-Sayre syndrome (KSS), diabetes and deafness (DAD), mitochondrial diabetes, Leber hereditary optic neuropathy (LHON), LHON-plus, neuropathy, ataxia, retinitis pigmentosa syndrome (NARP), maternally inherited Leigh syndrome (MILS), mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged-red fiber disease (MERRF), familial bilateral striatal necrosis/striatonigral degeneration (FBSN), Luft disease, aminoglycoside-induced Deafness (AID), or multiple deletions of mitochondrial DNA syndrome.

44. The method or cell of claim 42, wherein the mitochondrial disease or disorder is Mitochondrial DNA depletion syndrome-4A, mitochondrial recessive ataxia syndrome (MIRAS), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), mitochondrial DNA depletion syndrome (MTDPS), DNA polymerase gamma (POLG)-related disorders, sensory ataxia neuropathy dysarthria ophthalmoplegia (SANDO), leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL), co-enzyme Q10 deficiency, Leigh syndrome, mitochondrial complex abnormalities, fumarase deficiency,  $\alpha$ -ketoglutarate dehydrogenase complex (KGDHC) deficiency, succinyl-CoA ligase deficiency, pyruvate dehydrogenase complex deficiency (PDHC), pyruvate carboxylase deficiency (PCD), carnitine palmitoyltransferase I (CPT I) deficiency, carnitine palmitoyltransferase II (CPT II) deficiency, carnitine-acyl-carnitine (CACT) deficiency, autosomal dominant-/ autosomal recessive-

progressive external ophthalmoplegia (ad-/ar-PEO), infantile onset spinal cerebellar atrophy (IOSCA), mitochondrial myopathy (MM) spinal muscular atrophy (SMA), growth retardation, aminoaciduria, cholestasis, iron overload, early death (GRACILE), or Charcot-Marie-Tooth disease type 2 A (CMT2A).

45. The method or cell of any one of claims 37 to 44, wherein the cell is from a subject having a neurodegenerative disease.

46. The method or cell of claim 45, wherein the neurodegenerative disease is amyotrophic lateral sclerosis (ALS), Huntington's disease, Alzheimer's disease, Parkinson's disease, Friedreich's ataxia, Charcot Marie Tooth disease or leukodystrophy.

47. The method or cell of any one of claims 37 to 46, wherein the cell is from a subject having a retinal disease, diabetes, a hearing disorder, a genetic disease, heart failure, immunodeficiency, cancer, or infectious disease.

48. The method or cell of any one of claims 37 to 47, wherein the cell is an *ex vivo* cell.

49. The method of claim 48, further comprising administering the cell to a subject, optionally wherein the subject is the same subject from which the cell originated.

50. A method of treating a subject having an age-related disease, mitochondrial disease or disorder, neurodegenerative disease, retinal disease, diabetes, hearing disorder, genetic disease, heart failure, immunodeficiency, cancer, or infectious disease, the method comprising administering a therapeutically effective amount of cells according to any one of claims 35 to 48 to the subject.

51. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 27, the nucleic acid of claim 28, the particle of claim 29, or the cell of any one of claims 35 to 48 and a pharmaceutically acceptable excipient.

52. A kit comprising (a) the polypeptide of any one of claims 1 to 27, the nucleic acid of claim 28, or the particle of claim 29 and (b) a stabilizing agent.