



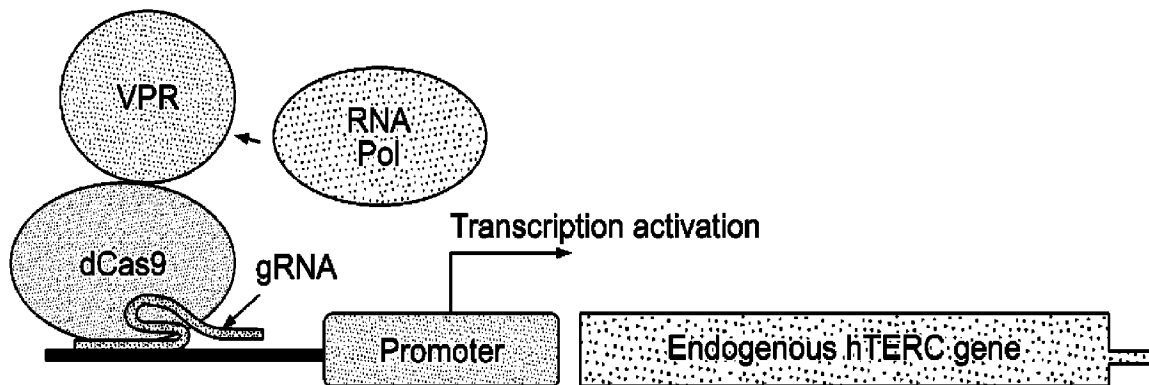
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 (54) Title: METHODS AND COMPOSITIONS FOR CELL AND TISSUE REJUVENATION



**FIG. 1**

(57) **Abrégé/Abstract:**

The present disclosure provides compositions, methods and kits for the rejuvenation of target cells. In some aspects, the compositions, methods and kits comprise mRNAs that promote the expression of TERT and/or TERC.

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**Abstract:**

The present disclosure provides compositions, methods and kits for the rejuvenation of target cells. In some aspects, the compositions, methods and kits comprise mRNAs that promote the expression of TERT and/or TERC.

## **METHODS AND COMPOSITIONS FOR CELL AND TISSUE REJUVENATION**

### **RELATED APPLICATIONS**

[0001] This application claims priority to, and the benefit of, U.S. Provisional Application No. 62/899,861, filed September 13, 2019, the contents of which is incorporated herein by reference in their entirety.

### **SEQUENCE LISTING**

[0002] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on August 31, 2020, is named "UNCO-028\_001WO\_SeqList.txt" and is about 241 KB in size.

### **BACKGROUND OF THE INVENTION**

[0003] The shortening of telomeres, repetitive DNA sequences at the ends of linear chromosomes, can lead to cellular senescence, apoptosis, or malignancy. In particular, the shortening of telomeres in cells cultured *in vitro* is an obstacle to the production of therapeutic cell populations, as shortened telomeres can limit further expansions of the therapeutic cell populations as well as degrade the cells' biological activity, leading to a decrease clinical efficacy. Increasing telomere length in cells can lead to cellular rejuvenation, but can also cause deleterious side-effects such as oncogenic cellular immortalization. Thus, there is a need in the art for compositions, kits and methods directed to effectively and safely increasing the length of telomeres in cells in a controllable way, thereby rejuvenating the cells.

### **SUMMARY OF THE INVENTION**

[0004] The present disclosure provides a composition comprising: a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0005] The present disclosure provides a composition comprising: a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0006] A first polynucleotide molecule can comprise an mRNA molecule encoding at least a portion of TERT. A first polynucleotide molecule can comprise a plasmid comprising a nucleic acid sequence encoding at least a portion of TERT operably linked to at least one promoter sufficient to drive expression of the at least one portion of TERT.

[0007] A second polynucleotide molecule can comprise an mRNA molecule encoding at least a portion of at least one DNA targeting polypeptide. A second polynucleotide molecule can comprise a plasmid comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide operably linked to at least one promoter sufficient to drive expression of the at least one portion of the at least one DNA targeting polypeptide.

[0008] A DNA targeting polypeptide can comprise at least one Cas9 molecule, at least one Cas9 variant molecule, at least one Cas9 ortholog molecule or any combination thereof. A Cas9 molecule, a Cas9 variant molecule or a Cas9 ortholog molecule can be nuclease-deficient or nuclease-dead. A Cas9 variant molecule can comprise eSpCas9 (K855A), eSpCas9 (1.0), eSpCas9 (1.1), SpCas9-HF1 (VP12), HypaCas9, xCas9, SpyFi Cas9, iSpy Cas9, iSpyMac, Cas9 (VQR), Cas9 (EQR), Cas9 (VRER), Cas9 (D1135E), Cas9(QQR1), SaCas9 (KKH), Nme1Cas9, Nme2Cas9, Nme3Cas9 or any combination thereof. A Cas9 ortholog molecule can comprise *Streptococcus pyogenes* Cas9 (spCas9), *Francisella novicida* Cas9 (FnCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Neisseria meningitidis* Cas9 (NmCas9; NmeCas9), *Streptococcus thermophilus* CRISPR1-Cas9 (St1Cas9), *Streptococcus thermophilus* CRISPR3-Cas9 (St3Cas9), *Campylobacter jejuni* Cas9 (CjCas9), *Acidaminococcus* sp. BV3L6 Cpf1 (AsCpf1), *Lachnospiraceae* bacterium ND2006 Cpf1 (LbCpf1), *Streptococcus canis* Cas9 (ScCas9), *Treponema denticola* Cas9 (TdCas9), *Streptococcus macacae* Cas9 (SmacCas9), Cas $\phi$  (Cas12j), *Francisella tularensis* subsp. *novicida* Cas9, *Pasteurella multocida* Cas9, *Campylobacter lari* CF89-12 Cas9, *Mycoplasma gallisepticum* str. F Cas9, *Nitratifactor salsuginis* str DSM 16511 Cas9, *Parvibaculum lavamentivorans* Cas9, *Roseburia intestinalis* Cas9, *Neisseria cinerea* Cas9, *Gluconacetobacter diazotrophicus* Cas9, *Azospirillum* B510 Cas9, *Sphaerochaeta globus* str. Buddy Cas9, *Flavobacterium columnare* Cas9, *Fluviicola taffensis* Cas9, *Bacteroides*

coprophilus Cas9, Mycoplasma mobile Cas9, Lactobacillus farciminis Cas9, Streptococcus pasteurianus Cas9, Lactobacillus johnsonii Cas9, Staphylococcus pseudintermedius Cas9, Filifactor alocis Cas9, Legionella pneumophila str. Paris Cas9, Sutterella wadsworthensis Cas9, Corynebacter diphtheriae Cas9 or any combination thereof.

[0009] A DNA targeting polypeptide can comprise at least one TALE molecule, at least one zinc-finger molecule, at least one meganuclease molecule or any combination thereof

[0010] A DNA targeting polypeptide can comprise at least one transactivation molecule. A transactivation molecule can comprise at least one P65 molecule, at least one Rta molecule, at least one VP16 molecule, at least one VP64 molecule, at least one VP160 molecule, at least one VP64-P65-Rta (VPR) molecule, at least one SunTag peptide, at least one single guide RNA-MS2 (sgRNA-MS2) molecule or any combination thereof. In some aspects, a DNA targeting polypeptide can be a DNA targeting ribonucleoprotein (RNP) complex. A DNA targeting ribonucleoprotein complex can comprise both at least one protein component and at least one nucleic acid component. A DNA targeting polypeptide can comprise at least one guide RNA. A transactivation molecule can comprise at least one single guide RNA-MS2 (sgRNA-MS2) molecule. An sgRNA-MS2 molecule can comprise a nucleic acid sequence complementary to a nucleic acid sequence located upstream, within, or downstream of the endogenous TERC gene and at least about one, or at least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten MS2 RNA aptamers.

[0011] A DNA targeting polypeptide can comprise a dCas9 molecule and a VPR molecule.

[0012] A DNA targeting polypeptide can bind upstream of, 5' to, within, downstream of or 3' to the endogenous TERC gene.

[0013] An mRNA molecule can be a modified mRNA molecule. A modified mRNA molecule can comprise at least one modified ribonucleoside base. A modified ribonucleoside base can comprise a pseudouridine (Ψ) residue, a 5-methylcytidine (m<sup>5</sup>C) residue or any combination thereof. A modified mRNA molecule can comprise at least one modified nucleoside. A modified nucleoside can comprise 5-methylcytidine (m<sup>5</sup>C), 5-methyluridine (m<sup>5</sup>U), N6-methyladenosine (m<sup>6</sup>A), inosine 2'-O-methylated nucleosides or any combination thereof.

[0014] Any composition of the present disclosure can further comprise a plurality of guide RNA (gRNA) molecules, wherein at least one gRNA in the plurality is complementary to a nucleic

acid sequence located upstream, within, or downstream of the endogenous TERC gene. A plurality of gRNA molecules can comprise at least about one, or at least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten distinct species of gRNA molecules, wherein each species has a different nucleic acid. Any composition of the present disclosure can further comprise at least one plasmid comprising at least one nucleic acid sequence encoding at least one species of gRNA operably linked to at least one promoter sufficient to drive expression of the at least one species gRNA. A plurality of gRNA molecules can comprise a plurality of single guide RNA (sgRNA) molecules, crRNA:tracrRNA molecules, truncated sgRNA molecules, high fidelity scaffold gRNA molecules or any combination thereof. A guide RNA molecule can be a modified guide RNA (mod gRNA) molecule. A guide RNA molecule can comprise any sequence recited in Table 1 or Table 2.

**[0015]** The present disclosure provides a composition comprising: a) at least one modified mRNA molecule comprising a nucleic acid sequence encoding at least a portion of human telomerase reverse transcriptase (hTERT); b) at least one modified mRNA molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the at least one DNA targeting polypeptide comprises dCas9 and a VP64-P65-Rta (VPR) molecule; and c) a plurality of guide RNA (gRNA) molecules, wherein at least one gRNA in the plurality is complementary to a nucleic acid sequence located upstream of the endogenous hTERT gene.

**[0016]** Any composition of the present disclosure can comprise at least one mRNA and/or polynucleotide encoding at least one rejuvenating factor. A rejuvenating factor can comprise telomerase RNA component (TERC), telomerase associated reverse-transcriptase (TERT), protection of telomeres 1 (POT1), insulin-like growth factor 1 (IGF1), WD repeat containing antisense to TP53 (WRAP53), nuclear protein family A, member 3 (NOP3), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), shelterin complex subunit and telomerase recruitment factor (ACD/TPP1), TRF-1 interacting ankyrin-related ADP-ribose polymerase (TNKS), telomeric repeat binding factor 1 (TRF-1), telomeric repeat binding factor 2 (TRF-2), TERF1 interacting nuclear factor 2 (TIN2), telomeric repeat binding factor 2 (Rap1), Dyskerin Pseudouridine Synthase 1 (DKC1), ribonucleoprotein NHP2 or any combination thereof.

**[0017]** TERT can be human TERT (hTERT). TERC can be human TERC (hTERC).

**[0018]** The present disclosure provides a composition comprising at least one viral particle comprising any composition of the present disclosure. A viral particle can be an adeno-associated virus (AAV) particle, adenovirus particle, lentivirus particle, foamy-virus particle, herpes simplex virus (HSV) particle, retrovirus particle, alphavirus particle, flavivirus particle, rhabdovirus particle, measles virus particle, Newcastle disease virus particle, poxvirus particle, picornavirus particle, or any combination thereof. An AAV particle can be an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV2/1, AAV2/2, AAV2/3, AAV2/4, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV-DJ or AAV-DJ8 particle. A retrovirus particle can be MMSV or MSCV particle. A lentivirus particle can be HIV-1 or HIV-2 particle. An alphavirus particle can be SFV, SIN, VEE, or M1 particle. A flavivirus particle can be Kunjin virus, West Nile virus, or Dengue virus particle.

**[0019]** The present disclosure provides a composition comprising at least one exosome, microvesicle or liposome, wherein the at least one exosome, microvesicle or liposome comprises any composition of the present disclosure. The present disclosure provides a composition comprising least one nanoparticle, wherein the at least one nanoparticle comprises any composition of the present disclosure. A nanoparticle can comprise a liposome, a micelle, a polymer-based nanoparticle, a lipid-polymer based nanoparticle, a metal based nanoparticle, a nanocrystal, a carbon nanotube based nanoparticle or a polymeric micelle.

**[0020]** The present disclosure provides a kit comprising any composition of the present disclosure.

**[0021]** The present disclosure provides a method of rejuvenating at least one cell, the method comprising contacting the at least one cell with any composition or kit of the present disclosure. The preceding method can further comprise expanding the at least one cell contacted with any composition or kit of the present disclosure to produce a plurality of rejuvenated cells.

**[0022]** The present disclosure provides a method of treating and/or preventing a disease in a subject comprising: a) contacting at least one cell with any composition or kit of the present disclosure; b) expanding the at least one cell contacted with any composition or kit of the present disclosure to produce a plurality of rejuvenated cells; and c) administering the plurality of rejuvenated cells to the subject.

**[0023]** The present disclosure provides a method of treating and/or preventing a disease in a subject comprising: a) contacting at least one cell with any composition or kit of the present

disclosure ; b) expanding the at least one cell contacted with any composition or kit of the present disclosure to produce a plurality of rejuvenated cells; c) culturing the plurality of rejuvenated cells under conditions sufficient to transform the plurality of rejuvenated cells into at least one tissue or organ; and d) administering the at least one tissue or organ to the subject.

[0024] The present disclosure provides a method of producing an *in vitro* tissue or organ comprising: a) contacting at least one cell with any composition or kit of the present disclosure; b) expanding the at least one cell contacted with any composition or kit of the present disclosure to produce a plurality of rejuvenated cells; c) culturing the plurality of rejuvenated cells under conditions sufficient to transform the plurality of rejuvenated cells into at least one tissue or organ.

[0025] The present disclosure provides a method of producing a plurality of rejuvenated edited cells comprising: a) contacting a plurality of cells with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with any composition or kit of the present disclosure; and d) expanding the at least one cell contacted with any composition or kit of the present disclosure to produce a plurality of rejuvenated edited cell.

[0026] The present disclosure provides a method of treating and/or preventing a disease in a subject comprising: a) contacting a plurality of cells with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with any composition or kit of the present disclosure; d) expanding the at least one cell contacted with any composition or kit of the present disclosure to produce a plurality of rejuvenated edited cells; and e) administering to the subject the plurality of rejuvenated edited cells.

[0027] The present disclosure provides a method of treating epidermolysis bullosa (EB) in a subject comprising: a) contacting a plurality of cells comprising keratinocytes, dermal fibroblasts, mesenchymal stem/stromal cells or any combination thereof with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with any composition or kit of the present disclosure; d)

expanding the at least one cell contacted with any composition or kit of the present disclosure to produce a plurality of rejuvenated edited cells; and e) administering to the subject the plurality of rejuvenated edited cells.

**[0028]** Expanding the at least one cell can comprise culturing the at least one cell using adjusted Opti-MEM, non-adjusted Opti-MEM, human serum, fetal bovine serum (FBS) or any combination thereof.

**[0029]** Rejuvenating at least one cell comprises can increase the expression of TERC in the at least one cell, increasing the expression of TERT in the at least one cell, increasing the total number of population doublings exhibited by the at least one cell, increasing the length of telomeres in the at least one cell, increasing the mitochondrial DNA copy number in the at least one cell, increasing the amount of mitochondrial DNA in the at least one cell, increasing the number of mitochondria in the at least one cell, increasing the migration activity of the at least one cell, restoring the young-like state of thiol group oxidation levels in proteins in the at least one cell, reducing senescence-associated DNA methylation in the at least one cell or any combination thereof.

**[0030]** An at least one cell can be a fibroblast, a keratinocyte, a mesenchymal stem/stromal cell, a peripheral blood mononuclear cell, a chimeric antigen receptor T cell (CAR-T cell), an endothelial cell, a chondrocyte, a muscle stem cell, a neural stem cell, a hepatocyte, a limbal stem cell, a retinal pigmented epithelial cell, a hematopoietic stem cell, a macrophage, a cardiomyocyte, a pancreatic cell, a  $\beta$ -cell or any combination thereof.

**[0031]** A disease can comprise graft-vs-host diseases (GvHD), autoimmune diseases, epidermolysis bullosa (EB), recessive dystrophic form of EB (RDEB), junctional EB (JEB), EB simplex (EBS), congenital ichthyosis, congenital dyskeratosis, macular degeneration, Parkinson's disease, Alzheimer's disease, aging, Type I and II diabetes, burns, chronic skin wounds, diabetes-associated ulcers/wounds, heart disease, osteoporosis, cancer, connective tissue diseases such as Ehlers-Danlos Syndrome (EDS) or Marfan syndrome, liver diseases, lung diseases, and any combination thereof.

**[0032]** Contacting at least one cell can comprise transfection, transduction, electroporation, nucleofection, at least one cell-penetrating peptide or any combination thereof.

[0033] The present disclosure provides a method for rejuvenating at least one cell in a subject comprising administering to the subject at least one therapeutically effective amount of any composition or kit of the present disclosure.

[0034] The present disclosure provides a method for rejuvenating at least one subject comprising administering to the subject at least one therapeutically effective amount of any composition or kit of the present disclosure.

[0035] A subject can be a mammal. A subject can be a human, a primate, a mouse, a rat, a dog, a cat, a cow, a horse, a goat, a camel, a sheep, a pig or any other mammal. A subject can be a bird.

[0036] Any of the above aspects can be combined with any other aspect.

[0037] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In the Specification, the singular forms also include the plural unless the context clearly dictates otherwise; as examples, the terms “a,” “an,” and “the” are understood to be singular or plural and the term “or” is understood to be inclusive. By way of example, “an element” means one or more element. Throughout the specification the word “comprising,” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term “about.”

[0038] Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The references cited herein are not admitted to be prior art to the claimed invention. In the case of conflict, the present Specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting. Other features and advantages of the disclosure will be apparent from the following detailed description and claim.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0039] The above and further features will be more clearly appreciated from the following detailed description when taken in conjunction with the accompanying drawings.

[0040] FIG. 1 is a schematic of a DNA-targeting molecule of the present disclosure binding upstream of an endogenous hTERC locus. In this non-limiting example, the DNA-targeting molecule comprises dCas9 and a transactivation molecule, wherein the transactivation molecule is a VP64-P65-Rta (VPR) molecule.

[0041] FIG. 2 is a schematic overview of a treatment method and/or a method of producing an *in vitro* tissue of the present disclosure

[0042] FIG. 3 is a schematic overview of a method of producing a plurality of rejuvenated edited cells of the present disclosure.

[0043] FIG. 4 is a chart showing the hTERC transcript level in various cell types.

[0044] FIG. 5 is a chart showing the level of human TERC RNA in F50 cells transfected with compositions of the present disclosure.

[0045] FIG. 6 is a series of charts showing the level of human TERC RNA in HEK<sub>n</sub> cells (left) and human Mesenchymal Stem/Stromal Cells (hMSCs) (right) transfected with compositions of the present disclosure (+dCas9-VPR+gmix) as compared to non-transfected HEK<sub>n</sub> cells, non-transfected hMSC cells and F50-derived induced pluripotent stem cells.

[0046] FIG. 7 is a series of charts showing the level of human TERC RNA in F50 cells (left) and hMSCs (right) transfected with compositions of the present disclosure lacking guide RNA (+dCas9-VPR (no guide)).

[0047] FIG. 8 is schematic overview of one of the transfection regimes of the present disclosure.

[0048] FIG. 9 is a chart showing the total population doubling of senescent F50S cells transfected with compositions of the present disclosure.

[0049] FIG. 10 is a schematic overview of an alternative transfection regime of the present disclosure.

[0050] FIG. 11 is a schematic overview of another transfection regime of the present disclosure.

[0051] FIG. 12 is a chart showing the relative telomere length in senescent F50S cells transfected with compositions of the present disclosure (+ TERT + dCas9-VPR/gmix) as compared to non-transfected F50S cells and F50-derived induced pluripotent stem cells.

[0052] FIG. 13 is a chart showing the relative telomere length in F50 cells transfected with compositions of the present disclosure (+ hTERT + dCas9-VPR/gmix) as compared to non-transfected F50 cells and F50-derived induced pluripotent stem cells.

[0053] FIG. 14 is a series of charts showing the relative telomere length in HEK293T cells (left) and hMSCs (right) transfected with compositions of the present disclosure (+TERT+dCas9-VPR/gmix) as compared to non-transfected HEK293T cells, non-transfected hMSCs and F50-derived induced pluripotent stem cells.

[0054] FIG. 15 is a series of charts showing the relative amount of mitochondrial DNA in F50 cells (left), HEK293T cells (middle) and hMSCs (right) transfected with compositions of the present disclosure (+TERT+dCas9-VPR/gmix) as compared to non-transfected F50 cells, non-transfected HEK293T cells and non-transfected hMSCs.

[0055] FIG. 16 is a gel image of the results of a telomerase activity assay in F50 cells transfected with various compositions of the present disclosure (F50 + TERT and F50 + TERT + dCas9-VPR/gmix) as well as non-transfected F50 cells and F50-derived induced pluripotent stem cells.

[0056] FIG. 17 is a series of representative microscopy images of adult human primary fibroblasts expanded from single cells that were not transfected (top two rows) or transfected with compositions of the present disclosure (+hTERT/dCas9-VPR+gRNA; bottom two rows).

[0057] FIG. 18 is a schematic overview of the transendothelial migration (TEM) assay.

[0058] FIG. 19 is chart showing the migration activity of hMSCs transfected with compositions of the present disclosure as measured using the TEM assay.

[0059] FIG. 20 is series chart showing the oxidation level of thiol groups detected in selected proteins in senescent hMSCs transfected with compositions of the present disclosure as compared to non-transfected young low passage and senescent high passage hMSCs.

[0060] FIG. 21 is a series chart showing the degree of methylation at 9 senescence-associated DNA methylation sites in senescent cells of different types transfected with compositions of the present disclosure as compared to non-transfected young low passage and senescent high passage cells.

#### **DETAILED DESCRIPTION OF THE INVENTION**

[0061] Telomeres comprise repetitive DNA sequences at the ends of linear chromosomes that, when sufficiently long, allow each chromosome end to form a loop that protects the ends from

acting as double-stranded or single-stranded DNA breaks. Telomeres shorten over time, due in part to oxidative damage and incomplete DNA replication, eventually leading to critically short telomeres unable to form the protective loop, exposure of the chromosome ends, chromosome-chromosome fusions, DNA damage responses, and cellular senescence, apoptosis, or malignancy.

[0062] The enzyme complex telomerase extends telomeres and comprises two essential components: the telomerase reverse transcriptase (TERT), and an RNA component known as telomerase RNA component (TERC). Other components of the telomerase complex include the proteins TCAB1, Dyskerin, Gar1, Nhp2, Nop10, and RHAU.

[0063] Due to the importance of telomere length maintenance in preventing cellular senescence and apoptosis and resulting cellular dysfunction, genetic mutations of TERT and TERC are linked to fatal inherited diseases of inadequate telomere maintenance, including forms of idiopathic pulmonary fibrosis, dyskeratosis congenita, and aplastic anemia. The effects of premature cellular senescence and apoptosis due to short telomeres in these diseases are devastating in themselves, and may be compounded by increased risk of cancer. Moreover, the shortening of telomeres in cells that are cultured *in vitro* results is also a major problem in the production of therapeutic cell populations, the creation of *in vitro* synthetic tissue and tumors and the *in vitro* creation of non-cancerous somatic cells lines for research and drug testing. Repeated passaging *in vitro* can lead to senescence and the lack of further expansion ability, and in the case of therapeutic cell populations, a decrease in clinically-relevant biological activity.

[0064] Thus, there is a clear need in the art for compositions, kits and methods directed to elongating telomeres in order to rejuvenate cells. Existing approaches directed to increasing the expression of TERT and/or TERC in target cells has relied on the use of integrating viruses to obtain the desired increase in TERT and/or TERC expression. However, these approaches suffer from safety concerns, as the integrating viruses can result in potentially dangerous, permanent genome modifications. Moreover, the sustained overexpression of TERT and/or TERC, and concomitant increases in telomere length, have been linked to cancer cell immortalization, making the integrating virus approach dangerous in a clinical context.

[0065] Without wishing to be bound by theory, the compositions, kits and methods of the present disclosure allow for the transient increase in TERT and/or TERC expression for a time period that is long enough to rejuvenate the target cells, but short enough to avoid deleterious and

dangerous off-target effects. The use of non-integrating RNA molecules in the present disclosure allows for fine-tuning of the expression levels and stoichiometry of rejuvenating factors in a clinically safe manner.

[0066] The compositions, kits and methods of the present disclosure can be used for a variety of different research and clinical applications, including, but not limited to, the production of therapeutic cell populations (*e.g.* CAR-T cell populations, mesenchymal stem/stromal cell populations), the production of *in vitro* tissue and organs for subsequent transplantation, research or drug testing, the production of genome-edited cell populations for therapeutic and research applications, the rejuvenation of senescent, aged and disease associated cell lines, etc.

[0067] Various compositions, kits and methods of the present disclosure are described in full detail herein.

[0068] Rejuvenating compositions

[0069] In some aspects, the present disclosure provides a composition comprising: a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0070] In some aspects, the present disclosure provides a composition comprising: a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0071] In some aspects, the at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT) can be an mRNA molecule encoding at least a portion of TERT. In some aspects, the at least one first polynucleotide molecule can be a plasmid comprising a nucleic acid sequence encoding at least a portion of TERT operably linked to at least one promoter sufficient to drive expression of the at least one portion of TERT.

[0072] In some aspects, the at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide can be an mRNA molecule encoding at least a portion of at least one DNA targeting polypeptide. In some

aspects, the at least one second polynucleotide molecule can be a plasmid comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide operably linked to at least one promoter sufficient to drive expression of the at least one portion of the at least one DNA targeting polypeptide.

[0073] Thus, the present disclosure provides a composition comprising: a) at least one first mRNA molecule encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one second mRNA molecule encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0074] The present disclosure also provides a composition comprising: a) at least one plasmid comprising a nucleic acid sequence encoding at least a portion of TERT operably linked to at least one promoter sufficient to drive expression of the at least one portion of TERT; and b) at least one second mRNA molecule encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0075] The present disclosure also provides a composition comprising: a) at least one first mRNA molecule encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one plasmid comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide operably linked to at least one promoter sufficient to drive expression of the at least one portion of the at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0076] The present disclosure also provides a composition comprising: a) at least one plasmid comprising a nucleic acid sequence encoding at least a portion of TERT operably linked to at least one promoter sufficient to drive expression of the at least one portion of TERT; and b) at least one plasmid comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide operably linked to at least one promoter sufficient to drive expression of the at least one portion of the at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

[0077] In some aspects, a DNA targeting polypeptide can comprise at least one Cas9 molecule, at least one Cas9 variant molecule, at least one Cas9 ortholog molecule or any combination thereof.

[0078] In some aspects, a Cas9 molecule, a Cas9 variant molecule or a Cas9 ortholog molecule can be nuclease-deficient or nuclease-dead. As used herein, the term “dCas9” is used in its broadest sense to refer to a Cas9 molecule, ortholog and/or variant that is nuclease-deficient or nuclease dead. In a non-limiting example, a Cas9 molecule, a Cas9 variant molecule or a Cas9 ortholog molecule can comprise at least one mutation, deletion or insertion which renders the Cas9 molecule, the Cas9 variant molecule or the Cas9 ortholog molecule nuclease-deficient or nuclease-dead.

[0079] In some aspects, a Cas9 variant molecule can comprise eSpCas9 (K855A), eSpCas9 (1.0), eSpCas9 (1.1), SpCas9-HF1 (VP12), HypaCas9, xCas9, SpyFi Cas9, iSpy Cas9, iSpyMac, Cas9 (VQR), Cas9 (EQR), Cas9 (VRER), Cas9 (D1135E), Cas9(QQR1), SaCas9 (KKH), Nme1Cas9, Nme2Cas9, Nme3Cas9 or any combination thereof.

[0080] In some aspects, a Cas9 ortholog molecule can comprise *Streptococcus pyogenes* Cas9 (spCas9), *Francisella novicida* Cas9 (FnCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Neisseria meningitidis* Cas9 (NmCas9; NmeCas9), *Streptococcus thermophilus* CRISPR1-Cas9 (St1Cas9), *Streptococcus thermophilus* CRISPR3-Cas9 (St3Cas9), *Campylobacter jejuni* Cas9 (CjCas9), *Acidaminococcus* sp. BV3L6 Cpf1 (AsCpf1), *Lachnospiraceae* bacterium ND2006 Cpf1 (LbCpf1), *Streptococcus canis* Cas9 (ScCas9), *Treponema denticola* Cas9 (TdCas9), *Streptococcus macacae* Cas9 (SmacCas9), Cas $\phi$  (Cas12j), *Francisella tularensis* subsp. *novicida* Cas9, *Pasteurella multocida* Cas9, *Campylobacter lari* CF89-12 Cas9, *Mycoplasma gallisepticum* str. F Cas9, *Nitratifactor salsuginis* str DSM 16511 Cas9, *Parvibaculum lavamentivorans* Cas9, *Roseburia intestinalis* Cas9, *Neisseria cinerea* Cas9, *Gluconacetobacter diazotrophicus* Cas9, *Azospirillum* B510 Cas9, *Sphaerochaeta globus* str. Buddy Cas9, *Flavobacterium columnare* Cas9, *Fluviicola taffensis* Cas9, *Bacteroides coprophilus* Cas9, *Mycoplasma mobile* Cas9, *Lactobacillus farciminis* Cas9, *Streptococcus pasteurianus* Cas9, *Lactobacillus johnsonii* Cas9, *Staphylococcus pseudintermedius* Cas9, *Filifactor alocis* Cas9, *Legionella pneumophila* str. Paris Cas9, *Sutterella wadsworthensis* Cas9, *Corynebacter diphtheriae* Cas9 or any combination thereof.

[0081] In some aspects, a Cas9 ortholog molecule can comprise a chimeric variant of *Streptococcus pyogenes* Cas9 (spCas9), *Francisella novicida* Cas9 (FnCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Neisseria meningitidis* Cas9 (NmCas9; NmeCas9), *Streptococcus thermophilus* CRISPR1-Cas9 (St1Cas9), *Streptococcus thermophilus* CRISPR3-Cas9 (St3Cas9),

Campylobacter jejuni Cas9 (CjCas9), Acidaminococcus sp. BV3L6 Cpf1 (AsCpf1), Lachnospiraceae bacterium ND2006 Cpf1 (LbCpf1), Streptococcus canis Cas9 (ScCas9), Treponema denticola Cas9 (TdCas9), Streptococcus macacae Cas9 (SmacCas9), Cas $\phi$  (Cas12j), Francisella tularensis subsp. novicida Cas9, Pasteurella multocida Cas9, Campylobacter lari CF89-12 Cas9, Mycoplasma gallisepticum str. F Cas9, Nitratifactor salsuginis str DSM 16511 Cas9, Parvibaculum lavamentivorans Cas9, Roseburia intestinalis Cas9, Neisseria cinerea Cas9, Gluconacetobacter diazotrophicus Cas9, Azospirillum B510 Cas9, Sphaerochaeta globus str. Buddy Cas9, Flavobacterium columnare Cas9, Fluvicola taffensis Cas9, Bacteroides coprophilus Cas9, Mycoplasma mobile Cas9, Lactobacillus farciminis Cas9, Streptococcus pasteurianus Cas9, Lactobacillus johnsonii Cas9, Staphylococcus pseudintermedius Cas9, Filifactor alocis Cas9, Legionella pneumophila str. Paris Cas9, Sutterella wadsworthensis Cas9, Corynebacter diphtheriae Cas9 or any combination thereof.

**[0082]** In some aspects, a DNA targeting polypeptide can comprise at least one TALE molecule, at least one zinc-finger molecule, at least one meganuclease molecule or any combination thereof.

**[0083]** In some aspects, a DNA targeting polypeptide can comprise at least one transactivation molecule. In some aspects, a transactivation molecule is a molecule that binds to transcription factors and/or transcriptional co-regulators that are capable of driving transcription of a target gene.

**[0084]** In some aspects, a transactivation molecule can comprise at least one P65 molecule, at least one Rta molecule, at least one VP16 molecule, at least one VP64 molecule, at least one VP160 molecule, at least one VP64-P65-Rta (VPR) molecule, at least one SunTag peptide, at least one single guide RNA-MS2 (sgRNA-MS2) molecule or any combination thereof.

**[0085]** In some aspects, a DNA targeting polypeptide can be a DNA targeting ribonucleoprotein (RNP) complex. A DNA targeting ribonucleoprotein complex can comprise both at least one protein component and at least one nucleic acid component. The at least one protein component can comprise any of the protein components described herein, including, but not limited to, a transactivation molecule, a Cas9 molecule, a Cas9 variant molecule or a Cas9 ortholog molecule, a TALE molecule, a zinc-finger molecule, a meganuclease molecule or any combination thereof. The at least one nucleic acid component can be a ribonucleic acid component. The at least one nucleic acid component can comprise any of the nucleic acid components described herein,

including, but not limited to, a guide RNA molecule, a single guide RNA molecule, a single guide RNA-MS2 (sgRNA-MS2) molecule or any combination thereof.

**[0086]** In some aspects, a DNA targeting polypeptide can further comprise at least one cell-penetrating peptide. A cell-penetrating peptide can comprise at least a portion of an HIV-derived TAT protein, polyarginine, any other cell-penetrating peptide known in the art or any combination thereof.

**[0087]** In some aspects, a DNA targeting polypeptide can comprise at least one guide RNA. In some aspects, a transactivation molecule can comprise at least one single guide RNA-MS2 (sgRNA-MS2) molecule. In some aspects, a sgRNA-MS2 molecule can comprise a nucleic acid sequence complementary to a nucleic acid sequence located upstream, within, or downstream of the endogenous TERC gene and at least about one, or at least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten MS2 RNA aptamers.

**[0088]** In some aspects, a DNA targeting polypeptide can comprise a dCas9 molecule and a VPR molecule.

**[0089]** In some aspects, a DNA targeting polypeptide can bind upstream of, 5' to, within, downstream of or 3' to the endogenous TERC gene, *e.g.* the endogenous human TERC gene.

**[0090]** In some aspects, an at least one DNA targeting polypeptide can bind at least about 0.1 kilobases (kb), or at least about 0.5 kb, or at least about 1.0 kb, or at least about 1.5 kb, or at least about 2.0 kb, or at least about 2.5 kb, or at least about 3.0 kb, or at least about 3.5 kb, or at least about 4.0 kb, or at least about 4.5 kb, or at least about 5.0 kb, or at least about 5.5 kb, or at least about 6.0 kb, or at least about 6.5 kb, or at least about 7.0 kb, or at least about 7.5 kb, or at least about 8.5 kb, or at least about 9.0 kb, or at least about 9.5 kb, or at least about 10.0 kb, or at least about 15 kb, or at least about 20 kb, or at least about 30 kb, or at least about 40 kb, or at least about 50 kb, or at least about 60 kb, or at least about 70 kb, or at least about 80 kb, or at least about 90 kb, or at least about 100 kb, or at least about 250 kb, or at least about 500 kb, or at least about 750 kb, or at least about 1000 kb, or at least 5000 kb or at least about 10,000 kb upstream of the endogenous TERC gene, *e.g.* the endogenous human TERC gene.

**[0091]** In some aspects, an at least one DNA targeting polypeptide can bind at least about 0.1 kilobases (kb), or at least about 0.5 kb, or at least about 1.0 kb, or at least about 1.5 kb, or at least

about 2.0 kb, or at least about 2.5 kb, or at least about 3.0 kb, or at least about 3.5 kb, or at least about 4.0 kb, or at least about 4.5 kb, or at least about 5.0 kb, or at least about 5.5 kb, or at least about 6.0 kb, or at least about 6.5 kb, or at least about 7.0 kb, or at least about 7.5 kb, or at least about 8.5 kb, or at least about 9.0 kb, or at least about 9.5 kb, or at least about 10.0 kb, or at least about 15 kb, or at least about 20 kb, or at least about 30 kb, or at least about 40 kb, or at least about 50 kb, or at least about 60 kb, or at least about 15 kb, or at least about 70 kb, or at least about 80 kb, or at least about 90 kb, or at least about 100 kb, or at least about 250 kb, or at least about 500 kb, or at least about 750 kb, or at least about 1000 kb, or at least 5000 kb or at least about 10,000 kb 3' to the endogenous TERC gene, *e.g.* the endogenous human TERC gene.

**[0092]** In some aspects, an at least one DNA targeting polypeptide can bind at least about 0.1 kilobases (kb), or at least about 0.5 kb, or at least about 1.0 kb, or at least about 1.5 kb, or at least about 2.0 kb, or at least about 2.5 kb, or at least about 3.0 kb, or at least about 3.5 kb, or at least about 4.0 kb, or at least about 4.5 kb, or at least about 5.0 kb, or at least about 5.5 kb, or at least about 6.0 kb, or at least about 6.5 kb, or at least about 7.0 kb, or at least about 7.5 kb, or at least about 8.5 kb, or at least about 9.0 kb, or at least about 9.5 kb, or at least about 10.0 kb, or at least about 15 kb, or at least about 20 kb, or at least about 30 kb, or at least about 40 kb, or at least about 50 kb, or at least about 60 kb, or at least about 15 kb, or at least about 70 kb, or at least about 80 kb, or at least about 90 kb, or at least about 100 kb, or at least about 250 kb, or at least about 500 kb, or at least about 750 kb, or at least about 1000 kb, or at least 5000 kb or at least about 10,000 kb downstream of the endogenous TERC gene, *e.g.* the endogenous human TERC gene.

**[0093]** In some aspects, an at least one DNA targeting polypeptide can bind at least about 0.1 kilobases (kb), or at least about 0.5 kb, or at least about 1.0 kb, or at least about 1.5 kb, or at least about 2.0 kb, or at least about 2.5 kb, or at least about 3.0 kb, or at least about 3.5 kb, or at least about 4.0 kb, or at least about 4.5 kb, or at least about 5.0 kb, or at least about 5.5 kb, or at least about 6.0 kb, or at least about 6.5 kb, or at least about 7.0 kb, or at least about 7.5 kb, or at least about 8.5 kb, or at least about 9.0 kb, or at least about 9.5 kb, or at least about 10.0 kb, or at least about 15 kb, or at least about 20 kb, or at least about 30 kb, or at least about 40 kb, or at least about 50 kb, or at least about 60 kb, or at least about 15 kb, or at least about 70 kb, or at least about 80 kb, or at least about 90 kb, or at least about 100 kb, or at least about 250 kb, or at least

about 500 kb, or at least about 750 kb, or at least about 1000 kb, or at least 5000 kb or at least about 10,000 kb 5' to the endogenous TERC gene, *e.g.* the endogenous human TERC gene.

[0094] In some aspects, an mRNA molecule of any composition of the present disclosure can be a modified mRNA molecule.

[0095] In some aspects, a modified mRNA molecule can comprise at least one modified ribonucleoside base. A modified ribonucleoside base can comprise a pseudouridine ( $\Psi$ ) residue, a 5-methylcytidine ( $m^5C$ ) residue or any combination thereof.

[0096] In some aspects, a modified mRNA molecule can comprise at least one modified nucleoside. A modified nucleoside can comprise 5-methylcytidine ( $m^5C$ ), 5-methyluridine ( $m^5U$ ), N<sup>6</sup>-methyladenosine ( $m^6A$ ), inosine and 2'-O-methylated nucleosides, in addition to N<sup>7</sup>-methylguanosine ( $m^7G$ ), 2-thiouridine ( $s^2U$ ), pseudouridine ( $\psi$ ), 2'-O-methyl-U,  $m^1A$  (1-methyladenosine);  $m^2A$  (2-methyladenosine); Am (2'-O-methyladenosine);  $ms^2m^6A$  (2-methylthio-N<sup>6</sup>-methyladenosine);  $i^6A$  (N<sup>6</sup>-isopentenyladenosine);  $ms^2i^6A$  (2-methylthio-N<sup>6</sup>-isopentenyladenosine);  $io^6A$  (N<sup>6</sup>-(cis-hydroxyisopentenyl)adenosine);  $ms^2io^6A$  (2-methylthio-N<sup>6</sup>-(cis-hydroxyisopentenyl)adenosine);  $g^6A$  (N<sup>6</sup>-glycylcarbamoyl-adenosine);  $t^6A$  (N<sup>6</sup>-threonylcarbamoyl-adenosine);  $ms^2t^6A$  (2-methylthio-N<sup>6</sup>-threonyl carbamoyl-adenosine);  $m^6t^6A$  (N<sup>6</sup>-methyl-N<sup>6</sup>-threonylcarbamoyl-adenosine);  $hn^6A$  (N<sup>6</sup>-hydroxynorvalylcarbamoyl-adenosine);  $ms^2hn^6A$  (2-methylthio-N<sup>6</sup>-hydroxynorvalyl carbamoyl-adenosine); Ar(p) (2'-O-ribosyladenosine(phosphate)); I (inosine);  $m^1I$  (1-methylinosine);  $m^1Im$  (1,2'-O-dimethylinosine);  $m^3C$  (3-methylcytidine); Cm (2'-O-methylcytidine);  $s^2C$  (2-thiocytidine);  $ac^4C(N^4-acetylcytidine)$ ;  $f^5C$  (5-formylcytidine);  $m^5Cm$  (5,2'-O-dimethylcytidine);  $ac^4Cm$  (N<sup>4</sup>-acetyl-2'-O-methylcytidine);  $k^2C$  (lysidine);  $m^1G$  (1-methylguanosine);  $m^2G$  (N<sup>2</sup>-methylguanosine);  $m^7G$  (7-methylguanosine); Gm (2'-O-methylguanosine);  $m^2_2G$  (N<sup>2</sup>,N<sup>2</sup>-dimethylguanosine);  $m^2Gm$  (N<sup>2</sup>,2'-O-dimethylguanosine);  $m^2_2Gm$  (N<sup>2</sup>,N<sup>2</sup>,2'-O-trimethylguanosine); Gr(p) (2'-O-ribosylguanosine (phosphate)); yW (wybutosine);  $o_2yW$  (peroxywybutosine); OHyW (hydroxywybutosine); OHyW\* (undermodified hydroxywybutosine); imG (wyosine); mimG (methylwyosine); Q (queuosine); oQ (epoxyqueuosine); galQ (galactosyl-queuosine); manQ (mannosyl-queuosine); preQ<sub>0</sub> (7-cyano-7-deazaguanosine); preQ<sub>1</sub> (7-aminomethyl-7-deazaguanosine); G<sup>+</sup> (archaeosine); D (dihydrouridine);  $m^5Um$  (5,2'-O-dimethyluridine);  $s^4U$  (4-thiouridine);  $m^5s^2U$  (5-methyl-2-thiouridine);  $s^2Um$  (2-thio-2'-O-methyluridine);  $acp^3U$  (3-(3-amino-3-carboxypropyl)uridine);  $ho^5U$  (5-hydroxyuridine);  $mo^5U$  (5-methoxyuridine);  $cmo^5U$  (uridine 5-oxyacetic acid);  $mcmo^5U$

(uridine 5-oxyacetic acid methyl ester); chm<sup>5</sup>U (5-(carboxyhydroxymethyl)uridine)); mchm<sup>5</sup>U (5-(carboxyhydroxymethyl)uridine methyl ester); mcm<sup>5</sup>U (5-methoxycarbonylmethyluridine); mcm<sup>5</sup>Um (5-methoxycarbonylmethyl-2'-0-methyluridine); mcm<sup>5</sup>s<sup>2</sup>U (5-methoxycarbonylmethyl-2-thiouridine); nm<sup>5</sup>s<sup>2</sup>U (5-aminomethyl-2-thiouridine); mnm<sup>5</sup>U (5-methylaminomethyluridine); mnm<sup>5</sup>s<sup>2</sup>U (5-methylaminomethyl-2-thiouridine); mnm<sup>5</sup>se<sup>2</sup>U (5-methylaminomethyl-2-selenouridine); ncm<sup>5</sup>U (5-carbamoylmethyluridine); ncm<sup>5</sup>Um (5-carbamoylmethyl-2'-0-methyluridine); cmnm<sup>5</sup>U (5-carboxymethylaminomethyluridine); cmnm<sup>5</sup>Um (5-carboxymethylaminomethyl-2'-0-methyluridine); cmnm<sup>5</sup>s<sup>2</sup>U (5-carboxymethylaminomethyl-2-thiouridine); m<sup>6</sup> 2A (N<sup>6</sup>,N<sup>6</sup>-dimethyladenosine); Im (2'-0-methylinosine); m<sup>4</sup>C(N<sup>4</sup>-methylcytidine); m<sup>4</sup>Cm (N<sup>4</sup>,2'-0-dimethylcytidine); hm<sup>5</sup>C (5-hydroxymethylcytidine); m<sup>3</sup>U (3-methyluridine); cm<sup>5</sup>U (5-carboxymethyluridine); m<sup>6</sup>Am (N<sup>6</sup>,2'-0-dimethyladenosine); m<sup>6</sup> 2Am (N<sup>6</sup>,N<sup>6</sup>,0-2'- 2 7 2 2 2 7 2 2trimethyladenosine); m<sup>2,7</sup>G (N<sup>2</sup>,7-dimethylguanosine); m<sup>2,2,7</sup>G (N<sup>2</sup>,N<sup>2</sup>,7-trimethylguanosine); m<sup>3</sup>Um (3,2'-0-dimethyluridine); m<sup>5</sup>D (5-methylidihydrouridine); f<sup>5</sup>Cm (5-formyl-2'-0-methylcytidine); m<sup>1</sup>Gm (1,2'-0-dimethylguanosine); m<sup>1</sup>Am (1,2'-0-dimethyladenosine); tm<sup>5</sup>U (5-taurinomethyluridine); tm<sup>5</sup>s<sup>2</sup>U (5-taurinomethyl-2-thiouridine)); imG-14 (4-demethylwyosine); imG2 (isowyosine); ac<sup>6</sup>A (N<sup>6</sup>-acetyladenosine), or any combination thereof.

**[0097]** In some aspects, an mRNA molecule can be chemically synthesized using methods standard in the art. In some aspects, an mRNA molecule can be chemically synthesized such that the mRNA molecule comprises at least one chemical modification. In some aspects, an mRNA molecule can be produced by *in vitro* transcription methods standard in the art, including, but not limited to, *in vitro* transcription using a plasmid template, *in vitro* transcription using a PCR-based template. In some aspects, *in vitro* transcription methods can be performed such that the produced mRNA molecules comprise at least one chemical modification.

**[0098]** In some aspects, a purified DNA targeting polypeptide can be produced using methods standard in the art, including, but not limited to, recombinant protein expression and purification in a bacterial, fungal, insect and/or mammalian system, ion-exchange chromatography, affinity chromatography, immunoaffinity chromatography, size exclusion chromatography, and/or other standard protein production/purification methods known in the art.

**[0099]** In some aspects, a purified DNA-targeting ribonucleoprotein (RNP) complex can be produced using methods standard in the art, including, but not limited to recombinant protein

expression and purification in a bacterial, fungal, insect and/or mammalian system, *in vitro* RNA transcription, ion-exchange chromatography, affinity chromatography, immunoaffinity chromatography, size exclusion chromatography, other standard protein production/purification methods known in the art, and/or other standard nucleic acid production/purification methods known in the art. In some aspects, a preassembled RNP complex that comprises both at least one protein component and at least one nucleic acid can be assembled *in vivo* (*i.e.* in a bacterial, fungal, insect and/or mammalian recombinant expression system) and co-purified. In some aspects, a RNP complex can be assembled *in vitro* after the individual purification of the at least one protein component and the at least one nucleic acid component.

[00100] In some aspects, any of the compositions of the present disclosure can further comprise a plurality of guide RNA (gRNA) molecules, wherein at least one gRNA in the plurality is complementary to a nucleic acid sequence located upstream, within, or downstream of the endogenous TERC gene. In some aspects, a plurality of gRNA molecules can comprise at least about one, or at least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten, or at least about 11, or at least about 12, or at least about 13, or at least about 14, or at least about 15, at least about 16, or at least about 17, or about at least 18, or at least about 19, or at least about 20, or at least about 30, or at least about 40, or at least about 50, or at least about 60, or at least about 70, or at least about 80, or at least about 90, or at least about 100, or at least about 500 or at least about 1000 distinct species of gRNA molecules, wherein each species has a different nucleic acid sequence.

[00101] In some aspects, any of the compositions of the present disclosure can further comprise at least one plasmid comprising at least one nucleic acid sequence encoding at least one species of gRNA operably linked to at least one promoter sufficient to drive expression of the at least one species gRNA. In some aspects, any of the compositions of the present disclosure can further comprise at least one plasmid comprising at least one nucleic acid sequence encoding least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten, or at least about 11, or at least about 12, or at least about 13, or at least about 14, or at least about 15, at least about 16, or at least about 17, or about at least 18, or at least about 19, or at least about 20, or at least about 30, or at least about 40, or at least about 50, or at least about 60, or at least about

70, or at least about 80, or at least about 90, or at least about 100, or at least about 500 or at least about 1000 distinct species of gRNA molecules operably linked to at least one promoter sufficient to drive expression of the gRNA species, wherein each species has a different nucleic acid sequence.

[00102] In some aspects, a plurality of gRNA molecules can comprise a plurality of single guide RNA (sgRNA) molecules, crRNA:tracrRNA molecules, truncated sgRNA molecules, high fidelity scaffold gRNA molecules or any combination thereof.

[00103] In some aspects, a plurality of gRNA molecules can comprise a plurality of single guide RNA (sgRNA) molecules. In some aspects, a sgRNA molecule can comprise a nucleic acid sequence complementary to a nucleic acid sequence located upstream, within, or downstream of the endogenous TERC gene and at least one MS2 RNA aptamer. In some aspects, a sgRNA molecule can comprise at least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten MS2 RNA aptamers.

[00104] In some aspects, a guide RNA molecule of any composition of the present disclosure can be a modified guide RNA (mod gRNA) molecule.

[00105] In some aspects, a modified guide RNA can comprise at least one modified ribonucleoside base. A modified ribonucleoside base can comprise a pseudouridine ( $\Psi$ ) residue, a 5-methylcytidine ( $m^5C$ ) residue or any combination thereof.

[00106] In some aspects, a modified guide RNA can comprise at least one modified nucleoside. A modified nucleoside can comprise 5-methylcytidine ( $m^5C$ ), 5-methyluridine ( $m^5U$ ), N6-methyladenosine ( $m^6A$ ), inosine and 2'-O-methylated nucleosides, in addition to N7-methylguanosine ( $m^7G$ ), 2-thiouridine ( $s^2U$ ), pseudouridine ( $\psi$ ), 2'-O-methyl-U,  $m^1A$  (1-methyladenosine);  $m^2A$  (2-methyladenosine); Am (2'-O-methyladenosine);  $ms^2m^6A$  (2-methylthio- $N^6$ -methyladenosine);  $i^6A$  ( $N^6$ -isopentenyladenosine);  $ms^2i^6A$  (2-methylthio- $N^6$ -isopentenyladenosine);  $io^6A$  ( $N^6$ -(cis-hydroxyisopentenyl)adenosine);  $ms^2io^6A$  (2-methylthio- $N^6$ -(cis-hydroxyisopentenyl)adenosine);  $g^6A$  ( $N^6$ -glycylcarbamoyladenosine);  $t^6A$  ( $N^6$ -threonylcarbamoyladenosine);  $ms^2t^6A$  (2-methylthio- $N^6$ -threonyl carbamoyladenosine);  $m^6t^6A$  ( $N^6$ -methyl- $N^6$ -threonylcarbamoyladenosine);  $hn^6A$  ( $N^6$ -hydroxynorvalylcarbamoyladenosine);  $ms^2hn^6A$  (2-methylthio- $N^6$ -hydroxynorvalyl carbamoyladenosine); Ar(p) (2'-O-ribosyladenosine(phosphate)); I (inosine);  $m^1I$  (1-methylinosine);  $m^1Im$  (1,2'-O-dimethylinosine);

$m^3C$  (3-methylcytidine);  $Cm$  (2'-0-methylcytidine);  $s^2C$  (2-thiocytidine);  $ac^4C(N^4-$   
 acetylcytidine);  $f^5C$  (5-formylcytidine);  $m^5Cm$  (5,2'-0-dimethylcytidine);  $ac^4Cm$  ( $N^4$ -acetyl-2'-0-  
 methylcytidine);  $k^2C$  (lysidine);  $m^1G$  (1-methylguanosine);  $m^2G$  ( $N^2$ -methylguanosine);  $m^7G$  (7-  
 methylguanosine);  $Gm$  (2'-0-methylguanosine);  $m^2 \text{ } ^2G$  ( $N^2, N^2$ -dimethylguanosine);  $m^2Gm$  ( $N^2, 2'$ -  
 0-dimethylguanosine);  $m^2 \text{ } ^2Gm$  ( $N^2, N^2, 2'$ -0-trimethylguanosine);  $Gr(p)$  (2'-0-riboseylguanosine  
 (phosphate));  $yW$  (wybutosine);  $ozyW$  (peroxywybutosine);  $OHyW$  (hydroxywybutosine);  
 $OHyW^*$  (undermodified hydroxywybutosine);  $imG$  (wyosine);  $mimG$  (methylwyosine);  $Q$   
 (queuosine);  $oQ$  (epoxyqueuosine);  $galQ$  (galactosyl-queuosine);  $manQ$  (mannosyl-queuosine);  
 $preQ_0$  (7-cyano-7-deazaguanosine);  $preQ_1$  (7-aminomethyl-7-deazaguanosine);  $G^+$  (archaeosine);  
 $D$  (dihydrouridine);  $m^5Um$  (5,2'-0-dimethyluridine);  $s^4U$  (4-thiouridine);  $m^5s^2U$  (5-methyl-2-  
 thiouridine);  $s^2Um$  (2-thio-2'-0-methyluridine);  $acp^3U$  (3-(3-amino-3-carboxypropyl)uridine);  
 $ho^5U$  (5-hydroxyuridine);  $mo^5U$  (5-methoxyuridine);  $cmo^5U$  (uridine 5-oxyacetic acid);  $mcmo^5U$   
 (uridine 5-oxyacetic acid methyl ester);  $chm^5U$  (5-(carboxyhydroxymethyl)uridine);  $mchm^5U$   
 (5-(carboxyhydroxymethyl)uridine methyl ester);  $mcm^5U$  (5-methoxycarbonylmethyluridine);  
 $mcm^5Um$  (5-methoxycarbonylmethyl-2'-0-methyluridine);  $mcm^5s^2U$  (5-methoxycarbonylmethyl-  
 2-thiouridine);  $nm^5s^2U$  (5-aminomethyl-2-thiouridine);  $mnm^5U$  (5-methylaminomethyluridine);  
 $mnm^5s^2U$  (5-methylaminomethyl-2-thiouridine);  $mnm^5se^2U$  (5-methylaminomethyl-2-  
 selenouridine);  $ncm^5U$  (5-carbamoylmethyluridine);  $ncm^5Um$  (5-carbamoylmethyl-2'-0-  
 methyluridine);  $cmnm^5U$  (5-carboxymethylaminomethyluridine);  $cmnm^5Um$  (5-  
 carboxymethylaminomethyl-2'-0-methyluridine);  $cmnm^5s^2U$  (5-carboxymethylaminomethyl-2-  
 thiouridine);  $m^6 \text{ } ^2A$  ( $N^6, N^6$ -dimethyladenosine);  $Im$  (2'-0-methylinosine);  $m^4C(N^4-$   
 methylcytidine);  $m^4Cm$  ( $N^4, 2'$ -0-dimethylcytidine);  $hm^5C$  (5-hydroxymethylcytidine);  $m^3U$  (3-  
 methyluridine);  $cm^5U$  (5-carboxymethyluridine);  $m^6Am$  ( $N^6, 2'$ -0-dimethyladenosine);  $m^6 \text{ } ^2Am$   
 ( $N^6, N^6, 0-2'$ - 2 7 2 2 2 7 2 2trimethyladenosine);  $m^{2,7}G$  ( $N^2, 7$ -dimethylguanosine);  $m^{2,2,7}G$  ( $N^2,$   
 $N^2, 7$ -trimethylguanosine);  $m^3Um$  (3,2'-0-dimethyluridine);  $m^5D$  (5-methyldihydrouridine);  $f^5Cm$   
 (5-formyl-2'-0-methylcytidine);  $m^1Gm$  (1,2'-0-dimethylguanosine);  $m^1Am$  (1,2'-0-  
 dimethyladenosine);  $\tau m^5U$  (5-aurinomethyluridine);  $\tau m^5s^2U$  (5-aurinomethyl-2-thiouridine));  
 $imG-14$  (4-demethylwyosine);  $imG2$  (isowyosine);  $ac^6A$  ( $N^6$ -acetyladenosine), or any  
 combination thereof.

**[00107]** In some aspects, a guide RNA molecule can comprise any sequence recited in Table 1 or Table 2.

**Table 1. Guide RNA sequences**

| Seq Name | Sequence              | Seq ID NO | Seq Name | Sequence              | Seq ID NO |
|----------|-----------------------|-----------|----------|-----------------------|-----------|
| 1015rev  | UGUUCAUAAAUUUACUGACA  | 1         | 1934rev  | GAGAAGCCCCGGGCCGACCG  | 265       |
| 1025forw | AAAAAAAUCGUUACAAUUUA  | 2         | 1937forw | CGAACCCCGCCUGGAGGCCG  | 266       |
| 1028forw | AAAAUCGUUACAAUUUAUGG  | 3         | 1941forw | CCCCGCCUGGAGGCCGCGGU  | 267       |
| 1037rev  | UCUUGAUGAGGUAAAAAGAG  | 4         | 1944rev  | GGUGCCUCCGGAGAAGCCCC  | 268       |
| 1038rev  | GUCUUGAUGAGGUAAAAAGA  | 5         | 1945rev  | GGGUGCCUCCGGAGAAGCCC  | 269       |
| 1039rev  | UGUCUUGAUGAGGUAAAAAG  | 6         | 1946forw | CCUGGAGGCCCGCGGUCGGCC | 270       |
| 103rev   | AAUUUCUCUCCUUUGCAUUA  | 7         | 1947forw | CUGGAGGCCCGCGGUCGGCCC | 271       |
| 1049rev  | AGUAGUGCUGUGUCUUGAUG  | 8         | 1948forw | UGGAGGCCCGCGGUCGGCCCC | 272       |
| 1059rev  | AGGGGACCUACUUAAGGUAAU | 9         | 1956rev  | GCGGUGGCAGUGGGUGCCUC  | 273       |
| 1066rev  | CAAUUCCAGGGGACCUACUU  | 10        | 1957forw | CGGUCGGCCCCGGGGCUUCUC | 274       |
| 106forw  | ACGGAGCGAGUCCCCGCGCG  | 11        | 1960forw | UCGGCCCCGGGGCUUCUCCGG | 275       |
| 1073forw | UUUUAACCUAUUACCUAAGU  | 12        | 1965rev  | CAACUCUUCGCGGUGGCAGU  | 276       |
| 1077rev  | UAUCUGCUAGACAAUCCAG   | 13        | 1966rev  | CCAACUCUUCGCGGUGGCAG  | 277       |
| 1078rev  | GUAUCUGCUAGACAAUCCA   | 14        | 1986forw | CCACUGCCACCGCGAAGAGU  | 278       |
| 1079rev  | UGUAUCUGCUAGACAAUCC   | 15        | 1987forw | CACUGCCACCGCGAAGAGUU  | 279       |
| 1081forw | UAUUACCUAAGUAGGUCCCC  | 16        | 199forw  | UUUGGAGAAUAAUUGAAUG   | 280       |
| 1098rev  | UCCUUUUUAUUAGGAAAGAA  | 17        | 1rev     | CAGAGCCCAACUCUUCGCGG  | 281       |
| 1107rev  | GACUGAAUCUCCUUUUUAU   | 18        | 203forw  | GAGAAUAAAUUGAAUGAGGA  | 282       |
| 1116forw | CGCCUUUCUUCCUAAUAAA   | 19        | 203rev   | CCAUUGCCGGCGAGGGUGA   | 283       |
| 1117forw | GCCUUUCUUCCUAAUAAA    | 20        | 206rev   | AACUGAUCACCAAUUCUCCA  | 284       |
| 1129rev  | CUACUACAUAUUAUUCUUA   | 21        | 207rev   | U AACUGAUCACCAAUUCUCC | 285       |
| 1139rev  | CCAGCAACAGUGGACUCUAG  | 22        | 209forw  | AAAUUGAAUGAGGAAGGCC   | 286       |
| 1149rev  | GAGAACAUAUACCAGCAACAG | 23        | 209rev   | AAGCCCCCAUUGCCGGCGAG  | 287       |
| 114forw  | GCUAAAUAUCCAUAUGCAA   | 24        | 210rev   | CAAGCCCCCAUUGCCGGCGA  | 288       |
| 1159forw | CCUCUAGAGUCCACUGUUGC  | 25        | 211rev   | ACAAGCCCCCAUUGCCGGCG  | 289       |
| 1168rev  | GCCUCUCCUUGAGCAGAGGA  | 26        | 216rev   | GGUUCACAAGCCCCCAUUGC  | 290       |
| 116rev   | GGUGCACGUCCACAGCUCA   | 27        | 217forw  | UGAGGAAGGCCUGGAGAUU   | 291       |
| 1172rev  | UCCAGCCUCUCCUUGAGCAG  | 28        | 217forw  | GCGCAUCCGUCACCCUUCGC  | 292       |
| 1178forw | CUGGUAUUGUUCUCUAAAUA  | 29        | 223forw  | CCGUCACCCUUCGCCGGCAA  | 293       |
| 117rev   | GGGUGCACGUCCACAGCUC   | 30        | 224forw  | CGUCACCCUUCGCCGGCAAU  | 294       |
| 1182forw | UUAAAGCCAUCCUCUGCUCA  | 31        | 225forw  | GUCACCCUUCGCCGGCAAUG  | 295       |
| 1187forw | GCCAUCCUCUGCUCAAGGAG  | 32        | 226forw  | UCACCCUUCGCCGGCAAUGG  | 296       |
| 1191forw | UCCUCUGCUCAAGGAGAGGC  | 33        | 237rev   | GCCCAGUCAGUCAGGUUUGG  | 297       |
| 1193rev  | UUCCACAAAACCAUGCUGAU  | 34        | 238rev   | GGCCCAGUCAGUCAGGUUUG  | 298       |
| 1197forw | GCUCAAGGAGAGGCUGGAGA  | 35        | 239rev   | UGGCCAGUCAGUCAGGUUU   | 299       |
| 1203forw | AAAUAUUUUUCCUAUCAGCA  | 36        | 240rev   | CUGGCCAGUCAGUCAGGUU   | 300       |
| 1207forw | AGGUCGGAGAAGGCAUUCUA  | 37        | 243rev   | AAGACUUGGCACUUUAUAUG  | 301       |
| 1211forw | UCCUAUCAGCAUGGUUUUG   | 38        | 245rev   | GCACACUGGCCAGUCAGUC   | 302       |

|          |                      |    |         |                       |     |
|----------|----------------------|----|---------|-----------------------|-----|
| 1213forw | GAGAAGGCAUUCUAAGGAGA | 39 | 255forw | AACCCCCAAACCUGACUGAC  | 303 |
| 1214forw | AGAAGGCAUUCUAAGGAGAA | 40 | 256forw | ACCCCCAAACCUGACUGACU  | 304 |
| 1215forw | GAAGGCAUUCUAAGGAGAAG | 41 | 257rev  | AUAAUCUUGAGUACAAGACU  | 305 |
| 1216forw | AAGGCAUUCUAAGGAGAAGG | 42 | 259rev  | CCUGCCAAUUUGCAGCACAC  | 306 |
| 1220forw | CAUUCUAAGGAGAAGGGGGC | 43 | 275forw | UGGGCCAGUGUGCUGCAAU   | 307 |
| 1221forw | AUUCUAAGGAGAAGGGGGCA | 44 | 279forw | CCAGUGUGCUGCAAAUUGGC  | 308 |
| 1221forw | CAUGGUUUUGUGGAAAAGUA | 45 | 287forw | UACUCAAGAUUAUAGCAAU   | 309 |
| 1225forw | UAAGGAGAAGGGGGCAGGGU | 46 | 28rev   | CCUCGCCCCCGAGAGACCCG  | 310 |
| 1232forw | AAGGGGGCAGGGUAGGAACU | 47 | 290forw | CAAAUUGGCAGGAGACGUGA  | 311 |
| 1234rev  | CAAGACUCUAGACAAGUUCU | 48 | 295rev  | UUCAUUUUGGCCGACUUUGG  | 312 |
| 1241rev  | GAAUCUUGUCUCGGCUCAGU | 49 | 298rev  | CCAUUCAUUUUGGCCGACUU  | 313 |
| 1242rev  | AGAAUCUUGUCUCGGCUCAG | 50 | 305forw | CGUGAAGGCACCUCCAAGU   | 314 |
| 1250rev  | ACUACAGCAGAAUCUUGUCU | 51 | 308rev  | GGCUCACUGCCCAUUCAUUU  | 315 |
| 1257forw | AGAACUUGUCUAGAGUCUUG | 52 | 318forw | CCAAAGUCGGCCAAAUGAA   | 316 |
| 126forw  | CGGCGGAUUCUCCUGAGCUG | 53 | 319forw | CAAAGUCGGCCAAAUGAAU   | 317 |
| 127forw  | GGCGGAUUCUCCUGAGCUGU | 54 | 31forw  | GAGUUGGGCUCUGUCAGCCG  | 318 |
| 1281rev  | CUUUGUGAAAUAAGAUUCCC | 55 | 329rev  | GGAACGGCUCACAGGCAACCC | 319 |
| 1281rev  | AGAUCACCUUGAGUAAACUG | 56 | 32forw  | AGUUGGGCUCUGUCAGCCGC  | 320 |
| 1283forw | CUGCUGUAGUCAGUGCUGCC | 57 | 330forw | AAAUGAAUGGGCAGUGAGC   | 321 |
| 1284forw | UGCUGUAGUCAGUGCUGCCU | 58 | 331forw | AAAUGAAUGGGCAGUGAGCC  | 322 |
| 1295forw | AGUAAGCCUCAGUUUACUCA | 59 | 331rev  | UUUCCCCUUAUAUCUAAGU   | 323 |
| 1308rev  | GUUUUGAUCAUCACAUUUUU | 60 | 332forw | AAUGAAUGGGCAGUGAGCCG  | 324 |
| 1332forw | AAAUGUGAUGAUCAAAACU  | 61 | 338rev  | ACCCACGCAGGAACGGCUCC  | 325 |
| 1335forw | UUCUUCUCUUUCUUUGAGA  | 62 | 340forw | GGCAGUGAGCCGGGGUUGCC  | 326 |
| 1341rev  | CCAGCUCUGGGUGACAGAGU | 63 | 345rev  | CGGGAGAACCCACGCAGGAA  | 327 |
| 1342rev  | UCCAGCUCUGGGUGACAGAG | 64 | 346forw | UAGUGCCUACUUAGAUUGA   | 328 |
| 1353rev  | GGACACUGCACUCCAGCUCU | 65 | 347forw | AGUGCCUACUUAGAUUGAA   | 329 |
| 1354forw | GAAUUAGUGUUCUGUGUCUU | 66 | 348forw | GUGCCUACUUAGAUUGAAG   | 330 |
| 1354rev  | GGGACACUGCACUCCAGCUC | 67 | 350rev  | GAAGACGGGAGAACCCACGC  | 331 |
| 1357rev  | GAAUUCACAGGAAGAUUUUA | 68 | 356forw | UUAGAUUGAAGGGGAAAGA   | 332 |
| 1358rev  | GGAAUUCACAGGAAGAUUUU | 69 | 356forw | UGCCUGGAGCCGUUCCUGCG  | 333 |
| 1361forw | CCCACUCUGUCACCCAGAGC | 70 | 357forw | UAGAUUGAAGGGGAAAGAA   | 334 |
| 1369rev  | ACCUUAAAAAUGGAAUUCAC | 71 | 357forw | GCCUGGAGCCGUUCCUGCGU  | 335 |
| 1374rev  | GGUUGCAGUGAGCCAAGAUG | 72 | 364rev  | GGCAACAAAAGCGGAAGAC   | 336 |
| 1375rev  | AGGUUGCAGUGAGCCAAGAU | 73 | 365rev  | AGGCAACAAAAGCGGAAGA   | 337 |
| 1376rev  | GAGGUUGCAGUGAGCCAAGA | 74 | 372forw | AAGAAGGGUUUGAGAUAAUG  | 338 |
| 1379rev  | CACCUCGACUACCUAAAAAA | 75 | 372rev  | CCAUAAAAGGCAACAAAAG   | 339 |
| 137rev   | GCAUGUGUGAGCCGAGUCCU | 76 | 373forw | AGAAGGGUUUGAGAUAAUGU  | 340 |
| 1382forw | GGAGUGCAGUGUCCCCAUCU | 77 | 385rev  | AGUUGUAAUACAACCAUAAA  | 341 |
| 1388forw | UCCUGUGAAUCCAUUUUUA  | 78 | 388forw | AAUGUGGGAUGCUAAGAGAA  | 342 |
| 138rev   | UGCAUGUGUGAGCCGAGUCC | 79 | 391forw | GUGGGAUGCUAAGAGAAUGG  | 343 |

|          |                       |     |         |                       |     |
|----------|-----------------------|-----|---------|-----------------------|-----|
| 1395rev  | GCUAGAAACCGAGGAGGCAG  | 80  | 392forw | CCGCUUUUUGUUGCCUUUA   | 344 |
| 1397forw | UUCCAUUUUUAAGGUAGUCG  | 81  | 40forw  | UCUGUCAGCCGCGGGUCUCU  | 345 |
| 1401rev  | AAAAUCGCUAGAAACCGAGG  | 82  | 413rev  | CUCAACAAAUCUGCAGAGC   | 346 |
| 1404rev  | UAUCCUCUGCAGACCAGACG  | 83  | 41forw  | CUGUCAGCCGCGGGUCUCUC  | 347 |
| 1404rev  | GAGAAAUCGCUAGAAACCG   | 84  | 42forw  | UGUCAGCCGCGGGUCUCUCG  | 348 |
| 1407forw | CACUGCAACCUCUGCCUCCU  | 85  | 434forw | CUGCUCUGCAGAUUUUGUUG  | 349 |
| 140forw  | GAAAUAAGAUUUAAAAGC    | 86  | 439forw | UUUAGCAUCUACUCU AUGUA | 350 |
| 140forw  | GAGCUGUGGGACGUGCACCC  | 87  | 43forw  | GUCAGCCGCGGGUCUCUCGG  | 351 |
| 1411forw | UAGUCGAGGUGAACCGCGUC  | 88  | 448rev  | GACUGGUCGAGAU CUACCUU | 352 |
| 1421forw | GAACCGGUCUGGUCUGCAG   | 89  | 449rev  | GGACUGGUCGAGAU CUACCU | 353 |
| 1431rev  | UGUAAACCCAGCUACUUGGG  | 90  | 452forw | UGAGGUUUUUGCUUCUCCCA  | 354 |
| 1433forw | GUCUGCAGAGGAUAGAAAAA  | 91  | 465rev  | CCACACCCCGUUGAGGGGAC  | 355 |
| 1434rev  | GCCUGUAAACCCAGCUACUU  | 92  | 470rev  | UUCUCCCACACCCCGUUGAG  | 356 |
| 1435rev  | UGCCUGUAAACCCAGCUACU  | 93  | 471rev  | GUUCUCCCACACCCCGUUGA  | 357 |
| 1436rev  | AACUAAAUUGAGGU AUCAGA | 94  | 472forw | AGUGCAAUAGUGCUAAAAAC  | 358 |
| 1437rev  | AAACUAAAUUGAGGU AUCAG | 95  | 472rev  | UGUUCUCCCACACCCCGUUG  | 359 |
| 1444forw | CUCUCAGCCUCCCAAGUAGC  | 96  | 478forw | AUCUCGACCAGUCCCUCAA   | 360 |
| 1445forw | UCUCAGCCUCCCAAGUAGCU  | 97  | 479forw | UCUCGACCAGUCCCUCAAC   | 361 |
| 1446rev  | UUAAAGGUGAAACU AACUUG | 98  | 480forw | CUCGACCAGUCCCUCAACG   | 362 |
| 1453forw | UCCCAAGUAGCUGGGUUUAC  | 99  | 485forw | CCAGUCCCUCAACGGGGUG   | 363 |
| 145rev   | AUCAUAACAUAGUUUCCUUA  | 100 | 486forw | CAGUCCCUCAACGGGGUGU   | 364 |
| 1462rev  | UUACUUCGACCUUCUUUA    | 101 | 488rev  | CCAGGUUGUAAAGUUUUUA   | 365 |
| 1462rev  | AAAAAUCAGCCGGGUAUGG   | 102 | 48forw  | CCGCGGGUCUCUCGGGGGCG  | 366 |
| 1465rev  | ACAAAAAAUCAGCCGGGUA   | 103 | 49forw  | CGCGGGUCUCUCGGGGGCGA  | 367 |
| 146forw  | UGGGACGUGCACCCAGGACU  | 104 | 4rev    | UGACAGAGCCCAACUCUUG   | 368 |
| 1470rev  | AAAAUACAAAAAAUCAGCC   | 105 | 506rev  | UUUCUUUCAUAGCAUCUGCC  | 369 |
| 1471rev  | GAAAAUACAAAAAAUCAGC   | 106 | 508forw | CCGUAAAAACUUUACAACC   | 370 |
| 1472forw | GUUAGUUUACCUUUAAAGA   | 107 | 530forw | GCAGAUGCUAUGAAAGAAAA  | 371 |
| 1472forw | CAGGCACACACCACCAUACC  | 108 | 531forw | CAGAUGCUAUGAAAGAAAAA  | 372 |
| 1476forw | GUUUCACCUUUAAAGAAGGU  | 109 | 532forw | AGAUGCUAUGAAAGAAAAAG  | 373 |
| 1495rev  | CCCUUCCGCACGUCCGGGAA  | 110 | 536forw | GCUAUGAAAGAAAAAGGGGA  | 374 |
| 1500rev  | CGUUGCCCUUCCGCACGUCC  | 111 | 537forw | CUAUGAAAGAAAAAGGGGAU  | 375 |
| 1501rev  | ACGUUGCCCUUCCGCACGUC  | 112 | 549forw | AAGGGGAUGGGAGAGAGAGA  | 376 |
| 1502forw | UAAAGACGCAAAGCCUUUCC  | 113 | 54forw  | GUCUCUCGGGGGCGAGGGCG  | 377 |
| 1502forw | UUUUGUAUUUUCAGUAAAGU  | 114 | 552forw | GGGAUGGGAGAGAGAGAAGG  | 378 |
| 1503forw | UUUGUAUUUUCAGUAAAGUU  | 115 | 553forw | GGAUGGGAGAGAGAGAAGGA  | 379 |
| 1507forw | UAUUUCAGUAAAGUUGGGC   | 116 | 561forw | UAGAAGAUUUUUGAACAUCU  | 380 |
| 150forw  | AUUUAAAAGCAGGAGCCAUA  | 117 | 563forw | GAGAGAAGGAGGGAGAGAGA  | 381 |
| 1510forw | CAAAGCCUUUCCCGGACGUG  | 118 | 568forw | AAGGAGGGAGAGAGAUGGAG  | 382 |
| 1511forw | UUCAGUAAAGUUGGGCAGGC  | 119 | 569forw | AGGAGGGAGAGAGAUGGAGA  | 383 |
| 1514forw | GCCUUUCCCGGACGUGCGGA  | 120 | 574rev  | CAUAAACCGAUGACCAUUA   | 384 |

|          |                      |     |         |                       |     |
|----------|----------------------|-----|---------|-----------------------|-----|
| 1514rev  | CACCUGAGGUCAGGAGUUCG | 121 | 57forw  | AACAAGCGCUAUGACUAGCA  | 385 |
| 1515forw | CCUUUCCCGGACGUGCGGAA | 122 | 581forw | UGGAAAUUGUGUCCUUUAA   | 386 |
| 1523rev  | CGGGCGGAUCACCUGAGGUC | 123 | 588forw | UGUGUCCUUUAAUAGGUCAU  | 387 |
| 1524rev  | UCCAUUUCCGGCCAUGAGGA | 124 | 597rev  | AAAAAGAAACUUCUAACCUC  | 388 |
| 1528rev  | AAGUCCAUUUCCGGCCAUG  | 125 | 598forw | UUUACUUUUCUUUCAGAUCG  | 389 |
| 1528rev  | AGAAGCGGGCGGAUCACCUG | 126 | 601forw | UGGUCAUCGGUUAUGCCAG   | 390 |
| 1532forw | GGCCUCGAACUCCUGACCUC | 127 | 602rev  | CUCCGUGGAGUUUCGCGUGU  | 391 |
| 1533forw | AAGGGCAACGUCCUCCUCA  | 128 | 60forw  | CGGGGGCGAGGGCGAGGUUC  | 392 |
| 1536rev  | GGAAAUAAAAGUCCAUUUC  | 129 | 617rev  | AUUCAGUUAGAUAAACUCCG  | 393 |
| 1537forw | GCAACGUCCUCCUCAUGGC  | 130 | 620forw | GACCGACAGCGACAACUCCA  | 394 |
| 1539rev  | CUUUGGGAGGCAGAAGCGGG | 131 | 632rev  | ACUGCUC AAGGUCAUCGCCA | 395 |
| 1542rev  | GCACUUUGGGAGGCAGAAGC | 132 | 635forw | UUUUUUGAAAAUUAGACCU   | 396 |
| 1543forw | UCCUCCUCAUGGCCGAAA   | 133 | 63rev   | UCCUCUCCUGCGGCCUGAA   | 397 |
| 1543rev  | AGCACUUUGGGAGGCAGAAG | 134 | 644rev  | GGGUUAUAUCCUACUGCUC A | 398 |
| 1552rev  | UGUAAUCCAGCACUUUGGG  | 135 | 655forw | UGGCGAUGACCUUGAGCAGU  | 399 |
| 1555rev  | GCCUGUAAUCCAGCACUUU  | 136 | 663rev  | CAGUUUACAUAUAAAUGAC   | 400 |
| 1556rev  | CGCCUGUAAUCCAGCACUU  | 137 | 664rev  | UUGGAACGCUAAGCUUGUGG  | 401 |
| 1557rev  | GCGGGCUGGUUGGGGGGAAC | 138 | 665rev  | AUUGGAACGCUAAGCUUGUG  | 402 |
| 1558rev  | GGCGGGCUGGUUGGGGGGAA | 139 | 666rev  | UAUUGGAACGCUAAGCUUGU  | 403 |
| 1563rev  | UCUCGGGCGGGCUGGUUGGG | 140 | 667rev  | UUAUUGGAACGCUAAGCUUG  | 404 |
| 1564rev  | CUCUCGGGCGGGCUGGUUGG | 141 | 683rev  | UAUGCCUAGUGUCCGUUAU   | 405 |
| 1565forw | GCUUCUGCCUCCCAAAGUGC | 142 | 68forw  | UGACUAGCAAGGUUAAGUGA  | 406 |
| 1565rev  | UCUCUCGGGCGGGCUGGUUG | 143 | 690forw | CAAGCUUAGCGUCCAAUAA   | 407 |
| 1566forw | CUUCUGCCUCCCAAAGUGCU | 144 | 694forw | UAUGUAAAACUGCACUAUAC  | 408 |
| 1566rev  | CUCUCUCGGGCGGGCUGGUU | 145 | 697rev  | CCGGCCGCAAUUUUUUAA    | 409 |
| 1567rev  | ACUCUCUCGGGCGGGCUGGU | 146 | 699forw | CGUCCAAUAACGGAACACU   | 410 |
| 1571rev  | AGUCACUCUCUCGGGCGGGC | 147 | 69forw  | GGGCGAGGUUCAGGCCUUC   | 411 |
| 1574forw | UCCCAAAGUGCUGGGAUUAC | 148 | 713forw | CUGGCCAUUAUAAAAAUUCG  | 412 |
| 1575rev  | UGAGAGUCACUCUCUGGGC  | 149 | 714forw | ACACUAGGCAUAUGAAAGA   | 413 |
| 1576rev  | GUGAGAGUCACUCUCUGGG  | 150 | 716rev  | CAGGU AUGAGCCACCGACC  | 414 |
| 1579rev  | CUCGUGAGAGUCACUCUC   | 151 | 717forw | CCAUUAUAAAAAUUCGCGGC  | 415 |
| 1580rev  | UCUCGUGAGAGUCACUCUCU | 152 | 718forw | CAUUAUAAAAAUUCGCGGCC  | 416 |
| 1583rev  | GGAUCUAGUCCCCGCACGG  | 153 | 71rev   | ACUUUAAGCCUUCAGUCC    | 417 |
| 1586rev  | AAGGGAUCUAGUCCCCGCA  | 154 | 723forw | UAAAAAUUCGCGGCCGGGUG  | 418 |
| 1591forw | UACAGGCGUGAGCCACCGUG | 155 | 726forw | AAAUUCGCGGCCGGGUGCGG  | 419 |
| 1592forw | ACAGGCGUGAGCCACCGUGC | 156 | 72rev   | UCGCUCCGUUCCUCUCCUG   | 420 |
| 1593forw | CAGGCGUGAGCCACCGUGCG | 157 | 731rev  | AGGGUUGGGGGUGGGGGGUG  | 421 |
| 1604rev  | AUUGGCCAAGCUGACUCUCG | 158 | 735rev  | UCCCAAAGUGCUGGGAUUAC  | 422 |
| 1604rev  | GAGUCCCCGCCUUGCAAAA  | 159 | 736rev  | CUGGGAGGGUUGGGGGUGGG  | 423 |
| 1605rev  | GGAGUCCCCGCCUUGCAAAA | 160 | 737rev  | GCUGGGAGGGUUGGGGGUGG  | 424 |
| 1614forw | GGACU AAGAUCCUUUUGCA | 161 | 738rev  | GGCUGGGAGGGUUGGGGGUG  | 425 |

|          |                       |     |         |                       |     |
|----------|-----------------------|-----|---------|-----------------------|-----|
| 1615forw | GACUAAGAUCUUUUUGCAA   | 162 | 739rev  | CGGCUGGGAGGGUUGGGGGU  | 426 |
| 1618forw | UAAGAUCUUUUUGCAAGGG   | 163 | 73forw  | AGCAAGGUUAGUGAAGGCC   | 427 |
| 1619forw | GAGAGCCGCGAGAGUCAGCU  | 164 | 740rev  | CCGGCUGGGAGGGUUGGGGG  | 428 |
| 1619forw | AAGAUCUUUUUGCAAGGGC   | 165 | 743rev  | CUUCGGCCUCCCAAAGUGCU  | 429 |
| 1620forw | AGAUCUUUUUGCAAGGGCG   | 166 | 743rev  | CUGCCGGCUGGGAGGGUUGG  | 430 |
| 1622rev  | CGGCCGCCGACCGCACGGAU  | 167 | 744rev  | GCUUCGGCCUCCCAAAGUGC  | 431 |
| 1626rev  | AUGCACUUGUCUGUAGUUCA  | 168 | 744rev  | ACUGCCGGCUGGGAGGGUUG  | 432 |
| 1627rev  | GGGAGCGGCCGCCGACCGCA  | 169 | 745rev  | GACUGCCGGCUGGGAGGGUUG | 433 |
| 1632forw | GUCAGCUUGGCCAAUCCGUG  | 170 | 746rev  | AGACUGCCGGCUGGGAGGGU  | 434 |
| 1636forw | GCUUGGCCAAUCCGUGCGGU  | 171 | 74forw  | GCAAGGUUAGUGAAGGCCA   | 435 |
| 1639forw | UGGCCAAUCCGUGCGGUCGG  | 172 | 750rev  | UGGGAGACUGCCGGCUGGGA  | 436 |
| 1642rev  | GAGUCGGCUUAUAAAAGGGAG | 173 | 751rev  | GUGGGAGACUGCCGGCUGGG  | 437 |
| 1647rev  | CGGGCGAGUCGGCUUAUAAA  | 174 | 753forw | UACCGUAAUCCAGCACUU    | 438 |
| 1648rev  | CCGGGCGAGUCGGCUUAUAAA | 175 | 754forw | ACCUGUAAUCCAGCACUUU   | 439 |
| 1658rev  | CGGUGCGCUGCCGGGCGAGU  | 176 | 754rev  | CUUGUGGGAGACUGCCGGCU  | 440 |
| 1665rev  | GAAGCAAAGUACCACUAGA   | 177 | 755rev  | UCUUGUGGGAGACUGCCGGC  | 441 |
| 1666rev  | CCGCAACCCGGUGCGCUGCC  | 178 | 757forw | UGUAAUCCAGCACUUUGGG   | 442 |
| 1667rev  | UCCGCAACCCGGUGCGCUGC  | 179 | 759rev  | CAAUUCUUGUGGGAGACUGC  | 443 |
| 1668forw | CCUUUAUAGCCGACUCGCC   | 180 | 760forw | CCACCCCAACCCUCCAGC    | 444 |
| 1673forw | UUUGUUCUACUCCAUCUAG   | 181 | 760rev  | CUCAAGUGAUCCACCCGUU   | 445 |
| 1678rev  | CAGGCCACCCUCCGCAACC   | 182 | 766forw | AGCACUUGGGAGGCCGAAG   | 446 |
| 1679forw | CGACUCGCCCAGCAGCGCAC  | 183 | 767forw | GCACUUUGGGAGGCCGAAGC  | 447 |
| 1680forw | GACUCGCCCAGCAGCGCAC   | 184 | 769rev  | AAAUCAGAGCCAAUUCUUGU  | 448 |
| 1686forw | CCCGGACGCGACCCGGGUUG  | 185 | 76forw  | GUUCAGGCCUUUCAGGCCGC  | 449 |
| 1689forw | GGCAGCGCACCCGGGUUGCGG | 186 | 770forw | CUUUGGGAGGCCGAAGCGGG  | 450 |
| 1689rev  | CACCACAAUUGUUGUAAAUG  | 187 | 770rev  | GAAAUCAGAGCCAAUUCUUG  | 451 |
| 1690forw | GCAGCGCACCCGGGUUGCGGA | 188 | 780forw | CGGCAGUCUCCACAAGAAU   | 452 |
| 1693forw | GCGCACCCGGGUUGCGGAGGG | 189 | 783rev  | CAGGCUGGUCUCGAACGCCA  | 453 |
| 1694forw | CGCACCCGGGUUGCGGAGGGU | 190 | 784rev  | CCAGGCUGGUCUCGAACGCC  | 454 |
| 1697rev  | AAAUGGCCACCACCCUCCC   | 191 | 786forw | CGGGUGGAUCACUUGAGCCC  | 455 |
| 1699forw | CGGGUUGCGGAGGGUGGGCC  | 192 | 792rev  | CCAUAAGCUUAUUUCUUA    | 456 |
| 1700forw | GGGUUGCGGAGGGUGGGCCU  | 193 | 798rev  | UUUCACCAUGUUGCCCAGGC  | 457 |
| 1703forw | UUGCGGAGGGUGGGCCUGGG  | 194 | 802rev  | GGGGUUCACCAUGUUGCCC   | 458 |
| 1704forw | UGCGGAGGGUGGGCCUGGGA  | 195 | 804forw | CCUGGCGUUCGAGACCAGCC  | 459 |
| 1705forw | GCGGAGGGUGGGCCUGGGAG  | 196 | 805forw | CUGGCGUUCGAGACCAGCCU  | 460 |
| 1707forw | CUCCACAUUACAACAUUUG   | 197 | 812forw | CCUUUAAGAAAUAAGCUAA   | 461 |
| 1708forw | GAGGGUGGGCCUGGGAGGGG  | 198 | 813forw | CGAGACCAGCCUGGGCAACA  | 462 |
| 170rev   | UCGGCGUUCACCCACCAAC   | 199 | 815rev  | UUUGUUUCUUAACCUAGU    | 463 |
| 1710forw | CACAUUACAACAUUUGUGG   | 200 | 816rev  | GUUUGUUUCUUAACCUAG    | 464 |
| 1711forw | GGUGGGCCUGGGAGGGGUGG  | 201 | 821forw | AAAUAAGCUAAUGGCCACU   | 465 |
| 1714rev  | AGUUAGGGUAGACAAAAAA   | 202 | 821rev  | UGUGUUUUUAGUAGAGACGG  | 466 |

|          |                      |     |         |                       |     |
|----------|----------------------|-----|---------|-----------------------|-----|
| 1716forw | UACAACAUUUGUGGUGGUGC | 203 | 822rev  | UUGUGUUUUUAGUAGAGACG  | 467 |
| 1717forw | ACAACAUUUGUGGUGGUGCA | 204 | 823rev  | UUUGUGUUUUUAGUAGAGAC  | 468 |
| 1720rev  | CUGUGGCCAUUCUUGCUUCA | 205 | 824rev  | UUUUGUGUUUUUAGUAGAGA  | 469 |
| 1729rev  | GCCUACGCCCUUCUCAGUUA | 206 | 82forw  | GCCUUUCAGGCCCGCAGGAAG | 470 |
| 1730rev  | CGCCUACGCCCUUCUCAGUU | 207 | 839forw | CUAGGUUGAAAGAAACAAAC  | 471 |
| 1734forw | GCAGGGCCGUGAAGCAAGAA | 208 | 83forw  | AGUGAAGGCCAGGGACUGAA  | 472 |
| 1737rev  | AGAAAAACAUUCCCAGUCUG | 209 | 842rev  | GUCGUGAUAAAGUGGGCAGAA | 473 |
| 1741forw | UUGUCUAACCCUAACUGAGA | 210 | 850rev  | AUUACCUUGUCGUGAUAAAGU | 474 |
| 1742forw | UGUCUAACCCUAACUGAGAA | 211 | 851rev  | AAUUACCUUGUCGUGAUAAAG | 475 |
| 1745forw | AAGCAAGAAUGGCCACAGAC | 212 | 853forw | CUAAAAACACAAAAACUAGC  | 476 |
| 1746forw | AGCAAGAAUGGCCACAGACU | 213 | 854forw | UAAAAACACAAAAACUAGCU  | 477 |
| 1748forw | ACCCUAACUGAGAAGGGCGU | 214 | 859forw | ACACAAAAACUAGCUGGGCG  | 478 |
| 1753rev  | GCGCGCGGGGAGCAAAAGCA | 215 | 862forw | CAAAAACUAGCUGGGCGUGG  | 479 |
| 175forw  | CAUGCAGUUCGUUCCUGU   | 216 | 866forw | AACUAGCUGGGCGUGGUGGC  | 480 |
| 1766rev  | AGCGAGAAAAACAGCGCGCG | 217 | 866forw | UCUGCCACUUUACACGACA   | 481 |
| 1767rev  | CAGCGAGAAAAACAGCGCGC | 218 | 871rev  | UCCUGAGUAGCUGGGAUUAC  | 482 |
| 1768rev  | UCAGCGAGAAAAACAGCGCG | 219 | 874rev  | UUGAAGGUAUGGAUUUGGA   | 483 |
| 178forw  | GCAGUUCGCUUCCUGUUGG  | 220 | 878rev  | GGAAUUGAAGGUAUGGAUUU  | 484 |
| 1798forw | UUUUUCUCGCGACUUUCAG  | 221 | 879rev  | UCUCAGCCUCCUGAGUAGCU  | 485 |
| 1799forw | UUUUCUCGCGACUUUCAGC  | 222 | 879rev  | AGGAAUUGAAGGUAUGGAUU  | 486 |
| 179forw  | CAGUUCGCUUCCUGUUGGU  | 223 | 87forw  | UCAGGCCCGAGGAAGAGGAA  | 487 |
| 1802forw | UCUCGCGACUUUCAGCGGG  | 224 | 880rev  | GUCUCAGCCUCCUGAGUAGC  | 488 |
| 180forw  | AGUUCGCUUCCUGUUGGUG  | 225 | 885rev  | AUCCUAAGGAAUUGAAGGUA  | 489 |
| 1810rev  | CGGUGGAAGGCGGCAGGCCG | 226 | 890forw | GCCUGUAAUCCCAGCUACUC  | 490 |
| 1813forw | UUCAGCGGGCGGAAAAGCCU | 227 | 890rev  | AGAUGAUCCUAAGGAAUUGA  | 491 |
| 1816rev  | AAUGAACGGUGGAAGGCGGC | 228 | 893forw | UGUAAUCCCAGCUACUCAGG  | 492 |
| 181forw  | UUAUGAUGAAUGUGAUAGUU | 229 | 899rev  | ACUACCCCCAGAUGAUCCUA  | 493 |
| 181forw  | GUUCGCUUCCUGUUGGUGG  | 230 | 903forw | AUCCAUAACCUCAAUCCUU   | 494 |
| 1820rev  | CUAGAAUGAACGGUGGAAGG | 231 | 912forw | UUCAAUCCUUAGGAUCAUC   | 495 |
| 1823rev  | GCUCUAGAAUGAACGGUGGA | 232 | 913forw | UCAAUCCUUAGGAUCAUCU   | 496 |
| 1827rev  | GUUUGCUCUAGAAUGAACGG | 233 | 914forw | CAAUCCUUAGGAUCAUCUG   | 497 |
| 182forw  | UUCGCUUCCUGUUGGUGGG  | 234 | 915forw | AAUCCUUAGGAUCAUCUGG   | 498 |
| 1830rev  | UUUGUUUGCUCUAGAAUGAA | 235 | 919rev  | CACUGCAACCUCUGCCUCCC  | 499 |
| 1866forw | AAACAAAAAUGUCAGCUGC  | 236 | 920rev  | UCACUGCAACCUCUGCCUCC  | 500 |
| 1869rev  | GGUCCCCGGGAGGGGCGAAC | 237 | 921forw | ACACGAGAAUCGCUUGAACC  | 501 |
| 1870rev  | AGGUCCCCGGGAGGGGCGAA | 238 | 922forw | CACGAGAAUCGCUUGAACC   | 502 |
| 1877rev  | CCGCCGAGGUCCCCGGGAG  | 239 | 924rev  | CCCCUGGUCGUCUCUCUCU   | 503 |
| 1878rev  | CCCGCCGAGGUCCCCGGGA  | 240 | 925forw | GAGAAUCGCUUGAACCCGGG  | 504 |
| 1879rev  | ACCCGCCGAGGUCCCCGGG  | 241 | 931forw | CGCUUGAACCCGGGAGGCAG  | 505 |
| 1882rev  | GCGACCCGCCGAGGUCCCC  | 242 | 940rev  | GGCCUUUAUAUACACACCCC  | 506 |
| 1883rev  | GGCGACCCGCCGAGGUCCCC | 243 | 942forw | UGCCAAGAGAGAGAGCAGCC  | 507 |

|          |                       |     |         |                      |     |
|----------|-----------------------|-----|---------|----------------------|-----|
| 1884forw | GCUGGCCCCGUUCGCCCCUCC | 244 | 943forw | GCCAAGAGAGAGAGCAGCCA | 508 |
| 1885forw | CUGGCCCCGUUCGCCCCUCC  | 245 | 944forw | CCAAGAGAGAGAGCAGCCAG | 509 |
| 1886forw | UGGCCCCGUUCGCCCCUCCG  | 246 | 944rev  | GGAGUCUAGUGGCGUGAUCU | 510 |
| 1890rev  | CUGGGCAGGCGACCCGCCGC  | 247 | 955rev  | CCAGGCUGGAUGGAGUCUAG | 511 |
| 1894forw | UCGCCCCUCCCGGGACCUG   | 248 | 958forw | AGCCAGGGGUGUGUAUUA   | 512 |
| 1897forw | CCCCUCCCGGGGACCUGCGG  | 249 | 961rev  | CAGGCUAUCACCCUAAAGGU | 513 |
| 1898forw | CCUCCCGGGGACCUGCGGC   | 250 | 962rev  | UCAGGCUAUCACCCUAAAGG | 514 |
| 189rev   | GGGUGACGGAUGCGCACGAU  | 251 | 965rev  | GCUCUUUCGCCAGGCUGGA  | 515 |
| 1904rev  | GCGGGGUUCGGGGGCUGGGC  | 252 | 965rev  | GAUUCAGGCUAUCACCCUAA | 516 |
| 1908rev  | CCAGGCGGGGUUCGGGGCU   | 253 | 969rev  | UCUUGCUCUUUCGCCAGGC  | 517 |
| 1909rev  | UCCAGGCGGGGUUCGGGGC   | 254 | 970forw | GUUAUAAAGGCCACCUUU   | 518 |
| 1913rev  | GGCCUCCAGGCGGGGUUCGG  | 255 | 971forw | UAUAUAAAGGCCACCUUA   | 519 |
| 1914rev  | CGGCCUCCAGGCGGGGUUCG  | 256 | 973rev  | GGAGUCUUGCUCUUCGCC   | 520 |
| 1915rev  | GCGGCCUCCAGGCGGGGUUC  | 257 | 975forw | CCACUAGACUCCAUCAGCC  | 521 |
| 1916rev  | CGCGCCUCCAGGCGGGGUU   | 258 | 976forw | CACUAGACUCCAUCAGCCU  | 522 |
| 1921rev  | CCGACCGCGCCUCCAGGCG   | 259 | 97rev   | AGGGAAUCGCGCCGCGCG   | 523 |
| 1922rev  | GCCGACCGCGCCUCCAGGC   | 260 | 980rev  | ACUUUCAAUCAUCAGGAUUC | 524 |
| 1923rev  | GGCCGACCGCGCCUCCAGG   | 261 | 987rev  | ACUUCUGACUUUCAUAUC   | 525 |
| 1926rev  | CCGGGCCGACCGCGCCUCC   | 262 | 98rev   | CAGGGAAUCGCGCCGCGCG  | 526 |
| 1928forw | CCCAGCCCCGAACCCGCC    | 263 | 994rev  | AACGAUUUUUUUUUGAGA   | 527 |
| 1931forw | AGCCCCGAACCCGCCUGG    | 264 | 99rev   | UCAGGGAAUCGCGCCGCGCG | 528 |

**Table 2. Guide RNA sequences**

| Seq Name | Sequence               | Seq ID NO | Seq Name | Sequence              | Seq ID NO |
|----------|------------------------|-----------|----------|-----------------------|-----------|
| 1420forw | GUGAACCGCGUCUGGUCUGCA  | 529       | 967rev   | AGUCUUGCUCUUUCGCCAGG  | 584       |
| 1591rev  | GCUGACUCUCGCGGCUCUCGU  | 530       | 991rev   | UAACGAUUUUUUUUUGAGA   | 585       |
| 634forw  | AACUCCACGGAGUUUAUCUAA  | 531       | 94rev    | GCCCAACUCUUCGCGGUGGCA | 586       |
| 640forw  | ACGGAGUUUAUCUAAUCUGAAU | 532       | 302rev   | CCCCAUUGCCGCGAGGGGUG  | 587       |
| 1688forw | CCGGCAGCGCACCGGGUUGCG  | 533       | 997rev   | AACUACCCCAUGAUGAUCCUA | 588       |
| 716forw  | GGCCAUUAUAAAAUUCGCGG   | 534       | 237rev   | ACUGCAUGUGUGAGCCGAGUC | 589       |
| 1608forw | AGUGACUCUCACGAGAGCCGC  | 535       | 309rev   | CACAAGCCCCAUUGCCGGCG  | 590       |
| 1678forw | GCCGACUCGCCCGGCAGCGCA  | 536       | 579forw  | GAUCUCGACCAGUCCCCUCAA | 591       |
| 1728rev  | GGCGCCUACGCCCUUCUCAGU  | 537       | 110forw  | GAGGCACCCACUGCCACCGCG | 592       |
| 620forw  | GGACCGACAGCGACAACUCCA  | 538       | 1082forw | GCCCAUUUAGGGUGAUAGC   | 593       |

|          |                         |     |          |                       |     |
|----------|-------------------------|-----|----------|-----------------------|-----|
| 1239rev  | CAGAAUCUUGUCUCGGCUCAG   | 539 | 456forw  | GUUGCCUGGAGCCGUUCCUGC | 594 |
| 1229rev  | UCUCGGCUCAGUGGGAUGCGU   | 540 | 1391rev  | AAAAAGCGAUCUUAGAUCACC | 595 |
| 1584forw | CCCCCAACCAGCCCGCCCGA    | 541 | 414forw  | GCACCUCCAAGUCGGCCAAA  | 596 |
| 481rev   | GGUUGUAAAGUUUUUACGGA    | 542 | 1182forw | CUAUUACCUAAGUAGGUCCCC | 597 |
| 1625rev  | AAGGGAGCGGCCGCCGACCGC   | 543 | 192forw  | GGCCGCAGGAAGAGGAACGGA | 598 |
| 1896forw | CGCCCCUCCCGGGGACCUGCG   | 544 | 875forw  | UCCCAGCCGGCAGUCUCCAC  | 599 |
| 1661rev  | CGCAACCCGGUGCGCUGCCGG   | 545 | 1830forw | GGUGGUGCAGGGCCGUGAAGC | 600 |
| 383forw  | UGAGAUAAUGUGGGAUGC UAA  | 546 | 214rev   | GGGUGCACGUCCACAGCUCA  | 601 |
| 472forw  | AAGUGCAAUAGUGC UAAAAAC  | 547 | 1724rev  | CAUGCACUUGUCUGUAGUUCA | 602 |
| 614rev   | UAUUCAGUUAGAUAAACUCCG   | 548 | 1070forw | GUGUAUUAAGGCCACCUU    | 603 |
| 1376rev  | UCACCUCGACUACCUAAAAA    | 549 | 337rev   | CUGGCCAGUCAGUCAGGUUU  | 604 |
| 166forw  | CCAUAAGGAAACUAUGUUAUG   | 550 | 850rev   | UUGUGGAGACUGCCGGCUGG  | 605 |
| 409rev   | AUAGAGUAGAUGC UAAAUGC U | 551 | 984rev   | UGAUCCUAAGGAAUUGAAGGU | 606 |
| 1170rev  | UCUCCAGCCUCUCCUUGAGCA   | 552 | 1086rev  | UGACUUCUGACUUUCAUCAU  | 607 |
| 1122rev  | CUACAUUAUUAUCUUAAGGA    | 553 | 843rev   | AGACUGCCGGCUGGGAGGGUU | 608 |
| 1372forw | CUUAGGCCCUAAAAUCUCCU    | 554 | 1014forw | UUCAAUCCUAGGAUCAUCU   | 609 |
| 268rev   | AAAUCCU AUUGCUUAUAUC    | 555 | 1348forw | UGAUUUUGCCAAGAACUUGUC | 610 |
| 1836rev  | GCUGACAUUUUUUGUUGCUC    | 556 | 1703rev  | AGGAGUCCCCGCCUUGCAA   | 611 |
| 183forw  | UAUGAUGAAUGUGAUAGUUUG   | 557 | 244rev   | AAAGCGAACUGCAUGUGUGAG | 612 |
| 1260rev  | CCCAGGCAGCACUGACUACAG   | 558 | 945rev   | UACCUUGUCGUGAUAGUGGG  | 613 |
| 1034rev  | GUCUUGAUGAGGUAAAAAGAG   | 559 | 1847forw | AAGCAAGAAUGGCCACAGACU | 614 |
| 1027forw | AAAAAUUCGUUACAAUUUAUG   | 560 | 1043forw | UUGCCAAGAGAGAGAGCAGCC | 615 |
| 287forw  | GUACUCAAGAUUAUAAGCAAU   | 561 | 50rev    | GGGCCGACCGGCCUCCAGG   | 616 |
| 101rev   | UUAUUUCUCUCCUUGCAUA     | 562 | 131forw  | AAGAGUUGGGCUCUGUCAGCC | 617 |
| 1201rev  | CCUACCCUGCCCCUUCUCCU    | 563 | 1217forw | AGAUACAUUUCUAGCACUAU  | 618 |
| 1284forw | CUGCUGUAGUCAGUGCUGCCU   | 564 | 1763rev  | AGAAGCAAAGUACCACUAGA  | 619 |

|          |                        |     |          |                        |     |
|----------|------------------------|-----|----------|------------------------|-----|
| 429rev   | CACUUAGCACAGUACCUUACA  | 565 | 431forw  | CAAAAUGAAUUGGGCAGUGAGC | 620 |
| 355forw  | ACUUAGAUUAUGAAGGGGAAAG | 566 | 1322forw | GCAUGGUUUUGUGGAAAAGUA  | 621 |
| 661rev   | UGCAGUUUUACAUAUAAAUGA  | 567 | 972rev   | AUUGAAGGUAUGGAUUUGGGA  | 622 |
| 1703forw | GUUGCGGAGGGUGGGCCUGGG  | 568 | 629forw  | UGAGAGAUCAUUUAACAUUUA  | 623 |
| 1332forw | AAAAAUGUGAUGAUCAAAACU  | 569 | 1003forw | AAAUCCAUAACCUCAAUUCU   | 624 |
| 1219forw | GGCAUUCUAAGGAGAAGGGGG  | 570 | 1244forw | UAAGAAAUGUAAAAAAACCUC  | 625 |
| 372forw  | AAAGAAGGGUUUGAGAUAAUG  | 571 | 836rev   | CGGCUGGGAGGGUUGGGGGUG  | 626 |
| 192forw  | UGUGAUAGUUUGGAGAAUAAA  | 572 | 1462forw | UCCCACUCUGCACCCAGAGC   | 627 |
| 531forw  | GCAGAUGCUAUGAAAGAAAAA  | 573 | 764rev   | UUAUUGGAACGCUAAGCUUGU  | 628 |
| 708rev   | UAUGAGCCACCGCACCCGGCC  | 574 | 829rev   | GAGGGUUGGGGGUGGGGGGUG  | 629 |
| 741rev   | CGCUUCGGCCUCCCAAAGUGC  | 575 | 1445rev  | CUGCACUCCAGCUCUGGGUGA  | 630 |
| 765forw  | CCAGCACUUUGGGAGGCCGAA  | 576 | 755forw  | CUUGGCGAUGACCUUGAGCAG  | 631 |
| 769forw  | CACUUUGGGAGGCCGAAGCGG  | 577 | 1436forw | UUUCUUCUCUUUCUUUGAGA   | 632 |
| 820rev   | UUUUGUGUUUUUAGUAGAGAC  | 578 | 1453rev  | UGGGGACACUGCACUCCAGCU  | 633 |
| 877rev   | UGUCUCAGCCUCCUGAGUAGC  | 579 | 1544forw | UUCUCUCAGCCUCCAAGUAG   | 634 |
| 886rev   | CGAUUCUCGUGUCUCAGCCUC  | 580 | 1570rev  | CUGAAAAUACAAAAAAUCAG   | 635 |
| 905forw  | CUACUCAGGAGGCUGAGACAC  | 581 | 1621rev  | GCGGGCGGAUACCUGAGGUC   | 636 |
| 918rev   | GCUCACUGCAACCUCUGCCUC  | 582 | 1638rev  | CACUUUGGGAGGCAGAAGCGG  | 637 |
| 962rev   | UGCUCUUUCGCCCAGGCUGGA  | 583 | 1666forw | CGCUUCUGCCUCCCAAAGUGC  | 638 |

[00108] In some aspects, a guide RNA molecule can comprise a part of any sequence recited in Table 3 or Table 4. In some aspects, a guide RNA molecule can comprise the first about 20 nucleotides of any sequence recited in Table 3. In some aspects, a guide RNA molecule can comprise the first about 21 nucleotides of any sequence recited in Table 4.

**Table 3. Guide RNA sequences**

| Seq Name | Sequence                | Seq ID NO | Seq Name | Sequence                | Seq ID NO |
|----------|-------------------------|-----------|----------|-------------------------|-----------|
| 1015rev  | UGUUCAUAAAUUACUGACAUGG  | 639       | 1934rev  | GAGAAGCCCCGGGCCGACCGCGG | 903       |
| 1025forw | AAAAAAAUCGUUACAAUUUAUGG | 640       | 1937forw | CGAACCCCGCCUGGAGGCCGCGG | 904       |

|          |                          |     |          |                          |     |
|----------|--------------------------|-----|----------|--------------------------|-----|
| 1028forw | AAAAUCGUUACAAUUUAUGGUGG  | 641 | 1941forw | CCCCGCCUGGAGGCCGCGGUCGG  | 905 |
| 1037rev  | UCUUGAUGAGGUAAAAAGAGGGG  | 642 | 1944rev  | GGUGCCUCCGGAGAAGCCCCGGG  | 906 |
| 1038rev  | GUCUUGAUGAGGUAAAAAGAGGG  | 643 | 1945rev  | GGGUGCCUCCGGAGAAGCCCCGG  | 907 |
| 1039rev  | UGUCUUGAUGAGGUAAAAAGAGG  | 644 | 1946forw | CCUGGAGGCCGCGGUCGGCCCCGG | 908 |
| 103rev   | AAUUUCUCUCCUUUGCAUAUUGG  | 645 | 1947forw | CUGGAGGCCGCGGUCGGCCCCGGG | 909 |
| 1049rev  | AGUAGUGCUGUGUCUUGAUGAGG  | 646 | 1948forw | UGGAGGCCGCGGUCGGCCCCGGGG | 910 |
| 1059rev  | AGGGGACCUACUUAGGUAAUAGG  | 647 | 1956rev  | GCGGUGGCAGUGGGUGCCUCCGG  | 911 |
| 1066rev  | CAAUUCCAGGGGACCUACUUAGG  | 648 | 1957forw | CGGUCGGCCCCGGGGCUUCUCCGG | 912 |
| 106forw  | ACGGAGCGAGUCCCCGCGCGCGG  | 649 | 1960forw | UCGGCCCCGGGGCUUCUCCGAGG  | 913 |
| 1073forw | UUUUAACCUAUUACCUAAGUAGG  | 650 | 1965rev  | CAACUCUUCGCGGUGGCAGUGGG  | 914 |
| 1077rev  | UAUCUGCUAGACAAUCCAGGGG   | 651 | 1966rev  | CCAACUCUUCGCGGUGGCAGUGG  | 915 |
| 1078rev  | GUAUCUGCUAGACAAUCCAGGG   | 652 | 1986forw | CCACUGCCACCGCAAGAGAUUGG  | 916 |
| 1079rev  | UGUAUCUGCUAGACAAUCCAGG   | 653 | 1987forw | CACUGCCACCGCAAGAGAUUGG   | 917 |
| 1081forw | UAUUACCUAAGUAGGUCCCCUGG  | 654 | 199forw  | UUUGGAGAAUAAAUUGAAUGAGG  | 918 |
| 1098rev  | UCCUUUUUAUAGGAAAGAAAGG   | 655 | 1rev     | CAGAGCCCAACUCUUCGCGGUGG  | 919 |
| 1107rev  | GACUGAAUCUCCUUUUUAUAGG   | 656 | 203forw  | GAGAAUAAAUUGAAUGAGGAAGG  | 920 |
| 1116forw | CGCCUUUCUUUCCUAAAUAAGG   | 657 | 203rev   | CCAUUGCCGGCGAGGGGUGACGG  | 921 |
| 1117forw | GCCUUUCUUUCCUAAAUAAGGG   | 658 | 206rev   | AACUGAUCACCAAUUCUCCAGGG  | 922 |
| 1129rev  | CUACUACAUAUUAUUAUCUUAAGG | 659 | 207rev   | UAACUGAUCACCAAUUCUCCAGG  | 923 |
| 1139rev  | CCAGCAACAGUGGACUCUAGAGG  | 660 | 209forw  | AAAUUGAAUGAGGAAGGCCUGG   | 924 |
| 1149rev  | GAGAACAUAUACCAGCAACAGUGG | 661 | 209rev   | AAGCCCCCAUUGCCGGCGAGGGG  | 925 |
| 114forw  | GCUAAAUAUCCAAUUGCAAAGG   | 662 | 210rev   | CAAGCCCCCAUUGCCGGCGAGGG  | 926 |
| 1159forw | CCUCUAGAGUCCACUGUUGCUGG  | 663 | 211rev   | ACAAGCCCCCAUUGCCGGCGAGG  | 927 |
| 1168rev  | GCCUCUCCUUGAGCAGAGGAUGG  | 664 | 216rev   | GGUUCACAAGCCCCCAUUGCCGG  | 928 |
| 116rev   | GGUGCACGUCCCACAGCUCAGGG  | 665 | 217forw  | UGAGGAAGGCCUUGGAGAUUUGG  | 929 |
| 1172rev  | UCCAGCCUCUCCUUGAGCAGAGG  | 666 | 217forw  | GCGCAUCCGUCACCCUUCGCCGG  | 930 |
| 1178forw | CUGGUAAUGUUCUCUAAAUAAGG  | 667 | 223forw  | CCGUCACCCUUCGCCGGCAAUGG  | 931 |
| 117rev   | GGGUGCACGUCCCACAGCUCAGG  | 668 | 224forw  | CGUCACCCUUCGCCGGCAAUGGG  | 932 |
| 1182forw | UUAAAGCCAUCCUCUGCUCAAGG  | 669 | 225forw  | GUCACCCUUCGCCGGCAAUGGGG  | 933 |
| 1187forw | GCCAUCCUCUGCUCAAGGAGAGG  | 670 | 226forw  | UCACCCUUCGCCGGCAAUGGGGG  | 934 |
| 1191forw | UCCUCUGCUCAAGGAGAGGCUGG  | 671 | 237rev   | GCCCAGUCAGUCAGGUUUUGGGG  | 935 |
| 1193rev  | UCCACAAAACCAUGCUGAUAGG   | 672 | 238rev   | GGCCAGUCAGUCAGGUUUUGGGG  | 936 |
| 1197forw | GCUCAAGGAGAGGCUGGAGAAGG  | 673 | 239rev   | UGGCCAGUCAGUCAGGUUUUGGG  | 937 |
| 1203forw | AAAUUUUUUCCUUAUCAGCAUGG  | 674 | 240rev   | CUGGCCAGUCAGUCAGGUUUUGG  | 938 |
| 1207forw | AGGCUGGAGAAGGCAUUCUAAGG  | 675 | 243rev   | AAGACUUGGCACUUUAUAUGUGG  | 939 |
| 1211forw | UCCUAUCAGCAUGGUUUUUGUGG  | 676 | 245rev   | GCACACUGGCCAGUCAGUCAGG   | 940 |
| 1213forw | GAGAAGGCAUUCUAAGGAGAAGG  | 677 | 255forw  | AACCCCAAACCUGACUGACUGG   | 941 |
| 1214forw | AGAAGGCAUUCUAAGGAGAAGGG  | 678 | 256forw  | ACCCCAAACCUGACUGACUGGG   | 942 |
| 1215forw | GAAGGCAUUCUAAGGAGAAGGGG  | 679 | 257rev   | AUAUCUUGAGUACAAGACUUGG   | 943 |
| 1216forw | AAGGCAUUCUAAGGAGAAGGGGG  | 680 | 259rev   | CCUGCCAAUUGCAGCACACUGG   | 944 |
| 1220forw | CAUUCUAAGGAGAAGGGGGCAGG  | 681 | 275forw  | UGGGCCAGUGUGCUGCAAAUUGG  | 945 |

|          |                         |     |         |                          |     |
|----------|-------------------------|-----|---------|--------------------------|-----|
| 1221forw | AUUCUAAGGAGAAGGGGGCAGGG | 682 | 279forw | CCAGUGUGCUGCAAAUUGGCAGG  | 946 |
| 1221forw | CAUGGUUUUGUGGAAAAGUAAGG | 683 | 287forw | UACUCAAGAUUUAAGCAAUAGG   | 947 |
| 1225forw | UAAGGAGAAGGGGGCAGGGUAGG | 684 | 28rev   | CCUCGCCCCGAGAGACCCGCGG   | 948 |
| 1232forw | AAGGGGGCAGGGUAGGAACUCGG | 685 | 290forw | CAAUUGGCAGGAGACGUGAAGG   | 949 |
| 1234rev  | CAAGACUCUAGACAAGUUCUUGG | 686 | 295rev  | UUCAUUUUGGCCGACUUUGGAGG  | 950 |
| 1241rev  | GAAUCUUGUCUCGGCUCAGUGGG | 687 | 298rev  | CCAUUCAUUUUGGCCGACUUUGG  | 951 |
| 1242rev  | AGAAUCUUGUCUCGGCUCAGUGG | 688 | 305forw | CGUGAAGGCACCUCCAAAGUCGG  | 952 |
| 1250rev  | ACUACAGCAGAAUCUUGUCUCGG | 689 | 308rev  | GGCUCACUGCCCAUUCAUUUUGG  | 953 |
| 1257forw | AGAACUUGUCUAGAGUCUUGAGG | 690 | 318forw | CCAAAGUCGGCCAAAUGAAUGG   | 954 |
| 126forw  | CGGCGCAUUCUCCUGAGCUGUGG | 691 | 319forw | CAAAGUCGGCCAAAUGAAUGGG   | 955 |
| 127forw  | GGCGCAUUCUCCUGAGCUGUGGG | 692 | 31forw  | GAGUUGGGCUCUGUCAGCCGCGG  | 956 |
| 1281rev  | CUUUGUGAAAAUAGAUUCCAGG  | 693 | 329rev  | GGAACGGCUCACAGGCAACCCCGG | 957 |
| 1281rev  | AGAUCACCUUGAGUAAACUGAGG | 694 | 32forw  | AGUUGGGCUCUGUCAGCCGCGGG  | 958 |
| 1283forw | CUGCUGUAGUCAGUGCUGCCUGG | 695 | 330forw | AAAUGAAUGGGCAGUGAGCCGG   | 959 |
| 1284forw | UGCUGUAGUCAGUGCUGCCUGGG | 696 | 331forw | AAAUGAAUGGGCAGUGAGCCGGG  | 960 |
| 1295forw | AGUAAGCCUCAGUUACUCAAGG  | 697 | 331rev  | UUUCCCCUUCUAUCAAGUAGG    | 961 |
| 1308rev  | GUUUUGAUCAUCAUUAUUUUGG  | 698 | 332forw | AAUGAAUGGGCAGUGAGCCGGGG  | 962 |
| 1332forw | AAAUGUGAUGAUCAAAACUAGG  | 699 | 338rev  | ACCCACGCAGGAACGGCUCCAGG  | 963 |
| 1335forw | UUCUUCUCUUUCUUUGAGACGG  | 700 | 340forw | GGCAGUGAGCCGGGGUUGCCUGG  | 964 |
| 1341rev  | CCAGCUCUGGGUGACAGAGUGGG | 701 | 345rev  | CGGGAGAACCCACGCAGGAACGG  | 965 |
| 1342rev  | UCCAGCUCUGGGUGACAGAGUGG | 702 | 346forw | UAGUGCCUACUUAUAUGAAGG    | 966 |
| 1353rev  | GGACACUGCACUCCAGCUCUGGG | 703 | 347forw | AGUGCCUACUUAUAUGAAGGG    | 967 |
| 1354forw | GAAUUAGUGUUCUGUCUUAGG   | 704 | 348forw | GUGCCUACUUAUAUGAAGGGG    | 968 |
| 1354rev  | GGGACACUGCACUCCAGCUCUGG | 705 | 350rev  | GAAGACGGGAGAACCCACGCAGG  | 969 |
| 1357rev  | GAAUUCACAGGAAGAUUUUAGGG | 706 | 356forw | UUAGAUUAUGAAGGGGAAAGAAGG | 970 |
| 1358rev  | GGAAUUCACAGGAAGAUUUUAGG | 707 | 356forw | UGCCUGGAGCCGUUCCUGCGUGG  | 971 |
| 1361forw | CCCACUCUGUCACCCAGAGCUGG | 708 | 357forw | UAGAUUAUGAAGGGGAAAGAAGGG | 972 |
| 1369rev  | ACCUUAAAAAUGGAAUUCACAGG | 709 | 357forw | GCCUGGAGCCGUUCCUGCGUGGG  | 973 |
| 1374rev  | GGUUGCAGUGAGCCAAGAUGGGG | 710 | 364rev  | GGCAACAAAAAGCGGAAGACGGG  | 974 |
| 1375rev  | AGGUUGCAGUGAGCCAAGAUGGG | 711 | 365rev  | AGGCAACAAAAAGCGGAAGACGG  | 975 |
| 1376rev  | GAGGUUGCAGUGAGCCAAGAUGG | 712 | 372forw | AAGAAGGGUUUGAGAUAAUGUGG  | 976 |
| 1379rev  | CACCUCGACUACCUAAAAAUGG  | 713 | 372rev  | CCAUAAAAGGCAACAAAAAGCGG  | 977 |
| 137rev   | GCAUGUGUGAGCCGAGUCCUGGG | 714 | 373forw | AGAAGGGUUUGAGAUAAUGUGGG  | 978 |
| 1382forw | GGAGUGCAGUGUCCCCAUUUGG  | 715 | 385rev  | AGUUGUAAUACAACCAUAAAAGG  | 979 |
| 1388forw | UCCUGUGAAUUCAUUUUUAAGG  | 716 | 388forw | AAUGUGGGAUGCUAAGAGAAUGG  | 980 |
| 138rev   | UGCAUGUGUGAGCCGAGUCCUGG | 717 | 391forw | GUGGGAUGCUAAGAGAAUGGUGG  | 981 |
| 1395rev  | GCUAGAAACCGAGGAGGCAGAGG | 718 | 392forw | CCGCUUUUUGUUGCCUUUAUGG   | 982 |
| 1397forw | UUCCAUUUUUAAGGUAGUCGAGG | 719 | 40forw  | UCUGUCAGCCGCGGGUCUCUCGG  | 983 |
| 1401rev  | AAAUCGCUAGAAACCGAGGAGG  | 720 | 413rev  | CUCAACAAAAUCUGCAGAGCAGG  | 984 |
| 1404rev  | UAUCCUCUGCAGACCAGACGCGG | 721 | 41forw  | CUGUCAGCCGCGGGUCUCUCGGG  | 985 |
| 1404rev  | GAGAAAUCGCUAGAAACCGAGG  | 722 | 42forw  | UGUCAGCCGCGGGUCUCUCGGGG  | 986 |

|          |                          |     |         |                          |      |
|----------|--------------------------|-----|---------|--------------------------|------|
| 1407forw | CACUGCAACCUCUGCCUCCUCGG  | 723 | 434forw | CUGCUCUGCAGAUUUUGUUGAGG  | 987  |
| 140forw  | GAAAUUAAAAGAUUUAAAAGCAGG | 724 | 439forw | UUUAGCAUCUACUCUAUGUAAGG  | 988  |
| 140forw  | GAGCUGUGGGACGUGCACCCAGG  | 725 | 43forw  | GUCAGCCGCGGGUCUCUCGGGGG  | 989  |
| 1411forw | UAGUCGAGGUGAACCGGUCUGG   | 726 | 448rev  | GACUGGUCGAGAUUCUACCUUGG  | 990  |
| 1421forw | GAACCGGUCUGGUCUCAGAGG    | 727 | 449rev  | GGACUGGUCGAGAUUCUACCUUGG | 991  |
| 1431rev  | UGUAAAACCCAGCUACUUGGGAGG | 728 | 452forw | UGAGGUUUUUGCUUCUCCCAAGG  | 992  |
| 1433forw | GUCUGCAGAGGAUAGAAAAAAGG  | 729 | 465rev  | CCACACCCCGUUGAGGGGACUGG  | 993  |
| 1434rev  | GCCUGUAAAACCCAGCUACUUGGG | 730 | 470rev  | UUCUCCACACCCCGUUGAGGGG   | 994  |
| 1435rev  | UGCCUGUAAAACCCAGCUACUUGG | 731 | 471rev  | GUUCUCCACACCCCGUUGAGGG   | 995  |
| 1436rev  | AACUAACUUGAGGUUACAGAGGG  | 732 | 472forw | AGUGCAAUAGUGCUAAAAAACAGG | 996  |
| 1437rev  | AAACUAACUUGAGGUUACAGAGG  | 733 | 472rev  | UGUUCUCCACACCCCGUUGAGG   | 997  |
| 1444forw | CUCUCAGCCUCCCAAGUAGCUGG  | 734 | 478forw | AUCUCGACCAGUCCCUCAACCGG  | 998  |
| 1445forw | UCUCAGCCUCCCAAGUAGCUGGG  | 735 | 479forw | UCUCGACCAGUCCCUCAACGGG   | 999  |
| 1446rev  | UUAAAAGGUGAAACUAACUUGAGG | 736 | 480forw | CUCGACCAGUCCCUCAACGGGG   | 1000 |
| 1453forw | UCCCAAGUAGCUGGGUUUACAGG  | 737 | 485forw | CCAGUCCCUCAACGGGGUGUGG   | 1001 |
| 145rev   | AUCAUAACAUAGUUUCCUUAUGG  | 738 | 486forw | CAGUCCCUCAACGGGGUGUGGG   | 1002 |
| 1462rev  | UUACUCCGACCUUCUUUAAAGG   | 739 | 488rev  | CCAGGUUGUAAAGUUUUUACGG   | 1003 |
| 1462rev  | AAAAAAUCAGCCGGUAUGGUGG   | 740 | 48forw  | CCGCGGGUCUCUCGGGGGCGAGG  | 1004 |
| 1465rev  | ACAAAAAAUCAGCCGGUAUGG    | 741 | 49forw  | CGCGGGUCUCUCGGGGGCGAGGG  | 1005 |
| 146forw  | UGGGACGUGCACCCAGGACUCGG  | 742 | 4rev    | UGACAGAGCCCAACUCUUCGCGG  | 1006 |
| 1470rev  | AAAAUACAAAAAAUCAGCCGGG   | 743 | 506rev  | UUUCUUUCAUAGCAUCUGCCAGG  | 1007 |
| 1471rev  | GAAAAUACAAAAAAUCAGCCGG   | 744 | 508forw | CCGUAAAAACUUUACAACCUUG   | 1008 |
| 1472forw | GUUAGUUUCACCUUUAAAGAAGG  | 745 | 530forw | GCAGAUGCUAUGAAAGAAAAGG   | 1009 |
| 1472forw | CAGGCACACACCACCAUACCCGG  | 746 | 531forw | CAGAUGCUAUGAAAGAAAAGGG   | 1010 |
| 1476forw | GUUUCACCUUUAAAAGAAGGUCGG | 747 | 532forw | AGAUGCUAUGAAAGAAAAGGGG   | 1011 |
| 1495rev  | CCCUUCCGCACGUCCGGGAAAGG  | 748 | 536forw | GCUAUGAAAGAAAAGGGGAUGG   | 1012 |
| 1500rev  | CGUUGCCCUUCCGCACGUCCGGG  | 749 | 537forw | CUAUGAAAGAAAAGGGGAUGGG   | 1013 |
| 1501rev  | ACGUUGCCCUUCCGCACGUCCGG  | 750 | 549forw | AAGGGGAUGGGAGAGAGAAGG    | 1014 |
| 1502forw | UAAAGACGCAAAGCCUUUCCCGG  | 751 | 54forw  | GUCUCUCGGGGGCGAGGGCGAGG  | 1015 |
| 1502forw | UUUUGUAUUUUCAGUAAAGUUGG  | 752 | 552forw | GGGAUGGGAGAGAGAGAAGGAGG  | 1016 |
| 1503forw | UUUGUAUUUUCAGUAAAGUUGGG  | 753 | 553forw | GGAUGGGAGAGAGAGAAGGAGGG  | 1017 |
| 1507forw | UAUUUUCAGUAAAGUUGGGCAGG  | 754 | 561forw | UAGAAGAUCUAAAUGAACAUUGG  | 1018 |
| 150forw  | AUUUAAAAGCAGGAGCCAUAAAGG | 755 | 563forw | GAGAGAAGGAGGGAGAGAGAUGG  | 1019 |
| 1510forw | CAAAGCCUUUCCCGGACGUGCGG  | 756 | 568forw | AAGGAGGGAGAGAGAUGGAGAGG  | 1020 |
| 1511forw | UUCAGUAAAGUUGGGCAGGCUGG  | 757 | 569forw | AGGAGGGAGAGAGAUGGAGAGGG  | 1021 |
| 1514forw | GCCUUUCCCGGACGUGCGGAAGG  | 758 | 574rev  | CAUAAACCGAUGACCAUUAAAGG  | 1022 |
| 1514rev  | CACCUGAGGUCAGGAGUUCGAGG  | 759 | 57forw  | AACAAGCGCUAUGACUAGCAAGG  | 1023 |
| 1515forw | CCUUUCCCGGACGUGCGGAAGGG  | 760 | 581forw | UGGAAAUUGUGUCCUUUAAUGG   | 1024 |
| 1523rev  | CGGGCGGAUACCUAGAGGUCAGG  | 761 | 588forw | UGUGUCCUUUAAUGGUCUACGG   | 1025 |
| 1524rev  | UCCAUUUCCGGCCAUGAGGAAGG  | 762 | 597rev  | AAAAAGAAACUUCUAAACCUCUGG | 1026 |
| 1528rev  | AAGUCCAUUUCCGGCCAUGAGG   | 763 | 598forw | UUUACUUUUUCUUUCAGAUCGAGG | 1027 |

|          |                          |     |         |                          |      |
|----------|--------------------------|-----|---------|--------------------------|------|
| 1528rev  | AGAAGCGGGCGGAUCACCUGAGG  | 764 | 601forw | UGGUCAUCGGUUUAUGCCAGAGG  | 1028 |
| 1532forw | GGCCUCGAACUCCUGACCUCAGG  | 765 | 602rev  | CUCCGUGGAGUUGUCGUCGUCGG  | 1029 |
| 1533forw | AAGGGCAACGUCCUCCUCAUGG   | 766 | 60forw  | CGGGGGCGAGGGCGAGGUUCAGG  | 1030 |
| 1536rev  | GGAAAUUAAAGUUCCAUIUCCGG  | 767 | 617rev  | AUUCAGUUAGAUAAAUCUCCGUGG | 1031 |
| 1537forw | GCAACGUCCUCCUCAUGGCCGG   | 768 | 620forw | GACCGACAGCGACAACUCCACGG  | 1032 |
| 1539rev  | CUUUGGGAGGCAGAAGCGGGCGG  | 769 | 632rev  | ACUGCUCUAGGUCUAGCCAAAGG  | 1033 |
| 1542rev  | GCACUUUGGGAGGCAGAAGCGGG  | 770 | 635forw | UUUUUUGAAAAUUAGACCUUGG   | 1034 |
| 1543forw | UCCUCCUCAUGGCCGAAAUGG    | 771 | 63rev   | UCCUCUCCUGCGGCCUGAAAAGG  | 1035 |
| 1543rev  | AGCACUUUGGGAGGCAGAAGCGG  | 772 | 644rev  | GGGUUAUAUCCUACUGCUCUAGG  | 1036 |
| 1552rev  | UGUAAUCCAGCACUUUGGGAGG   | 773 | 655forw | UGGCGAUGACCUUGAGCAGUAGG  | 1037 |
| 1555rev  | GCCUGUAAUCCAGCACUUUGGG   | 774 | 663rev  | CAGUUUUACAUAUAAAUGACAGG  | 1038 |
| 1556rev  | CGCCUGUAAUCCAGCACUUUGG   | 775 | 664rev  | UUGGAACGCUAAGCUUGUGGGGG  | 1039 |
| 1557rev  | GCGGGCUGGUUGGGGGGAACGGG  | 776 | 665rev  | AUUGGAACGCUAAGCUUGUGGGG  | 1040 |
| 1558rev  | GGCGGGCUGGUUGGGGGGAACGG  | 777 | 666rev  | UAUUGGAACGCUAAGCUUGUGGG  | 1041 |
| 1563rev  | UCUCGGGCGGGCUGGUUGGGGGG  | 778 | 667rev  | UUAUUGGAACGCUAAGCUUGUGG  | 1042 |
| 1564rev  | CUCUCGGGCGGGCUGGUUGGGGG  | 779 | 683rev  | UAUGCCUAGUGUCCGUUAUUGG   | 1043 |
| 1565forw | GCUUCUGCCUCCAAAGUGCUGG   | 780 | 68forw  | UGACUAGCAAGGUUAAGUGAAGG  | 1044 |
| 1565rev  | UCUCUCGGGCGGGCUGGUUGGGG  | 781 | 690forw | CAAGCUUAGCGUCCAAUAACGG   | 1045 |
| 1566forw | CUUCUGCCUCCAAAGUGCUGGG   | 782 | 694forw | UAUGUAAAACUGCACUAUACUGG  | 1046 |
| 1566rev  | CUCUCUCGGGCGGGCUGGUUGGG  | 783 | 697rev  | CCGGCCGCAAUUUUUUAUAAUGG  | 1047 |
| 1567rev  | ACUCUCUCGGGCGGGCUGGUUGG  | 784 | 699forw | CGUCCAUAUACGGAACACUAGG   | 1048 |
| 1571rev  | AGUCACUCUCUCGGGCGGGCUGG  | 785 | 69forw  | GGGCGAGGUUCAGGCCUUUCAGG  | 1049 |
| 1574forw | UCCCAAAGUGCUGGGAUUCAGG   | 786 | 713forw | CUGGCCAUUAUAAAAAUUCGCGG  | 1050 |
| 1575rev  | UGAGAGUCACUCUCUCGGGCGGG  | 787 | 714forw | ACACUAGGCAUAAUGAAAAGACGG | 1051 |
| 1576rev  | GUGAGAGUCACUCUCUCGGGCGG  | 788 | 716rev  | CAGGU AUGAGCCACCGCACCCGG | 1052 |
| 1579rev  | CUCGUGAGAGUCACUCUCUCGGG  | 789 | 717forw | CCAUUAUAAAAAUUCGCGGCCGG  | 1053 |
| 1580rev  | UCUCGUGAGAGUCACUCUCUCGG  | 790 | 718forw | CAUUAUAAAAAUUCGCGGCCGGG  | 1054 |
| 1583rev  | GGAUCUUAAGUCCCCGCACGGUGG | 791 | 71rev   | ACUUUAAGCCUUUCAGUCCUGG   | 1055 |
| 1586rev  | AAGGGAUCUUAGUCCCCGCACGG  | 792 | 723forw | UAAAAAUUCGCGGCCGGGUGCGG  | 1056 |
| 1591forw | UACAGGCGUGAGCCACCGUGCGG  | 793 | 726forw | AAAUUCGCGGCCGGGUGCGGUGG  | 1057 |
| 1592forw | ACAGGCGUGAGCCACCGUGCGGG  | 794 | 72rev   | UCGCUCCGUUCCUCUCCUGCGG   | 1058 |
| 1593forw | CAGGCGUGAGCCACCGUGCGGGG  | 795 | 731rev  | AGGGUUGGGGGUGGGGGGUGUGG  | 1059 |
| 1604rev  | AUUGGCCAAGCUGACUCUCGCGG  | 796 | 735rev  | UCCCAAAGUGCUGGGAUUACAGG  | 1060 |
| 1604rev  | GAGUCCCCGCCUUGCAAAAAGGG  | 797 | 736rev  | CUGGGAGGGUUGGGGGUGGGGGG  | 1061 |
| 1605rev  | GGAGUCCCCGCCUUGCAAAAAGG  | 798 | 737rev  | GCUGGGAGGGUUGGGGGUGGGGG  | 1062 |
| 1614forw | GGACUAAGAUCUUUUGCAAGG    | 799 | 738rev  | GGCUGGGAGGGUUGGGGGUGGGG  | 1063 |
| 1615forw | GACUAAGAUCUUUUGCAAGGG    | 800 | 739rev  | CGGCUGGGAGGGUUGGGGGUGGG  | 1064 |
| 1618forw | UAAGAUCUUUUGCAAGGGCGG    | 801 | 73forw  | AGCAAGGUUAAGUGAAGGCCAGG  | 1065 |
| 1619forw | GAGAGCCGCGAGAGUCAGCUUGG  | 802 | 740rev  | CCGGCUGGGAGGGUUGGGGGUGG  | 1066 |
| 1619forw | AAGAUCUUUUGCAAGGGCGGG    | 803 | 743rev  | CUUCGGCCUCCAAAGUGCUGGG   | 1067 |
| 1620forw | AGAUCUUUUGCAAGGGCGGGG    | 804 | 743rev  | CUGCCGGCUGGGAGGGUUGGGGG  | 1068 |

|          |                          |     |         |                          |      |
|----------|--------------------------|-----|---------|--------------------------|------|
| 1622rev  | CGGCCGCCGACCGCACGGAUUGG  | 805 | 744rev  | GCUUCGGCCUCCCAAAGUGCUGG  | 1069 |
| 1626rev  | AUGCACUUGUCUGUAGUUCAAGG  | 806 | 744rev  | ACUGCCGGCUGGGAGGGUUGGGG  | 1070 |
| 1627rev  | GGGAGCGGCCCGCCGACCGCACGG | 807 | 745rev  | GACUGCCGGCUGGGAGGGUUGGG  | 1071 |
| 1632forw | GUCAGCUUGGCCAAUCCGUGCGGG | 808 | 746rev  | AGACUGCCGGCUGGGAGGGUUGG  | 1072 |
| 1636forw | GCUUGGCCAAUCCGUGCGGUCGG  | 809 | 74forw  | GCAAGGUUAAGUGAAGGCCAGGG  | 1073 |
| 1639forw | UGGCCAAUCCGUGCGGUCGGCGG  | 810 | 750rev  | UGGGAGACUGCCGGCUGGGAGGG  | 1074 |
| 1642rev  | GAGUCGGCUUAUAAAAGGGAGCGG | 811 | 751rev  | GUGGGAGACUGCCGGCUGGGAGG  | 1075 |
| 1647rev  | CGGGCGAGUCGGCUUAUAAAAGGG | 812 | 753forw | UACCUGUAAUCCAGCACUUUGG   | 1076 |
| 1648rev  | CCGGGCGAGUCGGCUUAUAAAAGG | 813 | 754forw | ACCUGUAAUCCAGCACUUUGGG   | 1077 |
| 1658rev  | CGGUGCGCUGCCGGGCGAGUCGG  | 814 | 754rev  | CUUGUGGGAGACUGCCGGCUGGG  | 1078 |
| 1665rev  | GAAGCAAAAGUACCACUAGAUGG  | 815 | 755rev  | UCUUGUGGGAGACUGCCGGCUGG  | 1079 |
| 1666rev  | CCGCAACCCGGUGCGCUGCCGGG  | 816 | 757forw | UGUAAUCCAGCACUUUGGGAGG   | 1080 |
| 1667rev  | UCCGCAACCCGGUGCGCUGCCGG  | 817 | 759rev  | CAAUUCUUGUGGGAGACUGCCGG  | 1081 |
| 1668forw | CCUUUAUAAGCCGACUCGCCCGG  | 818 | 760forw | CCACCCCAACCCUCCAGCCGG    | 1082 |
| 1673forw | UUUGUUCUUAACUCAUCUAGUGG  | 819 | 760rev  | CUCAAGUGAUCCACCCGCUUCGG  | 1083 |
| 1678rev  | CAGGCCACCCUCCGCAACCCGG   | 820 | 766forw | AGCACUUUGGGAGGCCGAAGCGG  | 1084 |
| 1679forw | CGACUCGCCCGGCAGCGCACCGG  | 821 | 767forw | GCACUUUGGGAGGCCGAAGCGGG  | 1085 |
| 1680forw | GACUCGCCCGGCAGCGCACCGGG  | 822 | 769rev  | AAAUCAGAGCCAAUUCUUGUGGG  | 1086 |
| 1686forw | CCCGGCAGCGCACCGGGUUGCGG  | 823 | 76forw  | GUUCAGGCCUUUCAGGCCCGCAGG | 1087 |
| 1689forw | GGCAGCGCACCGGGUUGCGGAGG  | 824 | 770forw | CUUUGGGAGGCCGAAGCGGGUGG  | 1088 |
| 1689rev  | CACCACAAAUGUUGUAAAUGUGG  | 825 | 770rev  | GAAAUAGAGCCAAUUCUUGUGG   | 1089 |
| 1690forw | GCAGCGCACCGGGUUGCGGAGGG  | 826 | 780forw | CGGCAGUCUCCACAAGAAUUGG   | 1090 |
| 1693forw | GCGCACCGGGUUGCGGAGGGUGG  | 827 | 783rev  | CAGGCUGGUCUCGAACGCCAGGG  | 1091 |
| 1694forw | CGCACCGGGUUGCGGAGGGUGGG  | 828 | 784rev  | CCAGGCUGGUCUCGAACGCCAGG  | 1092 |
| 1697rev  | AAAUGGCCACCACCCUCCAGG    | 829 | 786forw | CGGGUGGAUCACUUGAGCCCUUG  | 1093 |
| 1699forw | CGGGUUGCGGAGGGUGGGCCUGG  | 830 | 792rev  | CCAUUAGCUUAUUUUCUUAAGG   | 1094 |
| 1700forw | GGGUUGCGGAGGGUGGGCCUGGG  | 831 | 798rev  | UUUCACCAUGUUGCCCAGGCUGG  | 1095 |
| 1703forw | UUGCGGAGGGUGGGCCUGGGAGG  | 832 | 802rev  | GGGGUUUCACCAUGUUGCCCAGG  | 1096 |
| 1704forw | UGCAGGAGGGUGGGCCUGGGAGGG | 833 | 804forw | CCUGGCGUUCGAGACCAGCCUGG  | 1097 |
| 1705forw | GCGGAGGGUGGGCCUGGGAGGGG  | 834 | 805forw | CUGGCGUUCGAGACCAGCCUGGG  | 1098 |
| 1707forw | CUCCACAUUUACAACAUUUGUGG  | 835 | 812forw | CCUUUAAGAAAAUAAGCUAUGG   | 1099 |
| 1708forw | GAGGGUGGGCCUGGGAGGGGUGG  | 836 | 813forw | CGAGACCAGCCUGGGCAACAUGG  | 1100 |
| 170rev   | UCGGCGUUCUUUUUACCAACAGG  | 837 | 815rev  | UUUGUUUCUUAACCUAGUGGG    | 1101 |
| 1710forw | CACAUUUACAACAUUUGUGGUGG  | 838 | 816rev  | GUUUGUUUCUUAACCUAGUGG    | 1102 |
| 1711forw | GGUGGGCCUGGGAGGGGUGGUGG  | 839 | 821forw | AAAUAAAGCUAAUUGGCCACUAGG | 1103 |
| 1714rev  | AGUUAGGGUAGACAAAAAUGG    | 840 | 821rev  | UGUGUUUUUAGUAGAGACGGGG   | 1104 |
| 1716forw | UACAACAUUUGUGGUGGUGCAGG  | 841 | 822rev  | UUGUGUUUUUAGUAGAGACGGG   | 1105 |
| 1717forw | ACAACAUUUGUGGUGGUGCAGGG  | 842 | 823rev  | UUUGUGUUUUUAGUAGAGACGGG  | 1106 |
| 1720rev  | CUGUGGCCAUUCUUGCUUCACGG  | 843 | 824rev  | UUUUGUGUUUUUAGUAGAGACGG  | 1107 |
| 1729rev  | GCCUACGCCUUCUCAGUUAGGG   | 844 | 82forw  | GCCUUCAGGCCCGCAGGAAGAGG  | 1108 |
| 1730rev  | CGCCUACGCCUUCUCAGUUAGG   | 845 | 839forw | CUAGGUUGAAAGAAACAACAGG   | 1109 |

|          |                          |     |         |                          |      |
|----------|--------------------------|-----|---------|--------------------------|------|
| 1734forw | GCAGGGCCGUGAAGCAAGAAUGG  | 846 | 83forw  | AGUGAAGGCCAGGGACUGAAAGG  | 1110 |
| 1737rev  | AGAAAAACAUUCCAGUCUGUGG   | 847 | 842rev  | GUCGUGAU AAGUGGGCAGAAUGG | 1111 |
| 1741forw | UUGUCUAACCCU AACUGAGAAGG | 848 | 850rev  | AUUACCUUGUCGUGAU AAGUGGG | 1112 |
| 1742forw | UGUCUAACCCU AACUGAGAAGGG | 849 | 851rev  | AAUUACCUUGUCGUGAU AAGUGG | 1113 |
| 1745forw | AAGCAAGAAUGGCCACAGACUGG  | 850 | 853forw | CUAAAAACACAAAAACUAGCUGG  | 1114 |
| 1746forw | AGCAAGAAUGGCCACAGACUGGG  | 851 | 854forw | UAAAAACACAAAAACUAGCUGGG  | 1115 |
| 1748forw | ACCCU AACUGAGAAGGGCGUAGG | 852 | 859forw | ACACAAAAACUAGCUGGGCGUGG  | 1116 |
| 1753rev  | GCGCGCGGGGAGCAAAAGCACGG  | 853 | 862forw | CAAAAACUAGCUGGGCGUGGUGG  | 1117 |
| 175forw  | CAUGCAGUUCGUUCCUGUUGG    | 854 | 866forw | AACUAGCUGGGCGUGGUGGCAGG  | 1118 |
| 1766rev  | AGCGAGAAAAACAGCGCGCGGGG  | 855 | 866forw | UCUGCCCACUUAUCACGACAAGG  | 1119 |
| 1767rev  | CAGCGAGAAAAACAGCGCGCGGG  | 856 | 871rev  | UCCUGAGUAGCUGGGAUUACAGG  | 1120 |
| 1768rev  | UCAGCGAGAAAAACAGCGCGCGG  | 857 | 874rev  | UUGAAGGU AUGGAUUUGGGACGG | 1121 |
| 178forw  | GCAGUUCGUUCCUGUUGGUGG    | 858 | 878rev  | GGAAUUGAAGGU AUGGAUUUGGG | 1122 |
| 1798forw | UUUUUCUCGUGACUUCAGCGG    | 859 | 879rev  | UCUCAGCCUCCUGAGUAGCUGGG  | 1123 |
| 1799forw | UUUUCUCGUGACUUCAGCGGG    | 860 | 879rev  | AGGAUUGAAGGU AUGGAUUUGG  | 1124 |
| 179forw  | CAGUUCGUUCCUGUUGGUGGG    | 861 | 87forw  | UCAGGCCGCAGGAAGAGGAACGG  | 1125 |
| 1802forw | UCUCGUGACUUCAGCGGGCGG    | 862 | 880rev  | GUCUCAGCCUCCUGAGUAGCUGG  | 1126 |
| 180forw  | AGUUCGUUCCUGUUGGUGGGG    | 863 | 885rev  | AUCCUAAGGAUUGAAGGU AUGG  | 1127 |
| 1810rev  | CGGUGGAAGGCGGCAGGCCGAGG  | 864 | 890forw | GCCUGUAAUCCAGCUACUCAGG   | 1128 |
| 1813forw | UUCAGCGGGCGGAAAAGCCUCGG  | 865 | 890rev  | AGAUGAUCCU AAGGAAUUGAAGG | 1129 |
| 1816rev  | AAUGAACGGUGGAAGGCGGCAGG  | 866 | 893forw | UGUAAUCCAGCUACUCAGGAGG   | 1130 |
| 181forw  | UUAUGAUGAAUGUGAUAGUUUGG  | 867 | 899rev  | ACUACCCCCAGAU GAUCCUAAGG | 1131 |
| 181forw  | GUUCGUUCCUGUUGGUGGGGG    | 868 | 903forw | AUCCAUACCUUCAAUUCCUAGG   | 1132 |
| 1820rev  | CUAGAAUGAACGGUGGAAGGCGG  | 869 | 912forw | UUCAAUUCCU UAGGAUCAUCUGG | 1133 |
| 1823rev  | GCUCUAGAAUGAACGGUGGAAGG  | 870 | 913forw | UCAAUUCCU UAGGAUCAUCUGGG | 1134 |
| 1827rev  | GUUUGCUCUAGAAUGAACGGUGG  | 871 | 914forw | CAAUUCCU UAGGAUCAUCUGGGG | 1135 |
| 182forw  | UUCGUUCCUGUUGGUGGGGGG    | 872 | 915forw | AAUUCCU UAGGAUCAUCUGGGGG | 1136 |
| 1830rev  | UUUGUUUGCUCUAGAAUGAACGG  | 873 | 919rev  | CACUGCAACCUCUGCCUCCCGGG  | 1137 |
| 1866forw | AAACAAAAAUGUCAGCUGCUGG   | 874 | 920rev  | UCACUGCAACCUCUGCCUCCCGG  | 1138 |
| 1869rev  | GGUCCCCGGGAGGGGCGAACGGG  | 875 | 921forw | ACACGAGAAUCGCUUGAACCCGG  | 1139 |
| 1870rev  | AGGUCCCCGGGAGGGGCGAACGG  | 876 | 922forw | CACGAGAAUCGCUUGAACCCGGG  | 1140 |
| 1877rev  | CCGCCGCAGGUCCCCGGGAGGGG  | 877 | 924rev  | CCCUUGGUCGUCUCUCUCUUGG   | 1141 |
| 1878rev  | CCCGCCGCAGGUCCCCGGGAGGG  | 878 | 925forw | GAGAAUCGCUUGAACCCGGGAGG  | 1142 |
| 1879rev  | ACCCGCCGCAGGUCCCCGGGAGG  | 879 | 931forw | CGCUUGAACCCGGGAGGCAGAGG  | 1143 |
| 1882rev  | GCGACCCGCCGCAGGUCCCCGGG  | 880 | 940rev  | GGCCUUUAUAUACACACCCUUGG  | 1144 |
| 1883rev  | GGCGACCCGCCGCAGGUCCCCGG  | 881 | 942forw | UGCCAAGAGAGAGAGCAGCCAGG  | 1145 |
| 1884forw | GCUGGCCCGUUCGCCCCUCCCGG  | 882 | 943forw | GCCAAGAGAGAGAGCAGCCAGGG  | 1146 |
| 1885forw | CUGGCCCGUUCGCCCCUCCCGGG  | 883 | 944forw | CCAAGAGAGAGAGCAGCCAGGGG  | 1147 |
| 1886forw | UGGCCCGUUCGCCCCUCCCGGGG  | 884 | 944rev  | GGAGUCUAGUGGGGUGAUUCUGG  | 1148 |
| 1890rev  | CUGGGCAGGCGACCCGCCGCAGG  | 885 | 955rev  | CCAGGCUGGAUGGAGUCUAGUGG  | 1149 |
| 1894forw | UCGCCCCUCCCGGGGACCUUGCGG | 886 | 958forw | AGCCAGGGGUGUGUAUUA AAGG  | 1150 |

|          |                          |     |         |                          |      |
|----------|--------------------------|-----|---------|--------------------------|------|
| 1897forw | CCCCUCCCGGGGACCUGCGGCGG  | 887 | 961rev  | CAGGCUAUCACCCUAAAGGUGGG  | 1151 |
| 1898forw | CCCUCGCGGGGACCUGCGGCGGG  | 888 | 962rev  | UCAGGCUAUCACCCUAAAGGUGGG | 1152 |
| 189rev   | GGGUGACGGAU GCGCACGAUCGG | 889 | 965rev  | GCUCUUUCGCCAGGCUGGAUGG   | 1153 |
| 1904rev  | GCGGGGUUCGGGGGUCUGGGCAGG | 890 | 965rev  | GAUUCAGGCUAUCACCCUAAAGG  | 1154 |
| 1908rev  | CCAGGCGGGGUUCGGGGGUCUGGG | 891 | 969rev  | UCUUGCUCUUUCGCCAGGCUGG   | 1155 |
| 1909rev  | UCCAGGCGGGGUUCGGGGGUCUGG | 892 | 970forw | GUAUAUAAAGGCCACCUUAGG    | 1156 |
| 1913rev  | GGCCUCCAGGCGGGGUUCGGGGG  | 893 | 971forw | UAUAUAAAGGCCACCUUAGGG    | 1157 |
| 1914rev  | CGGCCUCCAGGCGGGGUUCGGGG  | 894 | 973rev  | GGAGUCUUGCUCUUUCGCCAGG   | 1158 |
| 1915rev  | GCGGCCUCCAGGCGGGGUUCGGG  | 895 | 975forw | CCACUAGACUCCAUCAGCCUGG   | 1159 |
| 1916rev  | CGCGGCCUCCAGGCGGGGUUCGG  | 896 | 976forw | CACUAGACUCCAUCAGCCUGGG   | 1160 |
| 1921rev  | CCGACCGCGGCCUCCAGGCGGGG  | 897 | 97rev   | AGGGAAUCGCGCCGCGCGGGG    | 1161 |
| 1922rev  | GCCGACCGCGGCCUCCAGGCGGG  | 898 | 980rev  | ACUUUCAAUCAUCAGGAUUCAGG  | 1162 |
| 1923rev  | GGCCGACCGCGGCCUCCAGGCGG  | 899 | 987rev  | ACUUCUGACUUUCAUCAUCAGG   | 1163 |
| 1926rev  | CCGGGCGGACCGCGGCCUCCAGG  | 900 | 98rev   | CAGGGAAUCGCGCCGCGCGGG    | 1164 |
| 1928forw | CCCAGCCCCGAACCCCGCCUGG   | 901 | 994rev  | AACGAUUUUUUUUUUGAGACGG   | 1165 |
| 1931forw | AGCCCCGAACCCCGCCUGGAGG   | 902 | 99rev   | UCAGGGAAUCGCGCCGCGCGGG   | 1166 |

**Table 4. Guide RNA sequences**

| Seq Name | Sequence                        | Seq ID NO | Seq Name | Sequence                        | Seq ID NO |
|----------|---------------------------------|-----------|----------|---------------------------------|-----------|
| 1420forw | GUGAACCGCGUCUGGUCUGC<br>AGAGGAU | 1167      | 967rev   | AGUCUUGCUCUUUCGCCAGG<br>CUGGAU  | 1222      |
| 1591rev  | GCUGACUCUCGCGGCUCUCG<br>UGAGAGU | 1168      | 991rev   | UAACGAUUUUUUUUUUGAG<br>ACGGAGU  | 1223      |
| 634forw  | AACUCCACGGAGUUUAUCUA<br>ACUGAAU | 1169      | 94rev    | GCCCAACUCUUCGCGGUGGCA<br>GUGGGU | 1224      |
| 640forw  | ACGGAGUUUAUCUAACUGAA<br>UACGAGU | 1170      | 302rev   | CCCCAUUGCCGCGAGGGGUG<br>ACGGAU  | 1225      |
| 1688forw | CCGGCAGCGCACCGGGUUGC<br>GGAGGGU | 1171      | 997rev   | AACUACCCCGAGAUGAUCCUA<br>AGGAAU | 1226      |
| 716forw  | GGCCAUUAUAAAAAUUCGCG<br>GCCGGGU | 1172      | 237rev   | ACUGCAUGUGUGAGCCGAGUC<br>CUGGGU | 1227      |
| 1608forw | AGUGACUCUCACGAGAGCCG<br>CGAGAGU | 1173      | 309rev   | CACAAGCCCCAUUGCCGGCG<br>AGGGGU  | 1228      |
| 1678forw | GCCGACUCGCGCCGAGCGC<br>ACCGGGU  | 1174      | 579forw  | GAUCUCGACCAGUCCCCUCAA<br>CGGGGU | 1229      |
| 1728rev  | GGCGCCUACGCCUUUCUCAG<br>UUAGGGU | 1175      | 110forw  | GAGGCACCCACUGCCACCGCG<br>AAGAGU | 1230      |
| 620forw  | GGACCGACAGCGACAACUCC<br>ACGGAGU | 1176      | 1082forw | GCCCACCUUUAGGGUGAUAGC<br>CUGAAU | 1231      |
| 1239rev  | CAGAAUCUUGUCUCGGCUCA<br>GUGGGAU | 1177      | 456forw  | GUUGCCUGGAGCCGUUCCUGC<br>GUGGGU | 1232      |
| 1229rev  | UCUCGGCUCAGUGGGAUGCG<br>UCCGAGU | 1178      | 1391rev  | AAAAAGCGAUCUUGAUCACC<br>UUGAGU  | 1233      |
| 1584forw | CCCCCAACCCAGCCCGCCCG<br>AGAGAGU | 1179      | 414forw  | GCACCUCCAAAGUCGGCCAAA<br>AUGAAU | 1234      |

|          |                                  |      |              |                                  |      |
|----------|----------------------------------|------|--------------|----------------------------------|------|
| 481rev   | GGUUGUAAAGUUUUUACG<br>GACAGAAU   | 1180 | 1182fo<br>rw | CUAUUACCUAAGUAGGUCCCC<br>UGGAAU  | 1235 |
| 1625rev  | AAGGGAGCGGCCGCCGACCG<br>CACGGAU  | 1181 | 192for<br>w  | GGCCGCAGGAAGAGGAACGG<br>AGCGAGU  | 1236 |
| 1896forw | CGCCCCUCCCGGGGACCUGC<br>GGCGGGU  | 1182 | 875for<br>w  | UCCCAGCCGGCAGUCUCCAC<br>AAGAAU   | 1237 |
| 1661rev  | CGCAACCCGGUGCGCUGCCG<br>GGCGAGU  | 1183 | 1830fo<br>rw | GGUGGUGCAGGGCCGUGAAG<br>CAAGAAU  | 1238 |
| 383forw  | UGAGAUAAUGUGGAUGCU<br>AAGAGAAU   | 1184 | 214rev       | GGGUGCACGUCCACAGCUCA<br>GGGAAU   | 1239 |
| 472forw  | AAGUGCAAUAGUGCUIAAA<br>ACAGGAGU  | 1185 | 1724re<br>v  | CAUGCACUUGUCUGUAGUUA<br>AGGAGU   | 1240 |
| 614rev   | UAUUCAGUUAGAUAACUCC<br>GUGGAGU   | 1186 | 1070fo<br>rw | GUGUAUAUAAAGGCCACCUU<br>UAGGGU   | 1241 |
| 1376rev  | UCACCUCGACUACCUUAAAA<br>AUGGAAU  | 1187 | 337rev       | CUGGCCCAGUCAGUCAGGUUU<br>GGGGGU  | 1242 |
| 166forw  | CCAUAAAGGAAACUAUGUUAU<br>GAUGAAU | 1188 | 850rev       | UUGUGGGAGACUGCCGGCUG<br>GGAGGGU  | 1243 |
| 409rev   | AUAGAGUAGAUGCUIAAAUG<br>CUUUGAGU | 1189 | 984rev       | UGAUCCUAAGGAAUUGAAGG<br>UAUGGAAU | 1244 |
| 1170rev  | UCUCCAGCCUCUCCUUGAGC<br>AGAGGAAU | 1190 | 1086re<br>v  | UGACUUCUGACUUCACAUCAU<br>CAGGAAU | 1245 |
| 1122rev  | CUACAUUAUUAAUCUUAAGG<br>ACUGAAU  | 1191 | 843rev       | AGACUGCCGGCUGGGAGGGU<br>UGGGGGU  | 1246 |
| 1372forw | CUUAGGCCCUAAAAUCUCC<br>UGUGAAU   | 1192 | 1014fo<br>rw | UUCAAUCCUUAAGGAUCAUCU<br>GGGGGU  | 1247 |
| 268rev   | AAAUUCCUAUUGCUUAUAAU<br>CUUGAGU  | 1193 | 1348fo<br>rw | UGAUUUUGCCAAGAACUUGU<br>CUAGAGU  | 1248 |
| 1836rev  | GCUGACAUUUUUUGUUUGCU<br>CUAGAAU  | 1194 | 1703re<br>v  | AGGAGUCCCCGCCUUGCAAA<br>AGGGAAU  | 1249 |
| 183forw  | UAUGAUGAAUGUGAUAGUU<br>UGGAGAAU  | 1195 | 244rev       | AAAGCGAACUGCAUGUGUGA<br>GCCGAGU  | 1250 |
| 1260rev  | CCCAGGCAGCACUGACUACA<br>GCAGAAU  | 1196 | 945rev       | UACCUUGUCGUGAUAAUGUGG<br>GCAGAAU | 1251 |
| 1034rev  | GUCUUGAUGAGGUAAAAAG<br>AGGGGAGU  | 1197 | 1847fo<br>rw | AAGCAAGA AUGGCCACAGACU<br>GGGAAU | 1252 |
| 1027forw | AAAAAUCGUUACAAUUUA<br>UGGUGGAAU  | 1198 | 1043fo<br>rw | UUGCCAAGAGAGAGAGCAGCC<br>AGGGGU  | 1253 |
| 287forw  | GUACUCAAGAUUAAGCAA<br>UAGGAAU    | 1199 | 50rev        | GGGCCGACCGGCCUCCAGG<br>CGGGGU    | 1254 |
| 101rev   | UUAUUUCUCUCCUUGCAU<br>AUUGGAAU   | 1200 | 131for<br>w  | AAGAGUUGGGCUCUGUCAGCC<br>GCGGGU  | 1255 |
| 1201rev  | CCUACCCUGCCCCUUCUCC<br>UUAGAAU   | 1201 | 1217fo<br>rw | AGAUACAUUUCUAGCACUA<br>UUAGAAU   | 1256 |
| 1284forw | CUGCUGUAGUCAGUCUGCC<br>UGGGAAU   | 1202 | 1763re<br>v  | AGAAGCAAAAGUACCACUAG<br>AUGGAGU  | 1257 |
| 429rev   | CACUUAGCACAGUACCUAC<br>AUAGAGU   | 1203 | 431for<br>w  | CAAAUUGAAUGGGCAGUGAG<br>CCGGGGU  | 1258 |
| 355forw  | ACUUAGAUUGAAGGGGAA<br>AGAAGGGU   | 1204 | 1322fo<br>rw | GCAUGGUUUUGUGGAAAAGU<br>AAGGAAU  | 1259 |
| 661rev   | UGCAGUUUACAUUAAA<br>GACAGGAAU    | 1205 | 972rev       | AUUGAAGGUAUGGAUUUGG<br>ACGGAAU   | 1260 |

|          |                                  |      |          |                                  |      |
|----------|----------------------------------|------|----------|----------------------------------|------|
| 1703forw | GUUGC GGAGGGUGGGCCUGG<br>GAGGGGU | 1206 | 629forw  | UGAGAGAUCAUUUAAACAUUU<br>AAUGAAU | 1261 |
| 1332forw | AAAAAUGUGAUGAUCAAAA<br>CUAGGAAU  | 1207 | 1003forw | AAAUCCAUAACCUCAAUCCU<br>UAGGAU   | 1262 |
| 1219forw | GGCAUUCUAAGGAGAAGGG<br>GGCAGGGU  | 1208 | 1244forw | UAAGAAAUGUAAAAAAACCU<br>CUAGAGU  | 1263 |
| 372forw  | AAAGAAGGGUUUGAGAUAA<br>UGUGGGAU  | 1209 | 836rev   | CGGCUGGGAGGGUUGGGGGU<br>GGGGGU   | 1264 |
| 192forw  | UGUGAUAGUUUGGAGAAUA<br>AAUUGAAU  | 1210 | 1462forw | UCCCACUCUGUCACCCAGAGC<br>UGGAGU  | 1265 |
| 531forw  | GCAGAUGCUAUGAAAGAAA<br>AAGGGGAU  | 1211 | 764rev   | UUAUUGGAACGCUAAGCUUG<br>UGGGGGU  | 1266 |
| 708rev   | UAUGAGCCACCGCACCCGGC<br>CGCGAAU  | 1212 | 829rev   | GAGGGUUGGGGGUGGGGGGU<br>GUGGAAU  | 1267 |
| 741rev   | CGCUUCGGCCUCCAAAGUG<br>CUGGGAU   | 1213 | 1445rev  | CUGCACUCCAGCUCUGGGUGA<br>CAGAGU  | 1268 |
| 765forw  | CCAGCACUUUGGGAGGCCGA<br>AGCGGGU  | 1214 | 755forw  | CUUGGCGAUGACCUUGAGCAG<br>UAGGAU  | 1269 |
| 769forw  | CACUUUGGGAGGCCGAAGCG<br>GGUGGAU  | 1215 | 1436forw | UUUCUUCUCUUUCUUUUGAG<br>ACGGAGU  | 1270 |
| 820rev   | UUUUGUGUUUUAGUAGAG<br>ACGGGGGU   | 1216 | 1453rev  | UGGGGACACUGCACUCCAGCU<br>CUGGGU  | 1271 |
| 877rev   | UGUCUCAGCCUCCUGAGUAG<br>CUGGGAU  | 1217 | 1544forw | UUCUCUCAGCCUCCCAAGUAG<br>CUGGGU  | 1272 |
| 886rev   | CGAUUCUCGUGUCUCAGCCU<br>CCUGAGU  | 1218 | 1570rev  | CUGAAAUAACAAAAAAUCA<br>GCCGGGU   | 1273 |
| 905forw  | CUACUCAGGAGGCUGAGACA<br>CGAGAAU  | 1219 | 1621rev  | GCGGGCGGAUACCUAGAGGUC<br>AGGAGU  | 1274 |
| 918rev   | GCUCACUGCAACCUCUGCCU<br>CCCGGGU  | 1220 | 1638rev  | CACUUUGGGAGGCAGAAGCG<br>GGCGGAU  | 1275 |
| 962rev   | UGCUCUUUCGCCCAGGCUGG<br>AUGGAGU  | 1221 | 1666forw | CGCUUCUGCCUCCAAAGUGC<br>UGGGAU   | 1276 |

[00109] In some aspects, a guide RNA molecule can be chemically synthesized using methods standard in the art. In some aspects, a guide RNA molecule can be chemically synthesized such that the guide RNA molecule comprises at least one chemical modification. In some aspects, a guide RNA molecule can be produced by in vitro transcription methods standard in the art, including, but not limited to, in vitro transcription using a plasmid template, in vitro transcription using a PCR-based template. In some aspects, in vitro transcription methods can be performed such that the produced guide RNA molecules comprise at least one chemical modification.

[00110] In some aspects, any of the compositions of the present disclosure can further comprise at least one mRNA and/or polynucleotide encoding a fusion protein comprising at least a portion of the MS2 coat protein (MCP) and at least one transactivation molecule. In some aspects, the at least one polynucleotide can be a plasmid comprising a nucleic acid encoding a fusion protein comprising at least a portion of the MS2 coat protein (MCP) and at least one transactivation

molecule operably linked to at least one promoter sufficient to drive expression of the fusion protein.

**[00111]** In some aspects, any of the compositions of the present disclosure can further comprise at least one mRNA and/or polynucleotide encoding a fusion protein comprising at least a portion of MS2 coat protein (MCP) and at least one VP64 transactivation molecule. In some aspects, the at least one polynucleotide can be a plasmid comprising a nucleic acid encoding a fusion protein comprising at least a portion of MS2 coat protein (MCP) and at least one VP64 transactivation molecule operably linked to at least one promoter sufficient to drive expression of the fusion protein.

**[00112]** In some aspects, any of the compositions of the present disclosure can further comprise at least one mRNA and/or polynucleotide encoding a fusion protein comprising at least a portion of MS2 coat protein (MCP) and at least one P65-HSF transactivation molecule. In some aspects, the at least one polynucleotide can be a plasmid comprising a nucleic acid encoding a fusion protein comprising at least a portion of MS2 coat protein (MCP) and at least one P65-HSF transactivation molecule operably linked to at least one promoter sufficient to drive expression of the fusion protein.

**[00113]** In some aspects, any of the compositions of the present disclosure can further comprise at least one mRNA and/or polynucleotide encoding a fusion protein comprising at least one antibody that binds to the SunTag peptide and at least one transactivation molecule. In some aspects, the at least one polynucleotide can be a plasmid comprising a nucleic acid encoding a fusion protein comprising at least one antibody that binds to the SunTag peptide and at least one transactivation molecule operably linked to at least one promoter sufficient to drive expression of the fusion protein.

**[00114]** In some aspects, any of the compositions of the present disclosure can further comprise at least one mRNA and/or polynucleotide encoding a fusion protein comprising at least one antibody that binds to the SunTag peptide and at least one P65-HSF transactivation molecule. In some aspects, the at least one polynucleotide can be a plasmid comprising a nucleic acid encoding a fusion protein comprising at least one antibody that binds to the SunTag peptide and at least one P65-HSF transactivation molecule operably linked to at least one promoter sufficient to drive expression of the fusion protein.

[00115] In some aspects, any of the compositions of the present disclosure can further comprise at least one mRNA and/or polynucleotide encoding a fusion protein comprising at least one antibody that binds to the SunTag peptide and at least one VP64 transactivation molecule. In some aspects, the at least one polynucleotide can be a plasmid comprising a nucleic acid encoding a fusion protein comprising at least one antibody that binds to the SunTag peptide and at least one VP64 transactivation molecule operably linked to at least one promoter sufficient to drive expression of the fusion protein.

[00116] In some aspects, any composition of the present disclosure can further comprise at least one mRNA and/or polynucleotide encoding at least one rejuvenating factor. In some aspects, the at least one polynucleotide can be a plasmid comprising a nucleic acid encoding at least one rejuvenating factor operably linked to at least one promoter sufficient to drive expression of the at least one rejuvenating factor. A rejuvenating factor can comprise telomerase RNA component (TERC), telomerase associated reverse-transcriptase (TERT), protection of telomeres 1 (POT1), insulin-like growth factor 1 (IGF1), WD repeat containing antisense to TP53 (WRAP53), nuclear protein family A, member 3 (NOP3), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), shelterin complex subunit and telomerase recruitment factor (ACD/TPP1), TRF-1 interacting ankyrin-related ADP-ribose polymerase (TNKS), telomeric repeat binding factor 1 (TRF-1), telomeric repeat binding factor 2 (TRF-2), TERF1 interacting nuclear factor 2 (TIN2), telomeric repeat binding factor 2 (Rap1), Dyskerin Pseudouridine Synthase 1 (DKC1), ribonucleoprotein NHP2 or any combination thereof.

[00117] The compositions of the present disclosure can be diluted in at least one cell culture medium. In some aspects, the at least one cell culture medium can comprise adjusted Opti-MEM (Opti-MEM with the pH adjusted to 8.2 or Opti-MEM with the pH adjusted to any value in the range between 7.4 and 8.6), non-adjusted Opti-MEM, human serum, fetal bovine serum (FBS), 1x phosphate-buffered saline (PBS) with the pH in the range between of 7.0 and 8.6 or any combination thereof.

[00118] In some aspects, any composition of the present disclosure can be packaged into any cellular delivery system known in the art. Cellular delivery systems can include, but are not limited to, adeno-associated virus (AAV; all serotypes, pseudotypes and hybrids), adenovirus, lentivirus, foamy-virus, herpes simplex virus (HSV) particle, retrovirus particle, alphavirus particle, flavivirus particle, rhabdovirus particle, measles virus particle, Newcastle disease virus

particle, poxvirus particle, picornavirus particle, nanoparticles, exosomes and any combination thereof.

[00119] In some aspects, adeno-associate virus can include, but are not limited to AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV2/1, AAV2/2, AAV2/3, AAV2/4, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV-DJ, AAV-DJ8 or any combination thereof.

[00120] The present disclosure provides at least one viral particle, wherein the at least one viral particle comprises any composition of the present disclosure. In some aspects, an at least one viral particle can be an adeno-associated virus (AAV) particle. In some aspects, the at least one viral particle can be an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV2/1, AAV2/2, AAV2/3, AAV2/4, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV-DJ, or AAV-DJ8 particle. In some aspects, the at least one viral particle can be an adenovirus particle. In some aspects, the at least one viral particle can be a foamy-virus particle. In some aspects, the at least one viral particle can be a lentivirus particle. A retrovirus particle can be MMSV or MSCV particle. A lentivirus particle can be HIV-1 or HIV-2 particle. An alphavirus particle can be SFV, SIN, VEE, or M1 particle. A flavivirus particle can be Kunjin virus, West Nile virus, or Dengue virus particle.

[00121] The present disclosure provides at least one exosome, microvesicle or liposome, wherein the at least one exosome, microvesicle or liposome comprises any composition of the present disclosure.

[00122] The present disclosure provides at least one nanoparticle, wherein the at least one nanoparticle comprises any composition of the present disclosure. In some aspects, a nanoparticle can comprise a liposome, a micelle, a polymer-based nanoparticle, a lipid-polymer based nanoparticle, a metal based nanoparticle, a nanocrystal, a carbon nanotube based nanoparticle or a polymeric micelle. In some aspects, a polymer-based nanoparticle can comprise a multiblock copolymer, a diblock copolymer, a polymeric micelle or a hyperbranched macromolecule. In some aspects, a polymer-based nanoparticle can comprise a multiblock copolymer a diblock copolymer. In some aspects, a polymer-based nanoparticle comprises a poly(lactic-co-glycolic acid) PLGA polymer.

[00123] In some aspects, the present disclosure provides a composition comprising: a) at least one modified mRNA molecule comprising a nucleic acid sequence encoding at least a portion of

human telomerase reverse transcriptase (hTERT); b) at least one modified mRNA molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the at least one DNA targeting polypeptide comprises dCas9 and a VP64-P65-Rta (VPR) molecule; and c) a plurality of guide RNA (gRNA) molecules, wherein at least one gRNA in the plurality is complementary to a nucleic acid sequence located upstream of the endogenous hTERC gene.

**[00124] Kits**

**[00125]** In some aspects, the present disclosure provides a kit comprising any composition of the present disclosure. In some aspects, the present disclosure provides a kit comprising any portion of any composition of the present disclosure. In some aspects, any kit of the present disclosure can be used in any method of the present disclosure.

**[00126]** In a non-limiting example, the present disclosure provides a kit comprising a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

**[00127] Rejuvenation methods of the present disclosure**

**[00128]** The present disclosure provides a method of rejuvenating at least one cell, the method comprising contacting the at least one cell with at least one composition of the present disclosure. The method can further comprise expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated cells.

**[00129]** Thus, the present disclosure provides a method of rejuvenating at least one cell, the method comprising contacting the at least one cell with a composition comprising a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and b) at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

**[00130]** The present disclosure provides a method of treating and/or preventing a disease in a subject comprising: a) contacting at least one cell with at least one composition of the present

disclosure; b) expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated cells; and c) administering the plurality of rejuvenated cells to the subject.

[00131] The present disclosure provides a method of treating and/or preventing a disease in a subject comprising: a) contacting at least one cell with at least one composition of the present disclosure; b) expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated cells; c) culturing the plurality of rejuvenated cells under conditions sufficient to transform the plurality of rejuvenated cells into at least one tissue or organ; and d) administering the at least one tissue or organ to the subject.

[00132] The present disclosure provides a method of producing an *in vitro* tissue or organ comprising: a) contacting at least one cell with a composition of the present disclosure; b) expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated cells; c) culturing the plurality of rejuvenated cells under conditions sufficient to transform the plurality of rejuvenated cells into at least one tissue or organ. The at least one tissue or organ can be used for further *in vitro* testing, including, but not limited to the testing of drugs and/or therapeutic compounds.

[00133] The present disclosure provides a method of producing a plurality of rejuvenated cells comprising: a) contacting at least one cell with at least one composition of the present disclosure; b) expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated cells.

[00134] The present disclosure provides a method of producing a plurality of rejuvenated edited cells comprising: a) contacting a plurality of cells with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with at least one composition of the present disclosure; and d) expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated edited cell.

[00135] The present disclosure provides a method of treating and/or preventing a disease in a subject comprising: a) contacting a plurality of cells with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one

edited cell with at least one composition of the present disclosure; d) expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated edited cells; and e) administering to the subject the plurality of rejuvenated edited cells.

[00136] In some aspects, the present disclosure provides a method of treating epidermolysis bullosa (EB) in a subject comprising: a) contacting a plurality of cells comprising keratinocytes, dermal fibroblasts, mesenchymal stem/stromal cells or any combination thereof with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with at least one composition of the present disclosure; d) expanding the at least one cell contacted with the at least one composition of the present disclosure to produce a plurality of rejuvenated edited cells; and e) administering to the subject the plurality of rejuvenated edited cells.

[00137] In some aspects, the present disclosure provides a method of rejuvenating at least one cell in a subject comprising administering to the subject at least one therapeutically effective amount of at least one composition of the present disclosure. In some aspects, the present disclosure provides a method of rejuvenating at least one cell in a subject comprising administering to the subject at least one therapeutically effective amount of at least one portion of at least one composition of the present disclosure.

[00138] In some aspects, the present disclosure provides a method of rejuvenating at least one subject comprising administering at least one therapeutically effective amount of at least one composition of the present disclosure. In some aspects, the present disclosure provides a method of rejuvenating at least one subject comprising administering at least one therapeutically effective amount of at least one portion of at least one composition of the present disclosure.

[00139] In some aspects of the methods of the present disclosure, contacting at least one cell with at least one composition of the present disclosure can comprise contacting the at least one cell with a first portion of the at least one composition of the present disclosure and then contacting the at least one cell with a second portion of the least one composition of the present disclosure at least about 1 hour, or at least about 2 hours, or at least about 3 hours, or at least about 4 hours, or at least about 5 hours, or at least about 6 hours, or at least about 7 hours, or at least about 8 hours, or at least about 9 hours, or at least about 10 hours, or at least about 11 hours, or at least

about 12 hours, or at least about 16 hours, or at least about 20 hours, or at least about 24 hours, or at least about 28 hours, or at least about 32 hours, or at least about 36 hours, or at least about 40 hours, or at least about 44 hours, or at least about 48 hours, or at least about 52 hours, or at least about 56 hours, or at least about 60 hours, or at least about 64 hours, or at least about 68 hours, or at least about 72 hours, or at least about 76 hours, or at least about 80 hours, or at least about 84 hours, or at least about 88 hours, or at least about 92 hours, or at least about 96 hours after contacting the at least one cell with the first portion of the at least one composition of the present disclosure.

[00140] Thus, contacting at least one cell with at least one composition of the present disclosure can comprise: a) contacting the at least one cell with at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and b) contacting the at least one cell with at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC), at least about 24 hours after step (a). Optionally, steps (a) and step (b) can be repeated about every 1, or about every 2, or about every 3, or about every 4, or about every 5, or about every 6, or about every 7, or about every 8, or about every 9, or about every 10 days.

[00141] In some aspects of the methods of the present disclosure, contacting at least one cell with at least one composition of the present disclosure can further comprise pretreating the at least one cell. In some aspects, pretreating a cell can comprise contacting the at least one cell with at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT) once about every 4 hours, or about every 8 hours, or about every 12 hours, or about every 16 hours, or about every 20 hours, or about every 24 hours, or about every 28 hours, or about every 32 hours, or about every 36 hours, or about every 40 hours, or about every 44 hours, or about every 48 hours. In some aspects, the at least one cell can be pretreated for at least about 2, or at least about 4, or at least about 6, or at least about 8, or at least about 10 days.

[00142] In some aspects, contacting at least one cell with at least one composition of the present disclosure can comprise: a) contacting the at least one cell with at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse

transcriptase (TERT) and at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC); and b) repeating step (a) about every 1, or about every 2, or about every 3, or about every 4, or about every 5, or about every 6, or about every 7, or about every 8, or about every 9, or about every 10 days.

[00143] Exemplary transfection regimes are shown in FIGs. 8, 10 and 11.

[00144] In some aspects of the methods of the present disclosure, contacting at least one cell with a composition of the present disclosure can comprise transfection. In some aspects, transfection can comprise the use of lipofectamine. In some aspects, transfection can comprise any standard transfection method known in the art. In some aspects of the methods of the present disclosure, contacting at least one cell with a composition of the present disclosure can comprise electroporation.

[00145] In some aspects of the methods of the present disclosure, contacting at least one cell can comprise transfection, transduction, electroporation, nucleofection, at least one cell-penetrating peptide or any combination thereof.

[00146] In some aspects of the methods of the present disclosure, contacting at least one cell with a composition of the present disclosure can comprise nucleofection. In some aspects, nucleofection can comprise any standard nucleofection method known in the art.

[00147] In some aspects of the methods of the present disclosure, contacting at least one cell with a composition of the present disclosure can comprise contacting the cell with at least one cell-penetrating peptides. In some aspects, a cell-penetrating peptide can be an HIV-derived TAT protein. In some aspects, a cell-penetrating peptide can comprise polyarginine. Without wishing to be bound by theory, the at least one cell-penetrating peptide can aid in the delivery of a protein or a RNP complex of the present disclosure to the cytoplasm of a target cell.

[00148] In some aspects, at least one composition or at least one portion of at least one composition of the present disclosure can be administered to a subject orally, nasally, transdermally, pulmonary, inhalationally, buccally, sublingually, intraperitoneally, subcutaneously, intramuscularly, intravenously, rectally, intrapleurally, intrathecally and/or parenterally.

[00149] In some aspects of the methods of the present disclosure, expanding at least one cell can comprise culturing the at least one cell using adjusted Opti-MEM, non-adjusted Opti-MEM, human serum, fetal bovine serum (FBS) or any combination thereof.

[00150] In some aspect of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the expression of TERC in the at least one cell. In some aspects, rejuvenating at least one cell can comprise increasing the expression of TERC by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1000%, or at least about 10,000%, or at least about 100,000%, or at least about 1,000,000%, or at least about 10,000,000%, or at least about 100,000,000%. In some aspects, rejuvenating at least one cell can comprise increasing the expression of TERC in the at least one cell such that the expression level of TERC after contacting the at least one cell with at least one composition of the present disclosure is at least about 0.5 times, or at least about 1.0 times, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 1000 times, or at least about 10,000 times, or at least about 20,000 times, or at least about 30,000 times, or at least about 40,000 times, or at least about 50,000 times, or at least about 60,000 times, or at least about 70,000 times, or at least about 80,000 times, or at least about 90,000, or at least about 100,000 times greater as compared to the expression level of

TERC prior to contacting the at least one cell with the at least one composition of the present disclosure.

**[00151]** In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the expression of TERC in the at least one cell such that the expression level of TERC is at least about the same as, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 1000 times, or at least about 10,000 times, or at least about 20,000 times, or at least about 30,000 times, or at least about 40,000 times, or at least about 50,000 times, or at least about 60,000 times, or at least about 70,000 times, or at least about 80,000 times, or at least about 90,000, or at least about 100,000 times the expression of TERC in a control cell.

**[00152]** In some aspect of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the expression of TERT in the at least one cell. In some aspects, rejuvenating at least one cell can comprise increasing the expression of TERT by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1000% or at least about 10,000%, or at least about 100,000%, or at least about 1,000,000%, or at least about 10,000,000%, or at least about 100,000,000%. In some aspects, rejuvenating at least one cell can comprise increasing the expression of TERT in the at least one cell such that the expression level of TERT after contacting the at least one cell with at least one

composition of the present disclosure is at least about 0.5 times, or at least about 1.0 times, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 1000 times, or at least about 10,000 times, or at least about 20,000 times, or at least about 30,000 times, or at least about 40,000 times, or at least about 50,000 times, or at least about 60,000 times, or at least about 70,000 times, or at least about 80,000 times, or at least about 90,000, or at least about 100,000 times greater as compared to the expression level of TERT prior to contacting the at least one cell with the at least one composition of the present disclosure.

[00153] In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the expression of TERT in the at least one cell such that the expression level of TERT is at least about the same as, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 1000 times, or at least about 10,000 times, or at least about 20,000 times, or at least about 30,000 times, or at least about 40,000 times, or at least about 50,000 times, or at least about 60,000 times, or at least about 70,000 times, or at least about 80,000 times, or at least about 90,000, or at least about 100,000 times the expression of TERT in a control cell.

[00154] In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the total number of population doublings exhibited by the at least one cell. In some aspects, rejuvenating at least one cell can comprise increasing the total number of population doublings exhibited by the at least one cell by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at

least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1,000%, or at least about 2,000%, or at least about 3,000%, or at least about 4,000%, or at least about 5,000%, or at least about 6,000%, or at least about 7,000%, or at least about 8,000%, or at least about 9,000%, or at least about 10,000%, or at least about 20,000%, or at least about 30,000%, or at least about 40,000%, or at least about 50,000%, or at least about 60,000%, or at least about 70,000%, or at least about 80,000%, or at least about 90,000%, or at least about 100,000%.

[00155] In some aspects, rejuvenating at least one cell can comprise increasing the total number of population doublings such that the number of population doublings exhibited by the at least one cell is at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 200 times, or at least about 300 times, or at least about 400 times, or at least about 500 times, or at least about 600 times, or at least about 700 times or at least about 800 times, or at least about 900 times, or at least about 1,000 times the total number of population doublings exhibited by at least one control cell.

[00156] In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the length of telomeres in the at least one cell. In some aspects, rejuvenating at least one cell can comprising increasing the length of telomeres in the at least one cell by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about

60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1000%, or at least about 2000%, or at least about 3000%, or at least about 4000%, or at least about 5000%, or at least about 6000%, or at least about 7000%, or at least about 8,000%, or at least about 9,000%, or at least about 10,000%, or at least about 20,000%, or at least about 30,000%, or at least about 40,000%, or at least about 50,000%, or at least about 60,000%, or at least about 70,000%, or at least about 80,000%, or at least about 90,000%, or at least about 100,000%.

[00157] In some aspects, rejuvenating at least one cell can comprise increasing the length of telomeres in the at least one cell such that the length of the telomeres in the at least one cell is the same as, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 200 times, or at least about 300 times, or at least about 400 times, or at least about 500 times, or at least about 600 times, or at least about 700 times, or at least about 800 times, or at least about 900 times or at least about 1,000 times the length of telomeres in at least one control cell.

[00158] In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the mitochondrial DNA copy number in the at least one cell. In some aspects, rejuvenating at least one cell can comprise increasing the mitochondrial DNA copy number in the at least one cell by a at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about

75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1,000%, or at least about 2,000%, or at least about 3,000%, or at least about 4,000%, or at least about 5,000%, or at least about 6,000%, or at least about 7,000%, or at least about 8,000%, or at least about 9,000%, or at least about 10,000%, or at least about 20,000%, or at least about 30,000%, or at least about 40,000%, or at least about 50,000%, or at least about 60,000%, or at least about 70,000%, or at least about 80,000%, or at least about 90,000%, or at least about 100,000%.

[00159] In some aspects, rejuvenating at least one cell can comprise increasing the mitochondrial DNA copy number in the at least one cell such that the mitochondrial DNA copy number is the same as, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 200 times, or at least about 300 times, or at least about 400 times, or at least about 500 times, or at least about 600 times, or at least about 700 times, or at least about 800 times, or at least about 900 times, or at least about 1000 times the mitochondrial DNA copy number in at least one control cell.

[00160] In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the amount of mitochondrial DNA in the at least one cell. In some aspects, rejuvenating at least one cell can comprise increasing the amount of mitochondrial DNA in the at least one cell by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about

100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1,000%, or at least about 2,000%, or at least about 3,000%, or at least about 4,000%, or at least about 5,000%, or at least about 6,000%, or at least about 7,000%, or at least about 8,000%, or at least about 9,000%, or at least about 10,000%, or at least about 20,000%, or at least about 30,000%, or at least about 40,000%, or at least about 50,000%, or at least about 60,000%, or at least about 70,000%, or at least about 80,000%, or at least about 90,000%, or at least about 100,000%.

**[00161]** In some aspects, rejuvenating at least one cell can comprise increasing the amount of mitochondrial DNA in the at least one cell such that the amount of mitochondrial DNA in the at least one cell is the same as, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.0 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 200 times, or at least about 300 times, or at least about 400 times, or at least about 500 times, or at least about 600 times, or at least about 700 times, or at least about 800 times, or at least about 900 times, or at least about 1000 times the amount of mitochondrial DNA in at least one control cell.

**[00162]** In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the number of mitochondria in the at least one cell. In some aspects, rejuvenating at least one cell can comprise increasing the number of mitochondria in the at least one cell by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or

at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1,000%, or at least about 2,000%, or at least about 3,000%, or at least about 4,000%, or at least about 5,000%, or at least about 6,000%, or at least about 7,000%, or at least about 8,000%, or at least about 9,000%, or at least about 10,000%, or at least about 20,000%, or at least about 30,000%, or at least about 40,000%, or at least about 50,000%, or at least about 60,000%, or at least about 70,000%, or at least about 80,000%, or at least about 90,000%, or at least about 100,000%.

[00163] In some aspects, rejuvenating at least one cell can comprise increasing the number of mitochondria in the at least one cell such that the number of mitochondria is the same as, or at least about 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 200 times, or at least about 300 times, or at least about 400 times, or at least about 500 times, or at least about 600 times, or at least about 700 times, or at least about 800 times, or at least about 900 times, or at least about 1,000 times the amount of mitochondria in at least one control cell.

[00164] In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise increasing the migration activity of the at least one cell. In some aspects, rejuvenating at least one cell can comprise increasing the migration activity of the at least one cell by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at

least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1,000%, or at least about 2,000%, or at least about 3,000%, or at least about 4,000%, or at least about 5,000%, or at least about 6,000%, or at least about 7,000%, or at least about 8,000%, or at least about 9,000%, or at least about 10,000%, or at least about 20,000%, or at least about 30,000%, or at least about 40,000%, or at least about 50,000%, or at least about 60,000%, or at least about 70,000%, or at least about 80,000%, or at least about 90,000%, or at least about 100,000%.

[00165] In some aspects, rejuvenating at least one cell can comprise increasing the migration activity of the at least one cell such that the migration activity of the at least one cell is the same as, or at least 1.5 times, or at least about 2.0 times, or at least about 2.5 times, or at least about 3.5 times, or at least about 4.0 times, or at least about 4.5 times, or at least about 5.0 times, or at least about 5.5 times, or at least about 6.0 times, or at least about 6.5 times, or at least about 7.0 times, or at least about 7.5 times, or at least about 8.0 times, or at least about 8.5 times, or at least about 9.0 times, or at least about 9.5 times, or at least about 10.0 times, or at least about 25 times, or at least about 50 times, or at least about 75 times, or at least about 100 times, or at least about 200 times, or at least about 300 times, or at least about 400 times, or at least about 500 times, or at least about 600 times, or at least about 700 times, or at least about 800 times, or at least about 900 times, or at least about 1000 times the migration activity of at least one control cell.

[00166] In some aspects, rejuvenating at least one cell can comprise restoring the young-like state of thiol group oxidation on at least one protein in the at least one cell. In a non-limiting example, rejuvenating can comprise increasing the thiol group oxidation of at least one protein in the at least one cell such that the thiol group oxidation of the at least one protein in the at least one cell is comparable to the thiol group oxidation of the same protein in a young cell. In a non-limiting example, rejuvenating can comprise decreasing the thiol group oxidation of at least one protein in the at least one cell such that the thiol group oxidation of the at least one protein in the at least one cell is comparable to the thiol group oxidation of the same protein in a young cell.

[00167] In some aspects, rejuvenating at least one cell can comprise decreasing the thiol group oxidation of at least one protein in the at least one cell by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about

70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%. In some aspects, the at least one protein can be EIF2S1, TM9F3 or USP14.

[00168] In some aspects, rejuvenating at least one cell can comprise increasing the thiol group oxidation of at least one protein in the at least one cell by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about 55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%, or at least about 150%, or at least about 200%, or at least about 250%, or at least about 300%, or at least about 350%, or at least about 400%, or at least about 450%, or at least about 500%, or at least about 550%, or at least about 600%, or at least about 650%, or at least about 700%, or at least about 750%, or at least about 800%, or at least about 850%, or at least about 900%, or at least about 950%, or at least about 1,000%, or at least about 2,000%, or at least about 3,000%, or at least about 4,000%, or at least about 5,000%, or at least about 6,000%, or at least about 7,000%, or at least about 8,000%, or at least about 9,000%, or at least about 10,000%, or at least about 20,000%, or at least about 30,000%, or at least about 40,000%, or at least about 50,000%, or at least about 60,000%, or at least about 70,000%, or at least about 80,000%, or at least about 90,000%, or at least about 100,000%. In some aspects, the at least one protein can be IGFB5.

[00169] In some aspects of the methods of the present disclosure, rejuvenating at least one cell can comprise reducing senescence-associated DNA methylation in the at least one cell. In some aspects, reducing senescence-associated DNA methylation in the at least one cell can comprise reducing DNA methylation at least one genomic location which is associated with senescence-related methylation. In some aspects, the at least one genomic location can be cg09780241, cg05099537, cg24541426, cg04316624, cg13180312, cg13316854, cg15726154, cg21507095, cg01697719 or any combination thereof.

[00170] In some aspects, rejuvenating at least one cell can comprise reducing DNA methylation at least one genomic location by at least about 1%, or at least about 5%, or at least about 10%, or at least about 15%, or at least about 20%, or at least about 25%, or at least about 30%, or at least about 35%, or at least about 40%, or at least about 45%, or at least about 50%, or at least about

55%, or at least about 60%, or at least about 65%, or at least about 70%, or at least about 75%, or at least about 80%, or at least about 85%, or at least about 90%, or at least about 95%, or at least about 100%.

[00171] In some aspects, an at least one control cell can comprise a cell that has not been contacted with a composition of the present disclosure. In some aspects, an at least one control cell can comprise a cell that has not been contacted with a composition of the present disclosure but has otherwise been grown under the same conditions as at least one cell contacted with a composition of the present disclosure. In some aspects, an at least one control cell can be a dermal fibroblast isolated from a human subject that is 50 years old. In some aspects, an at least one control cell can be a neonatal human epidermal keratinocyte (HEK<sub>n</sub>). In some aspects, an at least one control cell can be an induced pluripotent stem cell (iPSC).

[00172] In some aspects, editing at least one cell can comprise the correction of at least one gene in the at least one cell, the knockout of at least one gene in the at least one cell, the insertion of at least one DNA sequence into the genome of the at least one cell, the deletion of at least one DNA sequence in the genome of the at least one cell or any combination thereof. In some aspects, a gene editing system can comprise any system known in the art for modifying the genome of a target cell, including, but not limited to CRISPR methods, viral methods, etc..

[00173] An at least one cell can be obtained and/or isolated from a subject. In some aspects, an at least one cell can be any somatic cell. In some aspects, an at least one cell can be a fibroblast, a keratinocyte, a mesenchymal stem/stromal cell, a peripheral blood mononuclear cell, a chimeric antigen receptor T cell (CAR-T cell), an endothelial cell, a chondrocyte, a muscle stem cell, a neural stem cell, a hepatocyte, a limbal stem cell, a retinal pigmented epithelial cell, a hematopoietic stem cell, a macrophage, a cardiomyocyte, a pancreatic cell, a  $\beta$ -cell or any combination thereof.

[00174] In some aspects, an at least one cell can be an Exocrine secretory epithelial cell, a Brunner's gland cell, an insulated goblet cell of the respiratory and digestive tracts, a stomach foveolar cell, a chief cell, a parietal cell, a pancreatic acinar cell, a paneth cell of small intestine, a Type II pneumocyte of lung, a club cell of lung, a barrier cell, a type I pneumocyte, a gall bladder epithelial cell, a centroacinar cell, an intercalated duct cell, an intestinal brush border cell, a hormone-secreting cell, an enteroendocrine cell, a K cell, an L cell, an I cell, a G cell, an Enterochromaffin cell, an Enterochromaffin-like cell, an N cell, an S cell, a D cell, a Mo cell (or

M cell), a Thyroid gland cell, a Thyroid epithelial cell, a Parafollicular cell, a Parathyroid gland cell, a Parathyroid chief cell, an Oxyphil cell, a Pancreatic islets (islets of Langerhans), an Alpha cell, a Beta cell, a Delta cell, an Epsilon cell, a PP cell (gamma cell), an Exocrine secretory epithelial cell, a Salivary gland mucous cell, a Salivary gland serous cell, a Von Ebner's gland cell, a Mammary gland cell, a Lacrimal gland cell, a Ceruminous gland cell, an Eccrine sweat gland dark cell, a Eccrine sweat gland clear cell, an Apocrine sweat gland cell, a Gland of Moll cell, a Sebaceous gland cell, a Bowman's gland cell, an Anterior/Intermediate pituitary cell, a Corticotrope, a Gonadotrope, a Lactotrope, a Melanotrope, a Somatotrope, a thyrotrope, a magnocellular neurosecretory cell, a Parvocellular neurosecretory cell, a Chromaffin cell, an Epithelial cell, a Keratinocyte, an Epidermal basal cell, a Melanocyte, a Trichocyte, a hair shaft cell, a Cortical hair shaft cell, a Cuticular hair shaft cell, a Huxley's layer hair root sheath cell, a Henle's layer hair root sheath cell, an Outer root sheath hair cell, a Surface epithelial cell, a basal cell (stem cell), an Intercalated duct cell, a Striated duct cell, a Lactiferous duct cell, an Ameloblast, an Oral cell, an Odontoblast, a Cementoblast, a neuron, an Auditory inner hair cell, an auditory outer hair cell, a Basal cell of olfactory epithelium cell, a Cold-sensitive primary sensory neuron, a Heat-sensitive primary sensory neuron, a Merkel cell, a Olfactory receptor neuron, a Pain-sensitive primary sensory neuron, a Photoreceptor cell, a Photoreceptor rod cell, a Photoreceptor blue-sensitive cone cell, a Photoreceptor green-sensitive cone cell, a Photoreceptor red-sensitive cone cell, a Proprioceptive primary sensory neuron, a Touch-sensitive primary sensory neuron, a Chemoreceptor glomus cell, an Outer hair cell, an Inner hair cell, a Taste receptor cell, an autonomic neuron, a Cholinergic neuron, a Adrenergic neural cell, a Peptidergic neural cell, an Inner pillar cell, an Outer pillar cell, an Inner phalangeal cell, an Outer phalangeal cell, a Border cell, a Hensen's cell, a Vestibular apparatus supporting cell, a Taste bud supporting cell, a Olfactory epithelium supporting cell, a Schwann cell, a Satellite glial cell , a Enteric glial cell, a glial cell, an interneuron, , a Basket cell, a Cartwheel cell, a Stellate cell, a Golgi cell, a Granule cell, a Lugaro cell, a Unipolar brush cell, a Martinotti cell, a Chandelier cell, a Cajal–Retzius cell, a Double-bouquet cell, a Neurogliaform cell, a Retina horizontal cell, an Amacrine cell, a Spinal interneuron, a Renshaw cell, a principal cell, a Spindle neuron, a Fork neuron, a Pyramidal cell , a Place cell, a Grid cell, a Speed cell, a Head direction cell, a Betz cell, a Stellate cell , a Boundary cell, a Bushy cell, a Purkinje cell, a Medium spiny neuron, a Astrocyte (various types), a Oligodendrocyte, a Ependymal cell , a Tanycytes, a Pituicyte, a Lens cell, an Anterior

lens epithelial cell, a Crystallin-containing lens fiber cell, a Adipocytes: White fat cell, a Brown fat cell, a Liver lipocyte, a Theca interna cell, a Corpus luteum cell, a Granulosa lutein cell, a Theca lutein cell, a Leydig cell of testes secreting testosterone, a Seminal vesicle cell, a Prostate gland cell, a Bulbourethral gland cell, a Bartholin's gland cell, a Gland of Littre cell, a Uterus endometrium cell, a Juxtaglomerular cell, a Macula densa cell of kidney, a Peripolar cell of kidney, a Mesangial cell of kidney, a barrier cell, a Parietal epithelial cell, a Podocyte, a Proximal tubule brush border cell, a Loop of Henle thin segment cell, a Kidney distal tubule cell, a Kidney collecting duct cell Principal cell, a Intercalated cell, a Transitional epithelium, a Duct cell, a Efferent ducts cell, a Epididymal principal cell, a Epididymal basal cell, a Endothelial cell, a Planum semilunatum epithelial cell, a interdental epithelial cell, a Corneal fibroblasts, a Tendon fibroblasts, a Bone marrow reticular tissue fibroblasts, an Other nonepithelial fibroblasts, a Pericyte Hepatic stellate cell (Ito cell), a Nucleus pulposus cell of intervertebral disc, a Hyaline cartilage chondrocyte, a Fibrocartilage chondrocyte, an Elastic cartilage chondrocyte, a Osteoblast/osteocyte, a Osteoprogenitor cell, a Hyalocyte of vitreous body of eye, a Stellate cell of perilymphatic space of ear, a Pancreatic stellate cell, a Skeletal muscle cell, a Red skeletal muscle cell, a White skeletal muscle cell, an Intermediate skeletal muscle cell, a Nuclear bag cell of muscle spindle, a Nuclear chain cell of muscle spindle, a Myosatellite cell, a Cardiac muscle cell, a Cardiac muscle cell, a SA node cell, a Purkinje fiber cell, a Smooth muscle cell, a Myoepithelial cell, a Erythrocyte, a Megakaryocyte, a Platelets, a Monocyte, a Connective tissue macrophage, a Epidermal Langerhans cell, a Osteoclast, a Dendritic cell, a Microglial cell, a Neutrophil granulocyte, a myeloblast, a promyelocyte, a myelocyte, a metamyelocyte, a Eosinophil granulocyte, a Basophil granulocyte, a Mast cell, a Helper T cell, a Suppressor T cell, a Cytotoxic T cell, a Natural killer T cell, a B cell, a Plasma cell, a Natural killer cell, a Hematopoietic stem cell, a Germ cell, a Oogonium/Oocyte, a Spermatid, a Spermatoocyte, a Spermatoogonium cell, a Spermatozoon, a Nurse cell, a Granulosa cell, a Sertoli cell, a Epithelial reticular cell, a Interstitial cell, a Interstitial kidney cell or any combination thereof. [00175] In some aspects, a disease can comprise inflammatory disorder, an autoimmune disease, a degenerative disease, cardiovascular disease, ischemic disease, cancer, a genetic disease, a metabolic disorder, idiopathic disorder or any combination thereof. In some aspects, a disease can comprise any medical disorder, including, but not limited to those medical disorders initiated by direct tissue injury (e.g., burns, trauma, decubitus ulcers, etc.), ischemic/vascular events (e.g.,

myocardial infarct, stroke, shock, hemorrhage, coagulopathy, etc.), infections (e.g., cellulitis, pneumonia, meningitis, SIRS, etc.), neoplasia (e.g., breast cancer, lung cancer, lymphoma, etc.), immunologic/autoimmune conditions (e.g., graft vs. host disease, multiple sclerosis, diabetes, inflammatory bowel disease, lupus erythematosus, rheumatoid arthritis, psoriasis, etc.), degenerative diseases (e.g., osteoporosis, osteoarthritis, Alzheimer's disease, etc.), congenital/genetic diseases (e.g., epidermolysis bullosa, osteogenesis imperfecta, muscular dystrophies, lysosomal storage diseases, Huntington's disease, etc.), adverse drug effects (e.g., drug-induced hepatitis, drug-induced cardiac injury, etc.), toxic injuries (e.g., radiation exposure(s), chemical exposure(s), alcoholic hepatitis, alcoholic pancreatitis, alcoholic cardiomyopathy, cocaine cardiomyopathy, etc.), metabolic derangements (e.g., uremic pericarditis, metabolic acidosis, etc.), iatrogenic conditions (e.g., radiation-induced tissue injury, surgery-related complications, etc.), and/or idiopathic processes (e.g., amyotrophic lateral sclerosis, Parsonnage-Turner Syndrome, etc.) or any combination thereof. In some aspects, a disease can comprise graft-vs-host diseases (GvHD), Epidermolysis Bullosa (EB), junctional EB (JEB), EB simplex (EBS), congenital ichthyosis, congenital dyskeratosis, Recessive Dystrophic form of EB (RDEB), macular degeneration, Alzheimer's disease, aging, Type II diabetes, heart disease, osteoporosis, chronic skin wounds, diabetes-associated ulcers/wounds, connective tissue diseases such as Ehlers-Danlos Syndrome (EDS) or Marfan syndrome, cancer, or any combination thereof. In some aspects, a disease can also comprise an injury. An injury can comprise a burn, a broken bone, a concussion, a contusion, a fractured bone, a ruptured tendon, a torn ligament, punctured, scaped and/or cut skin, or any other injury known in the art. In some aspects, a disease can be Ehlers-Danlos Syndrome.

[00176] As used herein, the term "treating" or "treat" describes the management and care of a patient for the purpose of combating a disease, condition, or disorder and includes the administration of a compound of the present disclosure, or a pharmaceutically acceptable salt, polymorph or solvate thereof, to alleviate the symptoms or complications of a disease, condition or disorder, or to eliminate the disease, condition or disorder. The term "treat" can also include treatment of a cell *in vitro* or an animal model.

[00177] As used herein, the term "preventing," "prevent," or "protecting against" describes reducing or eliminating the onset of the symptoms or complications of such disease, condition or disorder.

[00178] As used herein, the terms "ameliorate", "ameliorating" and grammatical variations thereof mean to decrease the severity of the symptoms of a disease in a subject.

[00179] The terms "effective amount" and "therapeutically effective amount" of an agent or compound are used in the broadest sense to refer to a nontoxic but sufficient amount of an active agent or compound to provide the desired effect or benefit.

[00180] The term "benefit" is used in the broadest sense and refers to any desirable effect and specifically includes clinical benefit as defined herein. Clinical benefit can be measured by assessing various endpoints, e.g., inhibition, to some extent, of disease progression, including slowing down and complete arrest; reduction in the number of disease episodes and/or symptoms; reduction in lesion size; inhibition (i.e., reduction, slowing down or complete stopping) of disease cell infiltration into adjacent peripheral organs and/or tissues; inhibition (i.e. reduction, slowing down or complete stopping) of disease spread; decrease of auto-immune response, which may, but does not have to, result in the regression or ablation of the disease lesion; relief, to some extent, of one or more symptoms associated with the disorder; increase in the length of disease-free presentation following treatment, e.g., progression-free survival; increased overall survival; higher response rate; and/or decreased mortality at a given point of time following treatment.

[00181] In any method, composition or kit of the present disclosure, TERT can be human TERT (hTERT).

[00182] In any method, composition or kit of the present disclosure, TERC can be human TERC (hTERC).

[00183] As used herein, a "subject" includes a mammal. The mammal can be any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a cow, a horse, a goat, a camel, a sheep, a pig or any other mammal. In some aspects, a mammal can be a human. The subject can be a male or a female.

[00184] Examples

[00185] Example 1 – Levels of hTERC transcripts are higher in induced pluripotent stem cells as compared to fibroblasts

[00186] The levels of human telomerase RNA component (hTERC) transcripts in fibroblasts (FBs), induced pluripotent stem cells (iPSCs) were measured using the NanoString nCounter Gene Expression Assay. An approximately 2.4 – 4.6-fold upregulation of hTERC in iPSCs as compared

to the parental F50 (human dermal fibroblast derived from a 50 year old individual) and FN2 (neonatal fibroblasts) lines, as shown in FIG. 4.

**[00187] Example 2 – contacting somatic cells with compositions of the present disclosure increases the level of hTERC in the somatic cells**

**[00188]** In this example, various cell lines were transfected with various compositions of the present disclosure.

**[00189]** Human dermal fibroblasts derived from a 50 year old individual (F50), neonatal human epidermal keratinocytes (HEKn) and GFP-expressing human mesenchymal stem/stromal cells (hMSC-GFP) were subjected to one transfection with 500 ng modified mRNA (mod-mRNA) encoding dCas9-VPR and 500 ng hTERC guide RNA (gRNA) (either independently or as a mix of 4 guides at a 1:1:1:1 ratio) using Lipofectamine<sup>®</sup> RNAiMAX<sup>™</sup> (RNAiMAX) (Thermo Fisher Scientific). Cells were collected 24 post-transfection. Real time quantitative PCR reactions for human TERC RNA expression were then carried out using the Bio-Rad CFX Connect System. Data was analyzed using the  $\Delta\Delta C_t$  method.

**[00190]** To test the activation of the endogenous hTERC transcript, F50 cells were transfected with either 1 separate individual gRNA (g1, g2, g3, and g4) plus mod-mRNA encoding dCas9-VPR or a mix of all 4 guides at a 1:1:1:1 ratio (gmix) plus mod-mRNA encoding dCas9-VPR. Levels of hTERC transcript were quantified using quantitative reverse transcription PCR. As shown in FIG. 5, the results indicate that even as little as only 1 single guide is sufficient to activate endogenous hTERC (g2, g4) to levels comparable to those achieved by the mix of 4 gRNAs. Transfection with the mix of 4 gRNAs and mod-mRNA encoding dCas9-VPR displayed the greatest increase in expression of hTERC, as shown in FIG. 5.

**[00191]** To monitor endogenous hTERC activation by dCas9-VPR across other cell lines, HEKn and hMSC-GFP cell lines were transfected with a mix of all 4 gRNAs plus mod-mRNA encoding dCas9-VPR. The expression of hTERC was then measured using quantitative reverse transcription PCR. The results are shown in FIG. 6. As shown in FIG. 6, the level of hTERC activation observed in all tested cells lines was comparable to that observed in iPSCs.

**[00192] *Example 2 Methods***

**[00193] *Cell lines:*** 50 year-old human dermal fibroblast (F50, at passage 5), and neonatal human epidermal keratinocytes (HEKn, at passage 7) lines were obtained from ATCC. Human mesenchymal stem/stromal cells with GFP fluorescence (hMSC-GFP, at passage 15) were

obtained from Cyagen. The F50 line was cultured in fibroblast expansion medium (FEM) comprised of DMEM/F12 supplemented with 5% human serum, 1× MEM non-essential amino acids solution, 55 μM of 2-mercaptoethanol (β-ME), 1× GlutaMAX™ supplement, plus antibiotics (all from Thermo Fisher Scientific), with 50 ug/ml ascorbic acid, 1ng/ml hydrocortisone (both from Sigma), 12 ng/ml basic FGF (Gibco) and 5 ng/ml human EGF (Invitrogen). HEK<sub>n</sub> cells were cultured in EpiLife medium supplemented with EDGS and antibiotics (all from ThermoFisher). hMSCs were cultured in mesenchymal stem cell growth medium (MSCGM) (prepared as a kit from Cyagen).

**[00194] Transfections:** All transfections of fibroblasts and keratinocytes were performed using Opti-MEM®<sup>I</sup> Reduced Serum Medium (Opti-MEM) (Thermo Fisher Scientific) as a complexation buffer, while transfections of human mesenchymal stem/stromal cells (hMSCs) was performed using Opti-MEM with the pH adjusted to 8.2 (Opti-MEM-pH 8.2) as described in *Kogut et al. Nature Communications, 2018*. One transfection with 500 ng mod-mRNA encoding dCas9-VPR and 500 ng hTERC guide RNA (gRNA) (either individual (g1, g2, g3, g4) or a mix of 4 guides (gmix) at a 1:1:1:1 ratio) or 500 ng modified mRNA encoding dCas9-VPR alone was performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in either Opti-MEM for keratinocyte and fibroblast transfections, or Opti-MEM-pH 8.2 for hMSC transfections. For mod-mRNA and/or gRNA transfections, 100 ng/μl RNA was diluted 5×, and 5 μl of RNAiMAX per microgram of mod-mRNAs and/or gRNA was diluted 10× using either Opti-MEM (for keratinocytes and fibroblasts) or Opti-MEM-pH 8.2 (for hMSCs). After dilution, these components were combined together and incubated for 15 min at room temperature (RT). After incubation at RT, transfection mixtures of mod-RNA mix and/or gRNA and RNAiMAX were applied to the cell cultures, in their respective media supplemented with 200 ng/ml B18R (eBioscience).

**[00195] PCR:** F50, HEK<sub>n</sub>, and hMSC-GFP cells collected 24 hours post-transfection. RNA was extracted using the RNeasy Plus Minikit (Qiagen). cDNA was synthesized using the iScript™ cDNA Synthesis Kit (BioRad). Quantitative PCR (QPCR) reactions for human TERC RNA were performed using SsoAdvanced™ Universal SYBR® Green Supermix. Data was analyzed using the ΔΔCt method.

**[00196] Summary of Example 2:** transfecting somatic cells with compositions of the present disclosure, more specifically a mod-mRNA encoding dCas9-VPR in combination with a plurality

of gRNAs comprising 1 or more different gRNA species, can increase the expression of hTERC in the transfected cells, including to levels that are comparable to induced pluripotent stem cells.

**[00197] Example 3 – transfecting mod-RNA encoding dCas9-VPR alone does not induce hTERC expression in target cells**

**[00198]** In this example, various cell lines were transfected with various compositions of the present disclosure, specifically a composition comprising only a mod-RNA encoding dCas9-VPR.

**[00199]** 50 year-old human dermal fibroblasts (F50), and GFP-expressing human mesenchymal stem/stromal cells (hMSC-GFP) were subjected to one transfection with 500 ng mod-mRNA encoding dCas9-VPR using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). Cells were collected 24 hours (F50, hMSC-GFP) post-transfection. Quantitative reverse transcription PCR reactions for human TERC RNA expression were carried out using the Bio-Rad CFX Connect System and data was analyzed using the  $\Delta\Delta C_t$  method. The results are shown in FIG. 7. As shown in FIG. 7, the expression levels of hTERC does not increase when only a mod-RNA encoding dCas9-VPR is transfected into a target cell in the absence of any guide RNA.

**[00200] *Example 3 methods:***

**[00201] *Cell lines:*** 50 year-old human dermal fibroblast (F50, at passage 5) were obtained from ATCC. Human mesenchymal stem/stromal cells with GFP fluorescence (hMSC-GFP, at passage p11) were obtained from Cyagen. F50 line was cultured in fibroblast expansion medium (FEM) comprised of DMEM/F12 supplemented with 5% human serum, 1× MEM non-essential amino acids solution, 55  $\mu$ M of 2-mercaptoethanol ( $\beta$ -ME), 1× GlutaMAX™ supplement, plus antibiotics (all from Thermo Fisher Scientific), with 50  $\mu$ g/ml ascorbic acid, 1ng/ml hydrocortisone (both from Sigma), 12 ng/ml basic FGF (Gibco) and 5 ng/ml human EGF (Invitrogen). hMSCs were cultured in mesenchymal stem cell growth medium (MSCGM) (prepared as a kit from Cyagen).

**[00202] *Transfections:*** All transfections of fibroblasts were performed using Opti-MEM as a complexation buffer, while transfections of human mesenchymal stem/stromal cells (hMSCs) was performed Opti-MEM-pH 8.2 One transfection with 500 ng mod-mRNA encoding dCas9-VPR alone was performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in appropriate Opti-MEM. For mod-mRNA transfections, 100 ng/ $\mu$ l RNA was diluted 5×, and 5  $\mu$ l of RNAiMAX per microgram of mod-mRNAs was diluted 10× using appropriate Opti-MEM. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures

of mod-RNA mix and RNAiMAX were applied to the cell cultures, in their respective media supplemented with 200 ng/ml B18R (eBioscience).

[00203] *PCR*: F50 and hMSC-GFP cells were collected 24 hours post-transfection. RNA was extracted using the RNeasy Plus Minikit (Qiagen). cDNA was synthesized using the iScript™ cDNA Synthesis Kit (BioRad). QPCR reactions for human TERC RNA were performed using SsoAdvanced™ Universal SYBR® Green Supermix. Data was analyzed using the  $\Delta\Delta C_t$  method. *P* values were calculated using a paired, two-tailed Student's *t*-test. \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .

[00204] *Example 3 summary*: Increases in hTERC expression using the methods and the compositions of the present disclosure are dependent on specific targeting of a DNA-targeting molecule comprising a transactivation domain, for example through the co-administration of at least one guide RNA.

[00205] Example 4 – the methods and compositions of the present disclosure cause an increase in population doubling (PD) of senescent fibroblast cells

[00206] In this example, senescent fibroblast cells were contacted with compositions of the present disclosure using methods of the present disclosure.

[00207] 50 year-old human dermal fibroblast (F50) line was obtained from ATCC, and subsequently cultured until 90% of cells displayed the senescent phenotype as previously described in *Kogut et al, Nature Communications, 2018*. Briefly, the senescent phenotype can include an enlargement of cellular morphology and upwards of about 90% positivity for senescence-associated  $\beta$ -galactosidase. The F50 line was thawed (F50S, at passage 15, 32.5 PD) and cultured in FEM: DMEM/F12 supplemented with 5% human serum, 1× MEM non-essential amino acids solution, 55  $\mu$ M of 2-mercaptoethanol ( $\beta$ -ME), 1× GlutaMAX™ supplement, plus antibiotics (all from Thermo Fisher Scientific), with 50 ug/ml ascorbic acid, 1ng/ml hydrocortisone (both from Sigma), 12 ng/ml basic FGF (Gibco) and 5 ng/ml human EGF (Invitrogen). Initially, 10k fibroblasts (F50S p15, 32.5 PD) were seeded, per well.

[00208] FIG. 8 shows a schematic of the transfection regimen of fibroblasts using rejuvenating compositions of the present disclosure. Initially, 10k senescent fibroblasts (F50S, p15, 32.5 PD) were seeded, per well. The cells were first pre-treated with three sequential transfections with 500 ng mod-mRNA encoding hTERT. After pretreatment, four sequential transfection series with 500 ng mod-mRNA encoding hTERT followed by 500 ng mod-mRNA encoding dCas9-VPR and 500

ng human TERC guide RNA (gRNA) (4 guides, 1:1:1:1 ratio) the next day were performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM®I Reduced Serum Medium (Opti-MEM). For mod-mRNA and/or gRNA transfections, 100 ng/μl RNA was diluted 5×, and 5 μl of RNAiMAX per microgram of mod-mRNAs and/or gRNA were diluted 10× using Opti-MEM. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and/or gRNA and RNAiMAX were applied to the cell culture, in FEM supplemented with 200 ng/ml B18R (eBioscience). The medium was changed after overnight incubation after each transfection.

[00209] The cumulative population doubling of non-treated senescent fibroblasts (F50S, p15, 32.5 starting PD) and the same cells treated with the rejuvenating composition (using the regimen put forth in FIG. 8) was measured. When cells reached 70-80% confluence, they were trypsinized, counted using a hemocytometer, and passaged. PD was calculated as the log of the ratio of the final count (N) to the starting (baseline) count ( $X_0$ ) divided by the log of 2; that is:  $PD = [\log(N \div X_0)] \div \log 2$ . *P* values were calculated using a paired, two-tailed Student's *t*-test. \**P* ≤ 0.05, \*\**P* ≤ 0.01, \*\*\**P* ≤ 0.001. The results are shown in FIG. 9. As shown in FIG. 9, the treatment with the rejuvenating composition of the present disclosure results in the increased population doubling.

[00210] *Example 4 summary:* The compositions and methods of the present disclosure can be used to rejuvenate senescent cells, including senescent fibroblasts, leading to an increase in the total number of population doublings exhibited by the treated cells.

[00211] Example 5 – the methods and compositions of the present disclosure increase telomere length and mitochondrial DNA amount in transfected target cells

[00212] In this example, various cell lines (low passage and senescent 50 year-old human dermal fibroblasts (F50 and F50S respectively), human mesenchymal stem/stromal cells (hMSCs) and human keratinocytes) were transfected with various compositions of the present disclosure using various methods of the present disclosure. Changes in telomere length in each cell line were then measured.

[00213] *Low passage and senescent 50 year-old human dermal fibroblasts:* 50 year-old human dermal fibroblast (F50) lines were obtained from ATCC, and subsequently cultured until 90% of cells displayed the senescent phenotype as previously described in *Kogut et al, Nature Communications, 2018*. Briefly, the senescent phenotype can include an enlargement of cellular

morphology and upwards of about 90% positivity for senescence-associated  $\beta$ -galactosidase. The F50 lined was thawed (F50S, at passage 15, 32.5 PD) and cultured in FEM: DMEM/F12 supplemented with 5% human serum, 1 $\times$  MEM non-essential amino acids solution, 55  $\mu$ M of 2-mercaptoethanol ( $\beta$ -ME), 1 $\times$  GlutaMAX<sup>TM</sup> supplement, plus antibiotics (all from Thermo Fisher Scientific), with 50  $\mu$ g/ml ascorbic acid, 1 ng/ml hydrocortisone (both from Sigma), 12 ng/ml basic FGF (Gibco) and 5 ng/ml human EGF (Invitrogen). Initially, 10k fibroblasts (F50 p3-4 or F50S p15, 32.5 PD) were seeded, per well.

[00214] FIG. 8 shows a schematic of a transfection regimen of fibroblasts using a rejuvenating composition of the present disclosure. Initially, 10k senescent fibroblasts (F50S, p15, 32.5 PD) were seeded, per well. The cells were first pre-treated with three sequential transfections with 500 ng mod-mRNA encoding hTERT. After pretreatment, four sequential transfection series with 500 ng mod-mRNA encoding hTERT followed by 500 ng mod-mRNA encoding dCas9-VPR and 500 ng human TERC guide RNA (gRNA) (4 guides, 1:1:1:1 ratio) the next day were performed using Lipofectamine<sup>®</sup> RNAiMAX<sup>TM</sup> (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM<sup>®</sup>I Reduced Serum Medium (Opti-MEM). For mod-mRNA and/or gRNA transfections, 100 ng/ $\mu$ l RNA was diluted 5 $\times$ , and 5  $\mu$ l of RNAiMAX per microgram of mod-mRNAs and/or gRNA were diluted 10 $\times$  using Opti-MEM. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and/or gRNA and RNAiMAX were applied to the cell culture, in FEM supplemented with 200 ng/ml B18R (eBioscience). The medium was changed after overnight incubation after each transfection.

[00215] FIG. 10 shows a schematic of an alternative transfection regimen of fibroblasts using a rejuvenating composition of the present disclosure. F50 fibroblasts were plated in FEM at 15K cells per well of a 6-well format dish. Four transfection series with 500 ng mod-mRNA encoding hTERT together with 200 ng mod-mRNA encoding dCas9-VPR and 500 ng hTERC guide RNA (gRNA) (1 selected guide) were performed every 4 days using Lipofectamine<sup>®</sup> RNAiMAX<sup>TM</sup> (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM<sup>®</sup>I Reduced Serum Medium (Opti-MEM). For mod-mRNA and/or gRNA transfections, 100 ng/ $\mu$ l RNA was diluted 5 $\times$ , and 5  $\mu$ l of RNAiMAX per microgram of mod-mRNAs and/or gRNA were diluted 10 $\times$  using Opti-MEM. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and/or

gRNA and RNAiMAX were applied to the cell culture, in FEM supplemented with 200 ng/ml B18R (eBioscience). The medium was changed after overnight incubation after each transfection.

[00216] *Human mesenchymal stem/stromal cells:* Human mesenchymal stem/stromal cells (hMSCs) were cultured in mesenchymal stem cell growth medium (MSCGM) (prepared as a kit from Cyagen) under low O<sub>2</sub> (5%). All transfections of hMSCs were performed using Opti-MEM with the pH adjusted to 8.2 (Opti-MEM-pH 8.2) as described in *Kogut et al. Nature Communications, 2018*.

[00217] FIG. 11 shows a schematic of the transfection regimen of hMSCs using rejuvenating compositions of the present disclosure. A pre-treatment of 3 transfections with 500 ng mod-mRNA encoding human TERT was performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). Following pre-treatment with hTERT mod-mRNA transfections, four sequential transfection series with 500 ng mod-mRNA encoding hTERT followed by 500 ng mod-mRNA encoding dCas9-VPR and 500 ng human TERC guide RNA (gRNA) (4 guides, 1:1:1:1 ratio) the next day were performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM-pH 8.2. For mod-mRNA transfections, 100 ng/μl RNA was diluted 5×, and 5 μl of RNAiMAX per microgram of mod-mRNAs was diluted 10× using Opti-MEM-pH 8.2. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and RNAiMAX were applied to the cell culture, in respective media supplemented with 200 ng/ml B18R. The medium was changed after overnight incubation after each transfection.

[00218] *Human keratinocytes:* Human neonatal epidermal keratinocytes (HEKn) were cultured in EpiLife medium supplemented with EDGS and antibiotics (all from ThermoFisher). All transfections of HEKs were performed using Opti-MEM with no pH adjustment.

[00219] FIG. 11 shows a schematic of the transfection regimen of HEKn using rejuvenating compositions of the present disclosure. A pre-treatment of 3 transfections with 100 ng mod-mRNA encoding human human TERT was performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM®I Reduced Serum Medium (Opti-MEM) (Thermo Fisher Scientific). For mod-mRNA transfections, 100 ng/μl RNA was diluted 5×, and 5 μl of RNAiMAX per microgram of mod-mRNAs was diluted 10× using Opti-MEM. After dilution, these components were combined together and incubated for

15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and RNAiMAX were applied to the cell culture, in EpiLife medium supplemented with 200 ng/ml B18R (eBioscience). Following pre-treatment of the HEK<sub>n</sub> cell line, 4 sequential transfection series with 100 ng mod-mRNA encoding hTERT followed by 100 ng mod-mRNA encoding dCas9-VPR + 100 ng gRNA mix the next day were performed using Lipofectamine<sup>®</sup> RNAiMAX<sup>™</sup> (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM (Thermo Fisher Scientific). For mod-mRNA and/or gRNA transfections, 100 ng/ $\mu$ l RNA was diluted 5 $\times$ , and 5  $\mu$ l of RNAiMAX per microgram of mod-mRNAs and/or gRNA were diluted 10 $\times$  using Opti-MEM. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and/or gRNA and RNAiMAX were applied to the cell culture, in EpiLife supplemented with 200 ng/ml B18R (eBioscience). The medium was changed after overnight incubation after each transfection.

[00220] The F50S cells were collected 3 days after the last transfection. Genomic DNA (gDNA) was extracted using the DNeasy<sup>®</sup> Blood and Tissue Kit (Qiagen). Quantitative PCR reactions for relative telomere length in treated and untreated cells were performed using SsoAdvanced<sup>™</sup> Universal SYBR<sup>®</sup> Green Supermix. The results are shown in FIG. 12.

[00221] The F50 cells were collected 3 days after the last transfection. Genomic DNA (gDNA) was extracted using the Quick-DNA<sup>™</sup> Miniprep Kit (Zymo Research). Quantitative PCR was used to determine changes in average telomere length in treated and untreated cells based on ScienCell's Absolute Human Telomere Length Quantification and Mitochondrial DNA Copy Number qPCR Assay Kit (#8958). The telomere primer set recognizes and amplifies telomere length by comparing samples to reference genomic DNA containing a 100 base pair (bp) telomere sequence located on human chromosome 17 (provided by kit). Primer-probe real-time PCR was performed using BioRad's CFX96 Real-Time System (BioRad, Hercules, CA). The results are shown in FIG. 13.

[00222] The hMSCs and HEK<sub>n</sub> cells were collected 3 days after the last transfection. Genomic DNA (gDNA) was extracted using the DNeasy<sup>®</sup> Blood and Tissue Kit (Qiagen). Quantitative PCR was used to determine changes in average telomere length in treated and untreated cells based on ScienCell's Absolute Human Telomere Length Quantification and Mitochondrial DNA Copy Number qPCR Assay Kit (#8958). The telomere primer set recognizes and amplifies telomere length by comparing samples to reference genomic DNA containing a 100 base pair (bp) telomere

sequence located on human chromosome 17 (provided by kit). Primer-probe real-time PCR was performed using BioRad's CFX96 Real-Time System (BioRad, Hercules, CA). The results are shown in FIG. 14.

[00223] As shown in FIG. 12, FIG. 13 and FIG. 14, F50S, F50, hMSCs and HEK<sub>n</sub> cells treated with the rejuvenating compositions of the present disclosure displayed increased telomere length as compared to untreated control cells. Moreover, in the case of the treated F50S and HEK<sub>n</sub> cells, the telomere lengths exceeded the telomere lengths measured in F50-derived induced pluripotent stem cells (F50-iPSCs).

[00224] Quantitative PCR was used to determine changes in mitochondrial DNA copy number using ScienCell's Absolute Human Telomere Length Quantification and Mitochondrial DNA Copy Number Dual Quantification qPCR Assay Kit (#8958). The mtDNA primer set recognizes and amplifies one of the most conserved regions on human mtDNA and will not amplify any off-target sequence on nuclear genomic DNA. The single copy reference (SCR) primer set recognizes and amplifies a 100 bp-long region on human chromosome 17 and serves as reference for data normalization. Primer-probe real-time PCR was performed using BioRad's CFX96 Real-Time System (BioRad, Hercules, CA). The results are shown in FIG. 15. As shown in FIG. 15, F50S, hMSCs and HEK<sub>n</sub> cells treated with the rejuvenating compositions of the present disclosure displayed increased mitochondrial DNA copy number as compared to untreated cells.

[00225] *Summary of Example 5:* the compositions and methods of the present disclosure can be used to rejuvenate various cell types, including low passage and senescent fibroblasts, human mesenchymal stem/stromal cells and human epidermal keratinocytes, leading to an increase in telomere length and mitochondrial DNA amount in treated cells.

[00226] Example 6 – the methods and compositions of the present disclosure reactivate telomerase activity in fibroblasts

[00227] In this example, 50 year-old human fibroblasts (F50) were transfected with various compositions of the present disclosure. The telomerase activity in the transfected target cells, as well as control cells, was analyzed.

[00228] Fifty year-old human dermal fibroblast (F50 passage 6) were cultured in fibroblast expansion medium (FEM) comprised of DMEM/F12 supplemented with 5% human serum, 1 × MEM non-essential amino acids solution, 55 μM of 2-mercaptoethanol (β-ME), 1 × GlutaMAX™ supplement, plus antibiotics (all from Thermo Fisher Scientific), with 50 ug/ml ascorbic acid,

1 ng/ml hydrocortisone (both from Sigma), 12 ng/ml basic FGF (Gibco) and 5 ng/ml human EGF (Invitrogen). As untreated control lines, the F50-iPSC line was cultured in mTeSR™1 Media supplemented with 1x mTeSR™1 supplement (StemCell Technologies) plus antibiotics (Thermo Fisher Scientific) on plates coated with Matrigel coating matrix (Corning).

[00229] Two sequential transfections with either 3 ug mod-mRNA encoding hTERT or 3 ug mod-mRNA encoding hTERT with 3 ug mod-mRNA encoding dCas9-VPR + 500 ng gRNA mix were performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM®1 Reduced Serum Medium (Opti-MEM) (Thermo Fisher Scientific). For mod-mRNA transfections, 100 ng/μl RNA was diluted 5×, and 5 μl of RNAiMAX per microgram of mod-mRNAs was diluted 10× using Opti-MEM. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA and/or gRNA and RNAiMAX were applied to the cell culture, in FEM supplemented with 200 ng/ml B18R (eBioscience). The medium was changed after overnight incubation after each transfection.

[00230] Telomerase activity was measured with the TRAPeze® Telomerase Detection Kit (Millipore) according to the manufacturer's instructions. CHAPS (1×) lysis buffer was used to obtain extracts from, positive control cells (kit provided), an iPSC line derived from F50 (F50-iPSC), fibroblasts (F50), and fibroblasts (F50) treated with two sequential transfections of 3 ug hTERT only or 3ug hTERT with 3 ug dCas9-VPR + 3 ug gRNA mix. About 10,000 cells were assayed for each telomeric repeat amplification protocol assay, and 1,500 cell equivalents were loaded into each well of a 15% non-denaturing TBE (Tris borate, EDTA)- Urea polyacrylamide gel. Each sample was heat inactivated for 10 min at 85 °C to assess the background of the assay.

[00231] The results of the telomerase activity assay are shown in FIG. 16. Brighter products are indicative of higher activity. As show in in FIG. 16, the combined treatment with mod-mRNA encoding hTERT, mod-mRNA encoding dCas9-VPR and hTERC-specific gRNAs resulted in a higher level of telomerase activity as compared to untreated iPSCs or F50 cells transfected with only mod-mRNA encoding hTERT treatment alone.

[00232] *Example 6 summary:* The compositions and methods of the present disclosure can reactivate and increase telomerase activity in target cells, thereby rejuvenating the target cells.

[00233] Example 7—the compositions of the present disclosure facilitate single cell expansion

[00234] In this example, primary human adult fibroblasts were transfected with compositions of the present disclosure to determine if the methods and compositions of the present disclosure could support the expansion from a single cell.

[00235] Primary human adult fibroblasts were obtained from a skin biopsy. Adult fibroblasts were cultured in fibroblast expansion medium (FEM) comprised of DMEM/F12 supplemented with 5% human serum, 1× MEM non-essential amino acids solution, 55 μM of 2-mercaptoethanol (β-ME), 1× GlutaMAX™ supplement, plus antibiotics (all from Thermo Fisher Scientific), with 50 ug/ml ascorbic acid, 1ng/ml hydrocortisone (both from Sigma), 12 ng/ml basic FGF (Gibco) and 5 ng/ml human EGF (Invitrogen). Individual patient-derived fibroblasts were plated and single cells selected using a (10 × 10mm) PYREX® cloning cylinder.

[00236] FIG. 11 shows a schematic of the transfection regimen of the select, single fibroblasts using rejuvenating compositions of the present disclosure, with adjustments made for the reduced tissue culture surface area. The cells were first pre-treated with three sequential transfections with 50 ng mod-mRNA encoding hTERT. After pretreatment, four sequential transfection series with 50 ng mod-mRNA encoding hTERT followed by 50 ng mod-mRNA encoding dCas9-VPR and 50 ng hTERT guide RNA (gRNA) (4 guides, 1:1:1:1 ratio) the next day were performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM®I Reduced Serum Medium (Opti-MEM). For mod-mRNA and/or gRNA transfections, 100 ng/μl RNA was diluted 5×, and 5 μl of RNAiMAX per microgram of mod-mRNAs and/or gRNA were diluted 10× using Opti-MEM. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and/or gRNA and RNAiMAX were applied to the cell culture, in FEM supplemented with 200 ng/ml B18R (eBioscience). The medium was changed after overnight incubation after each transfection.

[00237] Following the transfection regimen, each well was trypsinized and cells were transferred into one well of a 6-well tissue culture plate for further expansion in FEM. As shown in FIG. 17, one week after the last transfection, 2/10 wells of untreated cells expanded successfully and were able to be collected for gDNA extraction while 9/10 wells of treated fibroblasts expanded and collected.

[00238] *Summary of Example 7:* The compositions and methods of the present disclosure can facilitate the expansion of even single cells.

**[00239] Example 8—the compositions and methods of the present disclosure increase the migration activity of high passage human mesenchymal stem/stromal cells (hMSCs)**

[00240] In this example, human mesenchymal stem/stromal cells (hMSCs) were transfected with compositions of the present disclosure to determine if the compositions and methods of the present disclosure can increase the migration activity of high passage hMSCs.

[00241] To measure migration activity of treated and untreated hMSCs, a Transendothelial Migration (TEM) assay was used. A schematic of the TEM assay is shown in FIG. 18. Briefly, Corning FluoroBlok cell culture inserts were pre-seeded with human endothelial cells (HUVEC). GFP+ hMSCs are plated, and their migration through the HUVEC layer and pores of the FluoroBlok membrane was quantified over time via bottom-reading fluorescence microscopes such as the CellInsight CX7 High-Content Screening (HCS) Platform.

[00242] 24-well Corning FluoroBlok™ Inserts were coated with collagen. After coating, human umbilical vein endothelial cells (HUVECs) were plated at 80K/cm<sup>2</sup> in ECM-2MV BulletKit™ media (Lonza) and allowed to attach in 5% CO<sub>2</sub> incubation overnight. Following overnight incubation and successful attachment, media in the basal chamber was changed to Human Mesenchymal Stem Cell Growth Medium (Cyagen) supplemented with human recombinant EGF [10 ng/mL] (Stemcell Technologies). Human Mesenchymal Stem/Stromal Cells labeled with Green Fluorescent Protein (GFP) purchased from Cyagen were cultured for twelve passages (P12) and treated with the rejuvenating compositions of the present disclosure as described in FIG. 11 and Example 5, while control hMSCs were cultured without mRNA treatment. The rejuvenation procedure lasted for two passages bringing the passage number to P14. A portion of the rejuvenated hMSCs were frozen in a CoolCell LX™ overnight at -80° while the remaining cells were allowed to remain in culture. After two passages, the frozen cells were thawed and allowed to culture for further two passages.

[00243] The four conditions were as follows; Old high passage hMSCs (P20) were never rejuvenated or frozen; Young low passage hMSCs (P5) were a fresh vial of GFP labeled hMSCs (Cyagen) that was thawed allowed to attached overnight then lifted and run on the Transendothelial Migration Assay, frozen rejuvenated hMSCs (P17) that were frozen at P15 then thawed and allowed to culture for two passages and rejuvenated hMSCs that were never frozen but underwent five passages following the rejuvenation protocol. These four conditions were then added to the apical chamber of the FluoroBlok on top of the layer of attached HUVECs. Four fields of view

from three replicates were obtained on ThermoScientific's CellInsight CX7 LED High-Content Screening (HCS) Platform. The CX7 HCS is designed to use brightfield, widefield and confocal microscopy for the entire fluorescence spectrum to rapidly capture and quantify high content data such as the kinetic analysis performed on Transendothelial Migration Assays (TEM). As shown in FIG. 19, the rejuvenated high passage hMSCs reached the saturation point significantly faster than untreated young hMSCs (40-50 ordinal time vs 130 ordinal time), while old high passage hMSCs (P20) showed poor migratory ability. A one-way ANOVA analysis exhibited significance of  $p < 0.0001$  between young and both groups rejuvenated hMSCs as compared to high passage hMSCs.

[00244] *Example 8 summary:* The compositions and methods of the present disclosure can rejuvenate hMSCs as evidenced by the increase in the migration activity of high passage hMSCs treated using the compositions and methods of the present disclosure.

[00245] Example 9—the compositions and methods of the present disclosure restore the level of thiol group oxidation of proteins in high passage senescent human mesenchymal stem/stromal cells (hMSCs) to that observed in young low passage hMSCs.

[00246] In this example, senescent high passage human mesenchymal stem/stromal cells (hMSCs) were transfected with compositions of the present disclosure to determine if the compositions and methods of the present disclosure can restore the level of thiol group oxidation of proteins in high passage senescent human mesenchymal stem/stromal cells (hMSCs) to that observed in young low passage hMSCs. Among amino acids, the sulphur-containing cysteine (Cys) is particularly prone to oxidation. This is due to the presence of the thiol moiety (-SH) in the side chain of Cys, which can easily form disulfide bonds with a different thiol moiety in response to oxidation. Reversible oxidation of Cys thiols regulate the activity of enzymes and ligand binding, as well as participate in redox signaling, which deregulation plays an essential role in the development of many human disease and aging.

[00247] Human mesenchymal stem/stromal cells (hMSCs) were cultured in mesenchymal stem cell growth medium (MSCGM) (prepared as a kit from Cyagen) under low O<sub>2</sub> (5%). The three conditions were as follows: Senescent high passage hMSCs (P14) were never rejuvenated; Young low passage hMSCs (P5) were a fresh vial of hMSCs that were thawed allowed to attached overnight then lifted and processed for the peptide analysis; Senescent high passage hMSCs treated at passage 12 with the rejuvenating compositions of the present disclosure. The rejuvenation

procedure lasted for two passages bringing the passage number of treated senescent hMSCs to P14 before the peptide analysis was performed, matching untreated senescent hMSCs.

[00248] For hMSCs, a pre-treatment of 3 transfections with 500 ng mod-mRNA encoding human TERT was performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). Following pre-treatment with hTERT mod-mRNA transfections, three sequential transfection series with 500 ng mod-mRNA encoding hTERT followed by 500 ng mod-mRNA encoding dCas9-VPR and 500 ng hTERC guide RNA (gRNA) (1 selected guide) the next day were performed using Lipofectamine® RNAiMAX™ (RNAiMAX) (Thermo Fisher Scientific). RNA and RNAiMAX were first diluted in Opti-MEM-pH 8.2. For mod-mRNA transfections, 100 ng/μl RNA was diluted 5×, and 5 μl of RNAiMAX per microgram of mod-mRNAs was diluted 10× using Opti-MEM-pH 8.2. After dilution, these components were combined together and incubated for 15 min at RT. After incubation at RT, transfection mixtures of mod-RNA mix and RNAiMAX were applied to the cell culture, in respective media supplemented with 200 ng/ml B18R. The medium was changed after overnight incubation after each transfection.

[00249] Transfected senescent and un-transfected senescent and low passage young hMSCs were processed using iodoTMTsixplex Isobaric Mass Tag Labeling Kit (ThermoScientific). Resulted iodoTMT labeled peptide mix was analyzed by QExactive HF Orbitrap mass spectrometer with an Easy nLC 1000 UPLC system (Thermo Fischer Scientific). Peptide identifications were performed using MaxQuant program. Each MS/MS spectrum was analyzed against a human specific database (Uniprot). After this analysis, data files were exported and additionally analyzed with Perseus software for data of interest. Each experiment was repeated twice. MaxQuant and Perseus software were downloaded from Max Planck Institute of Biochemistry website.

[00250] The level of thiol group oxidation in senescent high passage hMSCs increased in 88 proteins and decreased in 31 proteins as compared to young hMSCs. The transfection of senescent hMSCs with rejuvenating compositions of the present disclosure resulted in the restoration of thiol group oxidation levels in approximately 90% of target proteins to the level observed in young cells. FIG. 20 shows representative results of the thiol group analysis in proteins whose thiol group oxidation levels increased (EIF2S1, TM9F3 and USP14) and decreased (IGFB5) in senescent high passage hMSCs and the reversion of these thiol group oxidation levels to the young-like state in response to the treatment with the rejuvenating composition.

[00251] *Example 9 summary:* The compositions and methods of the present disclosure can rejuvenate hMSCs as evidenced by the restoration of the young-like level of protein thiol group oxidation in high passage hMSCs treated using the compositions and methods of the present disclosure.

[00252] Example 10—the compositions and methods of the present disclosure reduce senescence-associated DNA methylation in high passage senescent human mesenchymal stem/stromal cells (hMSCs) and human neonatal epidermal keratinocytes (HEKn)

[00253] In this example, senescent high passage human mesenchymal stem/stromal cells (hMSCs) and senescent high passage human neonatal epidermal keratinocytes (HEKn) were transfected with compositions of the present disclosure to determine if the compositions and methods of the present disclosure can reduce the level of senescence-associated DNA methylation in these cells. Changes in DNA methylation have been recognized as one of the most common molecular alterations in aging and cellular senescence.

[00254] Human fibroblasts of different origin were cultured in FEM; human keratinocytes of different origin were cultured in EpiLife medium supplemented with EDGS and hMSCs were cultured in mesenchymal stem cell growth medium (MSCGM). The following cell types used for DNA methylation analysis were not treated with rejuvenating compositions: young low passage neonatal fibroblasts (P3), young low passage adult F50 fibroblasts (P3), young low passage neonatal keratinocytes HEKn (P3), young low passage fetal keratinocyte (P2), young low passage adult keratinocytes (P3), young umbilical cord-derived hMSCs (P2), senescent high passage F50S fibroblasts (P15), senescent high passage hMSCs (P13) and senescent high passage HEKn (P10). The treated group included senescent high passage HEKn and senescent high passage hMSCs treated with rejuvenating compositions as described in FIG. 11 and Example 5. After completing the treatment with compositions, the treated cells were expanded for additional 6 days. Genomic DNA (gDNA) was extracted from each cell culture using the Quick-DNA™ Miniprep Kit (Zymo Research) and subjected to DNA methylation analysis using the Illumina Infinium MethylationEPIC BeadChip Kit.

[00255] The DNA methylation data generated for cells of different types were analyzed using the R package “IlluminaHumanMethylationEPICanno.ilm10b2.hg19” and combined into three groups as follows: young cells, senescent (high passage) cells and senescent cells treated with rejuvenating compositions of the present disclosure. The young group included young low passage neonatal

fibroblasts (P3), young low passage adult fibroblasts (P3), young low passage neonatal keratinocytes (P3), young low passage fetal keratinocyte (P2), young low passage adult keratinocytes (P3) and young umbilical cord-derived hMSCs (P2). The senescent group included senescent high passage F50S fibroblasts (P15), senescent high passage hMSCs (P13) and senescent high passage HEK293T (P10). The treated group included senescent high passage HEK293T and senescent high passage hMSCs treated with rejuvenating compositions. Cells of different type and origin were combined based on their senescence state to eliminate cell type-specific methylation differences among groups. The groups were compared using the 2-tailed t-test for two groups with unequal variance, and the degree of methylation was calculated as a fraction of methylated nucleotides at a site of interest, ranging from 0 to 1. Differential methylation sites were selected based on the largest difference in the degree of methylation for each group, and 9 DNA methylation sites were identified as the most methylated in senescent high passage cells irrespectively of the cell type of origin.

[00256] FIG. 21 depicts the location of 9 identified senescence-associated DNA marks and their associated genomic loci. All 9 sites showed an increase in DNA methylation levels in the senescence high passage group. The treatment of the cells from the senescent group with rejuvenating compositions of the present disclosure reduced the level of DNA methylation at all 9 sites to the level similar to that of the young cell group.

[00257] *Example 10 summary:* the compositions and methods of the present disclosure can be used to rejuvenate various cell types, including senescent human mesenchymal stem/stromal cells and human epidermal keratinocytes, leading to a reduction in senescence-association DNA methylation in treated cells.

What is claimed is:

1. A composition comprising:

- a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and
- b) at least one second polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

2. A composition comprising:

- a) at least one first polynucleotide molecule comprising a nucleic acid sequence encoding at least a portion of telomerase reverse transcriptase (TERT); and
- b) at least one DNA targeting polypeptide, wherein the DNA targeting polypeptide increases transcription of telomerase RNA component (TERC).

3. The composition of claim 1 or claim 2, wherein the at least one first polynucleotide molecule comprises an mRNA molecule encoding at least a portion of TERT.

4. The composition of claim 1 or claim 2, wherein the at least one first polynucleotide molecule comprises a plasmid comprising a nucleic acid sequence encoding at least a portion of TERT operably linked to at least one promoter sufficient to drive expression of the at least one portion of TERT.

5. The composition of claim 1, wherein the at least one second polynucleotide molecule comprises an mRNA molecule encoding at least a portion of at least one DNA targeting polypeptide.

6. The composition of claim 1, wherein the at least one second polynucleotide molecule comprises a plasmid comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide operably linked to at least one promoter sufficient to drive expression of the at least one portion of the at least one DNA targeting polypeptide.

7. The composition of any of claims 1-6, wherein the DNA targeting polypeptide comprises at least one Cas9 molecule, at least one Cas9 variant molecule, at least one Cas9 ortholog molecule or any combination thereof.
8. The composition of claim 7, wherein the Cas9 molecule, the Cas9 variant molecule or the Cas9 ortholog molecule is nuclease-deficient or nuclease-dead.
9. The composition of claim 8, wherein the Cas9 variant molecule comprises eSpCas9 (K855A), eSpCas9 (1.0), eSpCas9 (1.1), SpCas9-HF1 (VP12), HypaCas9, xCas9, SpyFi Cas9, iSpy Cas9, iSpyMac, Cas9 (VQR), Cas9 (EQR), Cas9 (VRER), Cas9 (D1135E), Cas9(QQR1), SaCas9 (KKH), Nme1Cas9, Nme2Cas9, Nme3Cas9 or any combination thereof.
10. The composition of claim 8, wherein the Cas9 ortholog molecule comprises *Streptococcus pyogenes* Cas9 (spCas9), *Francisella novicida* Cas9 (FnCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Neisseria meningitidis* Cas9 (NmCas9; NmeCas9), *Streptococcus thermophilus* CRISPR1-Cas9 (St1Cas9), *Streptococcus thermophilus* CRISPR3-Cas9 (St3Cas9), *Campylobacter jejuni* Cas9 (CjCas9), *Acidaminococcus* sp. BV3L6 Cpf1 (AsCpf1), *Lachnospiraceae* bacterium ND2006 Cpf1 (LbCpf1), *Streptococcus canis* Cas9 (ScCas9), *Treponema denticola* Cas9 (TdCas9), *Streptococcus macacae* Cas9 (SmacCas9), Cas $\phi$  (Cas12j), *Francisella tularensis* subsp. *novicida* Cas9, *Pasteurella multocida* Cas9, *Campylobacter lari* CF89-12 Cas9, *Mycoplasma gallisepticum* str. F Cas9, *Nitratifactor salsuginis* str DSM 16511 Cas9, *Parvibaculum lavamentivorans* Cas9, *Roseburia intestinalis* Cas9, *Neisseria cinerea* Cas9, *Gluconacetobacter diazotrophicus* Cas9, *Azospirillum* B510 Cas9, *Sphaerochaeta globus* str. Buddy Cas9, *Flavobacterium columnare* Cas9, *Fluviicola taffensis* Cas9, *Bacteroides coprophilus* Cas9, *Mycoplasma mobile* Cas9, *Lactobacillus farciminis* Cas9, *Streptococcus pasteurianus* Cas9, *Lactobacillus johnsonii* Cas9, *Staphylococcus pseudintermedius* Cas9, *Filifactor alocis* Cas9, *Legionella pneumophila* str. Paris Cas9, *Sutterella wadsworthensis* Cas9, *Corynebacter diphtheriae* Cas9 or any combination thereof.

11. The composition of claim 8, wherein the Cas9 ortholog molecule comprises a chimeric variant of *Streptococcus pyogenes* Cas9 (spCas9), *Francisella novicida* Cas9 (FnCas9), *Staphylococcus aureus* Cas9 (SaCas9), *Neisseria meningitidis* Cas9 (NmCas9; NmeCas9), *Streptococcus thermophilus* CRISPR1-Cas9 (St1Cas9), *Streptococcus thermophilus* CRISPR3-Cas9 (St3Cas9), *Campylobacter jejuni* Cas9 (CjCas9), *Acidaminococcus* sp. BV3L6 Cpf1 (AsCpf1), *Lachnospiraceae* bacterium ND2006 Cpf1 (LbCpf1), *Streptococcus canis* Cas9 (ScCas9), *Treponema denticola* Cas9 (TdCas9), *Streptococcus macacae* Cas9 (SmacCas9), Cas $\phi$  (Cas12j), *Francisella tularensis* subsp. *novicida* Cas9, *Pasteurella multocida* Cas9, *Campylobacter lari* CF89-12 Cas9, *Mycoplasma gallisepticum* str. F Cas9, *Nitratifactor salsuginis* str DSM 16511 Cas9, *Parvibaculum lavamentivorans* Cas9, *Roseburia intestinalis* Cas9, *Neisseria cinerea* Cas9, *Gluconacetobacter diazotrophicus* Cas9, *Azospirillum* B510 Cas9, *Sphaerochaeta globus* str. Buddy Cas9, *Flavobacterium columnare* Cas9, *Fluviicola taffensis* Cas9, *Bacteroides coprophilus* Cas9, *Mycoplasma mobile* Cas9, *Lactobacillus farciminis* Cas9, *Streptococcus pasteurianus* Cas9, *Lactobacillus johnsonii* Cas9, *Staphylococcus pseudintermedius* Cas9, *Filifactor alocis* Cas9, *Legionella pneumophila* str. Paris Cas9, *Sutterella wadsworthensis* Cas9, *Corynebacter diphtheriae* Cas9 or any combination thereof.
12. The composition of any of claims 1-11, wherein the DNA targeting polypeptide comprises at least one TALE molecule, at least one zinc-finger molecule, at least one meganuclease molecule or any combination thereof
13. The composition of any of claims 1-12, wherein the DNA targeting polypeptide comprises at least one transactivation molecule.
14. The composition of claim 13, wherein the at least one transactivation molecule comprises at least one P65 molecule, at least one Rta molecule, at least one VP16 molecule, at least one VP64 molecule, at least one VP160 molecule, at least one VP64-P65-Rta (VPR) molecule, at least one SunTag peptide, at least one single guide RNA-MS2 (sgRNA-MS2) molecule or any combination thereof.
15. The composition of any of claims 1-14, wherein the DNA targeting polypeptide comprises a DNA targeting ribonucleoprotein (RNP) complex.

16. The composition of any of claims 1-15, wherein the DNA targeting polypeptide comprises at least one guide RNA.
17. The composition of claim 13, wherein the transactivation molecule comprises at least one single guide RNA-MS2 (sgRNA-MS2) molecule.
18. The composition of claim 17, wherein the at least one sgRNA-MS2 molecule comprises a nucleic acid sequence complementary to a nucleic acid sequence located upstream, within, or downstream of the endogenous TERC gene and at least about one, or at least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten MS2 RNA aptamers.
19. The composition any of claims 1-18, wherein the DNA targeting polypeptide comprises a dCas9 molecule and a VPR molecule.
20. The composition any of claims 1-19, wherein the DNA targeting polypeptide binds upstream of, 5' to, within, downstream of or 3' to the endogenous TERC gene.
21. The composition any of claims 1-20, wherein the mRNA molecule is a modified mRNA molecule.
22. The composition of claim 21, wherein the modified mRNA molecule comprises at least one modified ribonucleoside base.
23. The composition of claim 22, wherein the modified ribonucleoside base comprises a pseudouridine ( $\Psi$ ) residue, a 5-methylcytidine ( $m^5C$ ) residue or any combination thereof.
24. The composition of claim 22, wherein the modified mRNA molecule comprises at least one modified nucleoside.

25. The composition of claim 24, wherein the modified nucleoside comprise 5-methylcytidine (m<sup>5</sup>C), 5-methyluridine (m<sup>5</sup>U), N6-methyladenosine (m<sup>6</sup>A), inosine 2'-O-methylated nucleosides or any combination thereof.
26. The composition of any of claims 1-25, further comprising a plurality of guide RNA (gRNA) molecules, wherein at least one gRNA in the plurality is complementary to a nucleic acid sequence located upstream, within, or downstream of the endogenous TERC gene.
27. The composition of claim 26, wherein the plurality of gRNA molecules comprises at least about one, or at least about two, or at least about three, or at least about four, or at least about five, or at least about six, or at least about seven, or at least about eight, or at least about nine, or at least about ten distinct species of gRNA molecules, wherein each species has a different nucleic acid.
28. The composition of any of claims 1-27, further comprising at least one plasmid comprising at least one nucleic acid sequence encoding at least one species of gRNA operably linked to at least one promoter sufficient to drive expression of the at least one species gRNA.
29. The composition any of claims 26-28, wherein the plurality of gRNA molecules comprises a plurality of single guide RNA (sgRNA) molecules, crRNA:tracrRNA molecules, truncated sgRNA molecules, high fidelity scaffold gRNA molecules or any combination thereof.
30. The composition of any of claims 26-29, wherein at least one guide RNA molecule is a modified guide RNA (mod gRNA) molecule.
31. The composition of any of claims 26-30, wherein at least one guide RNA molecule comprises any sequence recited in Table 1 or Table 2.
32. A composition comprising:
- a) at least one modified mRNA molecule comprising a nucleic acid sequence encoding at least a portion of human telomerase reverse transcriptase (hTERT);

b) at least one modified mRNA molecule comprising a nucleic acid sequence encoding at least a portion of at least one DNA targeting polypeptide, wherein the at least one DNA targeting polypeptide comprises dCas9 and a VP64-P65-Rta (VPR) molecule; and

c) a plurality of guide RNA (gRNA) molecules, wherein at least one gRNA in the plurality is complementary to a nucleic acid sequence located upstream of the endogenous hTERC gene.

33. The composition of any of claims 1-32, further comprising at least one mRNA and/or polynucleotide encoding at least one rejuvenating factor.

34. The composition of claim 33, wherein the rejuvenating factor comprises telomerase RNA component (TERC), telomerase associated reverse-transcriptase (TERT), protection of telomeres 1 (POT1), insulin-like growth factor 1 (IGF1), WD repeat containing antisense to TP53 (WRAP53), nuclear protein family A, member 3 (NOP3), heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), shelterin complex subunit and telomerase recruitment factor (ACD/TPP1), TRF-1 interacting ankyrin-related ADP-ribose polymerase (TNKS), telomeric repeat binding factor 1 (TRF-1), telomeric repeat binding factor 2 (TRF-2), TERF1 interacting nuclear factor 2 (TIN2), telomeric repeat binding factor 2 (Rap1), Dyskerin Pseudouridine Synthase 1 (DKC1), ribonucleoprotein NHP2 or any combination thereof.

35. A composition of any of claims 1-34, wherein TERT is human TERT (hTERT).

36. A composition of any of claims 1-35, wherein TERC is human TERC (hTERC).

37. A composition comprising at least one viral particle comprising the composition of any of claims 1-36.

38. The composition of claim 37, wherein the at least one viral particle is an adeno-associated virus (AAV) particle, adenovirus particle, lentivirus particle, foamy-virus particle, herpes simplex virus (HSV) particle, retrovirus particle, alphavirus particle, flavivirus particle,

rhabdovirus particle, measles virus particle, Newcastle disease virus particle, poxvirus particle, picornavirus particle, or any combination thereof.

39. The composition of claim 38, wherein the at least one AAV particle is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV2/1, AAV2/2, AAV2/3, AAV2/4, AAV2/5, AAV2/6, AAV2/7, AAV2/8, AAV2/9, AAV-DJ or AAV-DJ8 particle.

40. The composition of claim 38, wherein the at least one retrovirus particle is an MMSV particle or an MSCV particle.

41. The composition of claim 38, wherein the at least one lentivirus particle is an HIV-1 particle or an HIV-2 particle.

42. The composition of claim 38, wherein the at least one alphavirus particle is an SFV particle, an SIN particle, a VEE particle, or an M1 particle.

43. The composition of claim 38, wherein the at least one flavivirus particle is a Kunjin virus particle, a West Nile virus particle, or a Dengue virus particle.

44. A composition comprising at least one exosome, microvesicle or liposome, wherein the at least one exosome, microvesicle or liposome comprises the composition of any of claims 1-34.

45. A composition comprising at least one nanoparticle, wherein the at least one nanoparticle comprises the composition of any of claims 1-36.

46. The composition of claim 45, wherein the nanoparticle comprises a liposome, a micelle, a polymer-based nanoparticle, a lipid-polymer based nanoparticle, a metal based nanoparticle, a nanocrystal, a carbon nanotube based nanoparticle or a polymeric micelle.

47. A kit comprising the composition of any of claims 1-45.

48. A method of rejuvenating at least one cell, the method comprising contacting the at least one cell with the composition or kit of any of claims 1-47.
49. The method of claim 48, further comprising expanding the at least one cell contacted with the composition or kit of any of claims 1-47 to produce a plurality of rejuvenated cells.
50. A method of treating and/or preventing a disease in a subject comprising: a) contacting at least one cell with the composition or kit of any of claims 1-47; b) expanding the at least one cell contacted with the composition or kit of any of claims 1-47 to produce a plurality of rejuvenated cells; and c) administering the plurality of rejuvenated cells to the subject.
51. A method of treating and/or preventing a disease in a subject comprising: a) contacting at least one cell with the composition or kit of any of claims 1-47; b) expanding the at least one cell contacted with the composition or kit of any of claims 1-47 to produce a plurality of rejuvenated cells; c) culturing the plurality of rejuvenated cells under conditions sufficient to transform the plurality of rejuvenated cells into at least one tissue or organ; and d) administering the at least one tissue or organ to the subject.
52. A method of producing an *in vitro* tissue or organ comprising: a) contacting at least one cell with the composition or kit of any of claims 1-47; b) expanding the at least one cell contacted with the composition or kit of any of claims 1-47 to produce a plurality of rejuvenated cells; c) culturing the plurality of rejuvenated cells under conditions sufficient to transform the plurality of rejuvenated cells into at least one tissue or organ.
53. A method of producing a plurality of rejuvenated edited cells comprising: a) contacting a plurality of cells with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with the composition or kit of any of claims 1-47; and d) expanding the at least one cell contacted with the composition or kit of any of claims 1-47 to produce a plurality of rejuvenated edited cell.

54. A method of treating and/or preventing a disease in a subject comprising: a) contacting a plurality of cells with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with the composition or kit of any of claims 1-47; d) expanding the at least one cell contacted with the composition or kit of any of claims 1-47 to produce a plurality of rejuvenated edited cells; and e) administering to the subject the plurality of rejuvenated edited cells.

55. A method of treating epidermolysis bullosa (EB) in a subject comprising: a) contacting a plurality of cells comprising keratinocytes, dermal fibroblasts, mesenchymal/stromal stem cells or any combination thereof with a gene editing system such that at least one gene in the genome of at least one cell in the plurality is edited, thereby producing at least one edited cell; b) isolating the at least one edited cell; c) contacting the isolated at least one edited cell with the composition or kit of any of claims 1-47; d) expanding the at least one cell contacted with the composition or kit of any of claims 1-47 to produce a plurality of rejuvenated edited cells; and e) administering to the subject the plurality of rejuvenated edited cells.

56. The method of any of claims 48-55, wherein expanding the at least one cell comprises culturing the at least one cell using adjusted Opti-MEM, non-adjusted Opti-MEM, human serum, fetal bovine serum (FBS) or any combination thereof.

57. The method of any of claim 48-56, wherein rejuvenating at least one cell comprises increasing the expression of TERC in the at least one cell, increasing the expression of TERT in the at least one cell, increasing the total number of population doublings exhibited by the at least one cell, increasing the length of telomeres in the at least one cell, increasing the mitochondrial DNA copy number in the at least one cell, increasing the amount of mitochondrial DNA in the at least one cell, increasing the number of mitochondria in the at least one cell, increasing the migration activity of the at least one cell, restoring the young-like state of thiol group oxidation levels in proteins in the at least one cell, reducing senescence-associated DNA methylation in the at least one cell or any combination thereof.

58. The method of any of claim 48-57, wherein the at least one cell is a fibroblast, a keratinocyte, a mesenchymal stem/stromal cell, a peripheral blood mononuclear cell, a chimeric antigen receptor T cell (CAR-T cell), an endothelial cell, a chondrocyte, a muscle stem cell, a neural stem cell, a hepatocyte, a limbal stem cell, a retinal pigmented epithelial cell, a hematopoietic stem cell, a macrophage, a cardiomyocyte, a pancreatic cell, a  $\beta$ -cell or any combination thereof

59. The method of any of claim 48-58, wherein the disease comprises graft-vs-host diseases (GvHD), autoimmune diseases, epidermolysis bullosa (EB), recessive dystrophic form of EB (RDEB), junctional EB (JEB), EB simplex (EBS), congenital ichthyosis, congenital dyskeratosis, macular degeneration, Parkinson's disease, Alzheimer's disease, aging, Type I and II diabetes, burns, chronic skin wounds, diabetes-associated ulcers/wounds, heart disease, osteoporosis, cancer, connective tissue diseases such as Ehlers-Danlos Syndrome (EDS) or Marfan syndrome, liver diseases, lung diseases, and any combination thereof.

60. The method of any of claims 48-59, wherein contacting at least one cell comprises transfection, transduction, electroporation, nucleofection, at least one cell-penetrating peptide or any combination thereof.

61. A method for rejuvenating at least one cell in a subject comprising administering to the subject at least one therapeutically effective amount of the composition of any of claims 1-46.

62. A method for rejuvenating at least one subject comprising administering to the subject at least one therapeutically effective amount of the composition of any of claims 1-46.

63. The method of any of claims 48-62, wherein the subject is a mammal.

64. The method of claim 63, wherein the mammal is a human, a primate, a mouse, a rat, a dog, a cat, a cow, a horse, a goat, a camel, a sheep, a pig or any other mammal.

65. The method of claim 64, wherein the mammal is a human.

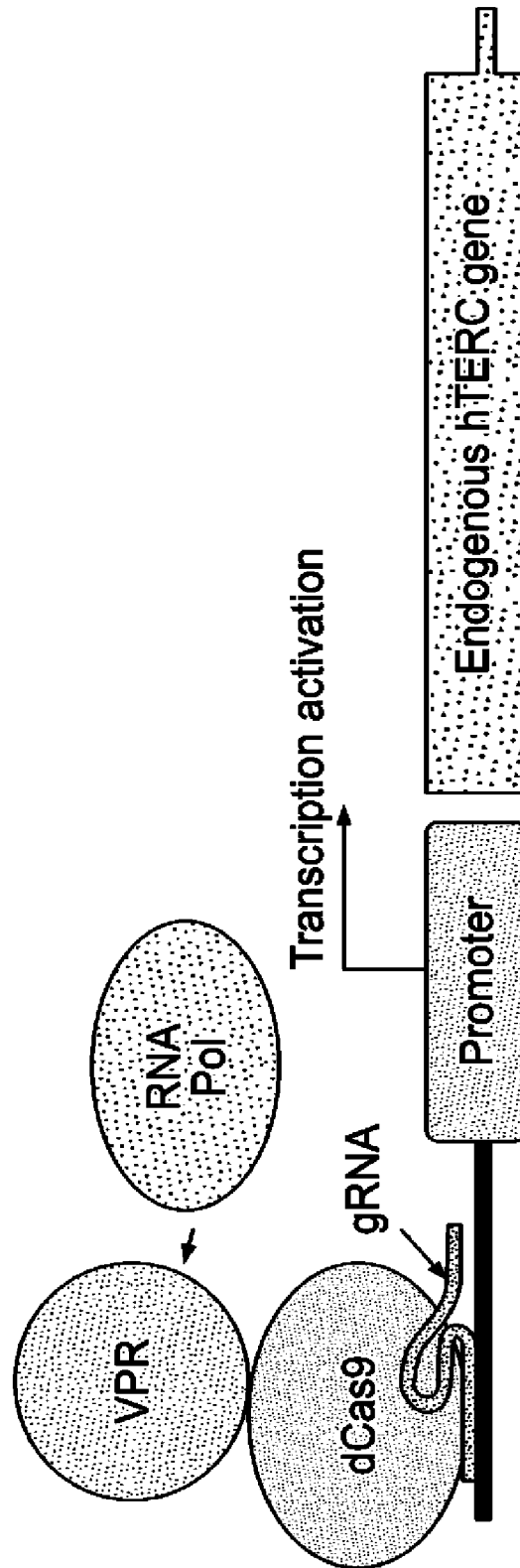
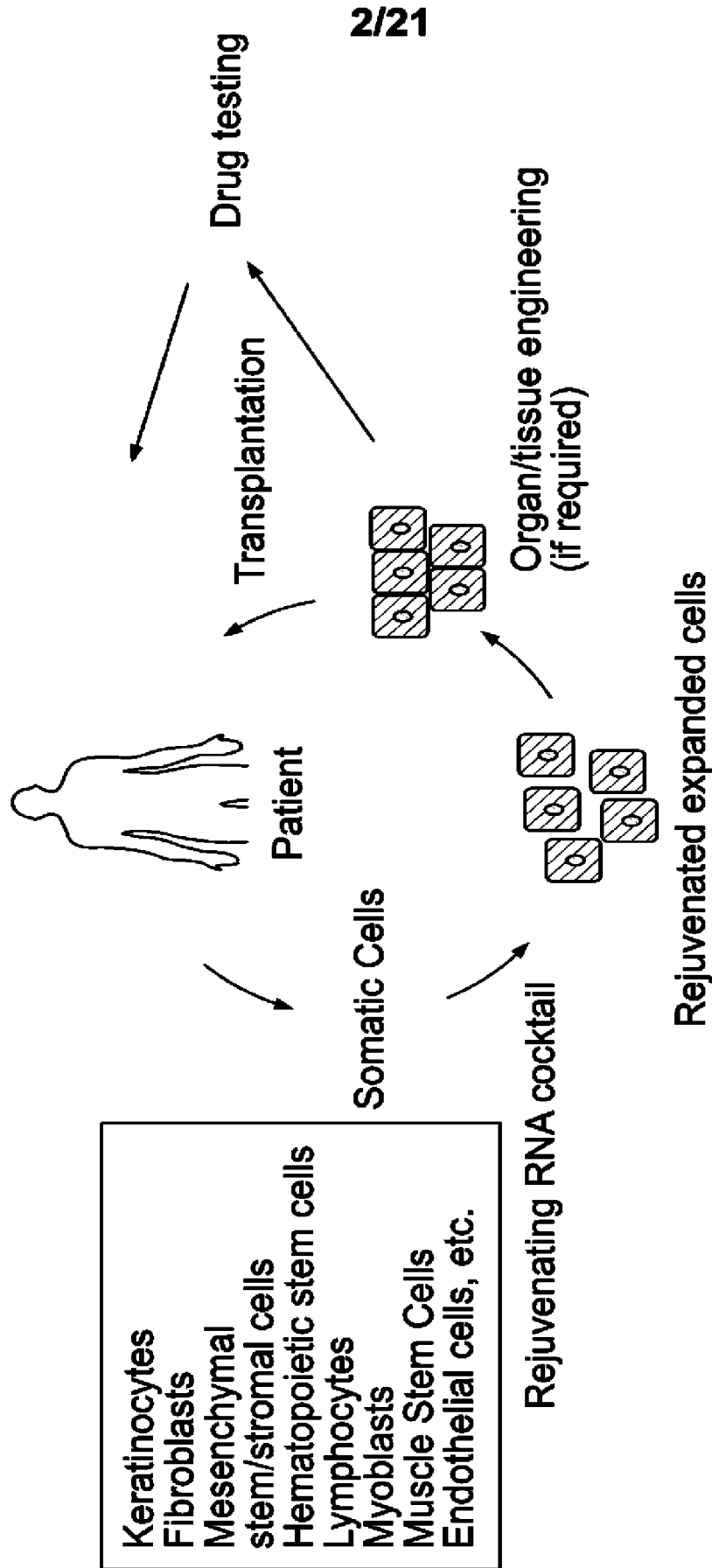
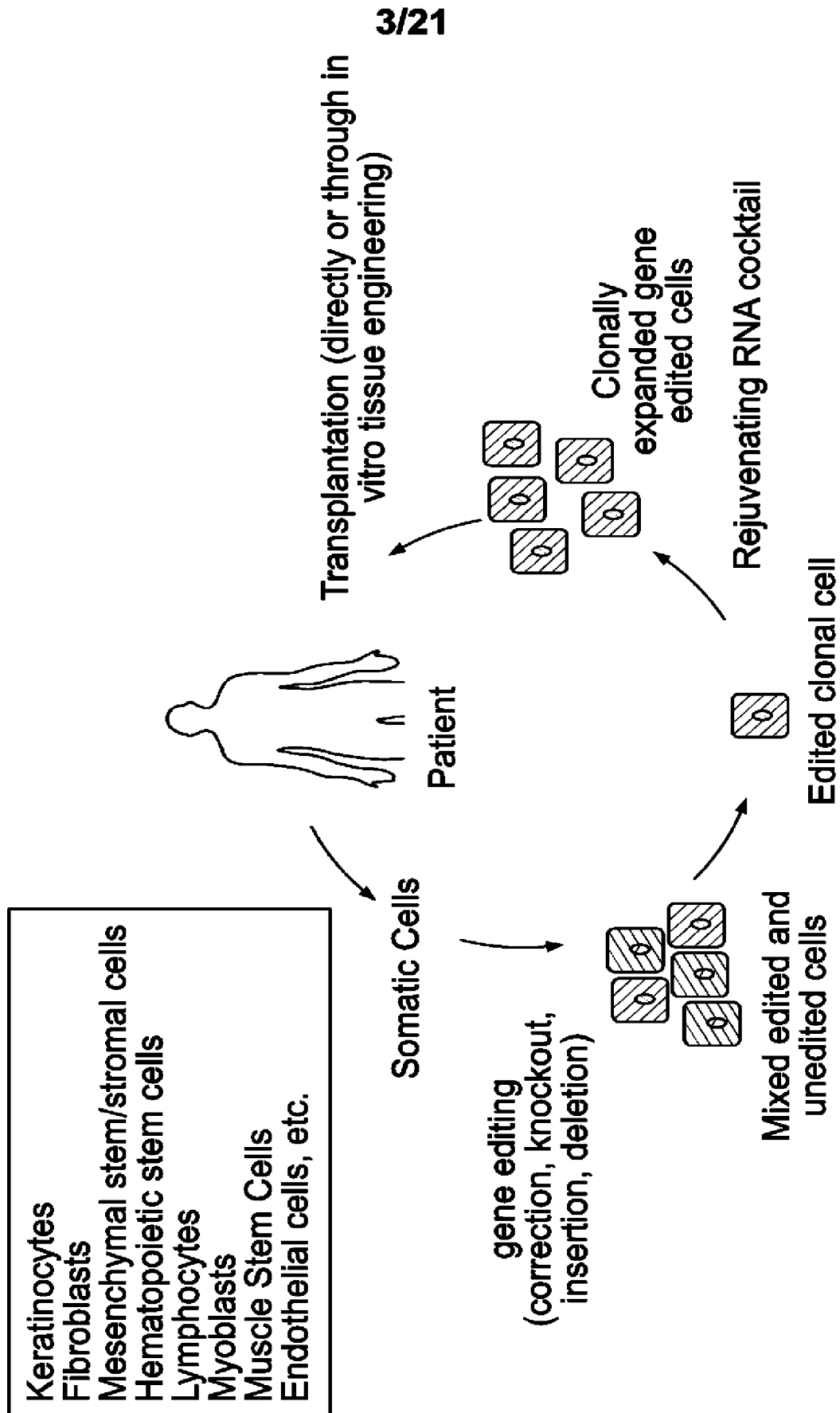


FIG. 1



**FIG. 2**



**FIG. 3**

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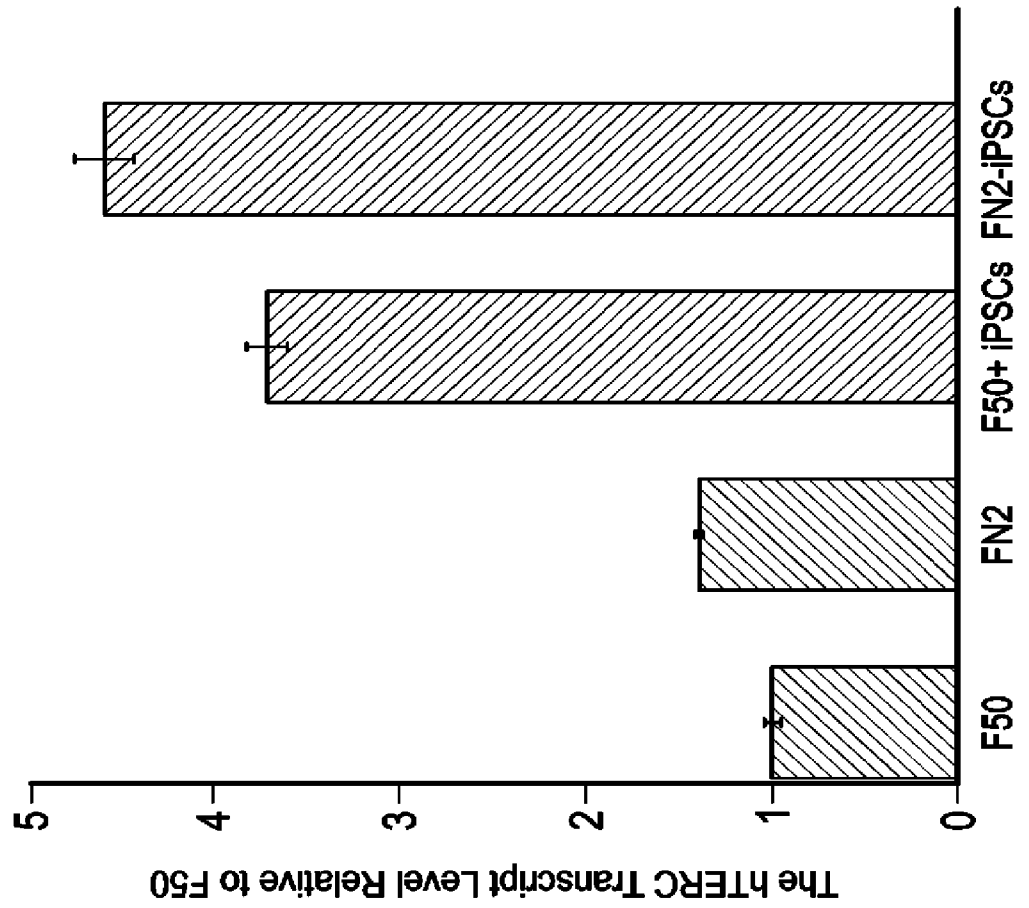


FIG. 4

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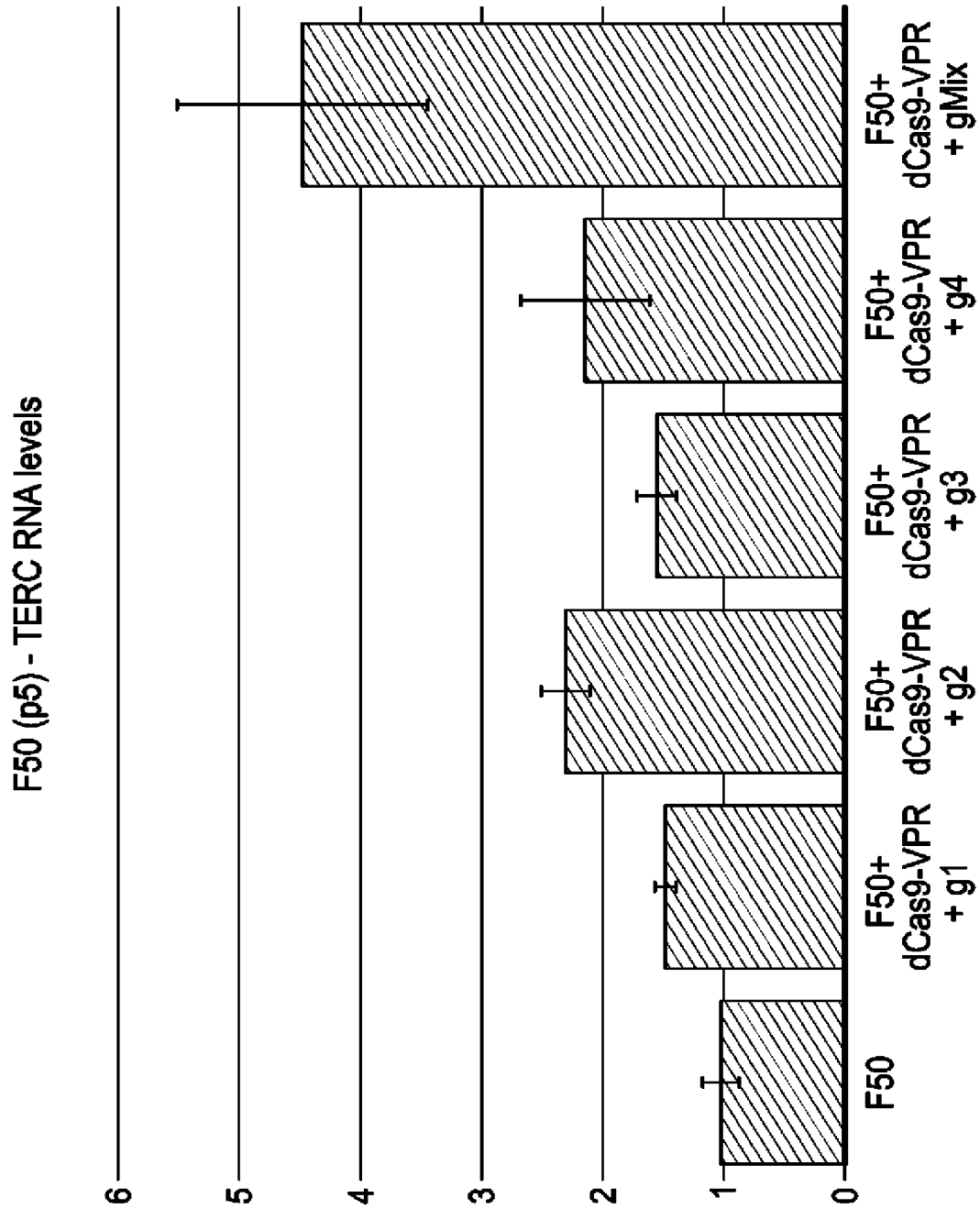


FIG. 5

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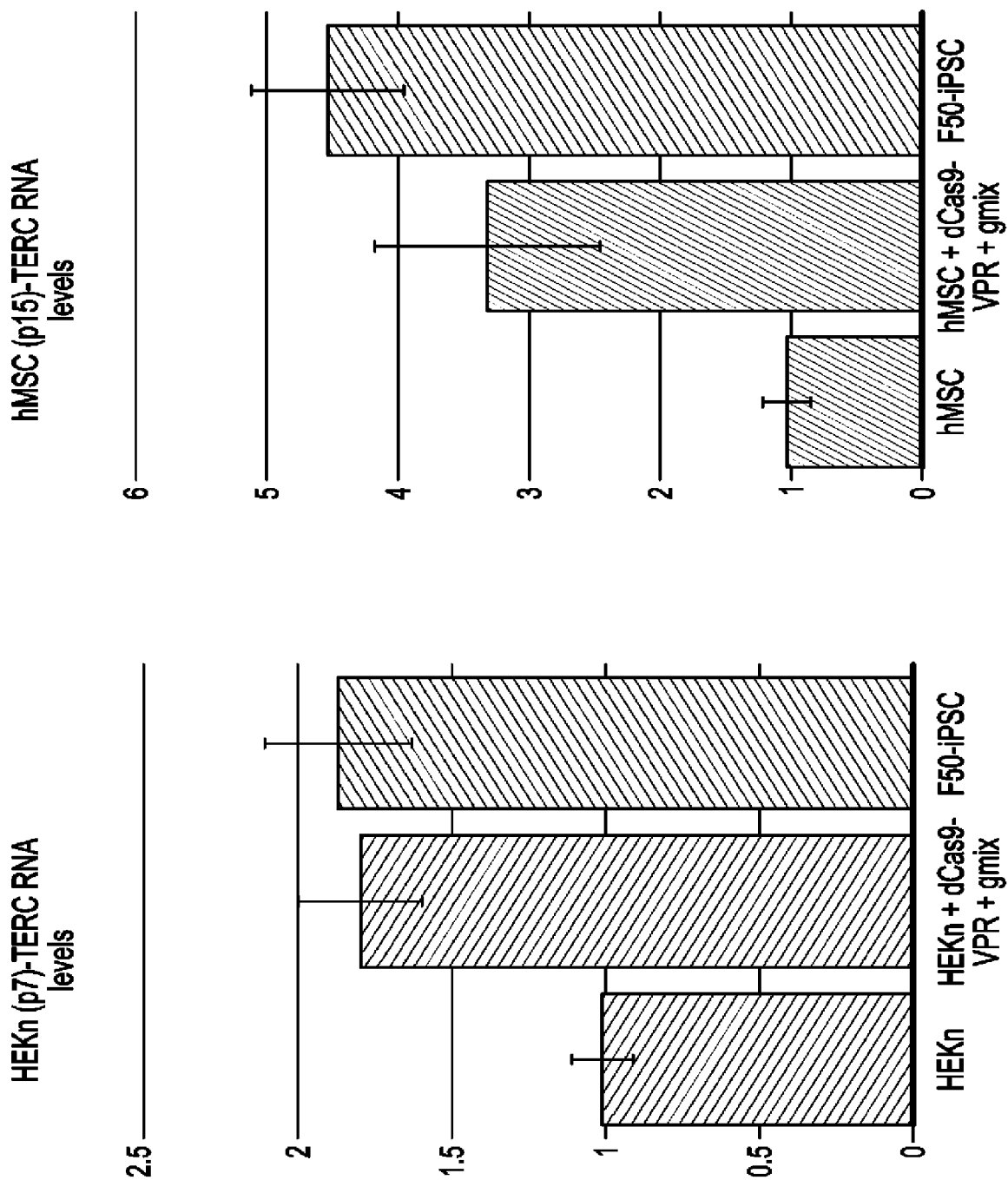


FIG. 6

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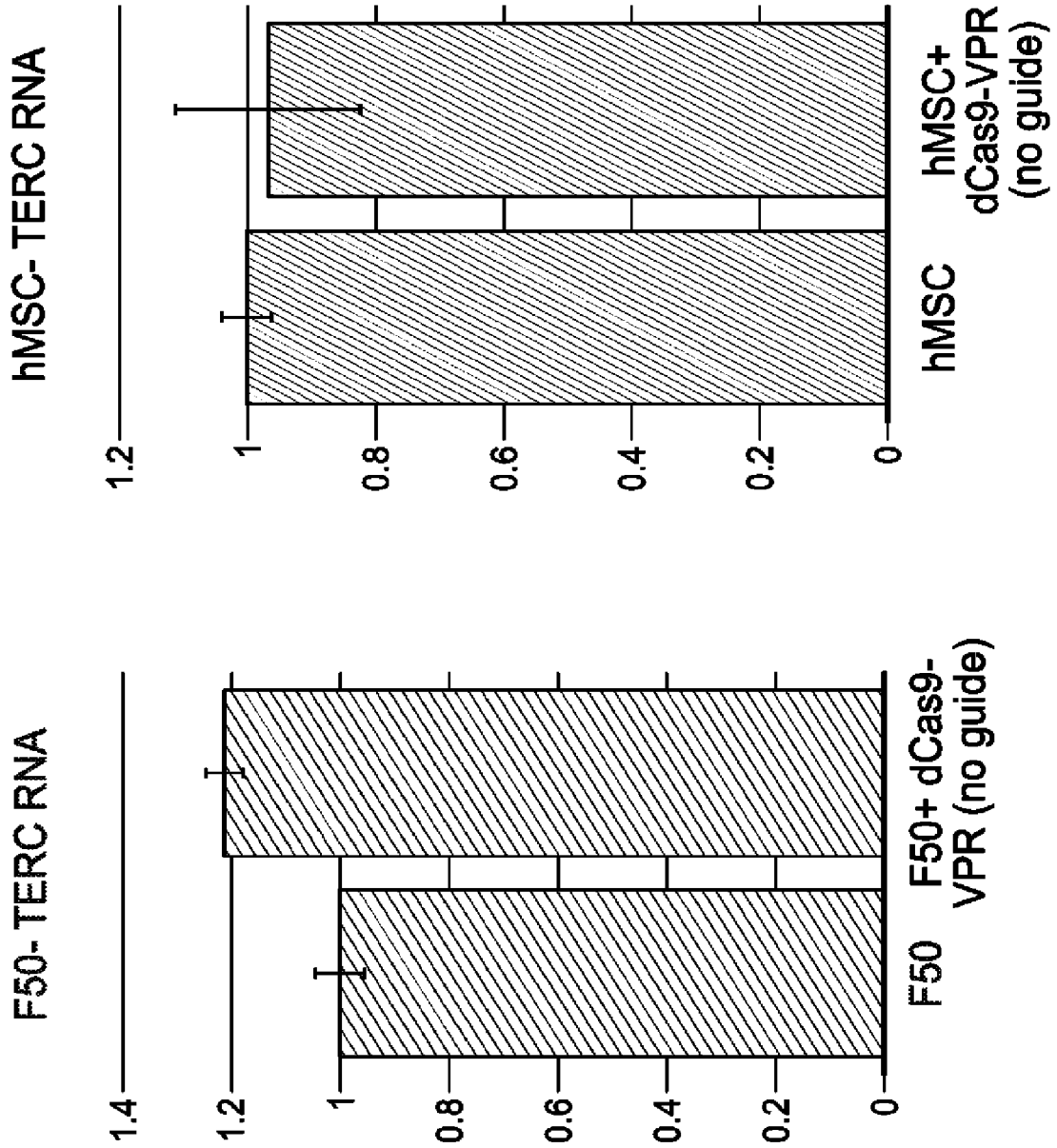


FIG. 7

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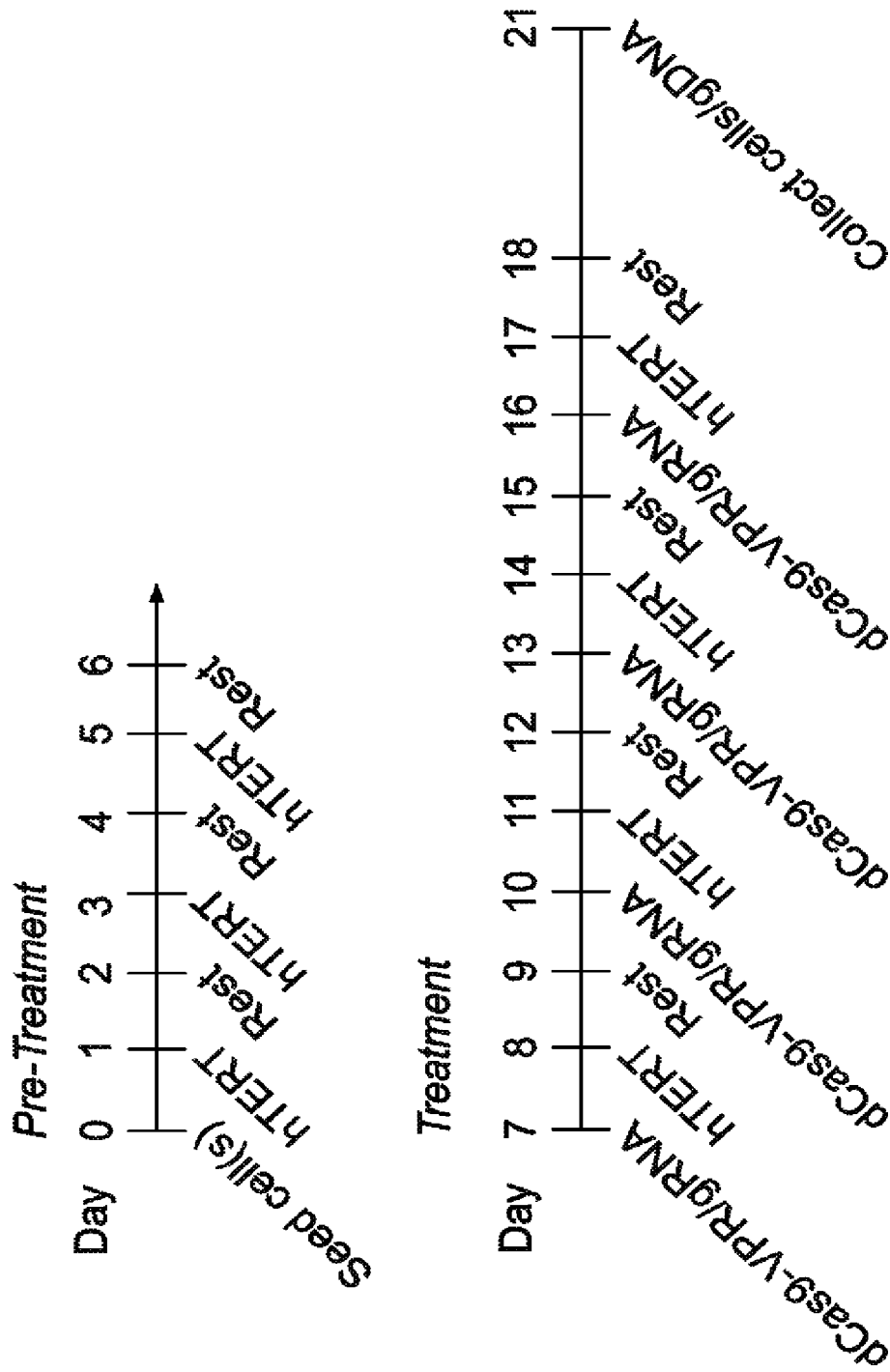


FIG. 8

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F50S Total Population Doublings

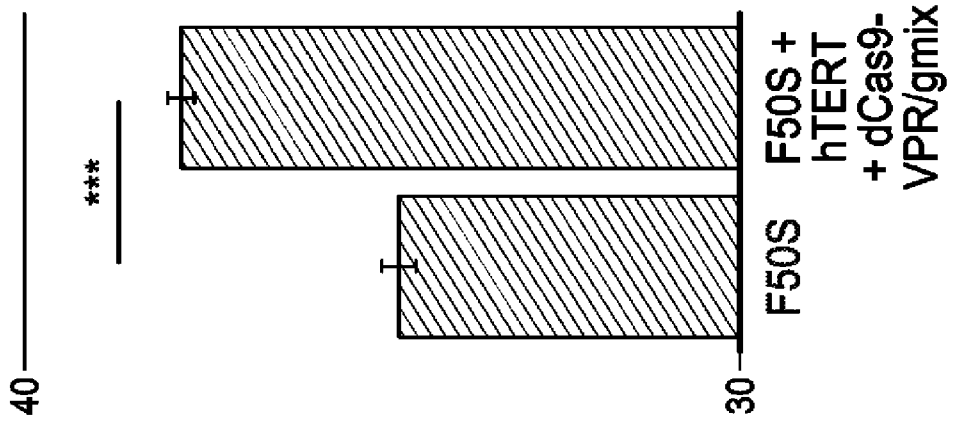
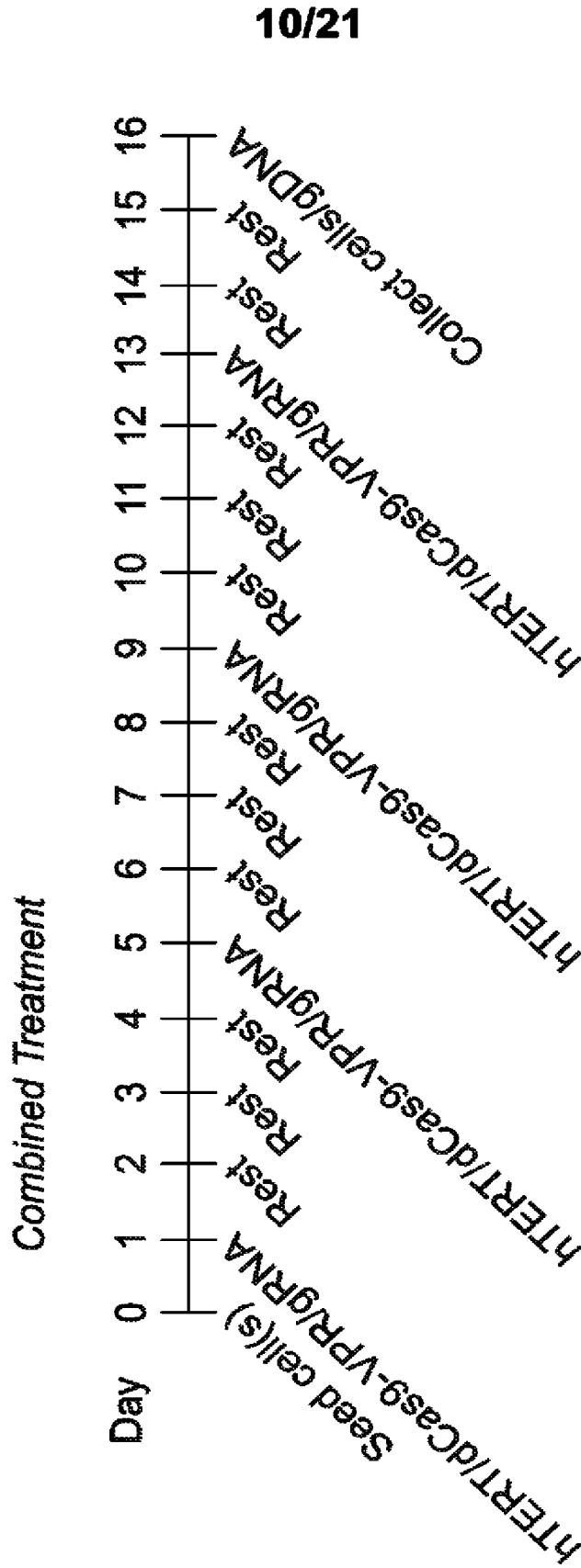


FIG. 9

Schematic overview of the transfection regimen where all factors are delivered together in one transfection



**FIG. 10**

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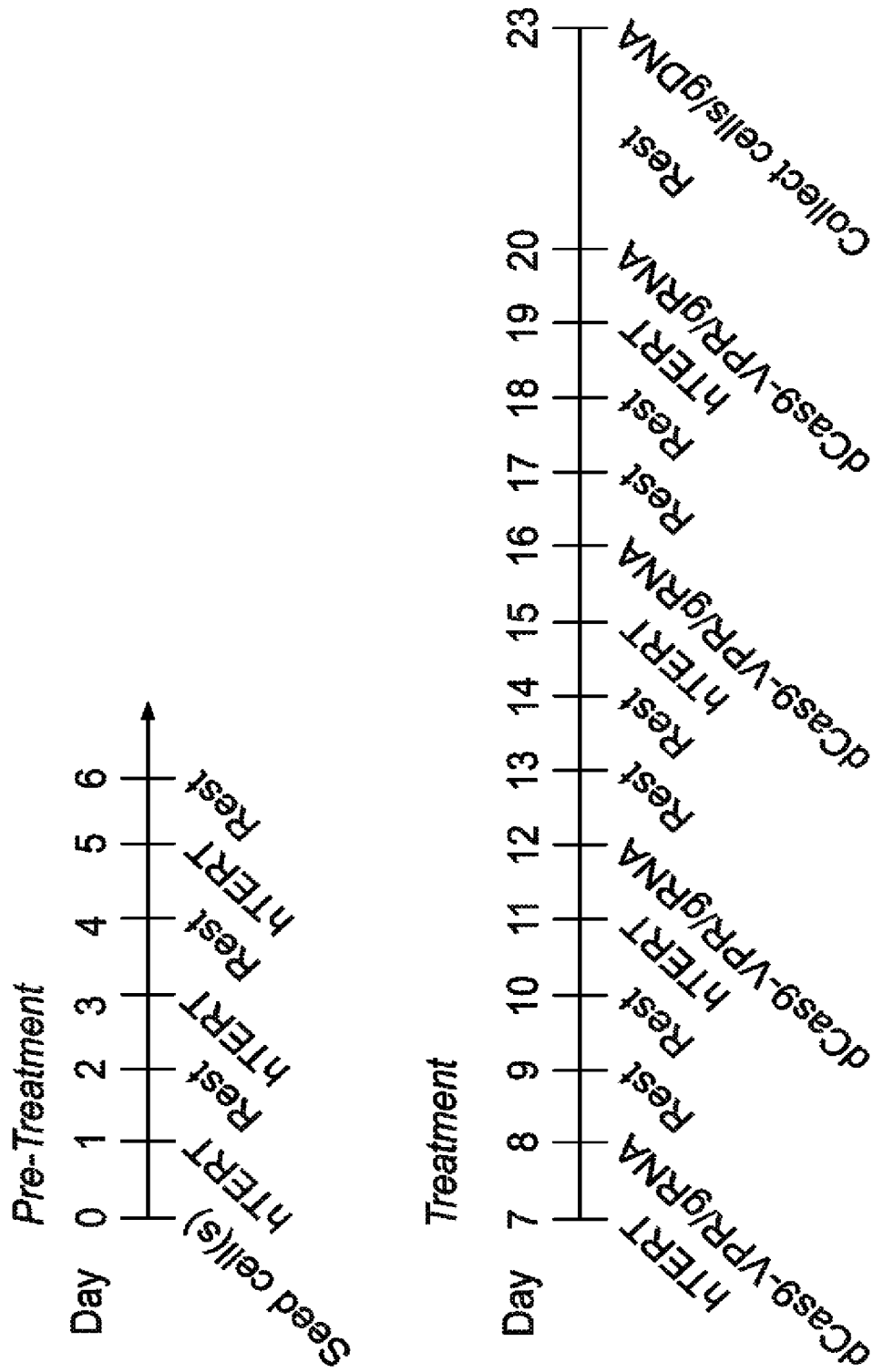


FIG. 11

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F50S – Relative Telomere Length

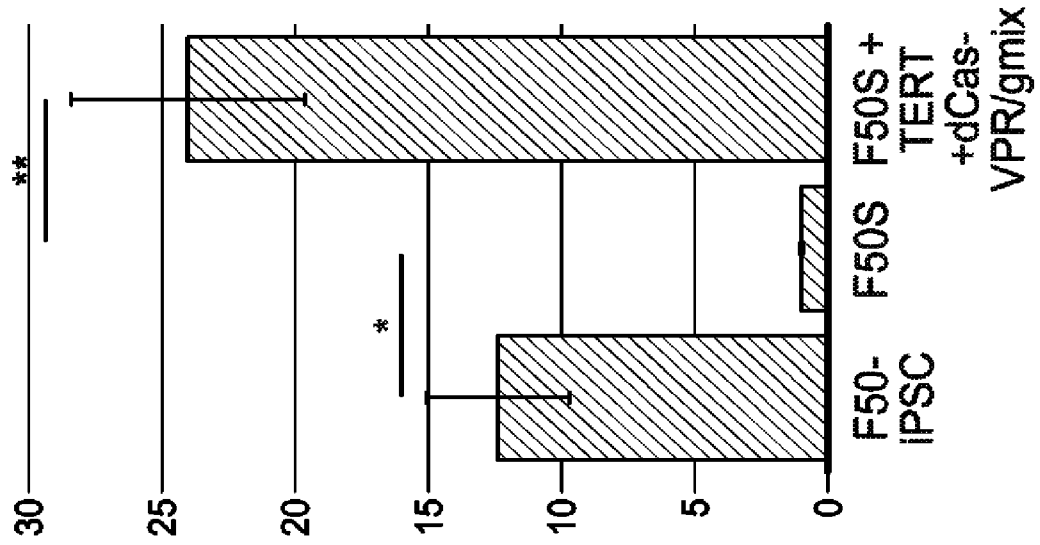


FIG. 12

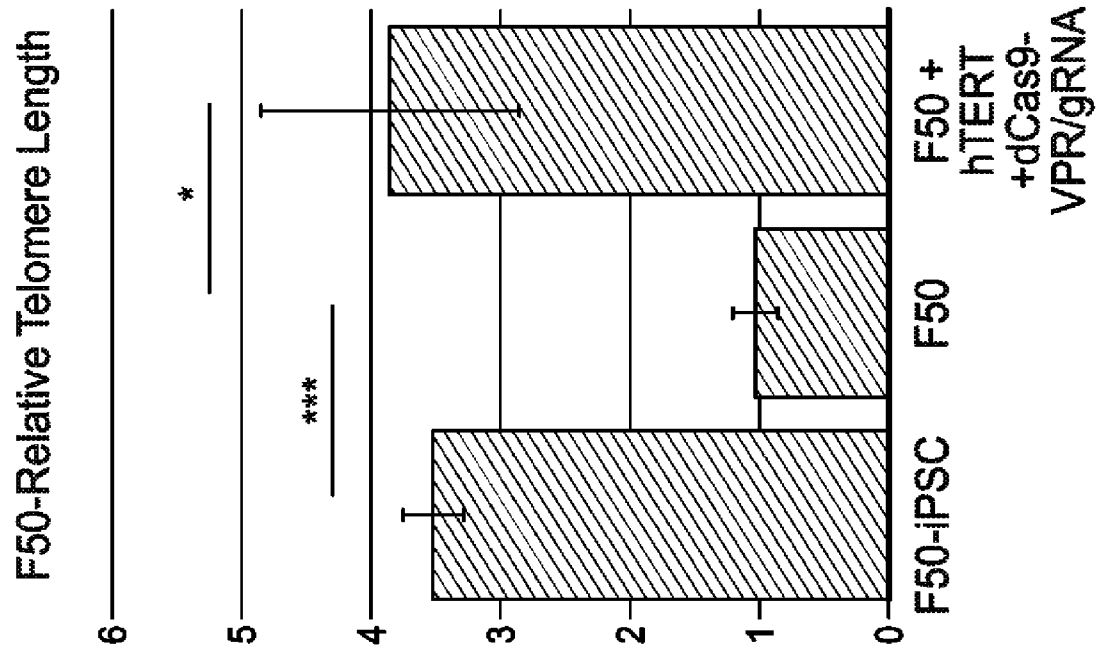
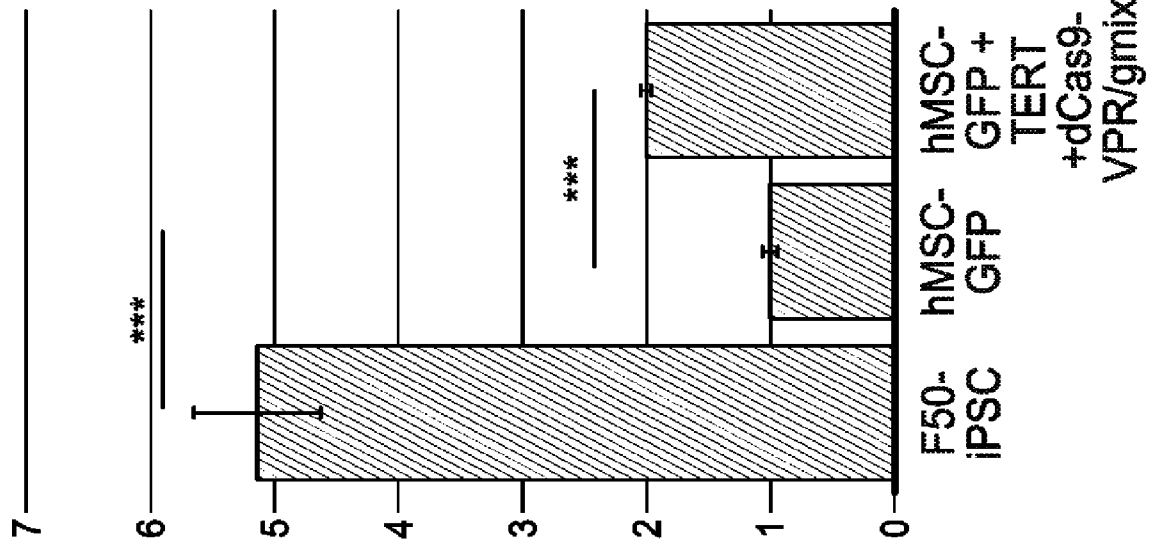


FIG. 13

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hMSC-GFP - Relative Telomere Length



HEKn-Relative Telomere Length

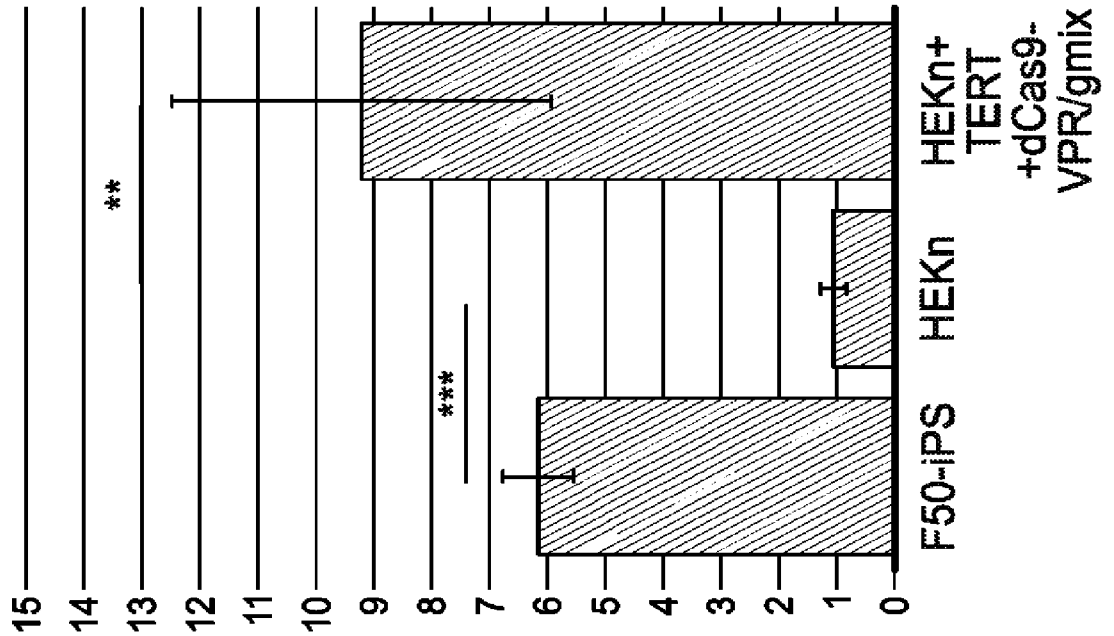


FIG. 14

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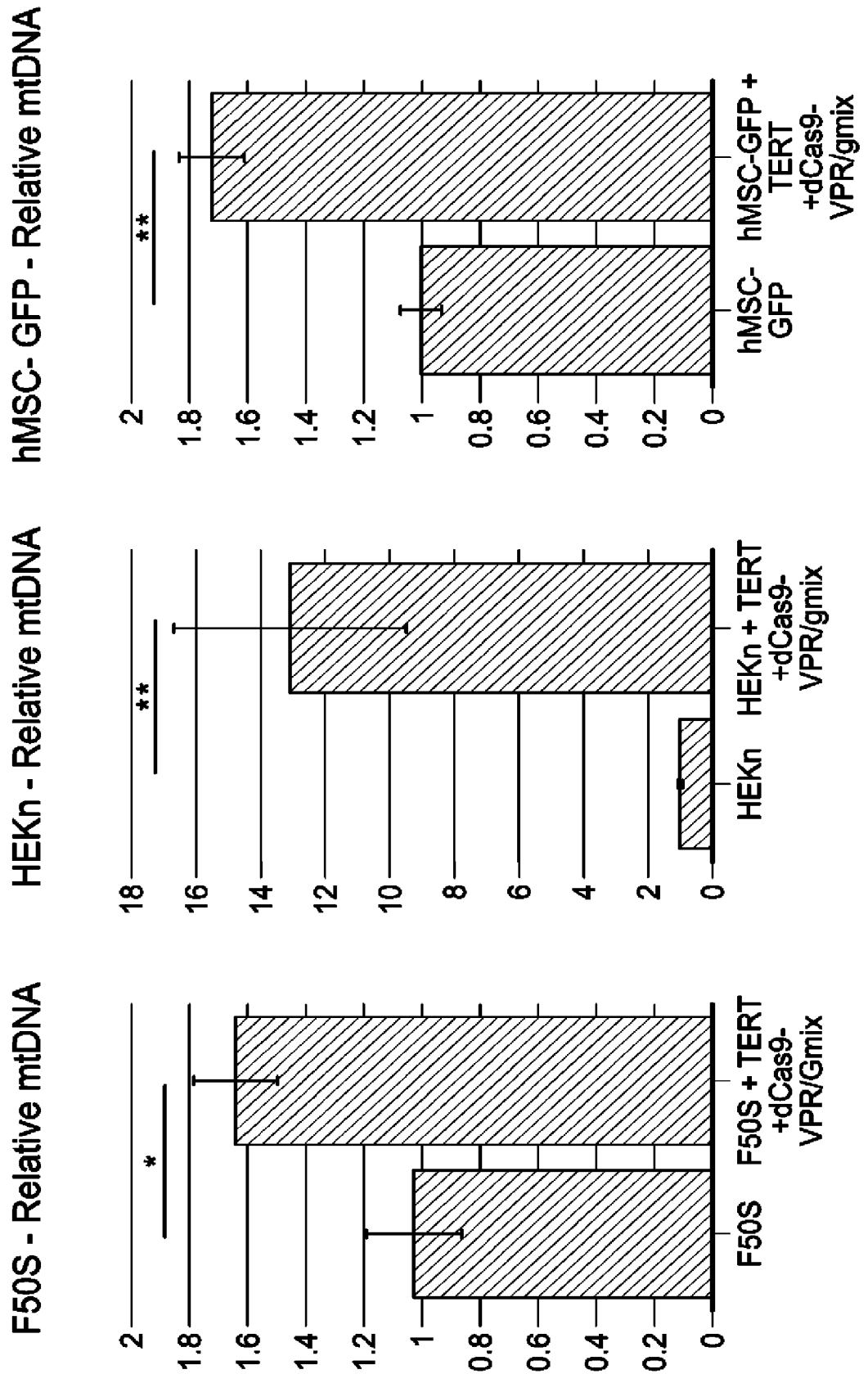


FIG. 15

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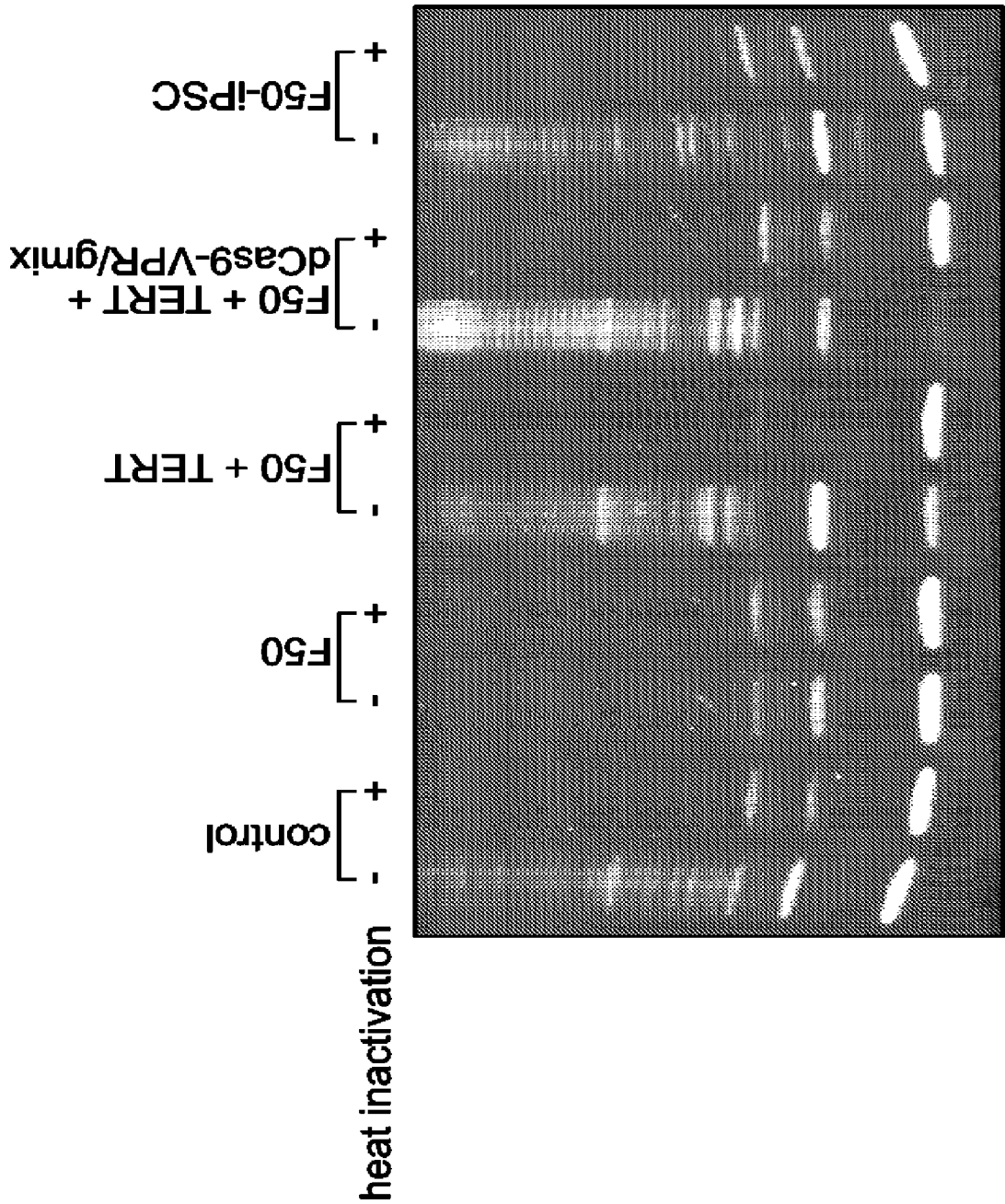


FIG. 16

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Adult Fibroblasts +  
hTERT/dCas9-  
VPR+gRNA

Adult Fibroblasts

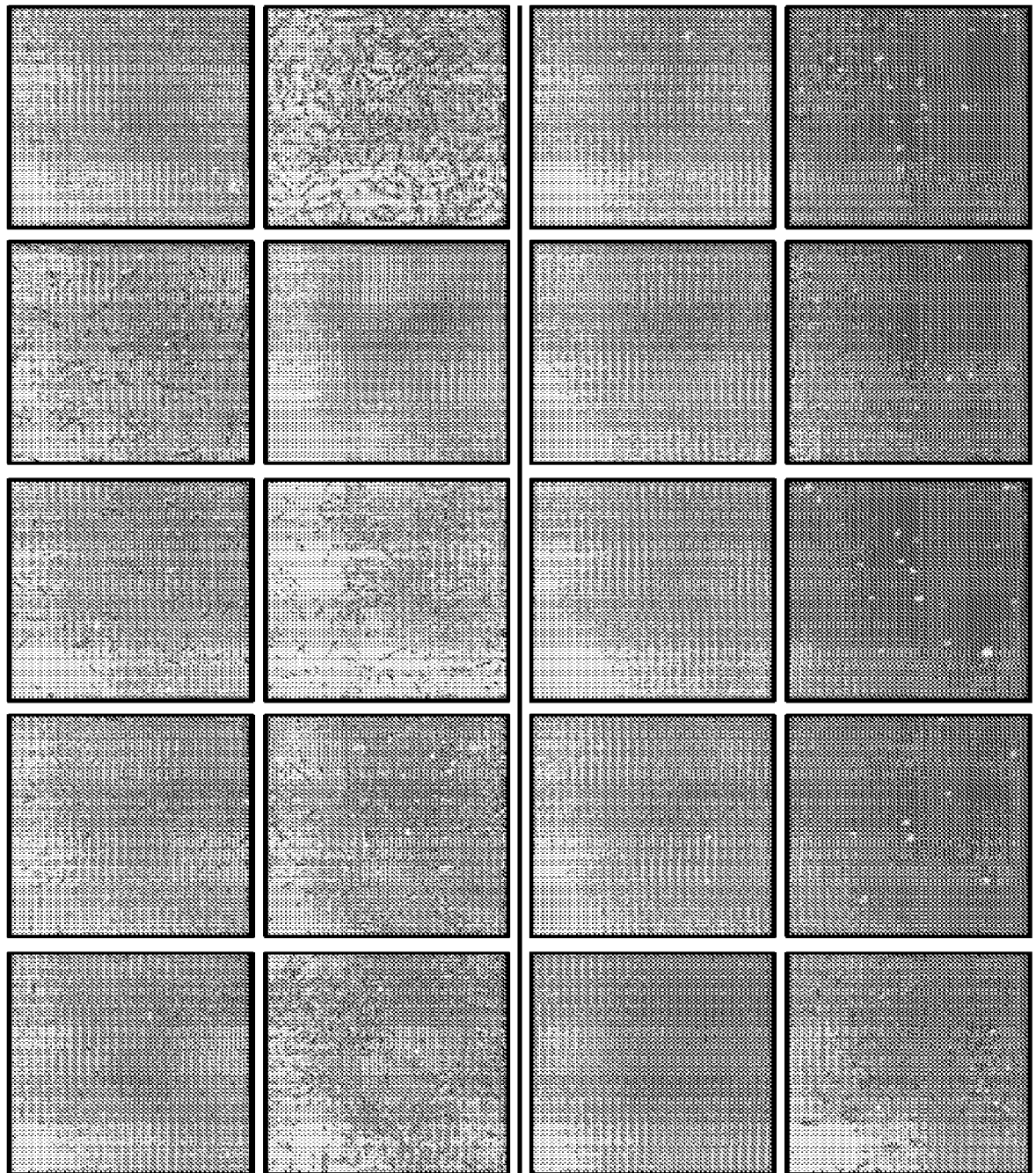


FIG. 17

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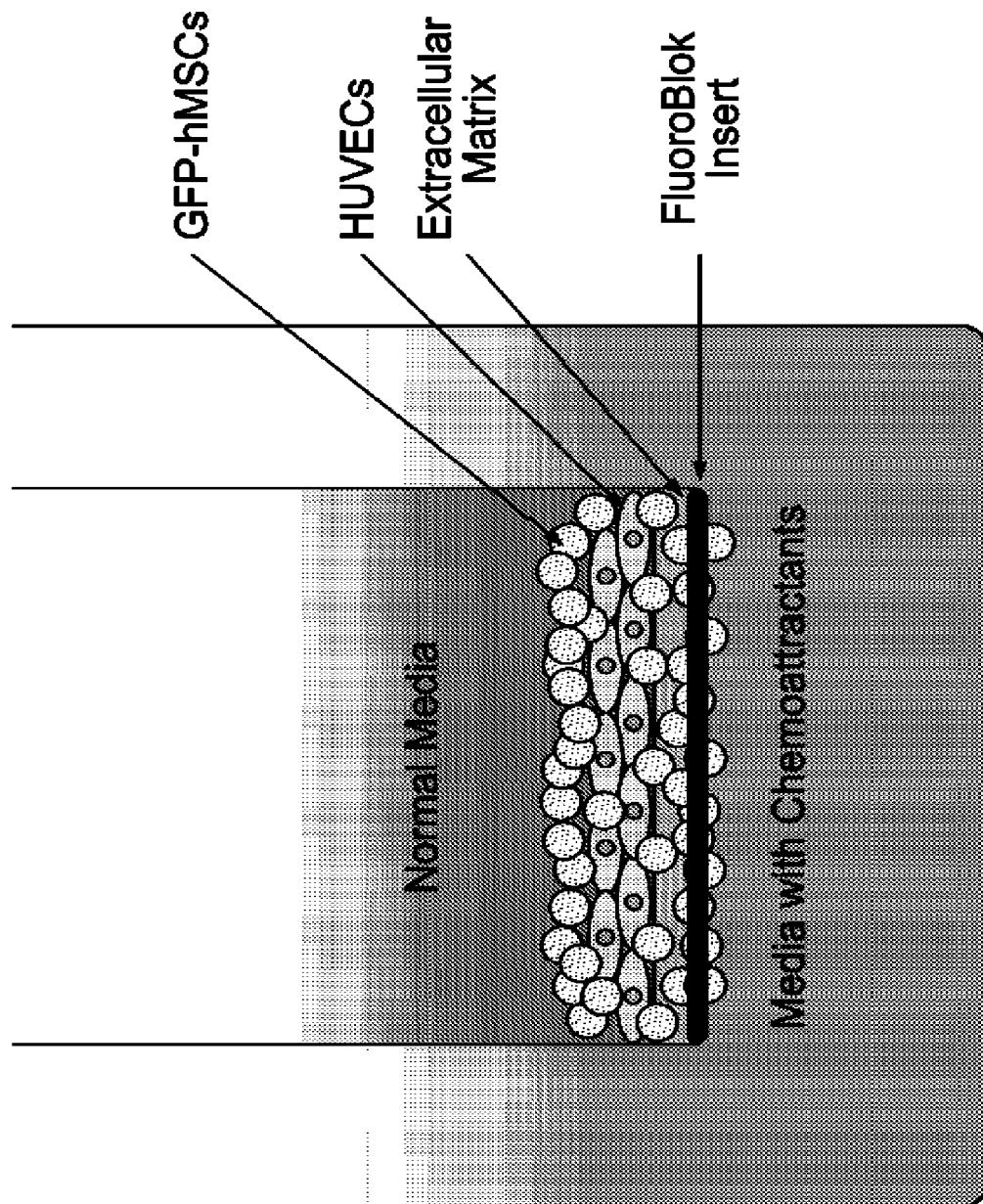


FIG. 18

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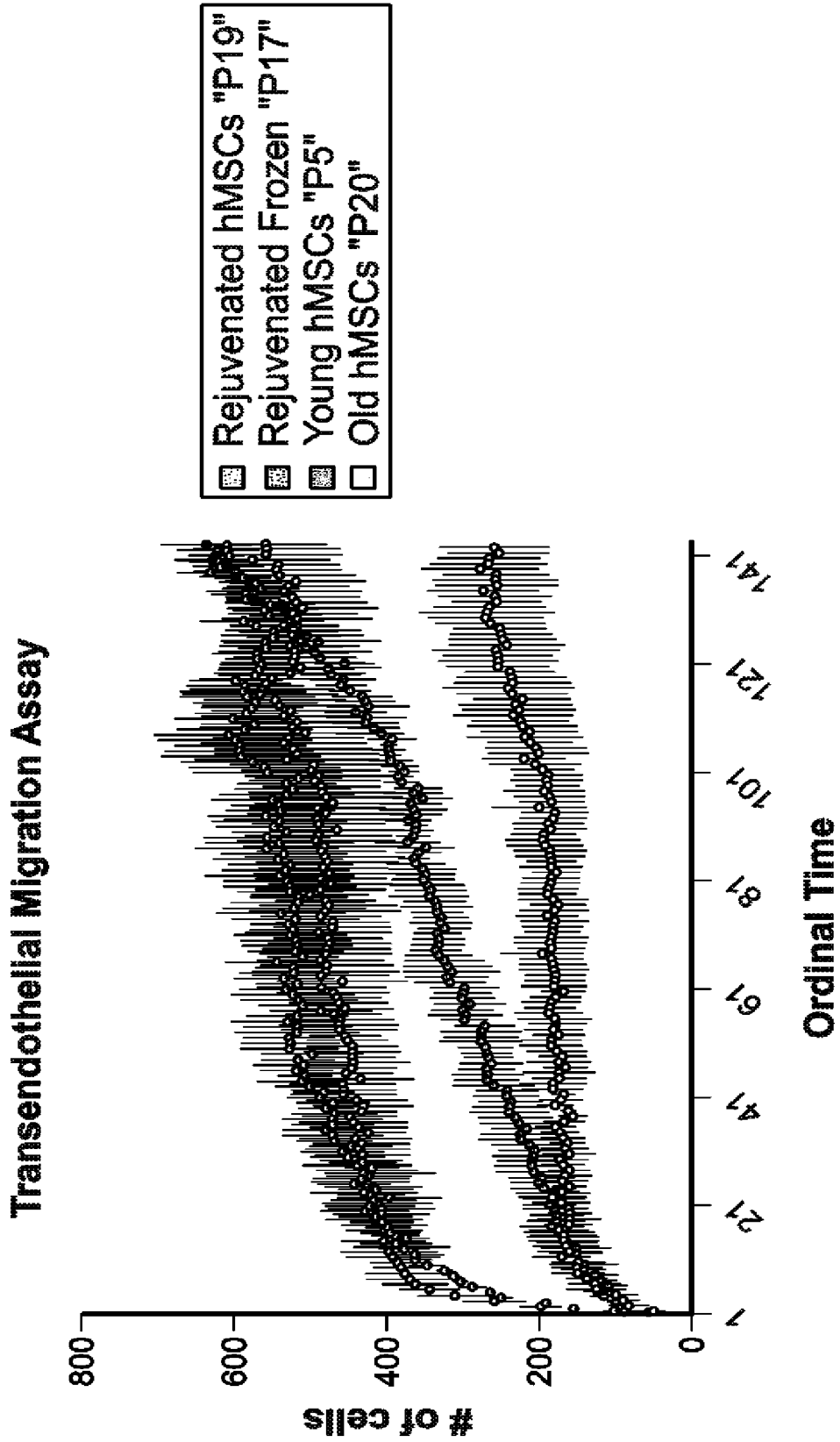


FIG. 19

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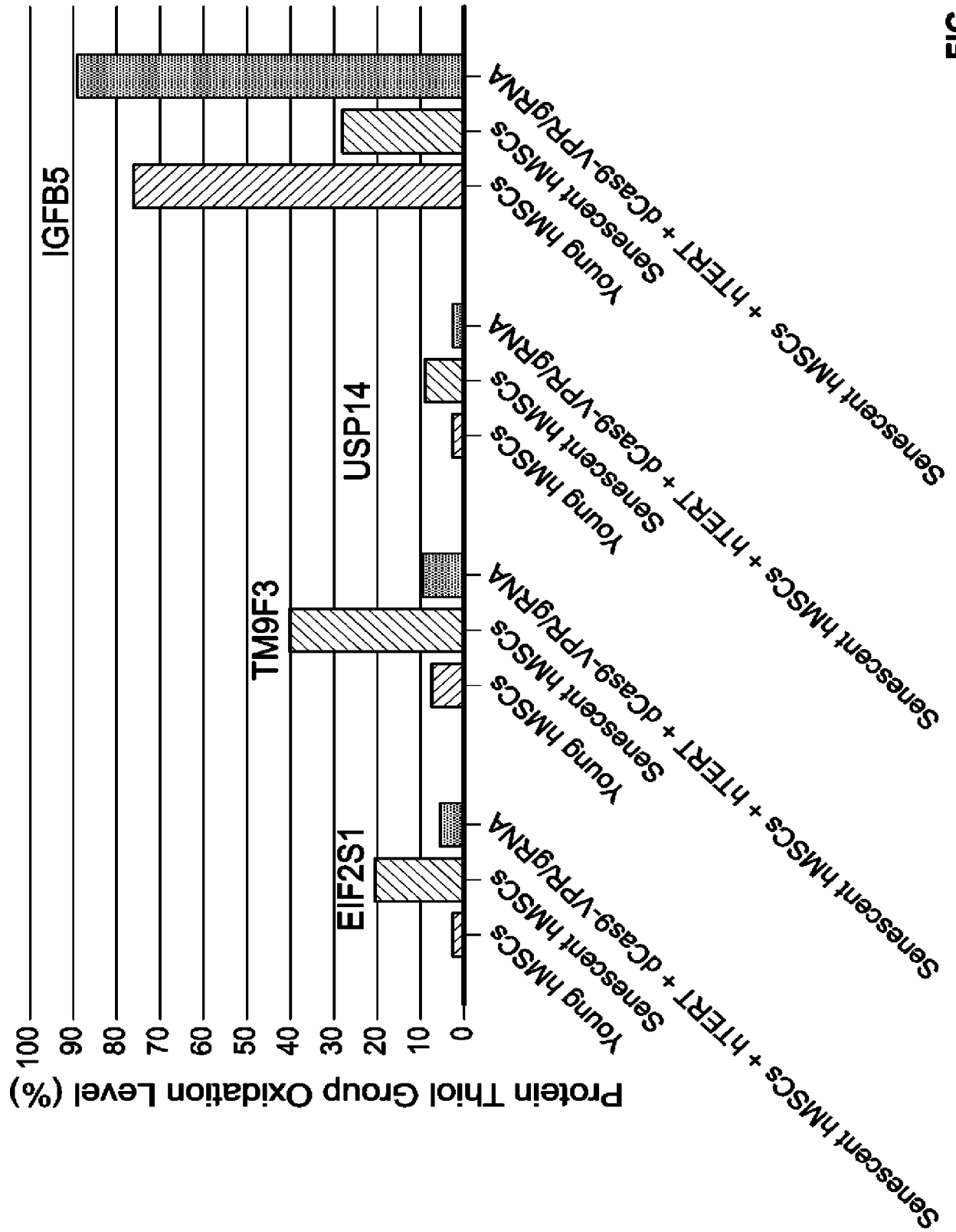


FIG. 20

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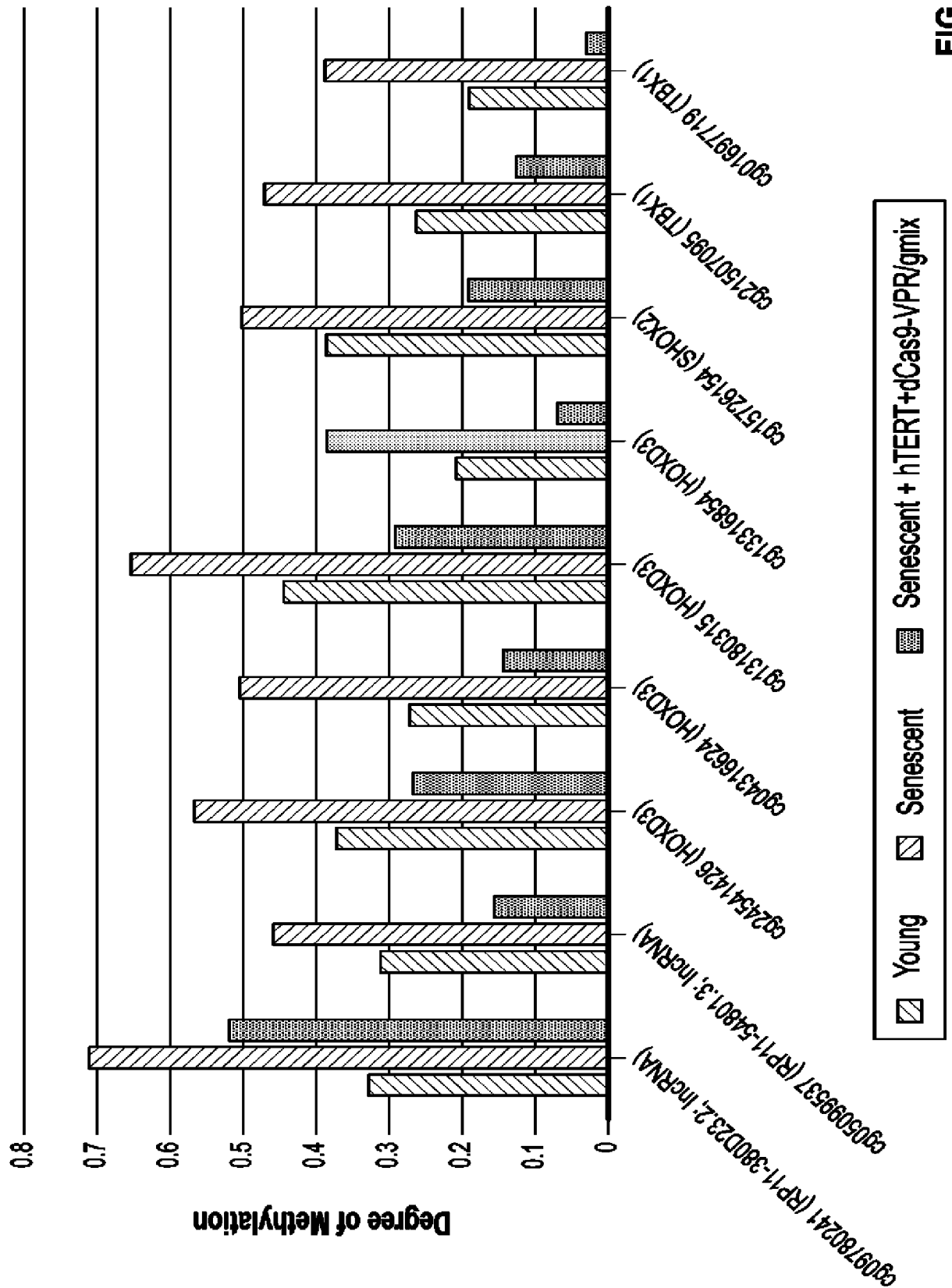
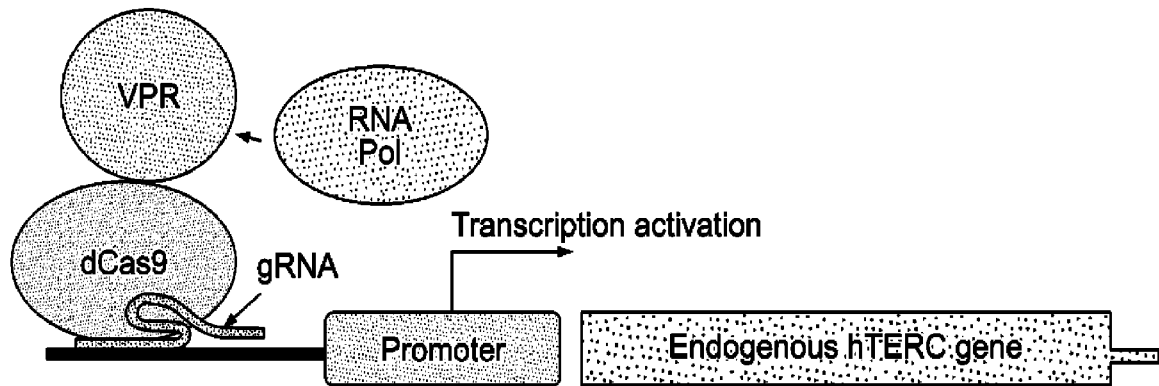


FIG. 21



**FIG. 1**