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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 March 2021 (18.03.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/051054 A1

(51) International Patent Classification:
C12N 9/22 (2006.01) *A61K 48/00* (2006.01)
C12N 9/12 (2006.01)

(21) International Application Number:
PCT/US2020/050665

(22) International Filing Date:
14 September 2020 (14.09.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/899,861 13 September 2019 (13.09.2019) US

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

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

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

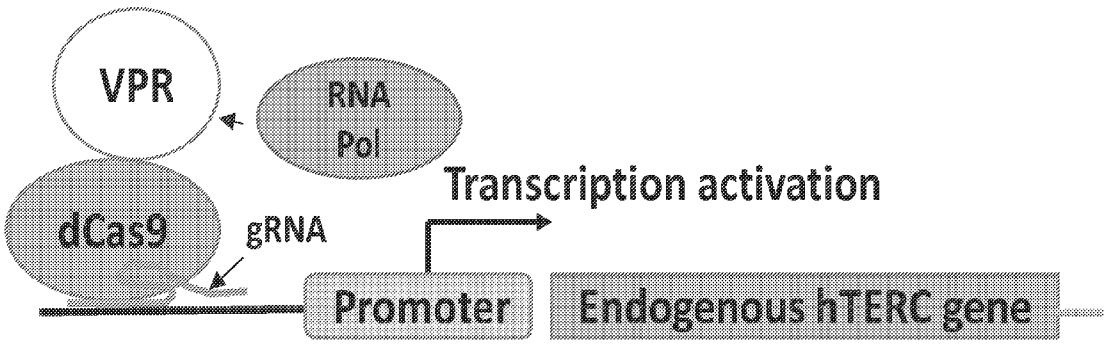

FIG. 1

(57) 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 the promote the expression of TERT and/or TERC.

[Continued on next page]

WO 2021/051054 A1

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

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), Casϕ (Cas12j), Francisella tularensis subsp. novicida Cas9, Pasteurella multocida Cas9, Campylobacter lari CF89-12 Cas9, Mycoplasma gallisepticum str. F Cas9, Nitratifractor 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^5C) residue or any combination thereof. A modified mRNA molecule 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 2'-0-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 hTERC 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, measle 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 β-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 HEKn 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 HEKn 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 HEKn cells (left) and hMSCs (right) transfected with compositions of the present disclosure (+TERT+dCas9-VPR/gmix) as compared to non-transfected HEKn 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), HEKn 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 HEKn 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, Garl, 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), Casϕ (Cas12j), Francisella tularensis subsp. novicida Cas9, Pasteurella multocida Cas9, Campylobacter lari CF89-12 Cas9, Mycoplasma gallisepticum str. F Cas9, Nitratifractor 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), Casϕ (Cas12j), Francisella tularensis subsp. novicida Cas9, Pasteurella multocida Cas9, Campylobacter lari CF89-12 Cas9, Mycoplasma gallisepticum str. F Cas9, Nitratifractor 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.

[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 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 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 (Ψ) 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), N6-methyladenosine (m^6A), inosine and 2'-0-methylated nucleosides, in addition to N7-methylguanosine (m^7G), 2-thiouridine (s^2U), pseudouridine (ψ), 2'-0-methyl-U, m^1A (1-methyladenosine); m^2A (2-methyladenosine); Am (2'-0-methyladenosine); ms^2m^6A (2-methylthio-N^6-methyladenosine); i^6A (N^6-isopentenyladenosine); ms^2i6A (2-methylthio-N^6isopentenyladenosine); io^6A (N^6-(cis-hydroxyisopentenyl)adenosine); ms^2i^6A (2-methylthio-N^6-(cis-hydroxyisopentenyl)adenosine); g^6A (N^6-glycinylcarbamoyladenosine); 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'-0-ribosyladenosine(phosphate)); I (inosine); m^1I (1-methylinosine); m^1Im (l,2'-0-dimethylinosine); m^3C (3-methylcytidine); Cm (2'-0-methylcytidine); s^2C (2-thiocytidine); ac^4C(N^4-acetylcytidine); f^5C (5-formylcytidine); m^5 Cm (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); $m^2{}_2G$ (N^2,N^2-dimethylguanosine); m^2Gm (N^2,2'-0-dimethylguanosine); $m^2{}_2Gm$ (N^2,N^2,2'-0-trimethylguanosine); Gr(p) (2'-0-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); $preQ_0$ (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^{5}U (5-(carboxyhydroxymethyl)uridine)); mchm^{5}U (5-(carboxyhydroxymethyl)uridine methyl ester); mcm^{5}U (5-methoxycarbonylmethyluridine); mcm^{5}Um (5-methoxycarbonylmethyl-2'-0-methyluridine); mcm^{5}s^{2}U (5-methoxycarbonylmethyl-2-thiouridine); nm^{5}s^{2}U (5-aminomethyl-2-thiouridine); mnm^{5}U (5-methylaminomethyluridine); mnm^{5}s^{2}U (5-methylaminomethyl-2-thiouridine); mnm^{5}se^{2}U (5-methylaminomethyl-2-selenouridine); ncm^{5}U (5-carbamoylmethyluridine); ncm^{5}Um (5-carbamoylmethyl-2'-0-methyluridine); cmnm^{5}U (5-carboxymethylaminomethyluridine); cmnm^{5}Um (5-carboxymethylaminomethyl-2'-0-methyluridine); cmnm^{5}s^{2}U (5-carboxymethylaminomethyl-2-thiouridine); m^6 $_2$A (N^6,N^6-dimethyladenosine); Im (2'-0-methylinosine); m^{4}C(N^4-methylcytidine); m^4 Cm (N^4,2'-0-dimethylcytidine); hm^{5}C (5-hydroxymethylcytidine); m^{3}U (3-methyluridine); cm^{5}U (5-carboxymethyluridine); m^{6}Am (N^6,2'-0-dimethyladenosine); m^6 $_2$Am (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^{3}Um (3,2'-0-dimethyluridine); m^{5}D (5-methyldihydrouridine); f^{5}Cm (5-formyl-2'-0-methylcytidine); m^{1}Gm (1,2'-0-dimethylguanosine); m^{1}Am (1,2'-0-dimethyladenosine); τm^{5}U (5-taurinomethyluridine); τm^{5}s^{2}U (5-taurinomethyl-2-thiouridine)); imG-14 (4-demethylwyosine); imG2 (isowyosine); ac^{6}A (N^6-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 (Ψ) 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'-0-methylated nucleosides, in addition to N7-methylguanosine (m^7G), 2-thiouridine (s^2U), pseudouridine (ψ), 2'-0-methyl-U, m^1A (1-methyladenosine); m^2A (2-methyladenosine); Am (2'-0-methyladenosine); ms^2m^6A (2-methylthio-N^6-methyladenosine); i^6A (N^6-isopentenyladenosine); ms^2i6A (2-methylthio-N^6isopentenyladenosine); io^6A (N^6-(cis-hydroxyisopentenyl)adenosine); ms^2i^6A (2-methylthio-N^6-(cis-hydroxyisopentenyl)adenosine); g^6A (N^6-glycinylcarbamoyladenosine); 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'-0-ribosyladenosine(phosphate)); I (inosine); m^1I (1-methylinosine); m^1Im (l,2'-0-dimethylinosine);

m^3C (3-methylcytidine); Cm (2'-0-methylcytidine); s^2C (2-thiocytidine); ac^4C(N^4-acetylcytidine); f^5C (5-formylcytidine); m^5 Cm (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); $m^2{}_2G$ (N^2,N^2-dimethylguanosine); m^2Gm (N^2,2'-0-dimethylguanosine); $m^2{}_2Gm$ (N^2,N^2,2'-0-trimethylguanosine); Gr(p) (2'-0-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); $preQ_0$ (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); $m^6{}_2A$ (N^6,N^6-dimethyladenosine); Im (2'-0-methylinosine); m^4C(N^4-methylcytidine); m^4 Cm (N^4,2'-0-dimethylcytidine); hm^5C (5-hydroxymethylcytidine); m^3U (3-methyluridine); cm^5U (5-carboxymethyluridine); m^6Am (N^6,2'-0-dimethyladenosine); $m^6{}_2Am$ (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); τm^5U (5-taurinomethyluridine); τm^5s^2U (5-taurinomethyl-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	CCUGGAGGCCGCGGUCGGCC	270
103rev	AAUUUCUCUCCUUUGCAUAU	7	1947forw	CUGGAGGCCGCGGUCGGCCC	271
1049rev	AGUAGUGCUGUGUCUUGAUG	8	1948forw	UGGAGGCCGCGGUCGGCCCG	272
1059rev	AGGGGACCUACUUAGGUAAU	9	1956rev	GCGGUGGCAGUGGGUGCCUC	273
1066rev	CAAUUCCAGGGGACCUACUU	10	1957forw	CGGUCGGCCCGGGGCUUCUC	274
106forw	ACGGAGCGAGUCCCCGCGCG	11	1960forw	UCGGCCCGGGGCUUCUCCGG	275
1073forw	UUUUAACCUAUUACCUAAGU	12	1965rev	CAACUCUUCGCGGUGGCAGU	276
1077rev	UAUCUGCUAGACAAUUCCAG	13	1966rev	CCAACUCUUCGCGGUGGCAG	277
1078rev	GUAUCUGCUAGACAAUUCCA	14	1986forw	CCACUGCCACCGCGAAGAGU	278
1079rev	UGUAUCUGCUAGACAAUUCC	15	1987forw	CACUGCCACCGCGAAGAGUU	279
1081forw	UAUUACCUAAGUAGGUCCCC	16	199forw	UUUGGAGAAUAAAUUGAAUG	280
1098rev	UCCCUUUUAUUAGGAAAGAA	17	1rev	CAGAGCCCAACUCUUCGCGG	281
1107rev	GACUGAAUCUCCCUUUUAUU	18	203forw	GAGAAUAAAUUGAAUGAGGA	282
1116forw	CGCCUUUCUUUCCUAAUAAA	19	203rev	CCAUUGCCGGCGAGGGGUGA	283
1117forw	GCCUUUCUUUCCUAAUAAAA	20	206rev	AACUGAUCACCAAAUCUCCA	284
1129rev	CUACUACAUUAUUAAUCUUA	21	207rev	UAACUGAUCACCAAAUCUCC	285
1139rev	CCAGCAACAGUGGACUCUAG	22	209forw	AAAUUGAAUGAGGAAGGCCC	286
1149rev	GAGAACAUUACCAGCAACAG	23	209rev	AAGCCCCCAUUGCCGGCGAG	287
114forw	GCUAAAUAUCCAAUAUGCAA	24	210rev	CAAGCCCCCAUUGCCGGCGA	288
1159forw	CCUCUAGAGUCCACUGUUGC	25	211rev	ACAAGCCCCCAUUGCCGGCG	289
1168rev	GCCUCUCCUUGAGCAGAGGA	26	216rev	GGUUCACAAGCCCCCAUUGC	290
116rev	GGUGCACGUCCCACAGCUCA	27	217forw	UGAGGAAGGCCCUGGAGAUU	291
1172rev	UCCAGCCUCUCCUUGAGCAG	28	217forw	GCGCAUCCGUCACCCCUCGC	292
1178forw	CUGGUAAUGUUCUCUAAAUA	29	223forw	CCGUCACCCCUCGCCGGCAA	293
117rev	GGGUGCACGUCCCACAGCUC	30	224forw	CGUCACCCCUCGCCGGCAAU	294
1182forw	UUAAAGCCAUCCUCUGCUCA	31	225forw	GUCACCCCUCGCCGGCAAUG	295
1187forw	GCCAUCCUCUGCUCAAGGAG	32	226forw	UCACCCCUCGCCGGCAAUGG	296
1191forw	UCCUCUGCUCAAGGAGAGGC	33	237rev	GCCCAGUCAGUCAGGUUUGG	297
1193rev	UUCCACAAAACCAUGCUGAU	34	238rev	GGCCCAGUCAGUCAGGUUUG	298
1197forw	GCUCAAGGAGAGGCUGGAGA	35	239rev	UGGCCCAGUCAGUCAGGUUU	299
1203forw	AAAUAUUUUUCCUAUCAGCA	36	240rev	CUGGCCCAGUCAGUCAGGUU	300
1207forw	AGGCUGGAGAAGGCAUUCUA	37	243rev	AAGACUUGGCACUUUAUAUG	301
1211forw	UUCCUAUCAGCAUGGUUUUG	38	245rev	GCACACUGGCCCAGUCAGUC	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	UGGGCCAGUGUGCUGCAAAU	307
1221forw	AUUCUAAGGAGAAGGGGGCA	44	279forw	CCAGUGUGCUGCAAAUUGGC	308
1221forw	CAUGGUUUUGUGGAAAAGUA	45	287forw	UACUCAAGAUUAUAAGCAAU	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	CGUGAAGGCACCUCCAAAGU	314
1250rev	ACUACAGCAGAAUCUUGUCU	51	308rev	GGCUCACUGCCCAUUCAUUU	315
1257forw	AGAACUUGUCUAGAGUCUUG	52	318forw	CCAAAGUCGGCCAAAAUGAA	316
126forw	CGGCGCGAUUCCCUGAGCUG	53	319forw	CAAAGUCGGCCAAAAUGAAU	317
127forw	GGCGCGAUUCCCUGAGCUGU	54	31forw	GAGUUGGGCUCUGUCAGCCG	318
1281rev	CUUUGUGAAAAUAGAUUCCC	55	329rev	GGAACGGCUCCAGGCAACCC	319
1281rev	AGAUCACCUUGAGUAAACUG	56	32forw	AGUUGGGCUCUGUCAGCCGC	320
1283forw	CUGCUGUAGUCAGUGCUGCC	57	330forw	AAAAUGAAUGGGCAGUGAGC	321
1284forw	UGCUGUAGUCAGUGCUGCCU	58	331forw	AAAUGAAUGGGCAGUGAGCC	322
1295forw	AGUAAGCCUCAGUUUACUCA	59	331rev	UUUCCCCUUCAUAUCUAAGU	323
1308rev	GUUUUGAUCAUCACAUUUUU	60	332forw	AAUGAAUGGGCAGUGAGCCG	324
1332forw	AAAAUGUGAUGAUCAAAACU	61	338rev	ACCCACGCAGGAACGGCUCC	325
1335forw	UUCUUCUCUUUCUUUUGAGA	62	340forw	GGCAGUGAGCCGGGGUUGCC	326
1341rev	CCAGCUCUGGGUGACAGAGU	63	345rev	CGGGAGAACCCACGCAGGAA	327
1342rev	UCCAGCUCUGGGUGACAGAG	64	346forw	UAGUGCCUACUUAGAUAUGA	328
1353rev	GGACACUGCACUCCAGCUCU	65	347forw	AGUGCCUACUUAGAUAUGAA	329
1354forw	GAAUUAGUGUUCUGUGUCUU	66	348forw	GUGCCUACUUAGAUAUGAAG	330
1354rev	GGGACACUGCACUCCAGCUC	67	350rev	GAAGACGGGAGAACCCACGC	331
1357rev	GAAUUCACAGGAAGAUUUUA	68	356forw	UUAGAUAUGAAGGGGAAAGA	332
1358rev	GGAAUUCACAGGAAGAUUUU	69	356forw	UGCCUGGAGCCGUUCCUGCG	333
1361forw	CCCACUCUGUCACCCAGAGC	70	357forw	UAGAUAUGAAGGGGAAAGAA	334
1369rev	ACCUUAAAAAUGGAAUUCAC	71	357forw	GCCUGGAGCCGUUCCUGCGU	335
1374rev	GGUUGCAGUGAGCCAAGAUG	72	364rev	GGCAACAAAAAGCGGAAGAC	336
1375rev	AGGUUGCAGUGAGCCAAGAU	73	365rev	AGGCAACAAAAAGCGGAAGA	337
1376rev	GAGGUUGCAGUGAGCCAAGA	74	372forw	AAGAAGGGUUUGAGAUAAUG	338
1379rev	CACCUCGACUACCUUAAAAA	75	372rev	CCAUAAAAGGCAACAAAAAG	339
137rev	GCAUGUGUGAGCCGAGUCCU	76	373forw	AGAAGGGUUUGAGAUAAUGU	340
1382forw	GGAGUGCAGUGUCCCCAUCU	77	385rev	AGUUGUAAUACAACCAUAAA	341
1388forw	UCCUGUGAAUUCCAUUUUUA	78	388forw	AAUGUGGGAUGCUAAGAGAA	342
138rev	UGCAUGUGUGAGCCGAGUCC	79	391forw	GUGGGAUGCUAAGAGAAUGG	343

1395rev	GCUAGAAACCGAGGAGGCAG	80	392forw	CCGCUUUUUGUUGCCUUUUA	344
1397forw	UUCCAUUUUUAAGGUAGUCG	81	40forw	UCUGUCAGCCGCGGGUCUCU	345
1401rev	AAAAUCGCUAGAAACCGAGG	82	413rev	CUCAACAAAAUCUGCAGAGC	346
1404rev	UAUCCUCUGCAGACCAGACG	83	41forw	CUGUCAGCCGCGGGUCUCUC	347
1404rev	GAGAAAAUCGCUAGAAACCG	84	42forw	UGUCAGCCGCGGGUCUCUCG	348
1407forw	CACUGCAACCUCUGCCUCCU	85	434forw	CUGCUCUGCAGAUUUUGUUG	349
140forw	GAAAUUAAAGAUUUAAAAGC	86	439forw	UUUAGCAUCUACUCUAUGUA	350
140forw	GAGCUGUGGGACGUGCACCC	87	43forw	GUCAGCCGCGGGUCUCUCGG	351
1411forw	UAGUCGAGGUGAACCGCGUC	88	448rev	GACUGGUCGAGAUCUACCUU	352
1421forw	GAACCGCGUCUGGUCUGCAG	89	449rev	GGACUGGUCGAGAUCUACCU	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	AACUAACUUGAGGUAUCAGA	94	472forw	AGUGCAAUAGUGCUAAAAAC	358
1437rev	AAACUAACUUGAGGUAUCAG	95	472rev	UGUUCUCCCACACCCCGUUG	359
1444forw	CUCUCAGCCUCCCAAGUAGC	96	478forw	AUCUCGACCAGUCCCCUCAA	360
1445forw	UCUCAGCCUCCCAAGUAGCU	97	479forw	UCUCGACCAGUCCCCUCAAC	361
1446rev	UUAAAGGUGAAACUAACUUG	98	480forw	CUCGACCAGUCCCCUCAACG	362
1453forw	UCCCAAGUAGCUGGGUUUAC	99	485forw	CCAGUCCCCUCAACGGGGUG	363
145rev	AUCAUAACAUAGUUUCCUUA	100	486forw	CAGUCCCCUCAACGGGGUGU	364
1462rev	UUACUUCCGACCUUCUUUAA	101	488rev	CCAGGUUGUAAAGUUUUUUA	365
1462rev	AAAAAAUCAGCCGGGUAUGG	102	48forw	CCGCGGGUCUCUCGGGGGCG	366
1465rev	ACAAAAAAAAUCAGCCGGGUA	103	49forw	CGCGGGUCUCUCGGGGGCGA	367
146forw	UGGGACGUGCACCCAGGACU	104	4rev	UGACAGAGCCCAACUCUUCG	368
1470rev	AAAAUACAAAAAAAAUCAGCC	105	506rev	UUUCUUUCAUAGCAUCUGCC	369
1471rev	GAAAAUACAAAAAAAAUCAGC	106	508forw	CCGUAAAAAACUUUACAACC	370
1472forw	GUUAGUUUCACCUUUAAAGA	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	UAUUUUCAGUAAAGUUGGGC	116	561forw	UAGAAGAUCUAAAUGAACAU	380
150forw	AUUUAAAAGCAGGAGCCAUA	117	563forw	GAGAGAAGGAGGGAGAGAGA	381
1510forw	CAAAGCCUUUCCCGGACGUG	118	568forw	AAGGAGGGAGAGAGAUGGAG	382
1511forw	UUCAGUAAAGUUGGGCAGGC	119	569forw	AGGAGGGAGAGAGAUGGAGA	383
1514forw	GCCUUUCCCGGACGUGCGGA	120	574rev	CAUAAACCGAUGACCAUUAA	384

1514rev	CACCUGAGGUCAGGAGUUCG	121	57forw	AACAAGCGCUAUGACUAGCA	385
1515forw	CCUUUCCCGGACGUGCGGAA	122	581forw	UGGAAAUUGUGUUCCUUUAA	386
1523rev	CGGGCGGAUCACCUGAGGUC	123	588forw	UGUGUUCCUUUAAUGGUCAU	387
1524rev	UCCAUUUCCGGCCAUGAGGA	124	597rev	AAAAAGAAACUUCUAACCUC	388
1528rev	AAGUUCCAUUUCCGGCCAUG	125	598forw	UUUACUUUUCUUUCAGAUCG	389
1528rev	AGAAGCGGGCGGAUCACCUG	126	601forw	UGGUCAUCGGUUUAUGCCAG	390
1532forw	GGCCUCGAACUCCUGACCUC	127	602rev	CUCCGUGGAGUUGUCGCUGU	391
1533forw	AAGGGCAACGUCCUUCCUCA	128	60forw	CGGGGGCGAGGGCGAGGUUC	392
1536rev	GGAAAUUAAAGUUCCAUUUC	129	617rev	AUUCAGUUAGAUAAACUCCG	393
1537forw	GCAACGUCCUUCCUCAUGGC	130	620forw	GACCGACAGCGACAACUCCA	394
1539rev	CUUUGGGAGGCAGAAGCGGG	131	632rev	ACUGCUCAAGGUCAUCGCCA	395
1542rev	GCACUUUGGGAGGCAGAAGC	132	635forw	UUUUUUGAAAAAUUAGACCU	396
1543forw	UCCUUCCUCAUGGCCGGAAA	133	63rev	UCCUCUUCCUGCGGCCUGAA	397
1543rev	AGCACUUUGGGAGGCAGAAG	134	644rev	GGGUUAUAUCCUACUGCUCA	398
1552rev	UGUAAUCCCAGCACUUUGGG	135	655forw	UGGCGAUGACCUUGAGCAGU	399
1555rev	GCCUGUAAUCCCAGCACUUU	136	663rev	CAGUUUUACAUAUAAAUGAC	400
1556rev	CGCCUGUAAUCCCAGCACUU	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	UAUGCCUAGUGUUCCGUUAU	405
1565forw	GCUUCUGCCUCCCAAAGUGC	142	68forw	UGACUAGCAAGGUUAAGUGA	406
1565rev	UCUCUCGGGCGGGCUGGUUG	143	690forw	CAAGCUUAGCGUUCCAAUAA	407
1566forw	CUUCUGCCUCCCAAAGUGCU	144	694forw	UAUGUAAAACUGCACUAUAC	408
1566rev	CUCUCUCGGGCGGGCUGGUU	145	697rev	CCGGCCGCGAAUUUUUAUAA	409
1567rev	ACUCUCUCGGGCGGGCUGGU	146	699forw	CGUUCCAAUAACGGAACACU	410
1571rev	AGUCACUCUCUCGGGCGGGC	147	69forw	GGGCGAGGUUCAGGCCUUUC	411
1574forw	UCCCAAAGUGCUGGGAUUAC	148	713forw	CUGGCCAUUAUAAAAAUUCG	412
1575rev	UGAGAGUCACUCUCUCGGGC	149	714forw	ACACUAGGCAUAAUGAAAGA	413
1576rev	GUGAGAGUCACUCUCUCGGG	150	716rev	CAGGUAUGAGCCACCGCACC	414
1579rev	CUCGUGAGAGUCACUCUCUC	151	717forw	CCAUUAUAAAAAUUCGCGGC	415
1580rev	UCUCGUGAGAGUCACUCUCU	152	718forw	CAUUAUAAAAAUUCGCGGCC	416
1583rev	GGAUCUUAGUCCCCGCACGG	153	71rev	ACUUUAAGCCUUUCAGUCCC	417
1586rev	AAGGGAUCUUAGUCCCCGCA	154	723forw	UAAAAAUUCGCGGCCGGGUG	418
1591forw	UACAGGCGUGAGCCACCGUG	155	726forw	AAAUUCGCGGCCGGGUGCGG	419
1592forw	ACAGGCGUGAGCCACCGUGC	156	72rev	UCGCUCCGUUCCUCUUCCUG	420
1593forw	CAGGCGUGAGCCACCGUGCG	157	731rev	AGGGUUGGGGGUGGGGGGUG	421
1604rev	AUUGGCCAAGCUGACUCUCG	158	735rev	UCCCAAAGUGCUGGGAUUAC	422
1604rev	GAGUCCCCGCCCUUGCAAAA	159	736rev	CUGGGAGGGUUGGGGGUGGG	423
1605rev	GGAGUCCCCGCCCUUGCAAA	160	737rev	GCUGGGAGGGUUGGGGGUGG	424
1614forw	GGACUAAGAUCCCUUUUGCA	161	738rev	GGCUGGGAGGGUUGGGGGUG	425

1615forw	GACUAAGAUCCCUUUUGCAA	162	739rev	CGGCUGGGAGGGUUGGGGGU	426
1618forw	UAAGAUCCCUUUUGCAAGGG	163	73forw	AGCAAGGUUAAGUGAAGGCC	427
1619forw	GAGAGCCGCGAGAGUCAGCU	164	740rev	CCGGCUGGGAGGGUUGGGGG	428
1619forw	AAGAUCCCUUUUGCAAGGGC	165	743rev	CUUCGGCCUCCCAAAGUGCU	429
1620forw	AGAUCCCUUUUGCAAGGGCG	166	743rev	CUGCCGGCUGGGAGGGUUGG	430
1622rev	CGGCCGCCGACCGCACGGAU	167	744rev	GCUUCGGCCUCCCAAAGUGC	431
1626rev	AUGCACUUGUCUGUAGUUCA	168	744rev	ACUGCCGGCUGGGAGGGUUG	432
1627rev	GGGAGCGGCCGCCGACCGCA	169	745rev	GACUGCCGGCUGGGAGGGUU	433
1632forw	GUCAGCUUGGCCAAUCCGUG	170	746rev	AGACUGCCGGCUGGGAGGGU	434
1636forw	GCUUGGCCAAUCCGUGCGGU	171	74forw	GCAAGGUUAAGUGAAGGCCA	435
1639forw	UGGCCAAUCCGUGCGGUCGG	172	750rev	UGGGAGACUGCCGGCUGGGA	436
1642rev	GAGUCGGCUUAUAAAGGGAG	173	751rev	GUGGGAGACUGCCGGCUGGG	437
1647rev	CGGGCGAGUCGGCUUAUAAA	174	753forw	UACCUGUAAUCCCAGCACUU	438
1648rev	CCGGGCGAGUCGGCUUAUAA	175	754forw	ACCUGUAAUCCCAGCACUUU	439
1658rev	CGGUGCGCUGCCGGGCGAGU	176	754rev	CUUGUGGGAGACUGCCGGCU	440
1665rev	GAAGCAAAAGUACCACUAGA	177	755rev	UCUUGUGGGAGACUGCCGGC	441
1666rev	CCGCAACCCGGUGCGCUGCC	178	757forw	UGUAAUCCCAGCACUUUGGG	442
1667rev	UCCGCAACCCGGUGCGCUGC	179	759rev	CAAUUCUUGUGGGAGACUGC	443
1668forw	CCUUUAUAAGCCGACUCGCC	180	760forw	CCACCCCCAACCCUCCCAGC	444
1673forw	UUUGUUCUUACUCCAUCUAG	181	760rev	CUCAAGUGAUCCACCCGCUU	445
1678rev	CAGGCCCACCCUCCGCAACC	182	766forw	AGCACUUUGGGAGGCCGAAG	446
1679forw	CGACUCGCCCGGCAGCGCAC	183	767forw	GCACUUUGGGAGGCCGAAGC	447
1680forw	GACUCGCCCGGCAGCGCACC	184	769rev	AAAUCAGAGCCAAUUCUUGU	448
1686forw	CCCGGCAGCGCACCGGGUUG	185	76forw	GUUCAGGCCUUUCAGGCCGC	449
1689forw	GGCAGCGCACCGGGUUGCGG	186	770forw	CUUUGGGAGGCCGAAGCGGG	450
1689rev	CACCACAAAUGUUGUAAAUG	187	770rev	GAAAUCAGAGCCAAUUCUUG	451
1690forw	GCAGCGCACCGGGUUGCGGA	188	780forw	CGGCAGUCUCCCACAAGAAU	452
1693forw	GCGCACCGGGUUGCGGAGGG	189	783rev	CAGGCUGGUCUCGAACGCCA	453
1694forw	CGCACCGGGUUGCGGAGGGU	190	784rev	CCAGGCUGGUCUCGAACGCC	454
1697rev	AAAUGGCCACCACCCCUCCC	191	786forw	CGGGUGGAUCACUUGAGCCC	455
1699forw	CGGGUUGCGGAGGGUGGGCC	192	792rev	CCAUUAGCUUAUUUUCUUAA	456
1700forw	GGGUUGCGGAGGGUGGGCCU	193	798rev	UUUCACCAUGUUGCCCAGGC	457
1703forw	UUGCGGAGGGUGGGCCUGGG	194	802rev	GGGGUUUCACCAUGUUGCCC	458
1704forw	UGCGGAGGGUGGGCCUGGGA	195	804forw	CCUGGCGUUCGAGACCAGCC	459
1705forw	GCGGAGGGUGGGCCUGGGAG	196	805forw	CUGGCGUUCGAGACCAGCCU	460
1707forw	CUCCACAUUUACAACAUUUG	197	812forw	CCUUUAAGAAAAUAAGCUAA	461
1708forw	GAGGGUGGGCCUGGGAGGGG	198	813forw	CGAGACCAGCCUGGGCAACA	462
170rev	UCGGCGUUCCCCCCACCAAC	199	815rev	UUUGUUUCUUUCAACCUAGU	463
1710forw	CACAUUUACAACAUUUGUGG	200	816rev	GUUUGUUUCUUUCAACCUAG	464
1711forw	GGUGGGCCUGGGAGGGGUGG	201	821forw	AAAUAAGCUAAUGGCCCACU	465
1714rev	AGUUAGGGUUAGACAAAAAA	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	GCCUUUCAGGCCGCAGGAAG	470
1730rev	CGCCUACGCCCUUCUCAGUU	207	839forw	CUAGGUUGAAAGAAACAAAC	471
1734forw	GCAGGGCCGUGAAGCAAGAA	208	83forw	AGUGAAGGCCAGGGACUGAA	472
1737rev	AGAAAAACAUUCCCAGUCUG	209	842rev	GUCGUGAUAAGUGGGCAGAA	473
1741forw	UUGUCUAACCCUAACUGAGA	210	850rev	AUUACCUUGUCGUGAUAAGU	474
1742forw	UGUCUAACCCUAACUGAGAA	211	851rev	AAUUACCUUGUCGUGAUAAG	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	CAUGCAGUUCGCUUUCCUGU	216	866forw	AACUAGCUGGGCGUGGUGGC	480
1766rev	AGCGAGAAAAACAGCGCGCG	217	866forw	UCUGCCCACUUAUCACGACA	481
1767rev	CAGCGAGAAAAACAGCGCGC	218	871rev	UCCUGAGUAGCUGGGAUUAC	482
1768rev	UCAGCGAGAAAAACAGCGCG	219	874rev	UUGAAGGUAUGGAUUUGGGA	483
178forw	GCAGUUCGCUUUCCUGUUGG	220	878rev	GGAAUUGAAGGUAUGGAUUU	484
1798forw	UUUUUCUCGCUGACUUUCAG	221	879rev	UCUCAGCCUCCUGAGUAGCU	485
1799forw	UUUUCUCGCUGACUUUCAGC	222	879rev	AGGAAUUGAAGGUAUGGAUU	486
179forw	CAGUUCGCUUUCCUGUUGGU	223	87forw	UCAGGCCGCAGGAAGAGGAA	487
1802forw	UCUCGCUGACUUUCAGCGGG	224	880rev	GUCUCAGCCUCCUGAGUAGC	488
180forw	AGUUCGCUUUCCUGUUGGUG	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	GUUCGCUUUCCUGUUGGUGG	230	903forw	AUCCAUACCUUCAAUUCCUU	494
1820rev	CUAGAAUGAACGGUGGAAGG	231	912forw	UUCAAUUCCUUAGGAUCAUC	495
1823rev	GCUCUAGAAUGAACGGUGGA	232	913forw	UCAAUUCCUUAGGAUCAUCU	496
1827rev	GUUUGCUCUAGAAUGAACGG	233	914forw	CAAUUCCUUAGGAUCAUCUG	497
182forw	UUCGCUUUCCUGUUGGUGGG	234	915forw	AAUUCCUUAGGAUCAUCUGG	498
1830rev	UUUGUUUGCUCUAGAAUGAA	235	919rev	CACUGCAACCUCUGCCUCCC	499
1866forw	AAACAAAAAAUGUCAGCUGC	236	920rev	UCACUGCAACCUCUGCCUCC	500
1869rev	GGUCCCCGGGAGGGGCGAAC	237	921forw	ACACGAGAAUCGCUUGAACC	501
1870rev	AGGUCCCCGGGAGGGGCGAA	238	922forw	CACGAGAAUCGCUUGAACCC	502
1877rev	CCGCCGCAGGUCCCCGGGAG	239	924rev	CCCCUGGCUGCUCUCUCUCU	503
1878rev	CCCGCCGCAGGUCCCCGGGA	240	925forw	GAGAAUCGCUUGAACCCGGG	504
1879rev	ACCCGCCGCAGGUCCCCGGG	241	931forw	CGCUUGAACCCGGGAGGCAG	505
1882rev	GCGACCCGCCGCAGGUCCCC	242	940rev	GGCCUUUAUAUACACACCCC	506
1883rev	GGCGACCCGCCGCAGGUCCC	243	942forw	UGCCAAGAGAGAGAGCAGCC	507

1884forw	GCUGGCCCGUUCGCCCCUCC	244	943forw	GCCAAGAGAGAGAGCAGCCA	508
1885forw	CUGGCCCGUUCGCCCCUCCC	245	944forw	CCAAGAGAGAGAGCAGCCAG	509
1886forw	UGGCCCGUUCGCCCCUCCCG	246	944rev	GGAGUCUAGUGGCGUGAUCU	510
1890rev	CUGGGCAGGCGACCCGCCGC	247	955rev	CCAGGCUGGAUGGAGUCUAG	511
1894forw	UCGCCCCUCCCGGGGACCUG	248	958forw	AGCCAGGGGUGUGUAUAUAA	512
1897forw	CCCCUCCCGGGGACCUGCGG	249	961rev	CAGGCUAUCACCCUAAAGGU	513
1898forw	CCCUCCCGGGGACCUGCGGC	250	962rev	UCAGGCUAUCACCCUAAAGG	514
189rev	GGGUGACGGAUGCGCACGAU	251	965rev	GCUCUUUCGCCCAGGCUGGA	515
1904rev	GCGGGGUUCGGGGGCUGGGC	252	965rev	GAUUCAGGCUAUCACCCUAA	516
1908rev	CCAGGCGGGGUUCGGGGGCU	253	969rev	UCUUGCUCUUUCGCCCAGGC	517
1909rev	UCCAGGCGGGGUUCGGGGGC	254	970forw	GUAUAUAAAGGCCCACCUUU	518
1913rev	GGCCUCCAGGCGGGGUUCGG	255	971forw	UAUAUAAAGGCCCACCUUUA	519
1914rev	CGGCCUCCAGGCGGGGUUCG	256	973rev	GGAGUCUUGCUCUUUCGCCC	520
1915rev	GCGGCCUCCAGGCGGGGUUC	257	975forw	CCACUAGACUCCAUCCAGCC	521
1916rev	CGCGGCCUCCAGGCGGGGUU	258	976forw	CACUAGACUCCAUCCAGCCU	522
1921rev	CCGACCGCGGCCUCCAGGCG	259	97rev	AGGGAAUCGCGCCGCGCGCG	523
1922rev	GCCGACCGCGGCCUCCAGGC	260	980rev	ACUUUCAAUCAUCAGGAUUC	524
1923rev	GGCCGACCGCGGCCUCCAGG	261	987rev	ACUUCUGACUUUCAAUCAUC	525
1926rev	CCGGGCCGACCGCGGCCUCC	262	98rev	CAGGGAAUCGCGCCGCGCGC	526
1928forw	CCCAGCCCCCGAACCCCGCC	263	994rev	AACGAUUUUUUUUUUUGAGA	527
1931forw	AGCCCCCGAACCCCGCCUGG	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	AGUCUUGCUCUUUCGCCCAGG	584
1591rev	GCUGACUCUCGCGGCUCUCGU	530	991rev	UAACGAUUUUUUUUUUUGAGA	585
634forw	AACUCCACGGAGUUUAUCUAA	531	94rev	GCCCAACUCUUCGCGGUGGCA	586
640forw	ACGGAGUUUAUCUAACUGAAU	532	302rev	CCCCAUUGCCGGCGAGGGGUG	587
1688forw	CCGGCAGCGCACCGGGUUGCG	533	997rev	AACUACCCCCAGAUGAUCCUA	588
716forw	GGCCAUUAUAAAAAUUCGCGG	534	237rev	ACUGCAUGUGUGAGCCGAGUC	589
1608forw	AGUGACUCUCACGAGAGCCGC	535	309rev	CACAAGCCCCCAUUGCCGGCG	590
1678forw	GCCGACUCGCCCGGCAGCGCA	536	579forw	GAUCUCGACCAGUCCCCUCAA	591
1728rev	GGCGCCUACGCCCUUCUCAGU	537	110forw	GAGGCACCCACUGCCACCGCG	592
620forw	GGACCGACAGCGACAACUCCA	538	1082forw	GCCCACCUUUAGGGUGAUAGC	593

1239rev	CAGAAUCUUGUCUCGGCUCAG	539	456forw	GUUGCCUGGAGCCGUUCCUGC	594
1229rev	UCUCGGCUCAGUGGGAUGCGU	540	1391rev	AAAAAGCGAUCUUAGAUCACC	595
1584forw	CCCCCCAACCAGCCCGCCCGA	541	414forw	GCACCUCCAAAGUCGGCCAAA	596
481rev	GGUUGUAAAGUUUUUUACGGA	542	1182forw	CUAUUACCUAAGUAGGUCCCC	597
1625rev	AAGGGAGCGGCCGCCGACCGC	543	192forw	GGCCGCAGGAAGAGGAACGGA	598
1896forw	CGCCCCUCCCGGGGACCUGCG	544	875forw	UCCCAGCCGGCAGUCUCCCAC	599
1661rev	CGCAACCCGGUGCGCUGCCGG	545	1830forw	GGUGGUGCAGGGCCGUGAAGC	600
383forw	UGAGAUAAUGUGGGAUGCUAA	546	214rev	GGGUGCACGUCCCACAGCUCA	601
472forw	AAGUGCAAUAGUGCUAAAAAC	547	1724rev	CAUGCACUUGUCUGUAGUUCA	602
614rev	UAUUCAGUUAGAUAAACUCCG	548	1070forw	GUGUAUAUAAAGGCCCACCUU	603
1376rev	UCACCUCGACUACCUUAAAAA	549	337rev	CUGGCCCAGUCAGUCAGGUUU	604
166forw	CCAUAAGGAAACUAUGUUAUG	550	850rev	UUGUGGGAGACUGCCGGCUGG	605
409rev	AUAGAGUAGAUGCUAAAUGCU	551	984rev	UGAUCCUAAGGAAUUGAAGGU	606
1170rev	UCUCCAGCCUCUCCUUGAGCA	552	1086rev	UGACUUCUGACUUUCAAUCAU	607
1122rev	CUACAUUAUUAAUCUUAAGGA	553	843rev	AGACUGCCGGCUGGGAGGGUU	608
1372forw	CUUAGGCCCUAAAAUCUUCCU	554	1014forw	UUCAAUUCCUUAGGAUCAUCU	609
268rev	AAAUUCCUAUUGCUUAUAAUC	555	1348forw	UGAUUUUGCCAAGAACUUGUC	610
1836rev	GCUGACAUUUUUUGUUUGCUC	556	1703rev	AGGAGUCCCCGCCCUUGCAAA	611
183forw	UAUGAUGAAUGUGAUAGUUUG	557	244rev	AAAGCGAACUGCAUGUGUGAG	612
1260rev	CCCAGGCAGCACUGACUACAG	558	945rev	UACCUUGUCGUGAUAAGUGGG	613
1034rev	GUCUUGAUGAGGUAAAAAGAG	559	1847forw	AAGCAAGAAUGGCCACAGACU	614
1027forw	AAAAAAUCGUUACAAUUUAUG	560	1043forw	UUGCCAAGAGAGAGAGCAGCC	615
287forw	GUACUCAAGAUUAUAAGCAAU	561	50rev	GGGCCGACCGCGGCCUCCAGG	616
101rev	UUAAUUUCUCUCCUUUGCAUA	562	131forw	AAGAGUUGGGCUCUGUCAGCC	617
1201rev	CCUACCCUGCCCCCUUCUCCU	563	1217forw	AGAUACAUUUCUUAGCACUAU	618
1284forw	CUGCUGUAGUCAGUGCUGCCU	564	1763rev	AGAAGCAAAAGUACCACUAGA	619

429rev	CACUUAGCACAGUACCUUACA	565	431forw	CAAAAUGAAUGGGCAGUGAGC	620
355forw	ACUUAGAUAUGAAGGGGAAAG	566	1322forw	GCAUGGUUUUGUGGAAAAGUA	621
661rev	UGCAGUUUUACAUAUAAAUGA	567	972rev	AUUGAAGGUAUGGAUUUGGGA	622
1703forw	GUUGCGGAGGGUGGGCCUGGG	568	629forw	UGAGAGAUCAUUUAACAUUUA	623
1332forw	AAAAAUGUGAUGAUCAAAACU	569	1003forw	AAAUCCAUACCUUCAAUUCCU	624
1219forw	GGCAUUCUAAGGAGAAGGGGG	570	1244forw	UAAGAAAUGUAAAAAAACCUC	625
372forw	AAAGAAGGGUUUGAGAUAAUG	571	836rev	CGGCUGGGAGGGUUGGGGGUG	626
192forw	UGUGAUAGUUUGGAGAAUAAA	572	1462forw	UCCCACUCUGUCACCCAGAGC	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	UUUCUUCUCUUUCUUUUGAGA	632
820rev	UUUUGUGUUUUUAGUAGAGAC	578	1453rev	UGGGGACACUGCACUCCAGCU	633
877rev	UGUCUCAGCCUCCUGAGUAGC	579	1544forw	UUCUCUCAGCCUCCCAAGUAG	634
886rev	CGAUUCUCGUGUCUCAGCCUC	580	1570rev	CUGAAAAUACAAAAAAAUCAG	635
905forw	CUACUCAGGAGGCUGAGACAC	581	1621rev	GCGGGCGGAUCACCUGAGGUC	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 nulceotides 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	UGUUCAUAAAUUUACUGACAUGG	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	CCUGGAGGCCGCGGUCGGCCCGG	908
103rev	AAUUUCUCUCCUUUGCAUAUUGG	645	1947forw	CUGGAGGCCGCGGUCGGCCCGGG	909
1049rev	AGUAGUGCUGUGUCUUGAUGAGG	646	1948forw	UGGAGGCCGCGGUCGGCCCGGGG	910
1059rev	AGGGGACCUACUUAGGUAAUAGG	647	1956rev	GCGGUGGCAGUGGGUGCCUCCGG	911
1066rev	CAAUUCCAGGGGACCUACUUAGG	648	1957forw	CGGUCGGCCCGGGGCUUCUCCGG	912
106forw	ACGGAGCGAGUCCCCGCGCGCGG	649	1960forw	UCGGCCCGGGGCUUCUCCGGAGG	913
1073forw	UUUUAACCUAUUACCUAAGUAGG	650	1965rev	CAACUCUUCGCGGUGGCAGUGGG	914
1077rev	UAUCUGCUAGACAAUUCCAGGGG	651	1966rev	CCAACUCUUCGCGGUGGCAGUGG	915
1078rev	GUAUCUGCUAGACAAUUCCAGGG	652	1986forw	CCACUGCCACCGCGAAGAGUUGG	916
1079rev	UGUAUCUGCUAGACAAUUCCAGG	653	1987forw	CACUGCCACCGCGAAGAGUUGGG	917
1081forw	UAUUACCUAAGUAGGUCCCCUGG	654	199forw	UUUGGAGAAUAAAUUGAAUGAGG	918
1098rev	UCCCUUUUAUUAGGAAAGAAAGG	655	1rev	CAGAGCCCAACUCUUCGCGGUGG	919
1107rev	GACUGAAUCUCCCUUUUAUUAGG	656	203forw	GAGAAUAAAUUGAAUGAGGAAGG	920
1116forw	CGCCUUUCUUUCCUAAUAAAAGG	657	203rev	CCAUUGCCGGCGAGGGGUGACGG	921
1117forw	GCCUUUCUUUCCUAAUAAAAGGG	658	206rev	AACUGAUCACCAAAUCUCCAGGG	922
1129rev	CUACUACAUUAUUAAUCUUAAGG	659	207rev	UAACUGAUCACCAAAUCUCCAGG	923
1139rev	CCAGCAACAGUGGACUCUAGAGG	660	209forw	AAAUUGAAUGAGGAAGGCCCUGG	924
1149rev	GAGAACAUUACCAGCAACAGUGG	661	209rev	AAGCCCCCAUUGCCGGCGAGGGG	925
114forw	GCUAAAUAUCCAAUAUGCAAAGG	662	210rev	CAAGCCCCCAUUGCCGGCGAGGG	926
1159forw	CCUCUAGAGUCCACUGUUGCUGG	663	211rev	ACAAGCCCCCAUUGCCGGCGAGG	927
1168rev	GCCUCUCCUUGAGCAGAGGAUGG	664	216rev	GGUUCACAAGCCCCCAUUGCCGG	928
116rev	GGUGCACGUCCCACAGCUCAGGG	665	217forw	UGAGGAAGGCCCUGGAGAUUUGG	929
1172rev	UCCAGCCUCUCCUUGAGCAGAGG	666	217forw	GCGCAUCCGUCACCCCUCGCCGG	930
1178forw	CUGGUAAUGUUCUCUAAAUAAGG	667	223forw	CCGUCACCCCUCGCCGGCAAUGG	931
117rev	GGGUGCACGUCCCACAGCUCAGG	668	224forw	CGUCACCCCUCGCCGGCAAUGGG	932
1182forw	UUAAAGCCAUCCUCUGCUCAAGG	669	225forw	GUCACCCCUCGCCGGCAAUGGGG	933
1187forw	GCCAUCCUCUGCUCAAGGAGAGG	670	226forw	UCACCCCUCGCCGGCAAUGGGGG	934
1191forw	UCCUCUGCUCAAGGAGAGGCUGG	671	237rev	GCCCAGUCAGUCAGGUUUGGGGG	935
1193rev	UUCCACAAAACCAUGCUGAUAGG	672	238rev	GGCCCAGUCAGUCAGGUUUGGGG	936
1197forw	GCUCAAGGAGAGGCUGGAGAAGG	673	239rev	UGGCCCAGUCAGUCAGGUUUGGG	937
1203forw	AAAUAUUUUUCCUAUCAGCAUGG	674	240rev	CUGGCCCAGUCAGUCAGGUUUGG	938
1207forw	AGGCUGGAGAAGGCAUUCUAAGG	675	243rev	AAGACUUGGCACUUUAUAUGUGG	939
1211forw	UUCCUAUCAGCAUGGUUUUGUGG	676	245rev	GCACACUGGCCCAGUCAGUCAGG	940
1213forw	GAGAAGGCAUUCUAAGGAGAAGG	677	255forw	AACCCCCAAACCUGACUGACUGG	941
1214forw	AGAAGGCAUUCUAAGGAGAAGGG	678	256forw	ACCCCCAAACCUGACUGACUGGG	942
1215forw	GAAGGCAUUCUAAGGAGAAGGGG	679	257rev	AUAAUCUUGAGUACAAGACUUGG	943
1216forw	AAGGCAUUCUAAGGAGAAGGGGG	680	259rev	CCUGCCAAUUUGCAGCACACUGG	944
1220forw	CAUUCUAAGGAGAAGGGGGCAGG	681	275forw	UGGGCCAGUGUGCUGCAAAUUGG	945

1221forw	AUUCUAAGGAGAAGGGGGCAGGG	682	279forw	CCAGUGUGCUGCAAAUUGGCAGG	946
1221forw	CAUGGUUUUGUGGAAAAGUAAGG	683	287forw	UACUCAAGAUUAUAAGCAAUAGG	947
1225forw	UAAGGAGAAGGGGGCAGGGUAGG	684	28rev	CCUCGCCCCCGAGAGACCCGCGG	948
1232forw	AAGGGGGCAGGGUAGGAACUCGG	685	290forw	CAAAUUGGCAGGAGACGUGAAGG	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	CCAAAGUCGGCCAAAAUGAAUGG	954
126forw	CGGCGCGAUUCCCUGAGCUGUGG	691	319forw	CAAAGUCGGCCAAAAUGAAUGGG	955
127forw	GGCGCGAUUCCCUGAGCUGUGGG	692	31forw	GAGUUGGGCUCUGUCAGCCGCGG	956
1281rev	CUUUGUGAAAAUAGAUUCCCAGG	693	329rev	GGAACGGCUCCAGGCAACCCCGG	957
1281rev	AGAUCACCUUGAGUAAACUGAGG	694	32forw	AGUUGGGCUCUGUCAGCCGCGGG	958
1283forw	CUGCUGUAGUCAGUGCUGCCUGG	695	330forw	AAAAUGAAUGGGCAGUGAGCCGG	959
1284forw	UGCUGUAGUCAGUGCUGCCUGGG	696	331forw	AAAUGAAUGGGCAGUGAGCCGGG	960
1295forw	AGUAAGCCUCAGUUUACUCAAGG	697	331rev	UUUCCCCUUCAUAUCUAAGUAGG	961
1308rev	GUUUUGAUCAUCACAUUUUUUGG	698	332forw	AAUGAAUGGGCAGUGAGCCGGGG	962
1332forw	AAAAUGUGAUGAUCAAAACUAGG	699	338rev	ACCCACGCAGGAACGGCUCCAGG	963
1335forw	UUCUUCUCUUUCUUUUGAGACGG	700	340forw	GGCAGUGAGCCGGGGUUGCCUGG	964
1341rev	CCAGCUCUGGGUGACAGAGUGGG	701	345rev	CGGGAGAACCCACGCAGGAACGG	965
1342rev	UCCAGCUCUGGGUGACAGAGUGG	702	346forw	UAGUGCCUACUUAGAUAUGAAGG	966
1353rev	GGACACUGCACUCCAGCUCUGGG	703	347forw	AGUGCCUACUUAGAUAUGAAGGG	967
1354forw	GAAUUAGUGUUCUGUGUCUUAGG	704	348forw	GUGCCUACUUAGAUAUGAAGGGG	968
1354rev	GGGACACUGCACUCCAGCUCUGG	705	350rev	GAAGACGGGAGAACCCACGCAGG	969
1357rev	GAAUUCACAGGAAGAUUUUAGGG	706	356forw	UUAGAUAUGAAGGGGAAAGAAGG	970
1358rev	GGAAUUCACAGGAAGAUUUUAGG	707	356forw	UGCCUGGAGCCGUUCCUGCGUGG	971
1361forw	CCCACUCUGUCACCCAGAGCUGG	708	357forw	UAGAUAUGAAGGGGAAAGAAGGG	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	CACCUCGACUACCUUAAAAAUGG	713	372rev	CCAUAAAAGGCAACAAAAAGCGG	977
137rev	GCAUGUGUGAGCCGAGUCCUGGG	714	373forw	AGAAGGGUUUGAGAUAAUGUGGG	978
1382forw	GGAGUGCAGUGUCCCCAUCUUGG	715	385rev	AGUUGUAAUACAACCAUAAAAGG	979
1388forw	UCCUGUGAAUUCCAUUUUUAAGG	716	388forw	AAUGUGGGAUGCUAAGAGAAUGG	980
138rev	UGCAUGUGUGAGCCGAGUCCUGG	717	391forw	GUGGGAUGCUAAGAGAAUGGUGG	981
1395rev	GCUAGAAACCGAGGAGGCAGAGG	718	392forw	CCGCUUUUUGUUGCCUUUUAUGG	982
1397forw	UUCCAUUUUUAAGGUAGUCGAGG	719	40forw	UCUGUCAGCCGCGGGUCUCUCGG	983
1401rev	AAAAUCGCUAGAAACCGAGGAGG	720	413rev	CUCAACAAAAUCUGCAGAGCAGG	984
1404rev	UAUCCUCUGCAGACCAGACGCGG	721	41forw	CUGUCAGCCGCGGGUCUCUCGGG	985
1404rev	GAGAAAAUCGCUAGAAACCGAGG	722	42forw	UGUCAGCCGCGGGUCUCUCGGGG	986

1407forw	CACUGCAACCUCUGCCUCCUCGG	723	434forw	CUGCUCUGCAGAUUUUGUUGAGG	987
140forw	GAAAUUAAAGAUUUAAAAGCAGG	724	439forw	UUUAGCAUCUACUCUAUGUAAGG	988
140forw	GAGCUGUGGGACGUGCACCCAGG	725	43forw	GUCAGCCGCGGGUCUCUCGGGGG	989
1411forw	UAGUCGAGGUGAACCGCGUCUGG	726	448rev	GACUGGUCGAGAUCUACCUUGGG	990
1421forw	GAACCGCGUCUGGUCUGCAGAGG	727	449rev	GGACUGGUCGAGAUCUACCUUGG	991
1431rev	UGUAAACCCAGCUACUUGGGAGG	728	452forw	UGAGGUUUUUGCUUCUCCCAAGG	992
1433forw	GUCUGCAGAGGAUAGAAAAAAGG	729	465rev	CCACACCCCGUUGAGGGGACUGG	993
1434rev	GCCUGUAAACCCAGCUACUUGGG	730	470rev	UUCUCCCACACCCCGUUGAGGGG	994
1435rev	UGCCUGUAAACCCAGCUACUUGG	731	471rev	GUUCUCCCACACCCCGUUGAGGG	995
1436rev	AACUAACUUGAGGUAUCAGAGGG	732	472forw	AGUGCAAUAGUGCUAAAAACAGG	996
1437rev	AAACUAACUUGAGGUAUCAGAGG	733	472rev	UGUUCUCCCACACCCCGUUGAGG	997
1444forw	CUCUCAGCCUCCCAAGUAGCUGG	734	478forw	AUCUCGACCAGUCCCCUCAACGG	998
1445forw	UCUCAGCCUCCCAAGUAGCUGGG	735	479forw	UCUCGACCAGUCCCCUCAACGGG	999
1446rev	UUAAAGGUGAAACUAACUUGAGG	736	480forw	CUCGACCAGUCCCCUCAACGGGG	1000
1453forw	UCCCAAGUAGCUGGGUUUACAGG	737	485forw	CCAGUCCCCUCAACGGGGUGUGG	1001
145rev	AUCAUAACAUAGUUUCCUUAUGG	738	486forw	CAGUCCCCUCAACGGGGUGUGGG	1002
1462rev	UUACUUCCGACCUUCUUUAAAGG	739	488rev	CCAGGUUGUAAAGUUUUUUACGG	1003
1462rev	AAAAAAUCAGCCGGGUAUGGUGG	740	48forw	CCGCGGGUCUCUCGGGGGCGAGG	1004
1465rev	ACAAAAAAAAUCAGCCGGGUAUGG	741	49forw	CGCGGGUCUCUCGGGGGCGAGGG	1005
146forw	UGGGACGUGCACCCAGGACUCGG	742	4rev	UGACAGAGCCCAACUCUUCGCGG	1006
1470rev	AAAAUACAAAAAAAUCAGCCGGG	743	506rev	UUUCUUUCAUAGCAUCUGCCAGG	1007
1471rev	GAAAAUACAAAAAAAUCAGCCGG	744	508forw	CCGUAAAAAACUUUACAACCUGG	1008
1472forw	GUUAGUUUCACCUUUAAAGAAGG	745	530forw	GCAGAUGCUAUGAAAGAAAAAGG	1009
1472forw	CAGGCACACACCACCAUACCCGG	746	531forw	CAGAUGCUAUGAAAGAAAAAGGG	1010
1476forw	GUUUCACCUUUAAAGAAGGUCGG	747	532forw	AGAUGCUAUGAAAGAAAAAGGGG	1011
1495rev	CCCUUCCGCACGUCCGGGAAAGG	748	536forw	GCUAUGAAAGAAAAAGGGGAUGG	1012
1500rev	CGUUGCCCUUCCGCACGUCCGGG	749	537forw	CUAUGAAAGAAAAAGGGGAUGGG	1013
1501rev	ACGUUGCCCUUCCGCACGUCCGG	750	549forw	AAGGGGAUGGGAGAGAGAGAAGG	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	AUUUAAAAGCAGGAGCCAUAAGG	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	UGGAAAUUGUGUUCCUUUAAUGG	1024
1523rev	CGGGCGGAUCACCUGAGGUCAGG	761	588forw	UGUGUUCCUUUAAUGGUCAUCGG	1025
1524rev	UCCAUUUCCGGCCAUGAGGAAGG	762	597rev	AAAAAGAAACUUCUAACCUCUGG	1026
1528rev	AAGUUCCAUUUCCGGCCAUGAGG	763	598forw	UUUACUUUUCUUUCAGAUCGAGG	1027

1528rev	AGAAGCGGGCGGAUCACCUGAGG	764	601forw	UGGUCAUCGGUUUAUGCCAGAGG	1028
1532forw	GGCCUCGAACUCCUGACCUCAGG	765	602rev	CUCCGUGGAGUUGUCGCUGUCGG	1029
1533forw	AAGGGCAACGUCCUUCCUCAUGG	766	60forw	CGGGGGCGAGGGCGAGGUUCAGG	1030
1536rev	GGAAAUUAAAGUUCCAUUUCCGG	767	617rev	AUUCAGUUAGAUAAACUCCGUGG	1031
1537forw	GCAACGUCCUUCCUCAUGGCCGG	768	620forw	GACCGACAGCGACAACUCCACGG	1032
1539rev	CUUUGGGAGGCAGAAGCGGGCGG	769	632rev	ACUGCUCAAGGUCAUCGCCAAGG	1033
1542rev	GCACUUUGGGAGGCAGAAGCGGG	770	635forw	UUUUUUGAAAAAUUAGACCUUGG	1034
1543forw	UCCUUCCUCAUGGCCGGAAAUGG	771	63rev	UCCUCUUCCUGCGGCCUGAAAGG	1035
1543rev	AGCACUUUGGGAGGCAGAAGCGG	772	644rev	GGGUUAUAUCCUACUGCUCAAGG	1036
1552rev	UGUAAUCCCAGCACUUUGGGAGG	773	655forw	UGGCGAUGACCUUGAGCAGUAGG	1037
1555rev	GCCUGUAAUCCCAGCACUUUGGG	774	663rev	CAGUUUUACAUAUAAAUGACAGG	1038
1556rev	CGCCUGUAAUCCCAGCACUUUGG	775	664rev	UUGGAACGCUAAGCUUGUGGGGGG	1039
1557rev	GCGGGCUGGUUGGGGGGGAACGGG	776	665rev	AUUGGAACGCUAAGCUUGUGGGGG	1040
1558rev	GGCGGGCUGGUUGGGGGGAACGG	777	666rev	UAUUGGAACGCUAAGCUUGUGGGG	1041
1563rev	UCUCGGGCGGGCUGGUUGGGGGG	778	667rev	UUAUUGGAACGCUAAGCUUGUGG	1042
1564rev	CUCUCGGGCGGGCUGGUUGGGGG	779	683rev	UAUGCCUAGUGUUCCGUUAUUGG	1043
1565forw	GCUUCUGCCUCCCAAAGUGCUGG	780	68forw	UGACUAGCAAGGUUAAGUGAAGG	1044
1565rev	UCUCUCGGGCGGGCUGGUUGGGG	781	690forw	CAAGCUUAGCGUUCCAAUAACGG	1045
1566forw	CUUCUGCCUCCCAAAGUGCUGGG	782	694forw	UAUGUAAAACUGCACUAUACUGG	1046
1566rev	CUCUCUCGGGCGGGCUGGUUGGG	783	697rev	CCGGCCGCGAAUUUUUAUAAUGG	1047
1567rev	ACUCUCUCGGGCGGGCUGGUUGG	784	699forw	CGUUCCAAUAACGGAACACUAGG	1048
1571rev	AGUCACUCUCUCGGGCGGGCUGG	785	69forw	GGGCGAGGUUCAGGCCUUUCAGG	1049
1574forw	UCCCAAAGUGCUGGGAUUACAGG	786	713forw	CUGGCCAUUAUAAAAAUUCGCGG	1050
1575rev	UGAGAGUCACUCUCUCGGGCGGG	787	714forw	ACACUAGGCAUAAUGAAAGACGG	1051
1576rev	GUGAGAGUCACUCUCUCGGGCGG	788	716rev	CAGGUAUGAGCCACCGCACCCGG	1052
1579rev	CUCGUGAGAGUCACUCUCUCGGG	789	717forw	CCAUUAUAAAAAUUCGCGGCCGG	1053
1580rev	UCUCGUGAGAGUCACUCUCUCGG	790	718forw	CAUUAUAAAAAUUCGCGGCCGGG	1054
1583rev	GGAUCUUAGUCCCCGCACGGUGG	791	71rev	ACUUUAAGCCUUUCAGUCCCUGG	1055
1586rev	AAGGGAUCUUAGUCCCCGCACGG	792	723forw	UAAAAAUUCGCGGCCGGGUGCGG	1056
1591forw	UACAGGCGUGAGCCACCGUGCGG	793	726forw	AAAUUCGCGGCCGGGUGCGGUGG	1057
1592forw	ACAGGCGUGAGCCACCGUGCGGG	794	72rev	UCGCUCCGUUCCUCUUCCUGCGG	1058
1593forw	CAGGCGUGAGCCACCGUGCGGGG	795	731rev	AGGGUUGGGGGUGGGGGGUGUGG	1059
1604rev	AUUGGCCAAGCUGACUCUCGCGG	796	735rev	UCCCAAAGUGCUGGGAUUACAGG	1060
1604rev	GAGUCCCCGCCCUUGCAAAAGGG	797	736rev	CUGGGAGGGUUGGGGGUGGGGGG	1061
1605rev	GGAGUCCCCGCCCUUGCAAAAGG	798	737rev	GCUGGGAGGGUUGGGGGUGGGGG	1062
1614forw	GGACUAAGAUCCCUUUUGCAAGG	799	738rev	GGCUGGGAGGGUUGGGGGUGGGG	1063
1615forw	GACUAAGAUCCCUUUUGCAAGGG	800	739rev	CGGCUGGGAGGGUUGGGGGUGGG	1064
1618forw	UAAGAUCCCUUUUGCAAGGGCGG	801	73forw	AGCAAGGUUAAGUGAAGGCCAGG	1065
1619forw	GAGAGCCGCGAGAGUCAGCUUGG	802	740rev	CCGGCUGGGAGGGUUGGGGGUGG	1066
1619forw	AAGAUCCCUUUUGCAAGGGCGGG	803	743rev	CUUCGGCCUCCCAAAGUGCUGGG	1067
1620forw	AGAUCCCUUUUGCAAGGGCGGGG	804	743rev	CUGCCGGCUGGGAGGGUUGGGGG	1068

1622rev	CGGCCGCCGACCGCACGGAUUGG	805	744rev	GCUUCGGCCUCCCAAAGUGCUGG	1069
1626rev	AUGCACUUGUCUGUAGUUCAAGG	806	744rev	ACUGCCGGCUGGGAGGGUUGGGG	1070
1627rev	GGGAGCGGCCGCCGACCGCACGG	807	745rev	GACUGCCGGCUGGGAGGGUUGGG	1071
1632forw	GUCAGCUUGGCCAAUCCGUGCGG	808	746rev	AGACUGCCGGCUGGGAGGGUUGG	1072
1636forw	GCUUGGCCAAUCCGUGCGGUCGG	809	74forw	GCAAGGUUAAGUGAAGGCCAGGG	1073
1639forw	UGGCCAAUCCGUGCGGUCGGCGG	810	750rev	UGGGAGACUGCCGGCUGGGAGGG	1074
1642rev	GAGUCGGCUUAUAAAGGGAGCGG	811	751rev	GUGGGAGACUGCCGGCUGGGAGG	1075
1647rev	CGGGCGAGUCGGCUUAUAAAGGG	812	753forw	UACCUGUAAUCCCAGCACUUUGG	1076
1648rev	CCGGGCGAGUCGGCUUAUAAAGG	813	754forw	ACCUGUAAUCCCAGCACUUUGGG	1077
1658rev	CGGUGCGCUGCCGGGCGAGUCGG	814	754rev	CUUGUGGGAGACUGCCGGCUGGG	1078
1665rev	GAAGCAAAAGUACCACUAGAUGG	815	755rev	UCUUGUGGGAGACUGCCGGCUGG	1079
1666rev	CCGCAACCCGGUGCGCUGCCGGG	816	757forw	UGUAAUCCCAGCACUUUGGGAGG	1080
1667rev	UCCGCAACCCGGUGCGCUGCCGG	817	759rev	CAAUUCUUGUGGGAGACUGCCGG	1081
1668forw	CCUUUAUAAGCCGACUCGCCCGG	818	760forw	CCACCCCCAACCCUCCCAGCCGG	1082
1673forw	UUUGUUCUUACUCCAUCUAGUGG	819	760rev	CUCAAGUGAUCCACCCGCUUCGG	1083
1678rev	CAGGCCCACCCUCCGCAACCCGG	820	766forw	AGCACUUUGGGAGGCCGAAGCGG	1084
1679forw	CGACUCGCCCGGCAGCGCACCGG	821	767forw	GCACUUUGGGAGGCCGAAGCGGG	1085
1680forw	GACUCGCCCGGCAGCGCACCGGG	822	769rev	AAAUCAGAGCCAAUUCUUGUGGG	1086
1686forw	CCCGGCAGCGCACCGGGUUGCGG	823	76forw	GUUCAGGCCUUUCAGGCCGCAGG	1087
1689forw	GGCAGCGCACCGGGUUGCGGAGG	824	770forw	CUUUGGGAGGCCGAAGCGGGUGG	1088
1689rev	CACCACAAAUGUUGUAAAUGUGG	825	770rev	GAAAUCAGAGCCAAUUCUUGUGG	1089
1690forw	GCAGCGCACCGGGUUGCGGAGGG	826	780forw	CGGCAGUCUCCCACAAGAAUUGG	1090
1693forw	GCGCACCGGGUUGCGGAGGGUGG	827	783rev	CAGGCUGGUCUCGAACGCCAGGG	1091
1694forw	CGCACCGGGUUGCGGAGGGUGGG	828	784rev	CCAGGCUGGUCUCGAACGCCAGG	1092
1697rev	AAAUGGCCACCACCCCUCCCAGG	829	786forw	CGGGUGGAUCACUUGAGCCCUGG	1093
1699forw	CGGGUUGCGGAGGGUGGGCCUGG	830	792rev	CCAUUAGCUUAUUUUCUUAAAGG	1094
1700forw	GGGUUGCGGAGGGUGGGCCUGGG	831	798rev	UUUCACCAUGUUGCCCAGGCUGG	1095
1703forw	UUGCGGAGGGUGGGCCUGGGAGG	832	802rev	GGGGUUUCACCAUGUUGCCCAGG	1096
1704forw	UGCGGAGGGUGGGCCUGGGAGGG	833	804forw	CCUGGCGUUCGAGACCAGCCUGG	1097
1705forw	GCGGAGGGUGGGCCUGGGAGGGG	834	805forw	CUGGCGUUCGAGACCAGCCUGGG	1098
1707forw	CUCCACAUUUACAACAUUUGUGG	835	812forw	CCUUUAAGAAAAUAAGCUAAUGG	1099
1708forw	GAGGGUGGGCCUGGGAGGGGUGG	836	813forw	CGAGACCAGCCUGGGCAACAUGG	1100
170rev	UCGGCGUUCCCCCCACCAACAGG	837	815rev	UUUGUUUCUUUCAACCUAGUGGG	1101
1710forw	CACAUUUACAACAUUUGUGGUGG	838	816rev	GUUUGUUUCUUUCAACCUAGUGG	1102
1711forw	GGUGGGCCUGGGAGGGGUGGUGG	839	821forw	AAAUAAGCUAAUGGCCCACUAGG	1103
1714rev	AGUUAGGGUUAGACAAAAAAUGG	840	821rev	UGUGUUUUUAGUAGAGACGGGGG	1104
1716forw	UACAACAUUUGUGGUGGUGCAGG	841	822rev	UUGUGUUUUUAGUAGAGACGGGG	1105
1717forw	ACAACAUUUGUGGUGGUGCAGGG	842	823rev	UUUGUGUUUUUAGUAGAGACGGG	1106
1720rev	CUGUGGCCAUUCUUGCUUCACGG	843	824rev	UUUUGUGUUUUUAGUAGAGACGG	1107
1729rev	GCCUACGCCCUUCUCAGUUAGGG	844	82forw	GCCUUUCAGGCCGCAGGAAGAGG	1108
1730rev	CGCCUACGCCCUUCUCAGUUAGG	845	839forw	CUAGGUUGAAAGAAACAAACAGG	1109

1734forw	GCAGGGCCGUGAAGCAAGAAUGG	846	83forw	AGUGAAGGCCAGGGACUGAAAGG	1110
1737rev	AGAAAAACAUUCCCAGUCUGUGG	847	842rev	GUCGUGAUAAGUGGGCAGAAUGG	1111
1741forw	UUGUCUAACCCUAACUGAGAAGG	848	850rev	AUUACCUUGUCGUGAUAAGUGGG	1112
1742forw	UGUCUAACCCUAACUGAGAAGGG	849	851rev	AAUUACCUUGUCGUGAUAAGUGG	1113
1745forw	AAGCAAGAAUGGCCACAGACUGG	850	853forw	CUAAAAACACAAAAACUAGCUGG	1114
1746forw	AGCAAGAAUGGCCACAGACUGGG	851	854forw	UAAAAACACAAAAACUAGCUGGG	1115
1748forw	ACCCUAACUGAGAAGGGCGUAGG	852	859forw	ACACAAAAACUAGCUGGGCGUGG	1116
1753rev	GCGCGCGGGGAGCAAAAGCACGG	853	862forw	CAAAAACUAGCUGGGCGUGGUGG	1117
175forw	CAUGCAGUUCGCUUUCCUGUUGG	854	866forw	AACUAGCUGGGCGUGGUGGCAGG	1118
1766rev	AGCGAGAAAAACAGCGCGCGGGG	855	866forw	UCUGCCCACUUAUCACGACAAGG	1119
1767rev	CAGCGAGAAAAACAGCGCGCGGG	856	871rev	UCCUGAGUAGCUGGGAUUACAGG	1120
1768rev	UCAGCGAGAAAAACAGCGCGCGG	857	874rev	UUGAAGGUAUGGAUUUGGGACGG	1121
178forw	GCAGUUCGCUUUCCUGUUGGUGG	858	878rev	GGAAUUGAAGGUAUGGAUUUGGG	1122
1798forw	UUUUUCUCGCUGACUUUCAGCGG	859	879rev	UCUCAGCCUCCUGAGUAGCUGGG	1123
1799forw	UUUUCUCGCUGACUUUCAGCGGG	860	879rev	AGGAAUUGAAGGUAUGGAUUUGG	1124
179forw	CAGUUCGCUUUCCUGUUGGUGGG	861	87forw	UCAGGCCGCAGGAAGAGGAACGG	1125
1802forw	UCUCGCUGACUUUCAGCGGGCGG	862	880rev	GUCUCAGCCUCCUGAGUAGCUGG	1126
180forw	AGUUCGCUUUCCUGUUGGUGGGG	863	885rev	AUCCUAAGGAAUUGAAGGUAUGG	1127
1810rev	CGGUGGAAGGCGGCAGGCCGAGG	864	890forw	GCCUGUAAUCCCAGCUACUCAGG	1128
1813forw	UUCAGCGGGCGGAAAAGCCUCGG	865	890rev	AGAUGAUCCUAAGGAAUUGAAGG	1129
1816rev	AAUGAACGGUGGAAGGCGGCAGG	866	893forw	UGUAAUCCCAGCUACUCAGGAGG	1130
181forw	UUAUGAUGAAUGUGAUAGUUUGG	867	899rev	ACUACCCCCAGAUGAUCCUAAGG	1131
181forw	GUUCGCUUUCCUGUUGGUGGGGG	868	903forw	AUCCAUACCUUCAAUUCCUUAGG	1132
1820rev	CUAGAAUGAACGGUGGAAGGCGG	869	912forw	UUCAAUUCCUUAGGAUCAUCUGG	1133
1823rev	GCUCUAGAAUGAACGGUGGAAGG	870	913forw	UCAAUUCCUUAGGAUCAUCUGGG	1134
1827rev	GUUUGCUCUAGAAUGAACGGUGG	871	914forw	CAAUUCCUUAGGAUCAUCUGGGG	1135
182forw	UUCGCUUUCCUGUUGGUGGGGGG	872	915forw	AAUUCCUUAGGAUCAUCUGGGGG	1136
1830rev	UUUGUUUGCUCUAGAAUGAACGG	873	919rev	CACUGCAACCUCUGCCUCCCGGG	1137
1866forw	AAACAAAAAAUGUCAGCUGCUGG	874	920rev	UCACUGCAACCUCUGCCUCCCGG	1138
1869rev	GGUCCCCGGGAGGGGCGAACGGG	875	921forw	ACACGAGAAUCGCUUGAACCCGG	1139
1870rev	AGGUCCCCGGGAGGGGCGAACGG	876	922forw	CACGAGAAUCGCUUGAACCCGGG	1140
1877rev	CCGCCGCAGGUCCCCGGGAGGGG	877	924rev	CCCCUGGCUGCUCUCUCUCUUGG	1141
1878rev	CCCGCCGCAGGUCCCCGGGAGGG	878	925forw	GAGAAUCGCUUGAACCCGGGAGG	1142
1879rev	ACCCGCCGCAGGUCCCCGGGAGG	879	931forw	CGCUUGAACCCGGGAGGCAGAGG	1143
1882rev	GCGACCCGCCGCAGGUCCCCGGG	880	940rev	GGCCUUUAUAUACACACCCCUGG	1144
1883rev	GGCGACCCGCCGCAGGUCCCCGG	881	942forw	UGCCAAGAGAGAGAGCAGCCAGG	1145
1884forw	GCUGGCCCGUUCGCCCCUCCCGG	882	943forw	GCCAAGAGAGAGAGCAGCCAGGG	1146
1885forw	CUGGCCCGUUCGCCCCUCCCGGG	883	944forw	CCAAGAGAGAGAGCAGCCAGGGG	1147
1886forw	UGGCCCGUUCGCCCCUCCCGGGG	884	944rev	GGAGUCUAGUGGCGUGAUCUCGG	1148
1890rev	CUGGGCAGGCGACCCGCCGCAGG	885	955rev	CCAGGCUGGAUGGAGUCUAGUGG	1149
1894forw	UCGCCCCUCCCGGGGACCUGCGG	886	958forw	AGCCAGGGGUGUGUAUAUAAAGG	1150

1897forw	CCCCUCCCGGGGACCUGCGGCGG	887	961rev	CAGGCUAUCACCCUAAAGGUGGG	1151
1898forw	CCCUCCCGGGGACCUGCGGCGGG	888	962rev	UCAGGCUAUCACCCUAAAGGUGG	1152
189rev	GGGUGACGGAUGCGCACGAUCGG	889	965rev	GCUCUUUCGCCCAGGCUGGAUGG	1153
1904rev	GCGGGGUUCGGGGGCUGGGCAGG	890	965rev	GAUUCAGGCUAUCACCCUAAAGG	1154
1908rev	CCAGGCGGGGUUCGGGGGCUGGG	891	969rev	UCUUGCUCUUUCGCCCAGGCUGG	1155
1909rev	UCCAGGCGGGGUUCGGGGGCUGG	892	970forw	GUAUAUAAAGGCCCACCUUUAGG	1156
1913rev	GGCCUCCAGGCGGGGUUCGGGGG	893	971forw	UAUAUAAAGGCCCACCUUUAGGG	1157
1914rev	CGGCCUCCAGGCGGGGUUCGGGG	894	973rev	GGAGUCUUGCUCUUUCGCCCAGG	1158
1915rev	GCGGCCUCCAGGCGGGGUUCGGG	895	975forw	CCACUAGACUCCAUCCAGCCUGG	1159
1916rev	CGCGGCCUCCAGGCGGGGUUCGG	896	976forw	CACUAGACUCCAUCCAGCCUGGG	1160
1921rev	CCGACCGCGGCCUCCAGGCGGGG	897	97rev	AGGGAAUCGCGCCGCGCGCGGGG	1161
1922rev	GCCGACCGCGGCCUCCAGGCGGG	898	980rev	ACUUUCAAUCAUCAGGAUUCAGG	1162
1923rev	GGCCGACCGCGGCCUCCAGGCGG	899	987rev	ACUUCUGACUUUCAAUCAUCAGG	1163
1926rev	CCGGGCCGACCGCGGCCUCCAGG	900	98rev	CAGGGAAUCGCGCCGCGCGCGGG	1164
1928forw	CCCAGCCCCCGAACCCCGCCUGG	901	994rev	AACGAUUUUUUUUUUUGAGACGG	1165
1931forw	AGCCCCCGAACCCCGCCUGGAGG	902	99rev	UCAGGGAAUCGCGCCGCGCGCGG	1166

Table 4. Guide RNA sequences

Seq Name	Sequence	Seq ID NO	Seq Name	Sequence	Seq ID NO
1420forw	GUGAACCGCGUCUGGUCUGC AGAGGAU	1167	967rev	AGUCUUGCUCUUUCGCCCAGG CUGGAU	1222
1591rev	GCUGACUCUCGCGGCUCUCG UGAGAGU	1168	991rev	UAACGAUUUUUUUUUUUGAG ACGGAGU	1223
634forw	AACUCCACGGAGUUUAUCUA ACUGAAU	1169	94rev	GCCCAACUCUUCGCGGUGGCA GUGGGU	1224
640forw	ACGGAGUUUAUCUAACUGAA UACGAGU	1170	302rev	CCCCAUUGCCGGCGAGGGGUG ACGGAU	1225
1688forw	CCGGCAGCGCACCGGGUUGC GGAGGGU	1171	997rev	AACUACCCCCAGAUGAUCCUA AGGAAU	1226
716forw	GGCCAUUAUAAAAAUUCGCG GCCGGGU	1172	237rev	ACUGCAUGUGUGAGCCGAGUC CUGGGU	1227
1608forw	AGUGACUCUCACGAGAGCCG CGAGAGU	1173	309rev	CACAAGCCCCCAUUGCCGGCG AGGGGU	1228
1678forw	GCCGACUCGCCCGGCAGCGC ACCGGGU	1174	579for w	GAUCUCGACCAGUCCCCUCAA CGGGGU	1229
1728rev	GGCGCCUACGCCCUUCUCAG UUAGGGU	1175	110for w	GAGGCACCCACUGCCACCGCG AAGAGU	1230
620forw	GGACCGACAGCGACAACUCC ACGGAGU	1176	1082fo rw	GCCCACCUUUAGGGUGAUAGC CUGAAU	1231
1239rev	CAGAAUCUUGUCUCGGCUCA GUGGGAU	1177	456for w	GUUGCCUGGAGCCGUUCCUGC GUGGGU	1232
1229rev	UCUCGGCUCAGUGGGAUGCG UCCGAGU	1178	1391re v	AAAAAGCGAUCUUAGAUCACC UUGAGU	1233
1584forw	CCCCCCAACCAGCCCGCCCG AGAGAGU	1179	414for w	GCACCUCCAAAGUCGGCCAAA AUGAAU	1234

481rev	GGUUGUAAAGUUUUUUACG GACAGAAU	1180	1182for w	CUAUUACCUAAGUAGGUCCCC UGGAAU	1235
1625rev	AAGGGAGCGGCCGCCGACCG CACGGAU	1181	192for w	GGCCGCAGGAAGAGGAACGG AGCGAGU	1236
1896forw	CGCCCCUCCCGGGGACCUGC GGCGGGU	1182	875for w	UCCCAGCCGGCAGUCUCCCAC AAGAAU	1237
1661rev	CGCAACCCGGUGCGCUGCCG GGCGAGU	1183	1830for w	GGUGGUGCAGGGCCGUGAAG CAAGAAU	1238
383forw	UGAGAUAAUGUGGGAUGCU AAGAGAAU	1184	214rev	GGGUGCACGUCCCACAGCUCA GGGAAU	1239
472forw	AAGUGCAAUAGUGCUAAAA ACAGGAGU	1185	1724rev	CAUGCACUUGUCUGUAGUUCA AGGAGU	1240
614rev	UAUUCAGUUAGAUAAACUCC GUGGAGU	1186	1070for w	GUGUAUAUAAAGGCCCACCUU UAGGGU	1241
1376rev	UCACCUCGACUACCUUAAAA AUGGAAU	1187	337rev	CUGGCCCAGUCAGUCAGGUUU GGGGGU	1242
166forw	CCAUAAGGAAACUAUGUUAU GAUGAAU	1188	850rev	UUGUGGGAGACUGCCGGCUG GGAGGGU	1243
409rev	AUAGAGUAGAUGCUAAAUG CUUUGAGU	1189	984rev	UGAUCCUAAGGAAUUGAAGG UAUGGAU	1244
1170rev	UCUCCAGCCUCUCCUUGAGC AGAGGAU	1190	1086rev	UGACUUCUGACUUUCAAUCAU CAGGAU	1245
1122rev	CUACAUUAUUAAUCUUAAGG ACUGAAU	1191	843rev	AGACUGCCGGCUGGGAGGGU UGGGGGU	1246
1372forw	CUUAGGCCCUAAAAUCUUCC UGUGAAU	1192	1014for w	UUCAAUUCCUUAGGAUCAUCU GGGGGU	1247
268rev	AAAUUCCUAUUGCUUAUAAU CUUGAGU	1193	1348for w	UGAUUUUGCCAAGAACUUGU CUAGAGU	1248
1836rev	GCUGACAUUUUUUGUUUGCU CUAGAAU	1194	1703rev	AGGAGUCCCCGCCCUUGCAAA AGGGAU	1249
183forw	UAUGAUGAAUGUGAUAGUU UGGAGAAU	1195	244rev	AAAGCGAACUGCAUGUGUGA GCCGAGU	1250
1260rev	CCCAGGCAGCACUGACUACA GCAGAAU	1196	945rev	UACCUUGUCGUGAUAAGUGG GCAGAAU	1251
1034rev	GUCUUGAUGAGGUAAAAAG AGGGGAGU	1197	1847for w	AAGCAAGAAUGGCCACAGACU GGGAAU	1252
1027forw	AAAAAAUCGUUACAAUUUA UGGUGGAU	1198	1043for w	UUGCCAAGAGAGAGAGCAGCC AGGGGU	1253
287forw	GUACUCAAGAUUAUAAGCAA UAGGAAU	1199	50rev	GGGCCGACCGCGGCCUCCAGG CGGGGU	1254
101rev	UUAAUUUCUCUCCUUUGCAU AUUGGAU	1200	131for w	AAGAGUUGGGCUCUGUCAGCC GCGGGU	1255
1201rev	CCUACCCUGCCCCCUUCUCC UUAGAAU	1201	1217for w	AGAUACAUUUCUUAGCACUA UUAGAAU	1256
1284forw	CUGCUGUAGUCAGUGCUGCC UGGGAAU	1202	1763rev	AGAAGCAAAAGUACCACUAG AUGGAGU	1257
429rev	CACUUAGCACAGUACCUUAC AUAGAGU	1203	431for w	CAAAAUGAAUGGGCAGUGAG CCGGGGU	1258
355forw	ACUUAGAUAUGAAGGGGAA AGAAGGGU	1204	1322for w	GCAUGGUUUUGUGGAAAAGU AAGGAAU	1259
661rev	UGCAGUUUUACAUAUAAAU GACAGGAU	1205	972rev	AUUGAAGGUAUGGAUUUGGG ACGGAAU	1260

1703forw	GUUGCGGAGGGUGGGCCUGG GAGGGGU	1206	629forw	UGAGAGAUCAUUUAACAUUU AAUGAAU	1261
1332forw	AAAAAUGUGAUGAUCAAAA CUAGGAAU	1207	1003forw	AAAUCCAUACCUUCAAUUCCU UAGGAU	1262
1219forw	GGCAUUCUAAGGAGAAGGG GGCAGGGU	1208	1244forw	UAAGAAAUGUAAAAAAACCU CUAGAGU	1263
372forw	AAAGAAGGGUUUGAGAUAA UGUGGGAU	1209	836rev	CGGCUGGGAGGGUUGGGGGU GGGGGGU	1264
192forw	UGUGAUAGUUUGGAGAAUA AAUUGAAU	1210	1462forw	UCCCACUCUGUCACCCAGAGC UGGAGU	1265
531forw	GCAGAUGCUAUGAAAGAAA AAGGGGAU	1211	764rev	UUAUUGGAACGCUAAGCUUG UGGGGGU	1266
708rev	UAUGAGCCACCGCACCCGGC CGCGAAU	1212	829rev	GAGGGUUGGGGGUGGGGGGU GUGGAAU	1267
741rev	CGCUUCGGCCUCCCAAAGUG CUGGGAU	1213	1445rev	CUGCACUCCAGCUCUGGGUGA CAGAGU	1268
765forw	CCAGCACUUUGGGAGGCCGA AGCGGGU	1214	755forw	CUUGGCGAUGACCUUGAGCAG UAGGAU	1269
769forw	CACUUUGGGAGGCCGAAGCG GGUGGAU	1215	1436forw	UUUCUUCUCUUUCUUUUGAG ACGGAGU	1270
820rev	UUUUGUGUUUUUAGUAGAG ACGGGGGU	1216	1453rev	UGGGGACACUGCACUCCAGCU CUGGGU	1271
877rev	UGUCUCAGCCUCCUGAGUAG CUGGGAU	1217	1544forw	UUCUCUCAGCCUCCCAAGUAG CUGGGU	1272
886rev	CGAUUCUCGUGUCUCAGCCU CCUGAGU	1218	1570rev	CUGAAAAUACAAAAAAAUCA GCCGGGU	1273
905forw	CUACUCAGGAGGCUGAGACA CGAGAAU	1219	1621rev	GCGGGCGGAUCACCUGAGGUC AGGAGU	1274
918rev	GCUCACUGCAACCUCUGCCU CCCGGGU	1220	1638rev	CACUUUGGGAGGCAGAAGCG GGCGGAU	1275
962rev	UGCUCUUUCGCCCAGGCUGG AUGGAGU	1221	1666forw	CGCUUCUGCCUCCCAAAGUGC 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, measle 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 or 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 leasta bout 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 leaset about 2000%, or at least about 3000%, or at least about 4000%, or at least about 5000%, or at leasta bout 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 abouat 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 abouat 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 abouat 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 abouat 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 abouat 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 (HEKn). 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 β-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 Spermatocyte, a Spermatogonium 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, scarped 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® RNAiMAX™ (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 ΔΔCt 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). HEKn 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®I 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, HEKn, 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 ΔΔCt 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 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). 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/μl RNA was diluted 5×, and 5 μl 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 ΔΔCt 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 β-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 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). 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 typsinized, 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 ÷ X_0)] ÷ log 2. *P* values were calculated using a paired, two-tailed Student's *t*-test. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. 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*. Breifly, the senescent phenotype can include an enlargement of cellular

morphology and upwards of about 90% positivity for senescence-associated β-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× 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). 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® 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.

[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® 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.

[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_2 (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 HEKn 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® RNAiMAX™ (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/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 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® Blood and Tissue Kit (Qiagen). Quantitative PCR reactions for relative telomere length in treated and untreated cells were performed using SsoAdvanced™ Universal SYBR® 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™ 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 HEKn cells were collected 3 days after the last transfection. Genomic DNA (gDNA) was extracted using the DNeasy® 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 HEKn 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 HEKn 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 HEKn 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,

1ng/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®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 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 x 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 hTERC 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^2 in ECM-2MV BulletKit™ media (Lonza) and allowed to attach in 5% CO2 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_2 (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, TM9F3and 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 MathylationEPIC 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 HEKn (P10). The treated group included senescent high passage HEKn 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), Casϕ (Cas12j), Francisella tularensis subsp. novicida Cas9, Pasteurella multocida Cas9, Campylobacter lari CF89-12 Cas9, Mycoplasma gallisepticum str. F Cas9, Nitratifractor 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, wherien 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), Casϕ (Cas12j), Francisella tularensis subsp. novicida Cas9, Pasteurella multocida Cas9, Campylobacter lari CF89-12 Cas9, Mycoplasma gallisepticum str. F Cas9, Nitratifractor 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 (Ψ) 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^5C), 5-methyluridine (m^5U), N6-methyladenosine (m^6A), inosine 2'-0-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, measle 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, wherien the at least one retrovirus particle is an MMSV particle or an MSCV particle.

41. The composition of claim 38, wherien 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, wherien the at least one flavivirus particle is a Kunjin virus particle, a West Nile virus particle, or a Dengue virus particle 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 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 β-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.

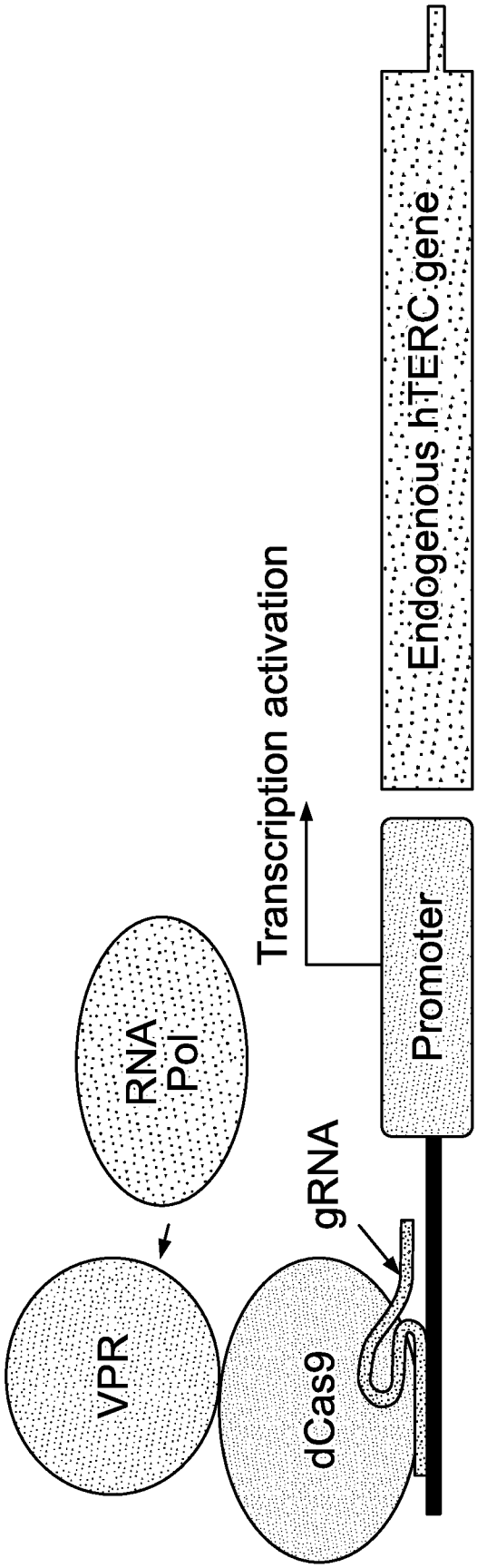
Endogenous hTERC gene
Transcription activation
Promoter
RNA Pol
gRNA
VPR
dCas9

FIG. 1

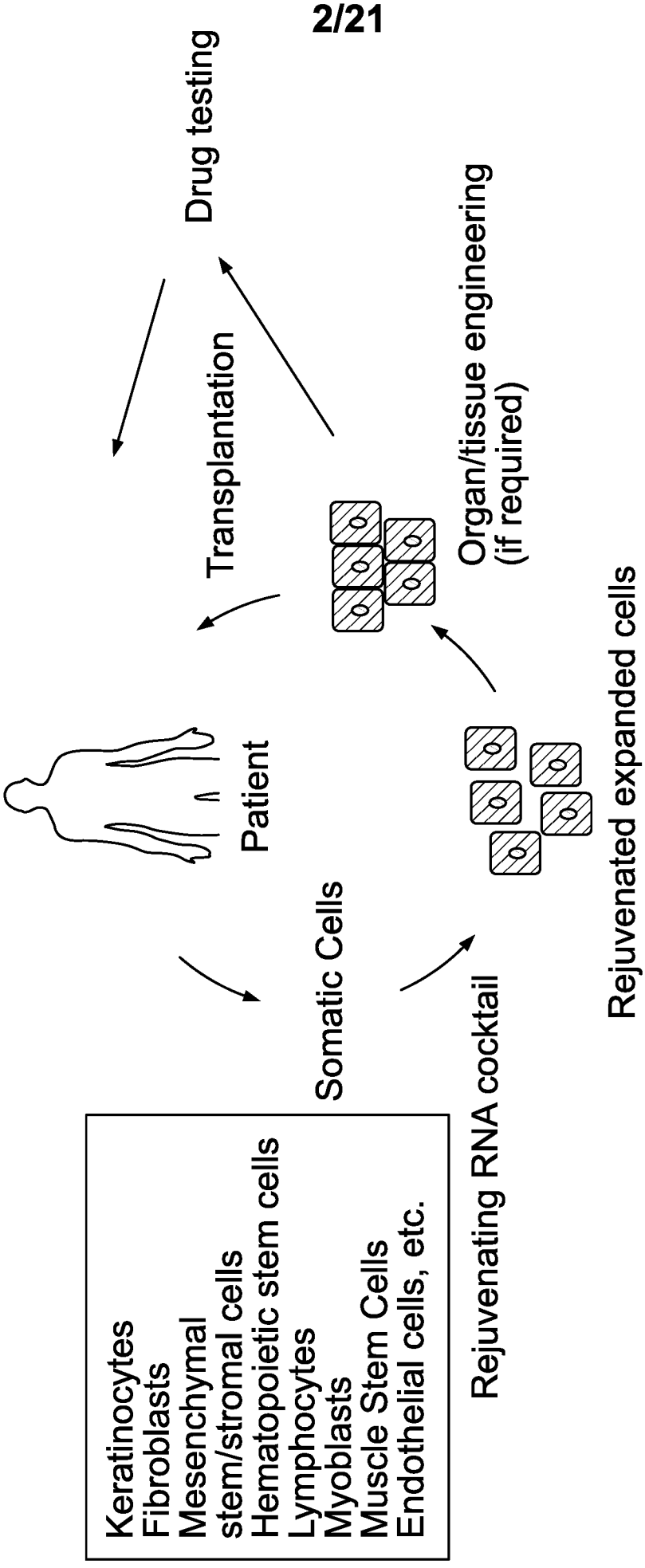
Keratinocytes
Fibroblasts
Mesenchymal stem/stromal cells
Hematopoietic stem cells
Lymphocytes
Myoblasts
Muscle Stem Cells
Endothelial cells, etc.
Somatic Cells
Rejuvenating RNA cocktail
Rejuvenated expanded cells
Patient
Transplantation
Drug testing
Organ/tissue engineering (if required)

FIG. 2

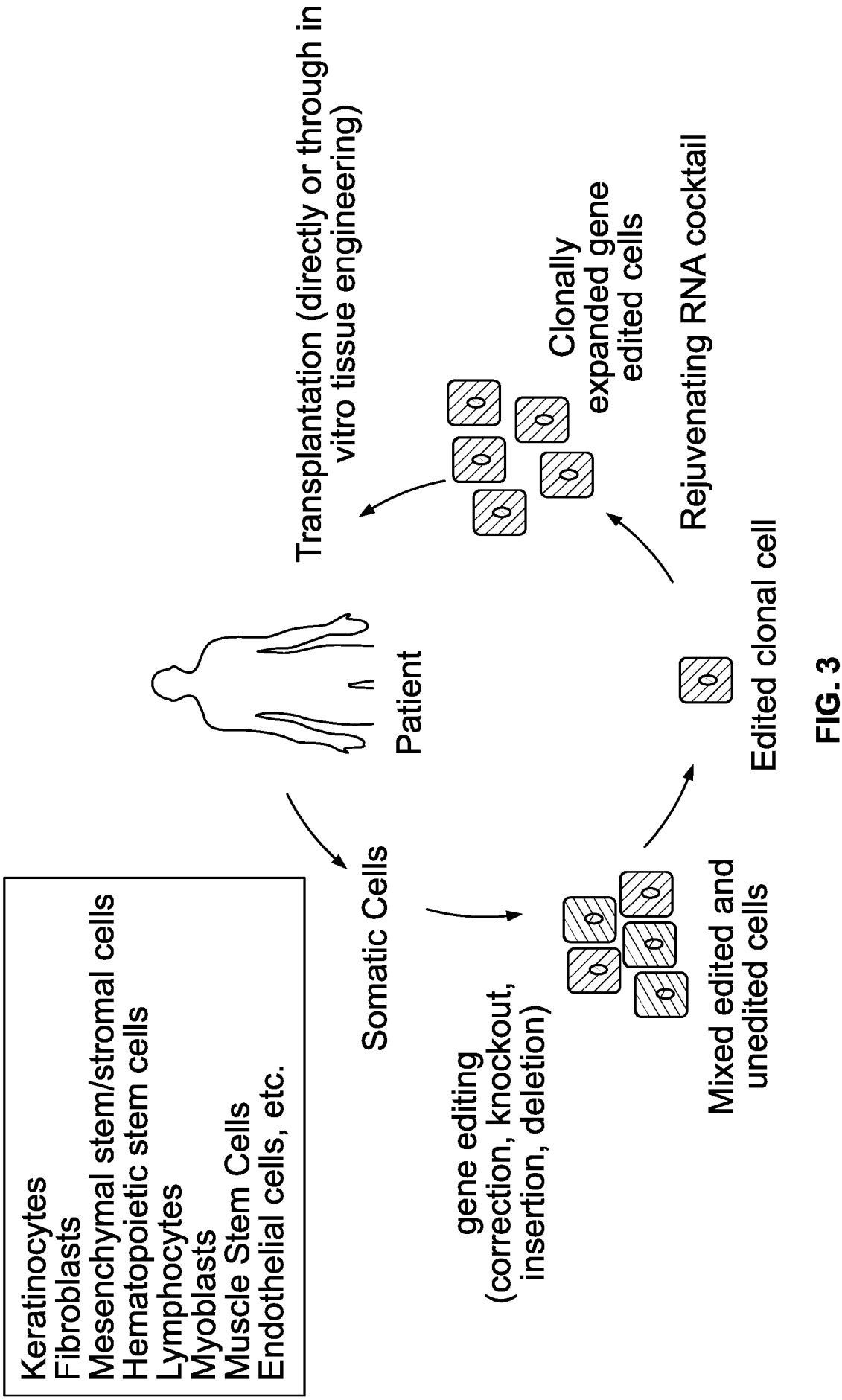
Keratinocytes
Fibroblasts
Mesenchymal stem/stromal cells
Hematopoietic stem cells
Lymphocytes
Myoblasts
Muscle Stem Cells
Endothelial cells, etc.
Somatic Cells
gene editing
(correction, knockout,
insertion, deletion)
Mixed edited and
unedited cells
Edited clonal cell
Rejuvenating RNA cocktail
Clonally
expanded gene
edited cells
Transplantation (directly or through in
vitro tissue engineering)
Patient

FIG. 3

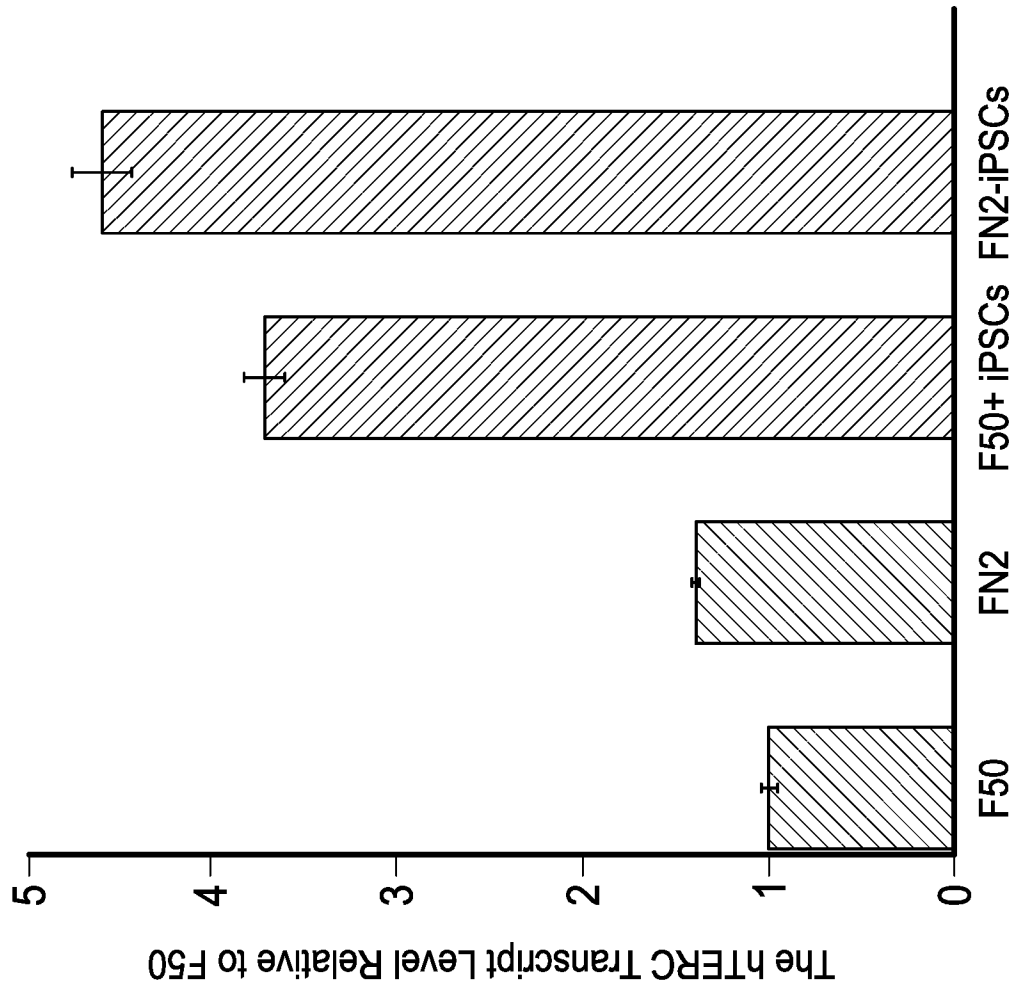
The hTERC Transcript Level Relative to F50
0
1
2
3
4
5
F50
FN2
F50+ iPSCs
FN2-iPSCs

FIG. 4

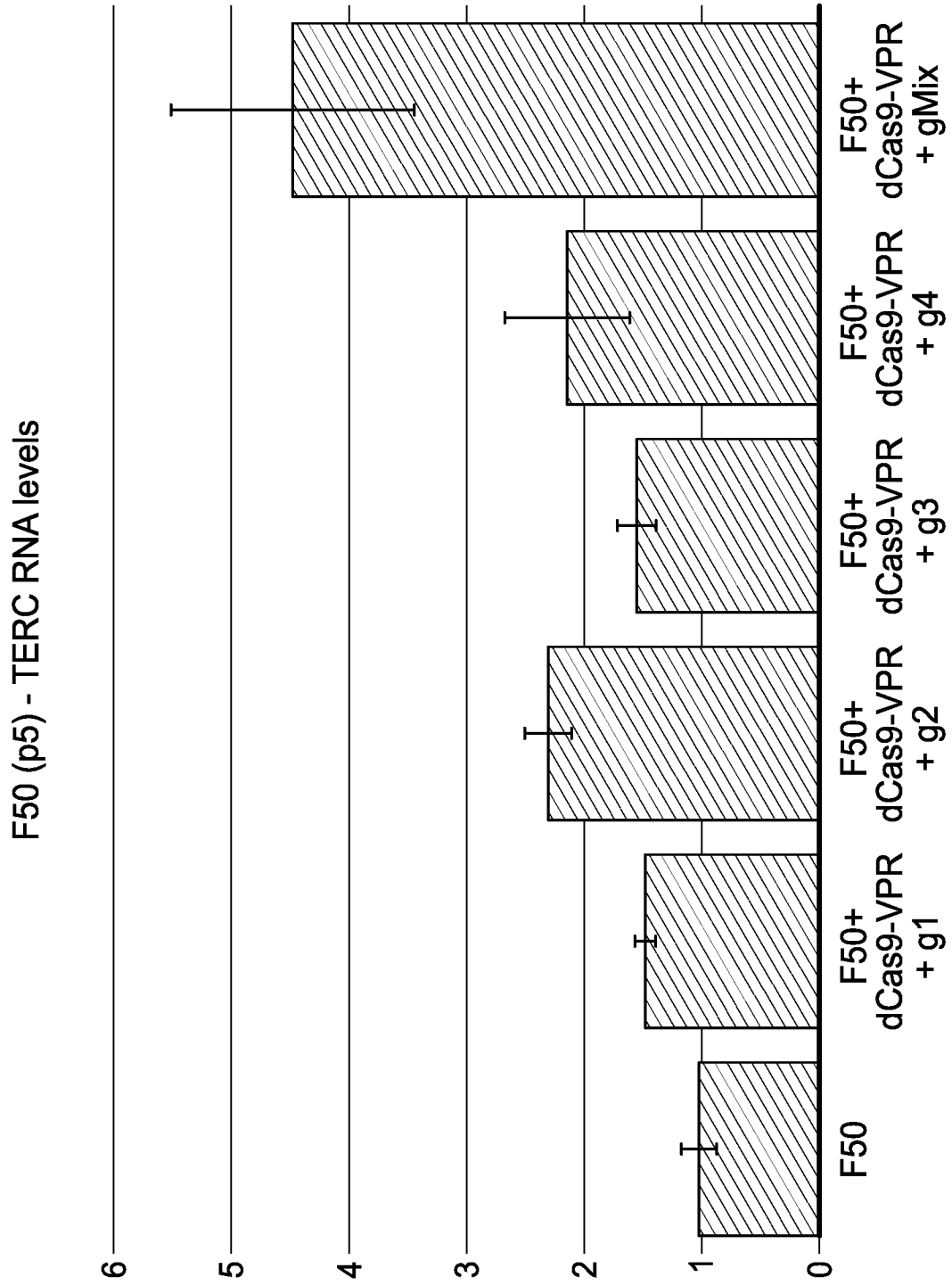
F50 (p5) - TERC RNA levels
0
1
2
3
4
5
6
F50
F50+ dCas9-VPR + g1
F50+ dCas9-VPR + g2
F50+ dCas9-VPR + g3
F50+ dCas9-VPR + g4
F50+ dCas9-VPR + gMix

FIG. 5

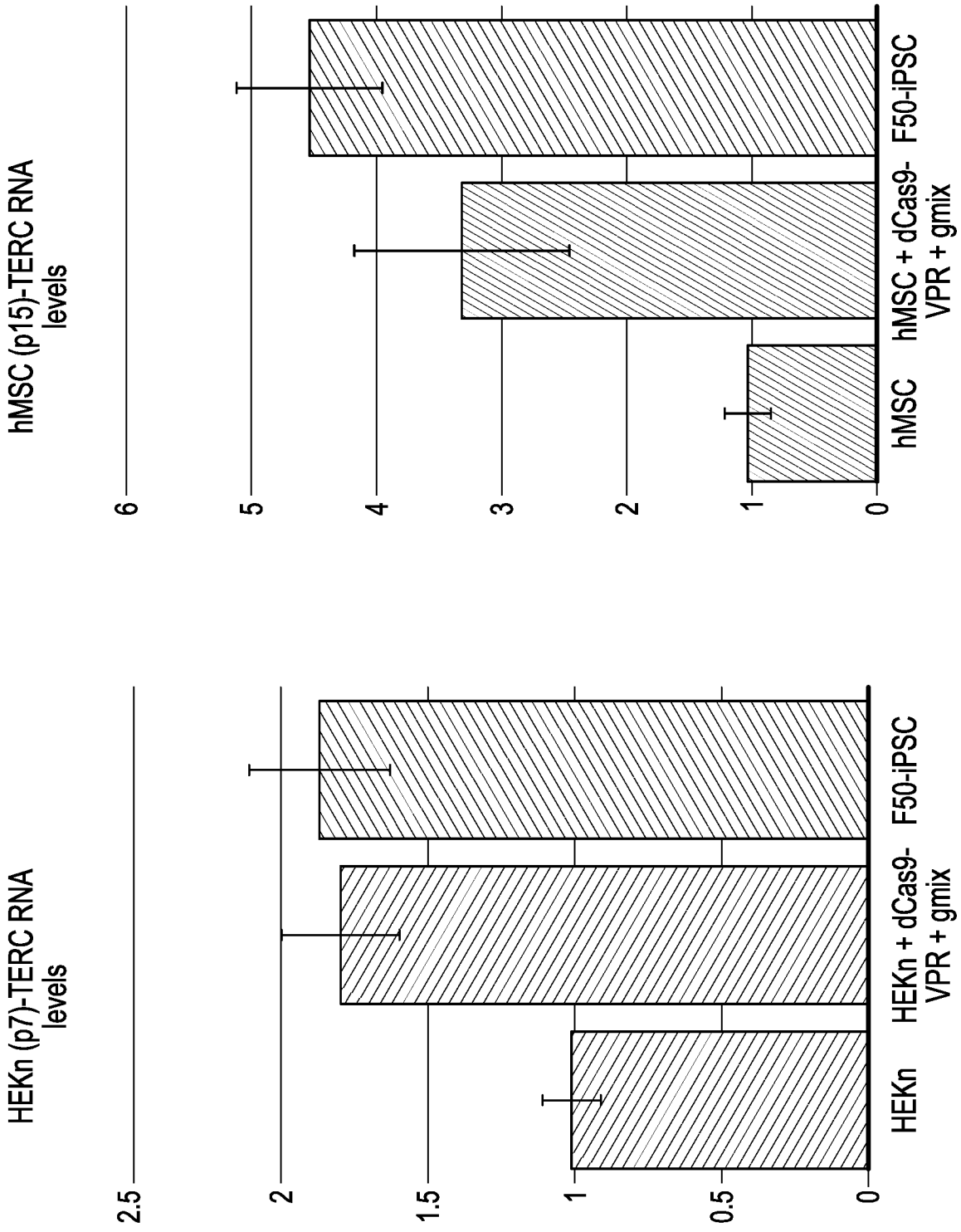
hMSC (p15)-TERC RNA levels
6
5
4
3
2
1
0
hMSC
hMSC + dCas9-VPR + gmix
F50-iPSC
HEKn (p7)-TERC RNA levels
2.5
2
1.5
1
0.5
0
HEKn
HEKn + dCas9-VPR + gmix
F50-iPSC

FIG. 6

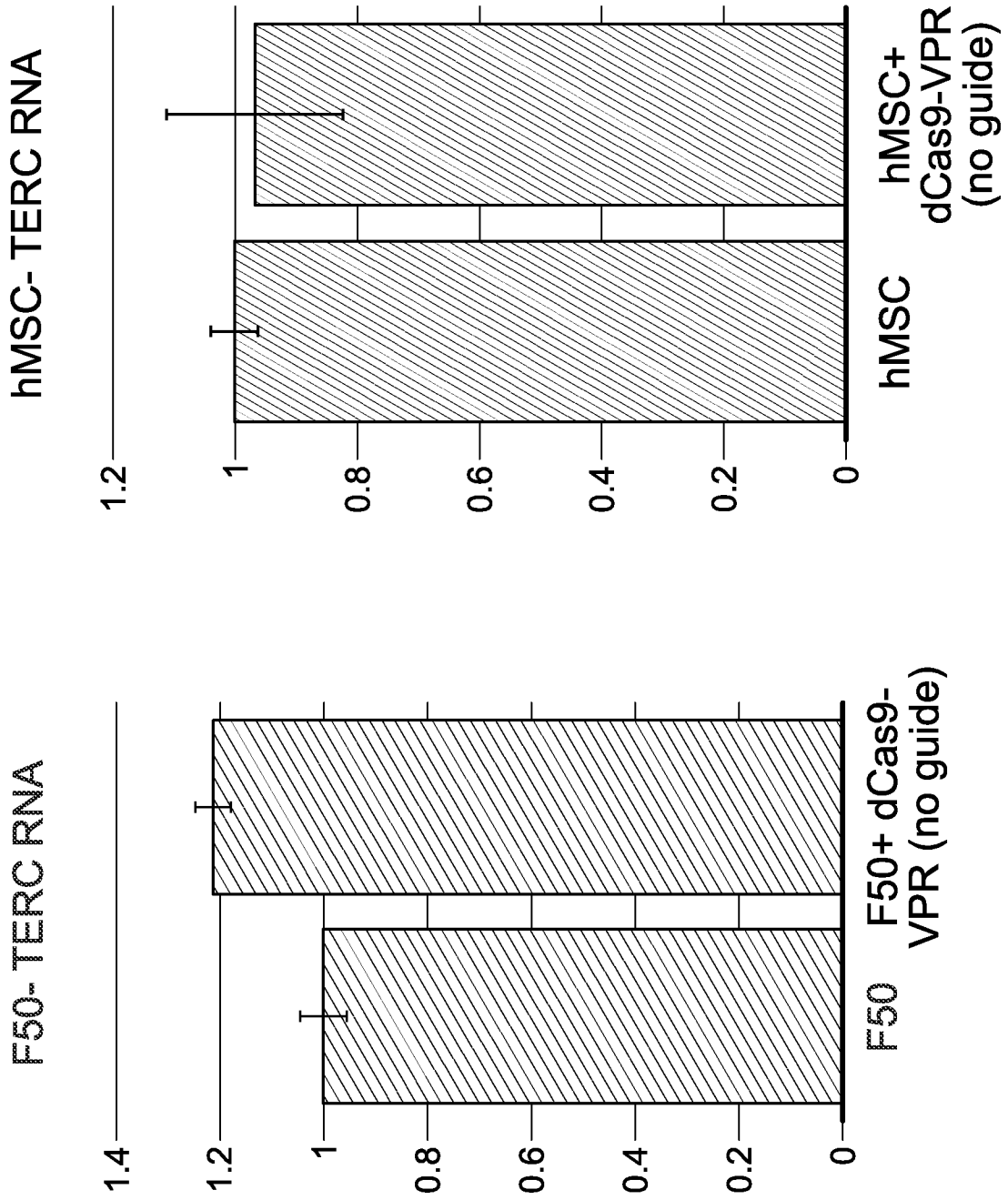
hMSC- TERC RNA
1.2
1
0.8
0.6
0.4
0.2
0
hMSC
hMSC+ dCas9-VPR (no guide)
F50- TERC RNA
1.4
1.2
1
0.8
0.6
0.4
0.2
0
F50
F50+ dCas9-VPR (no guide)

FIG. 7

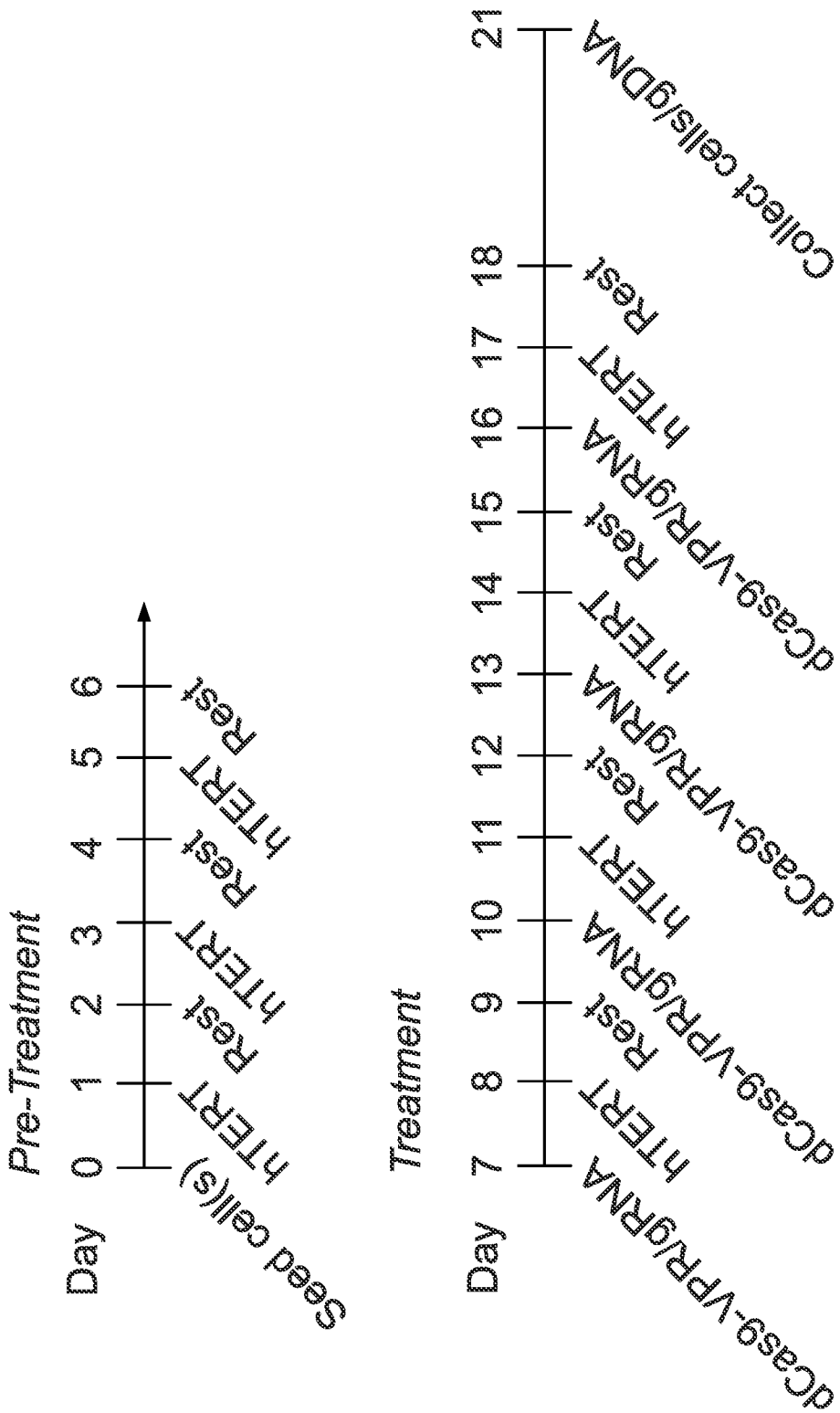
Pre-Treatment
Day
0
1
2
3
4
5
6
Seed cell(s)
hTERT
Rest
hTERT
Rest
hTERT
Rest
Treatment
Day
7
8
9
10
11
12
13
14
15
16
17
18
21
dCas9-VPR/gRNA
hTERT
Rest
dCas9-VPR/gRNA
hTERT
Rest
dCas9-VPR/gRNA
hTERT
Rest
dCas9-VPR/gRNA
hTERT
Rest
Collect cells/gDNA

FIG. 8

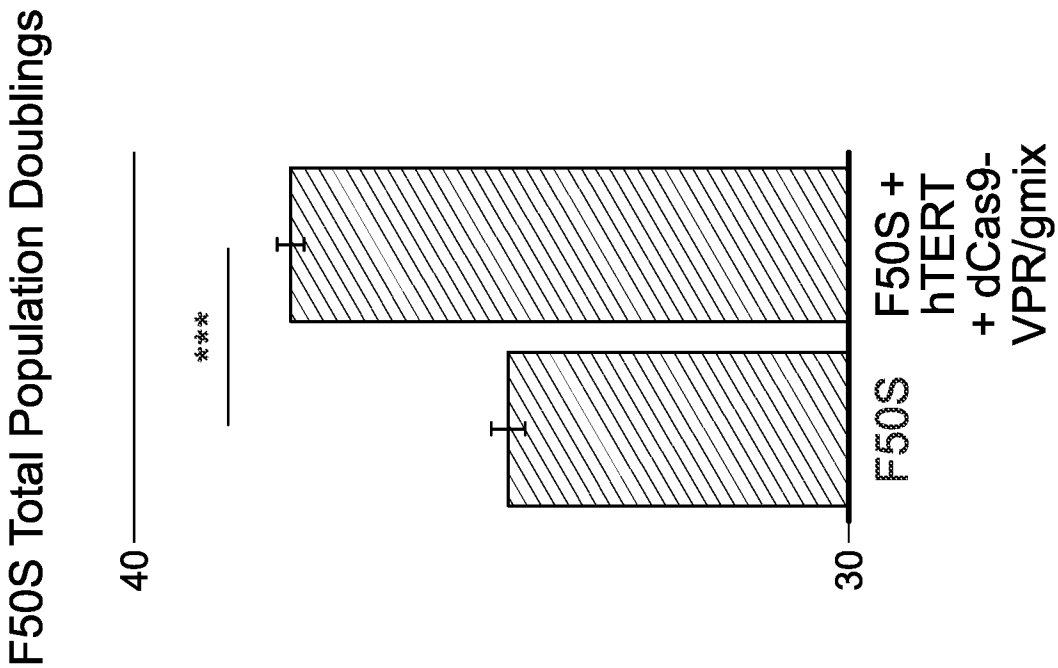
F50S Total Population Doublings
40
30
***
F50S
F50S + hTERT + dCas9-VPR/gmix

FIG. 9

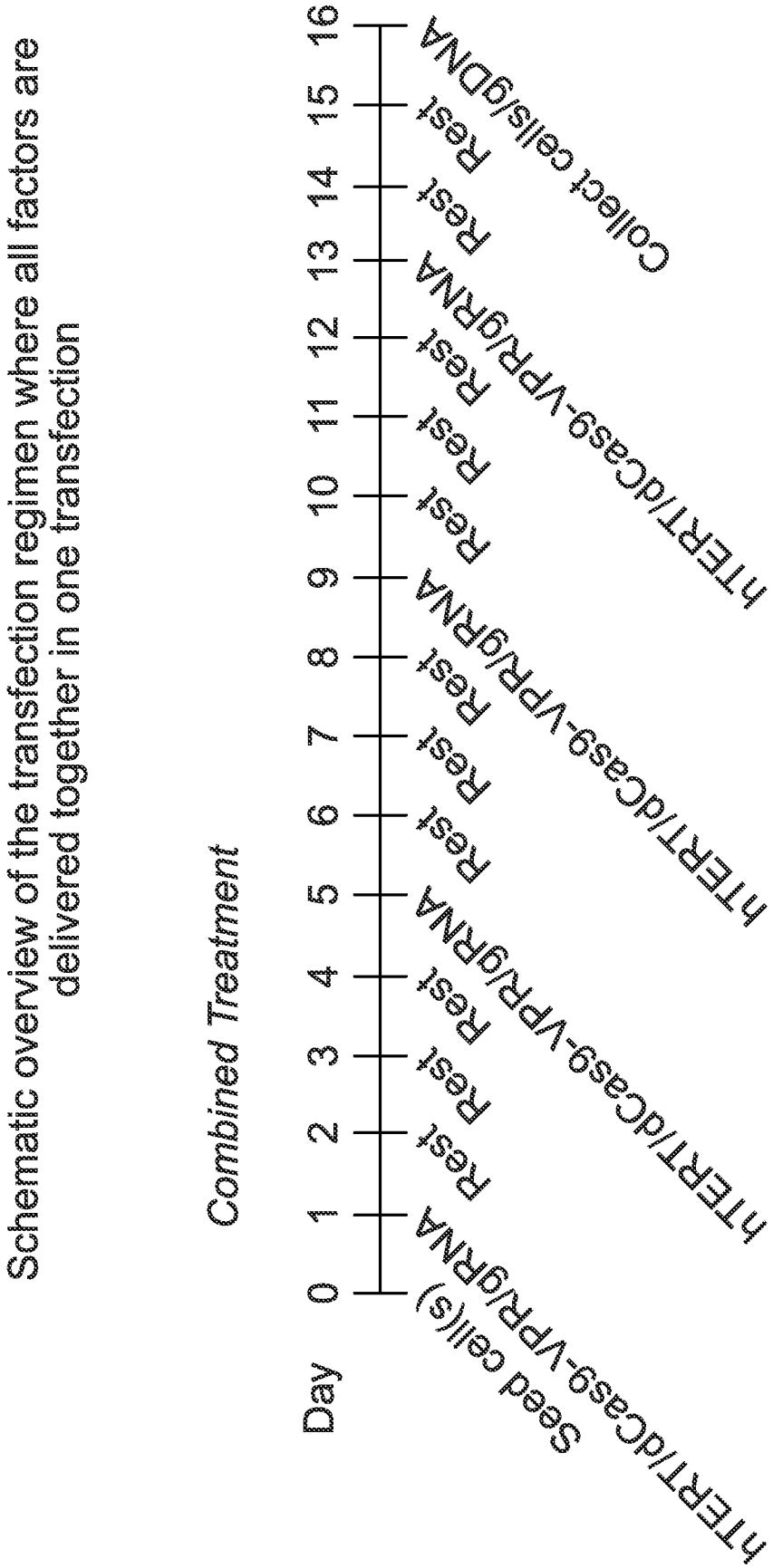
Schematic overview of the transfection regimen where all factors are delivered together in one transfection
Combined Treatment
Day
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Seed cell(s)
hTERT/dCas9-VPR/gRNA
Rest
hTERT/dCas9-VPR/gRNA
Rest
hTERT/dCas9-VPR/gRNA
Rest
hTERT/dCas9-VPR/gRNA
Rest
Collect cells/gDNA

FIG. 10

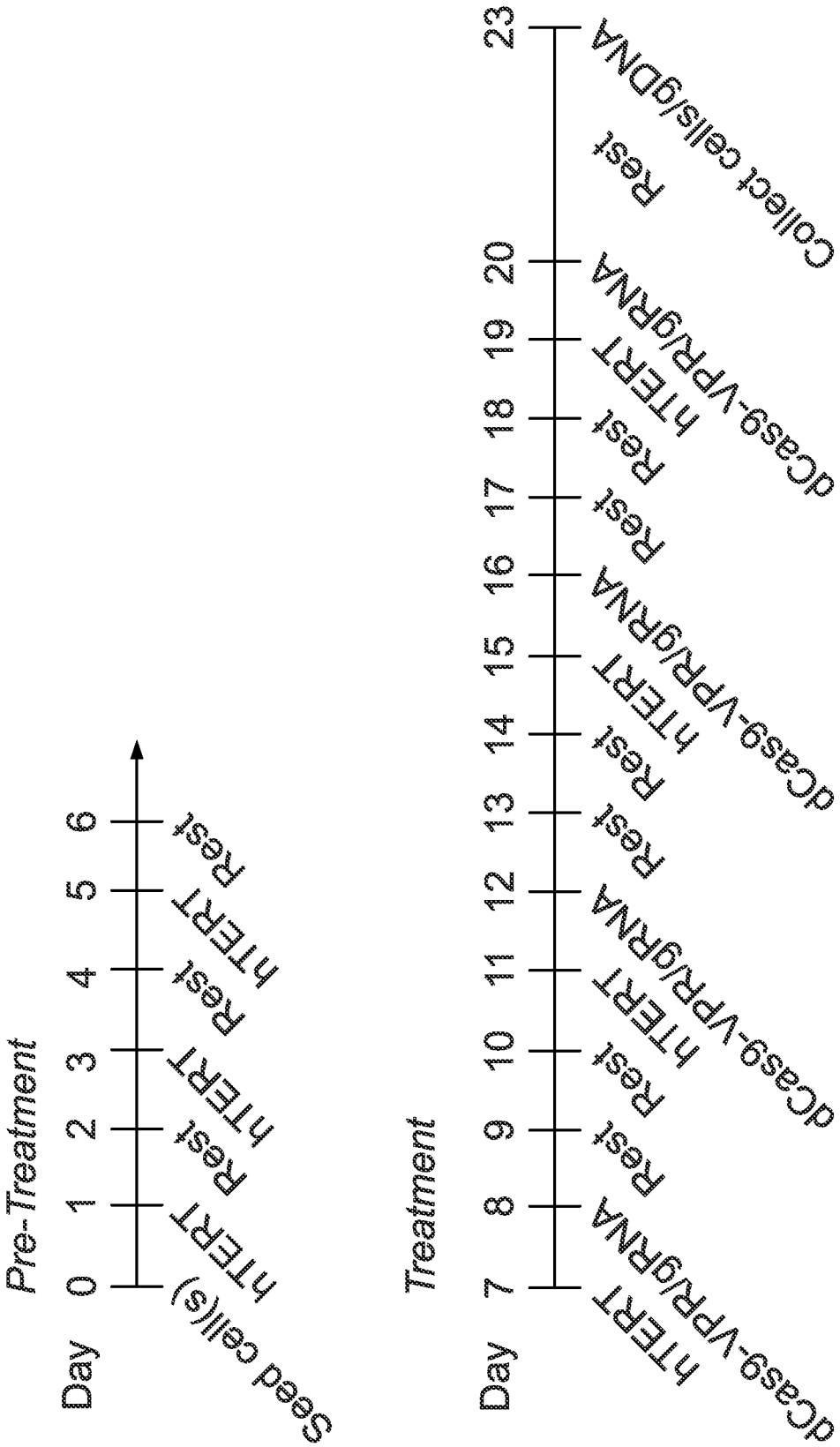
Pre-Treatment
Day
0
1
2
3
4
5
6
Seed cell(s)
hTERT
Rest
hTERT
Rest
hTERT
Rest
Treatment
Day
7
8
9
10
11
12
13
14
15
16
17
18
19
20
23
dCas9-VPR/gRNA
hTERT
Rest
dCas9-VPR/gRNA
hTERT
Rest
dCas9-VPR/gRNA
hTERT
Rest
dCas9-VPR/gRNA
hTERT
Rest
Collect cells/gDNA

FIG. 11

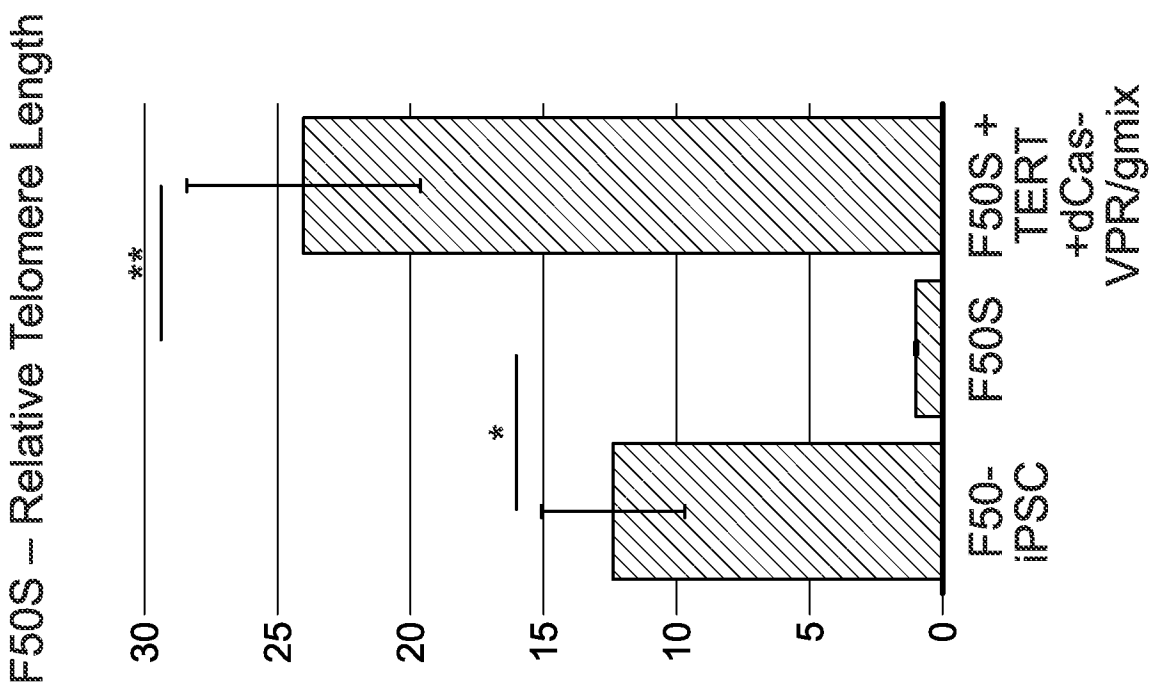
F50S – Relative Telomere Length
30
25
20
15
10
5
0
**
*
F50-iPSC
F50S
F50S + TERT +dCas-VPR/gmix

FIG. 12

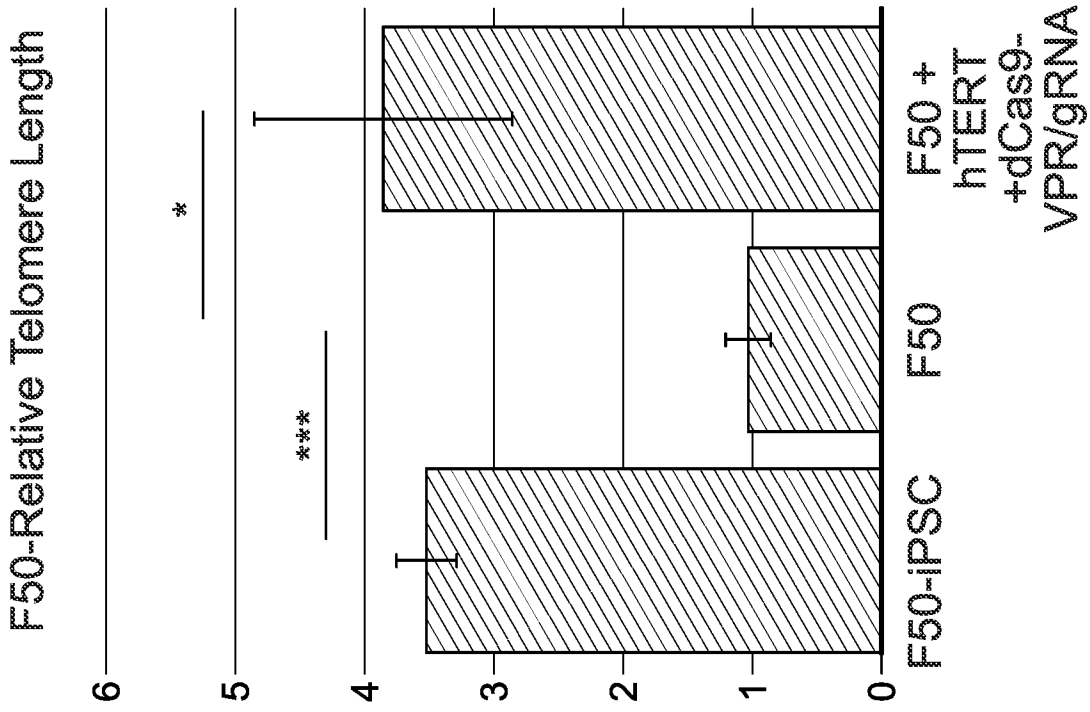
F50-Relative Telomere Length
6
5
4
3
2
1
0
*
***
F50-iPSC
F50
F50 + hTERT +dCas9-VPR/gRNA

FIG. 13

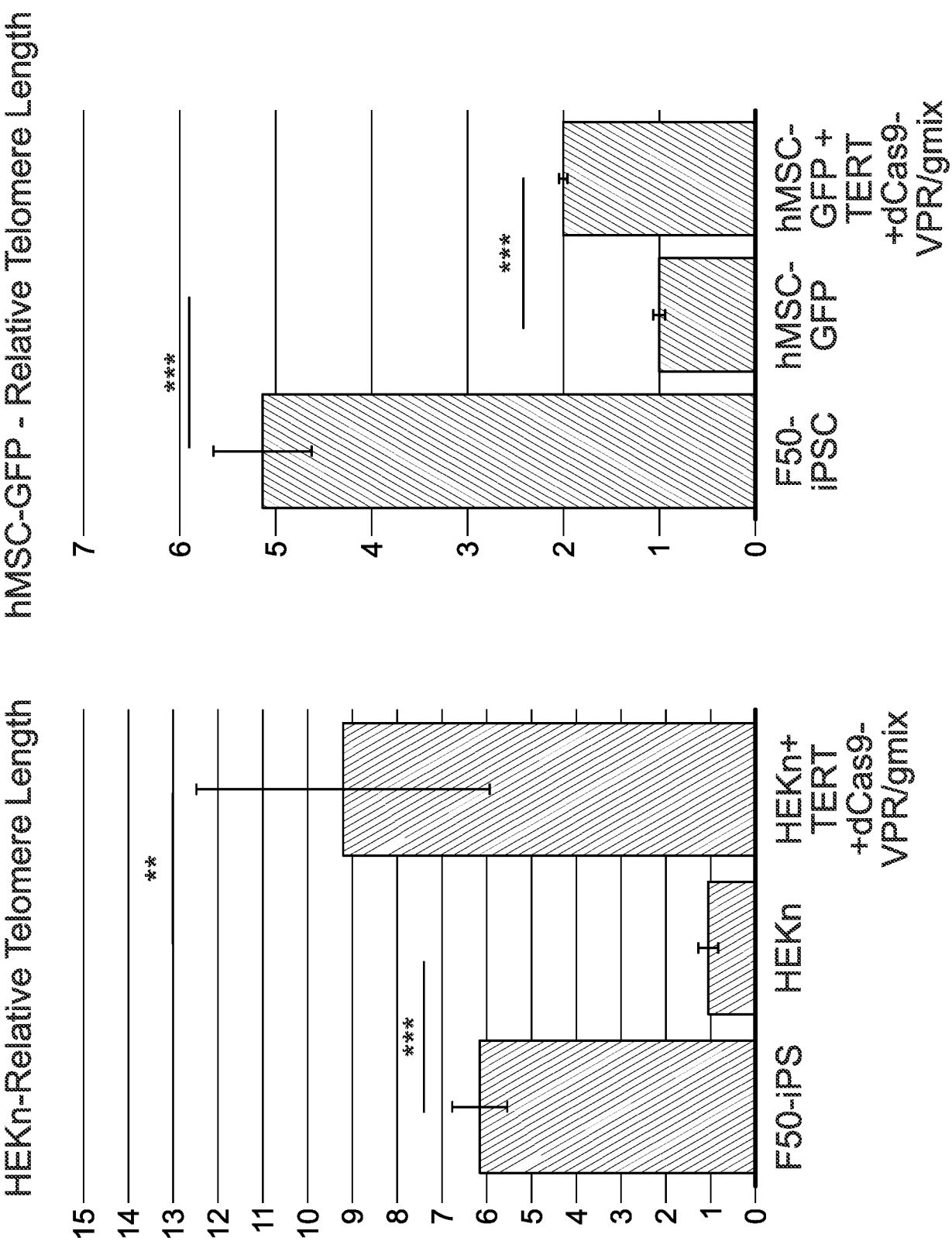
HEKn-Relative Telomere Length
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
**
***
F50-iPS
HEKn
HEKn+ TERT +dCas9-VPR/gmix
hMSC-GFP - Relative Telomere Length
7
6
5
4
3
2
1
0
***
***
F50-iPSC
hMSC-GFP
hMSC-GFP + TERT +dCas9-VPR/gmix

FIG. 14

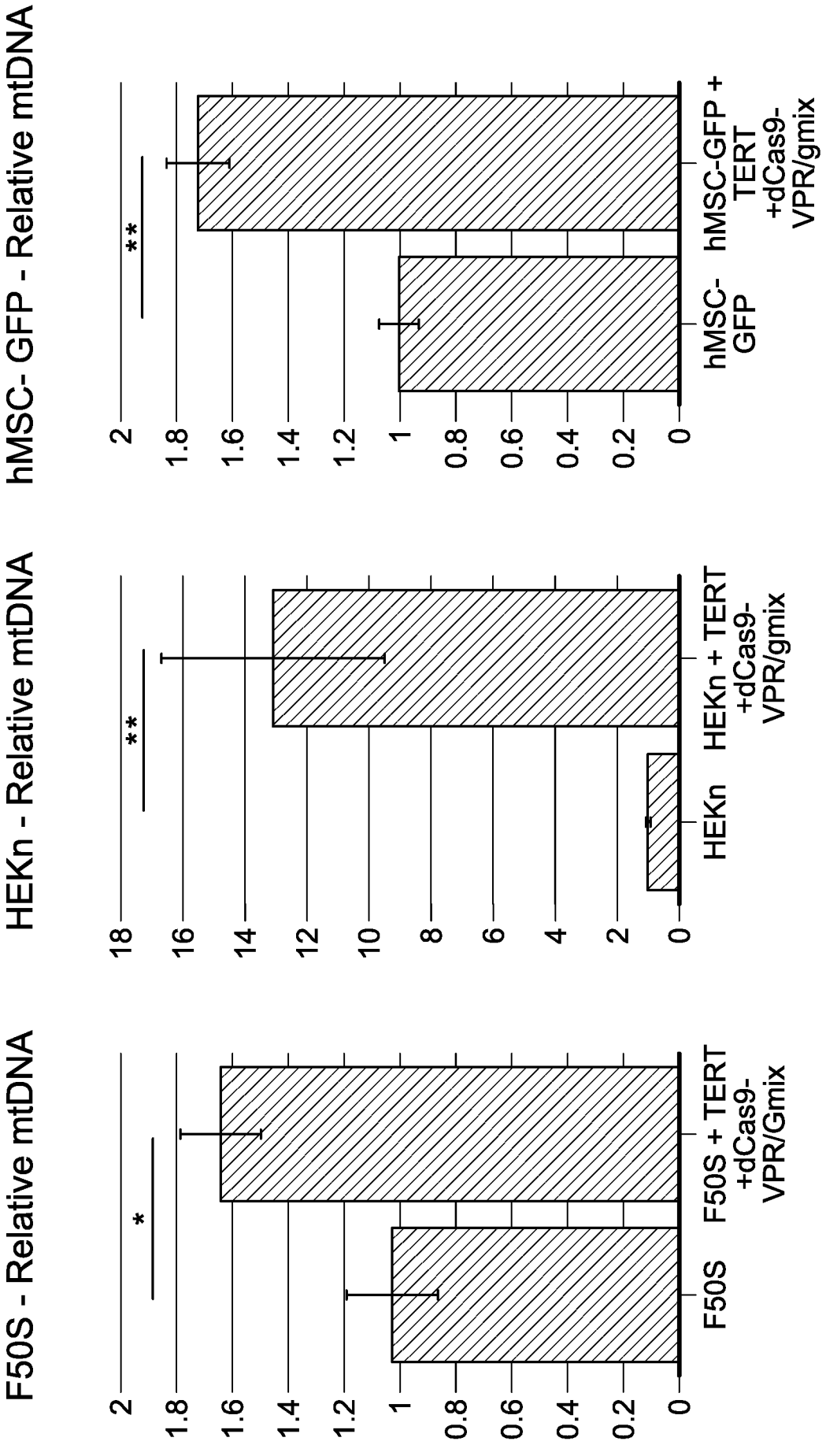
F50S - Relative mtDNA
HEKn - Relative mtDNA
hMSC- GFP - Relative mtDNA
*
**
**
F50S
F50S + TERT +dCas9-VPR/Gmix
HEKn
HEKn + TERT +dCas9-VPR/gmix
hMSC-GFP
hMSC-GFP + TERT +dCas9-VPR/gmix
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
0
2
4
6
8
10
12
14
16
18

FIG. 15

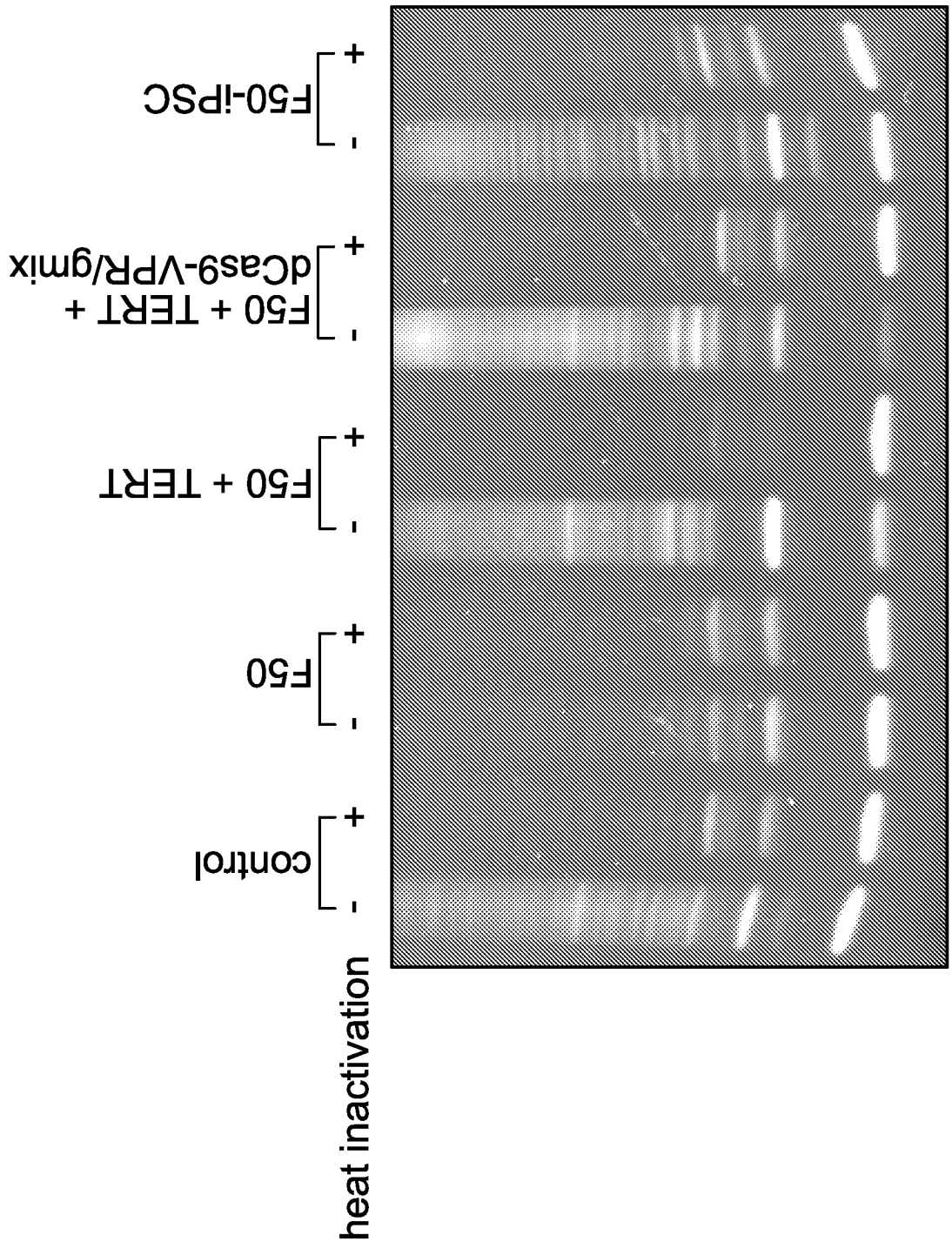
heat inactivation
control
F50
F50 + TERT
F50 + TERT +
dCas9-VPR/gmix
F50-iPSC
-
+

FIG. 16

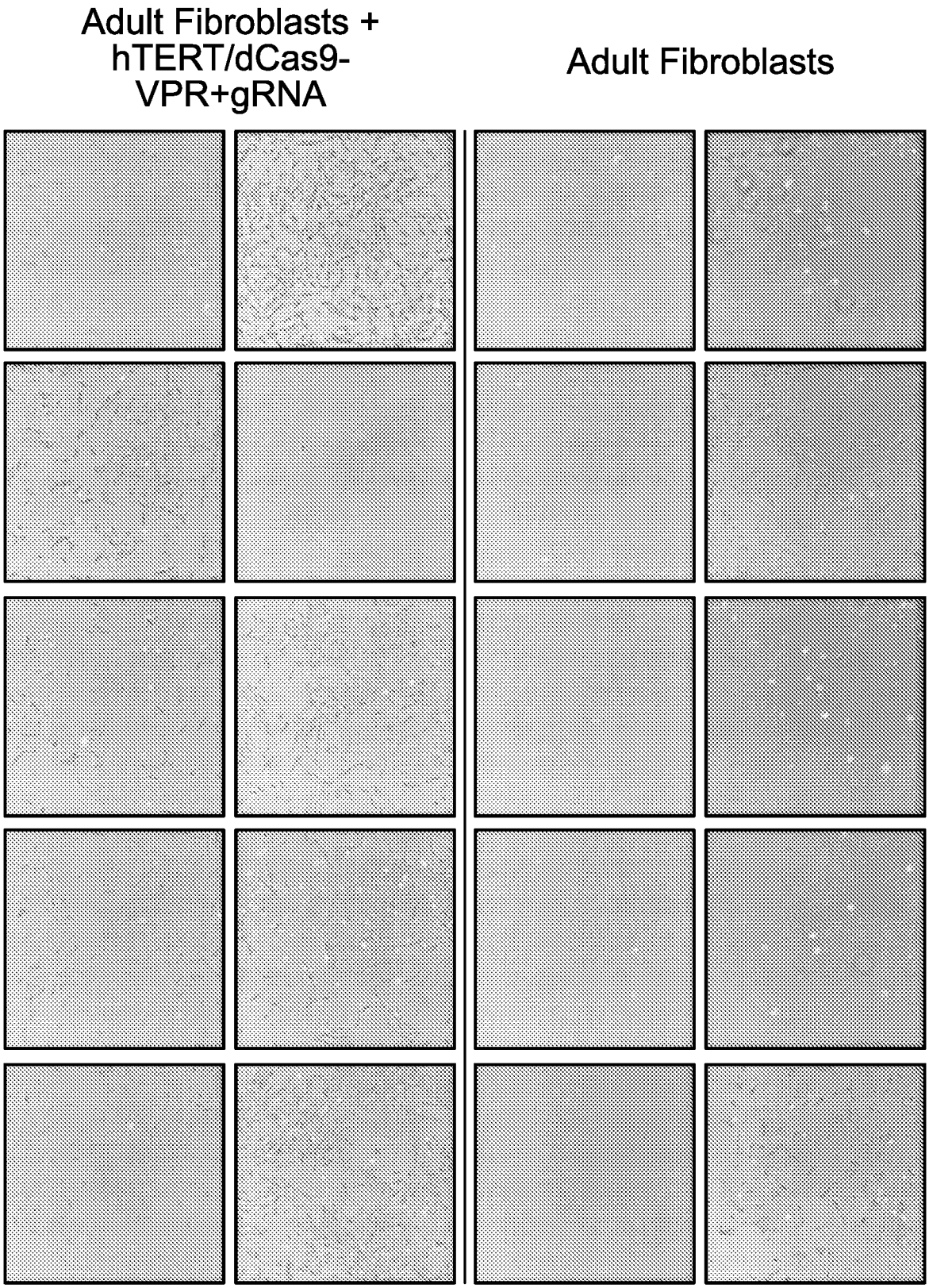

FIG. 17

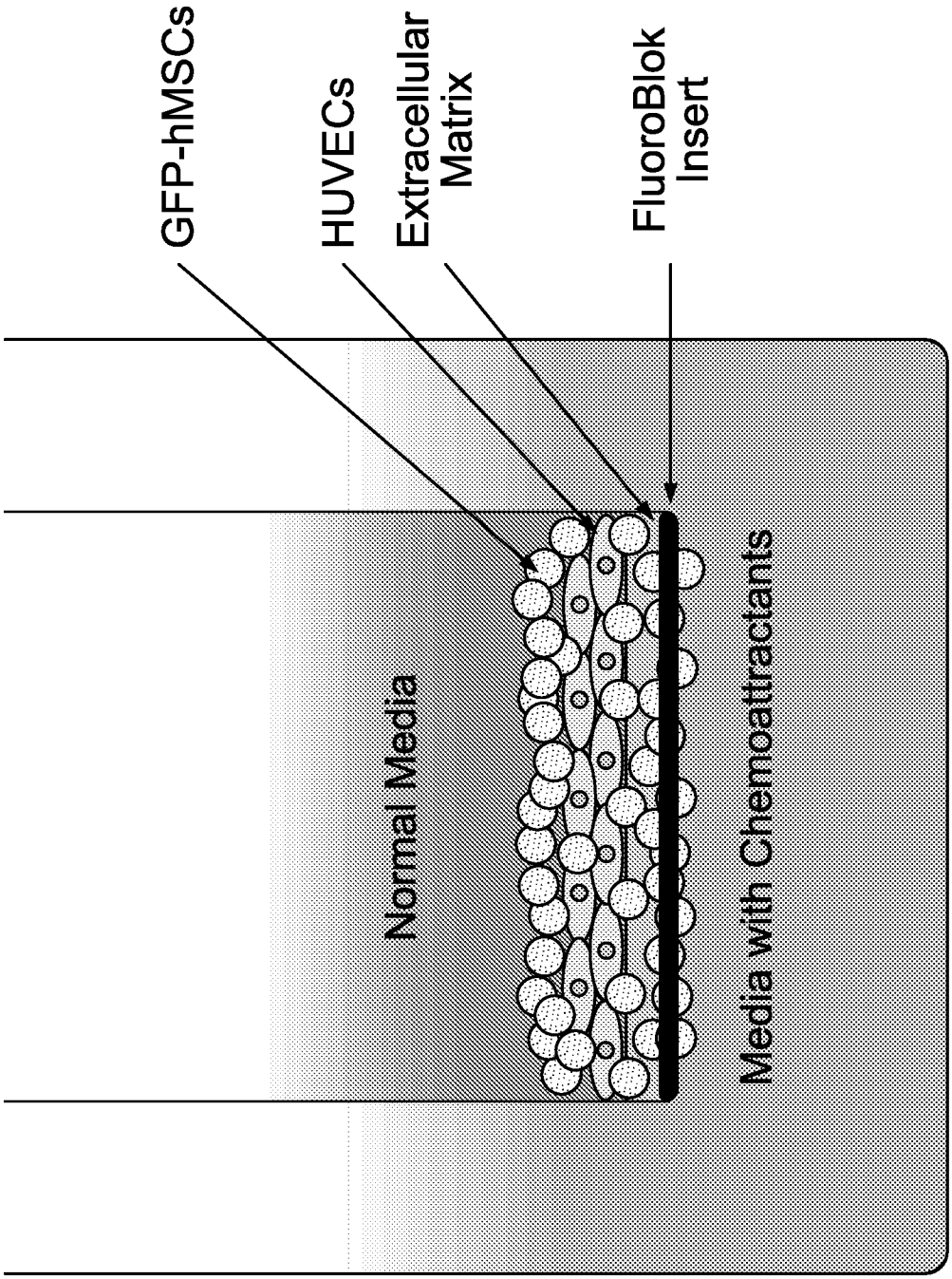
GFP-hMSCs
HUVECs
Extracellular Matrix
FluoroBlok Insert
Normal Media
Media with Chemoattractants

FIG. 18

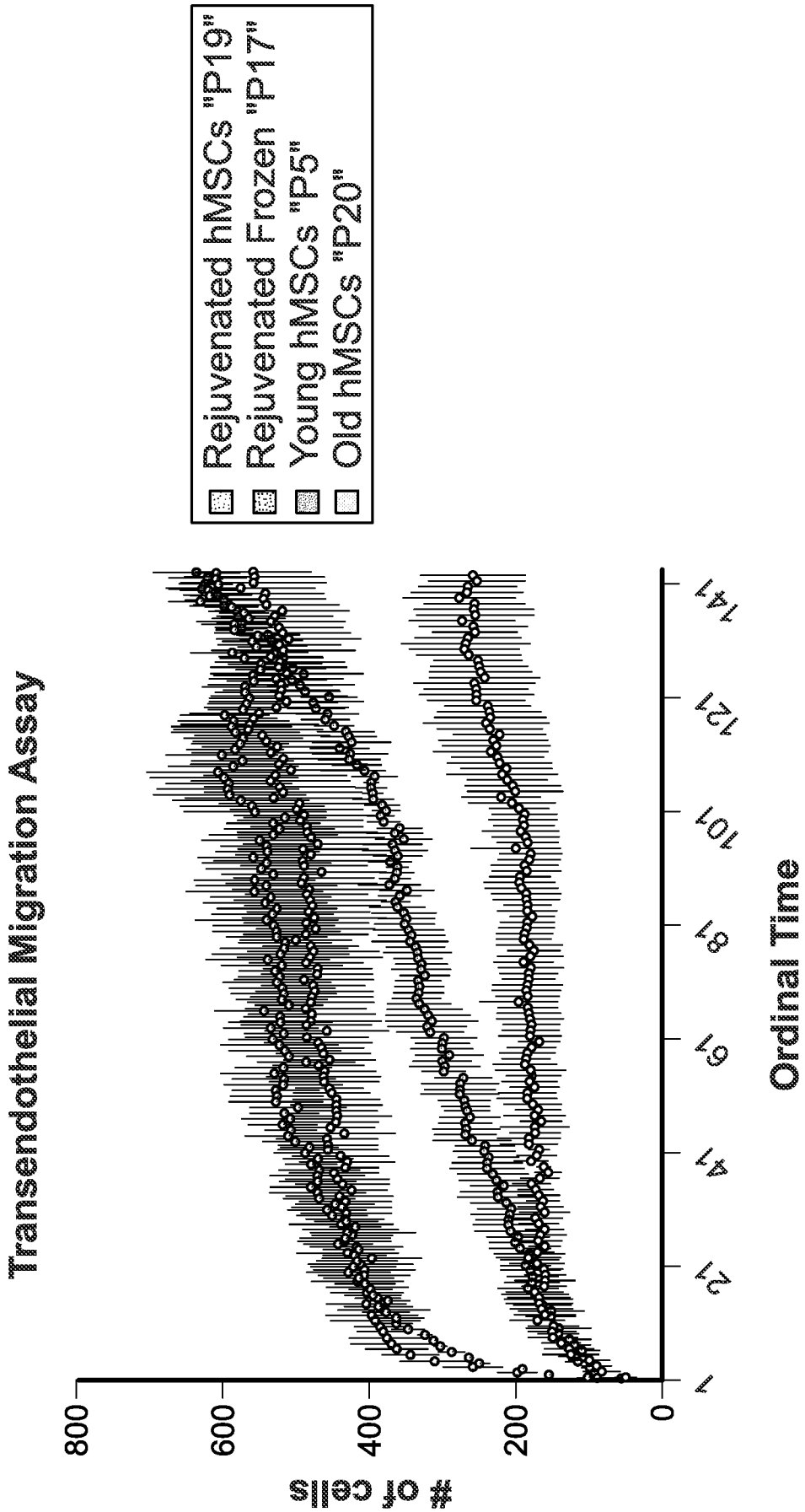
Rejuvenated hMSCs "P19"
Rejuvenated Frozen "P17"
Young hMSCs "P5"
Old hMSCs "P20"
Transendothelial Migration Assay
# of cells
0
200
400
600
800
1
21
41
61
81
101
121
141
Ordinal Time

FIG. 19

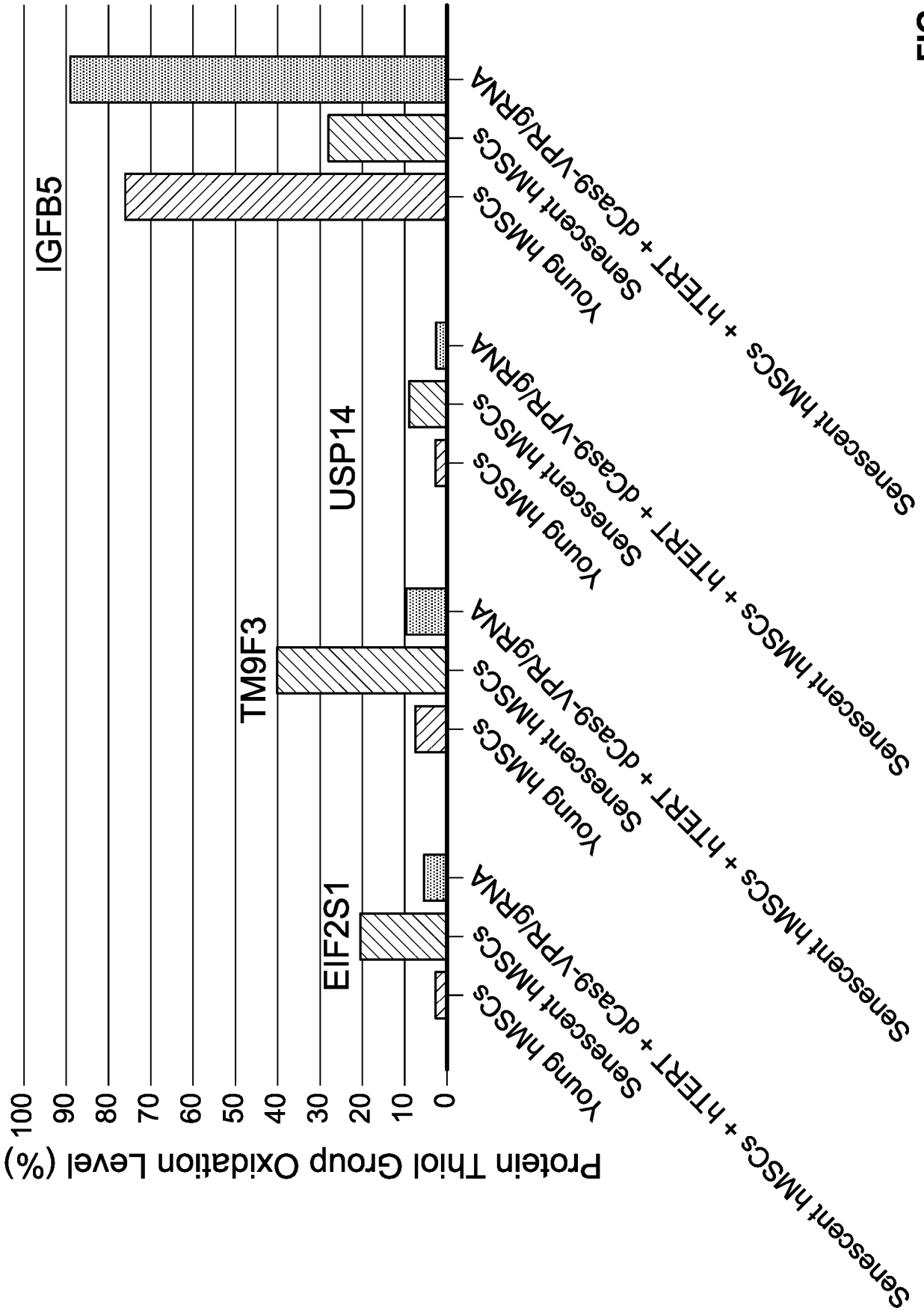
Protein Thiol Group Oxidation Level (%)
0
10
20
30
40
50
60
70
80
90
100
IGFB5
USP14
TM9F3
EIF2S1
Young hMSCs
Senescent hMSCs
Senescent hMSCs + hTERT + dCas9-VPR/gRNA
Young hMSCs
Senescent hMSCs
Senescent hMSCs + hTERT + dCas9-VPR/gRNA
Young hMSCs
Senescent hMSCs
Senescent hMSCs + hTERT + dCas9-VPR/gRNA
Young hMSCs
Senescent hMSCs
Senescent hMSCs + hTERT + dCas9-VPR/gRNA

FIG. 20

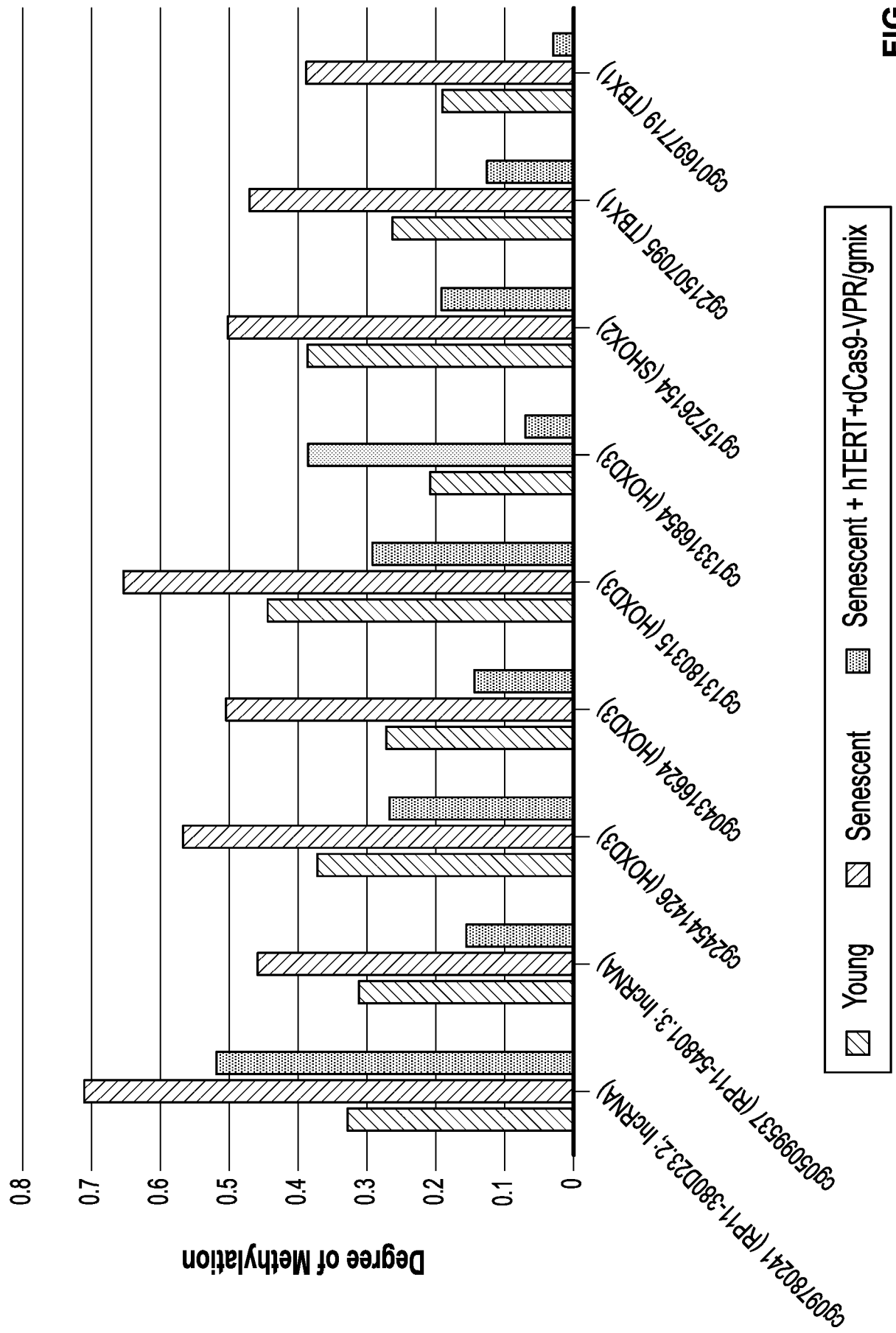
Young
Senescent
Senescent + hTERT+dCas9-VPR/gmix
Degree of Methylation
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
cg09780241 (RP11-380D23.2; lncRNA)
cg05099537 (RP11-548O1.3; lncRNA)
cg24541426 (HOXD3)
cg04316624 (HOXD3)
cg13180315 (HOXD3)
cg13316854 (HOXD3)
cg15726154 (SHOX2)
cg21507095 (TBX1)
cg01697719 (TBX1)

FIG. 21

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/050665

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/22 C12N9/12 A61K48/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/197748 A2 (UNIV DUKE [US]) 11 December 2014 (2014-12-11) claims 1-130; example 2; Fig. 2f -----	1-65
X	WO 2018/110471 A1 (ASTELLAS PHARMA INC) 21 June 2018 (2018-06-21) claims 1-15; Table 1; examples 1-8 -----	1,2,6, 12-14, 20, 47-49, 56-58,60
A	WO 2015/100269 A2 (DOUBLE HELIX CORP [CA]; COYLE DOUGLAS [CA]; FOSSEL MICHAEL [US]) 2 July 2015 (2015-07-02) claims 1-44 ----- -/--	1-65

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search	Date of mailing of the international search report
2 December 2020	10/12/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Behrens, Joyce

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