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(71) **Applicants: THE J. DAVID GLADSTONE INSTITUTES** [US/US]; 1650 Owens Street, San Francisco, California 94158 (US). **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, California 94607 (US).

(72) **Inventors: LIU, Ziqi;** 1650 Owens Street, San Francisco, California 94158 (US). **CONKLIN, Bruce R.;** 1650 Owens Street, San Francisco, California 94158 (US).

(74) **Agent: RUBIN, Michael B.;** Bozicevic, Field & Francis LLP, 201 Redwood Shores Parkway, Suite 200, Redwood City, California 94065 (US).

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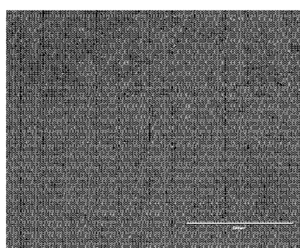
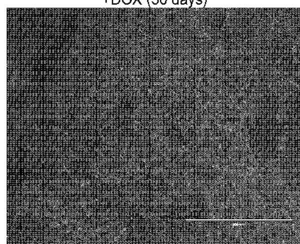
(54) **Title:** METHODS OF GENERATING RETINAL PIGMENT EPITHELIUM (RPE)

(57) **Abstract:** The present disclosure provides methods of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs). The present disclosure also provides RPE generated using a subject method as well as methods of using RPE generated according to the disclosed methods. Also provided are recombinant vectors useful for the generation of RPE according to the disclosed methods.

Figure 5
WTB6.13 hOPM RPE
-DOX (30 days)



+DOX (30 days)



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METHODS OF GENERATING RETINAL PIGMENT EPITHELIUM (RPE)

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/586,074, filed November 14, 2017, which application is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

[0002] A Sequence Listing is provided herewith as a text file, "GLAD-435WO_SeqList_ST25.txt" created on November 13, 2018 and having a size of 109 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

[0003] The naturally occurring retinal pigment epithelium (RPE) is a polarized monolayer of densely packed hexagonal cells in the mammalian eye that separates the neural retina from the choroid. The cells in the RPE contain pigment granules and perform an important role in retinal physiology by forming a blood-retinal barrier and closely interacting with photoreceptors to maintain visual function by absorbing the light energy focused by the lens on the retina. These cells also transport ions, water, and metabolic end products from the subretinal space to the blood and take up nutrients such as glucose, retinol, and fatty acids from the blood and deliver these nutrients to photoreceptors.

[0004] Many ophthalmic diseases, such as (age-related) macular degeneration, macular dystrophies such as Stargardf s and Stargardfs-like disease, Best disease (vitelliform macular dystrophy), and adult vitelliform dystrophy or subtypes of retinitis pigmentosa, are associated with a degeneration or deterioration of the retina itself or of the RPE.

[0005] Due to its location at the back of the eye, the RPE is difficult to access *in vivo,* making experimentation with RPE disease related treatments problematic. As an alternative to *in vivo* experimentation, RPE may be cultivated *in vitro.*

[0006] Despite improvements in RPE culturing techniques that have occurred over time, difficulties still arise with regard to the efficient production of a pigmented, epithelioid monolayer that best mimics native RPE tissue.

[0007] There is significant interest in the use of RPE in the context of drug testing and gene editing to develop therapies for the treatment of genetic disorders and diseases of the RPE, e.g., age-related macular degeneration. Accordingly, there is a need in the art for improved *in vitro* methods of generating RPE.

SUMMARY OF THE DISCLOSURE

[0008] The present disclosure provides methods of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs). The present disclosure also provides RPE generated using a subject method as well as methods of using RPE generated according to the disclosed methods. Also provided are recombinant vectors useful for the generation of RPE according to the disclosed methods.

[0009] In a first aspect, the present disclosure provides a method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs). The method includes introducing into the iPSCs an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs. The genetically modified iPSCs are cultured under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells. In some embodiments of the first aspect, the method does not include introducing into the iPSCs or genetically modified iPSCs a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide, or an exogenous nucleic acid including a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide. In this manner, RPE is generated.

[0010] In a second aspect, the present disclosure provides a method of generating RPE from iPSCs, wherein the method includes introducing into the iPSCs an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs. The genetically modified iPSCs

are cultured under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells. In the second aspect, the method does not include introducing into the iPSCs or genetically modified iPSCs an exogenous nucleic acid including a tyrosinase enhancer. In this manner, RPE is generated.

[0011] In a third aspect, the present disclosure provides a method of generating RPE from iPSCs, wherein the method includes introducing into the iPSCs a recombinant vector including an inducible promoter, e.g., a Tet-inducible promoter, an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein one or more of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide are operably linked to the promoter. The genetically modified iPSCs are cultured under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells. In this manner RPE is generated.

[0012] In a fourth aspect, the present disclosure provides a method of generating RPE from iPSCs, wherein the method includes introducing into the iPSCs a recombinant vector including an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein the recombinant vector is targeted to a genomic locus such as the AAVS1 locus or a CLYBL locus of the iPSCs. The genetically modified iPSCs are cultured under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells. In this manner RPE is generated.

[0013] In a fifth aspect, the present disclosure provides a method of generating RPE from iPSCs, wherein the method includes introducing into the iPSCs a recombinant vector including a promoter, an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein each of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide is operably linked to the promoter. The genetically modified iPSCs are cultured under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells. In this manner RPE is generated.

[0014] In additional aspects, the present disclosure provides RPE generated by the method of any one of aspects one through five as well as compositions including such RPE. Also provided, are methods of using the RPE and compositions prepared according to the methods of the present disclosure, e.g., screening methods including contacting the RPE with a test agent and assaying to determine the effect of the test agent on the RPE.

[0015] In still further aspects, the present disclosure provides recombinant vectors useful in the methods disclosed herein, e.g., a recombinant vector including a promoter, a nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide operably linked to the promoter, a nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide operably linked to the promoter, and a nucleic acid including a nucleotide sequence encoding an MITF polypeptide operably linked to the promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] **Figure 1** provides a vector map of a recombinant vector according to an aspect of the present disclosure.

[0017] **Figure 2** provides images showing the stage of RPE development at 5 weeks following co-transfection of viral vectors separately expressing OTX1, OTX2, MITF, PAX2, and PAX6 in human iPSCs. Scale bar = 200 pm.

[0018] **Figure** 3 provides a schematic illustrating a method for RPE generation according to an aspect of the present disclosure.

[0019] Figure 4 provides a schematic illustrating a timeline for RPE generation according to an aspect of the present disclosure as well as images showing the stage of RPE development at different time points.

[0020] Figure 5 provides images showing the stage of RPE development at 30 days in the presence (bottom) or absence (top) of induced expression of OTX2, PAX6 and MITF. Scale bar = 200 pm.

[0021] Figure 6 provides images showing the stage of RPE development at 5 weeks in the presence (bottom) or absence (top) of induced expression of OTX2, PAX6 and MITF. Scale bar = 200 pm.

[0022] Figure 7 provides images showing the stage of RPE development at 56 days in the presence (bottom) or absence (top) of induced expression of OTX2, PAX6 and MITF. Scale bar = 200 pm.

[0023] Figure 8 provides images showing the stage of RPE development at 85 days in the presence (bottom) or absence (top) of induced expression of OTX2, PAX6 and MITF. Scale bar = 200 pm.

[0024] Figure 9 provides images comparing results of the Zhu et al. protocol described herein (left) with RPE differentiation using the hOPM-AAVS vector of the present disclosure (right). Scale bar = 200 pm.

[0025] Figure 10 provides images comparing results of the protocol described in Figure 17 (top) with RPE differentiation using the hOPM-AAVS vector of the present disclosure (bottom). Scale bar = 200 pm.

[0026] Figure 11 provides a cDNA and corresponding amino acid sequence for a human OTX1 protein according to an aspect of the present disclosure (SEQ ID NOs.: 10-1 1).

[0027] Figure 12 provides a cDNA and corresponding amino acid sequence for a human OTX2 protein according to an aspect of the present disclosure (SEQ ID NOs.: 12-13).

[0028] Figure 13 provides a cDNA and corresponding amino acid sequence for a human PAX2 protein according to an aspect of the present disclosure (SEQ ID NOs.: 14-15).

[0029] Figure 14 provides a cDNA and corresponding amino acid sequence for a human PAX6 protein according to an aspect of the present disclosure (SEQ ID NOs.: 16- 17).

[0030] Figure 15 provides a cDNA and corresponding amino acid sequence for a human MITF protein according to an aspect of the present disclosure (SEQ ID NOs.:l8-l9).

[0031] **Figure 16** provides a summary of an RPE differentiation protocol adapted from Buchholz et al. *Stem Cells Transl Med.* 2013 May;2(5):384-93. doi:l0.5966/sctm.20l2-0l63. Epub 2013 Apr 18; and Leach et al. *Invest Ophthalmol Vis Sci.* 2015 Jan 20;56(2): 1002-13. doi:lO.H67/iovs. 14-15835, for comparison with the methods of the present disclosure.

[0032] **Figure 17** provides a CLYBL TALEN L vector map and sequence of a recombinant vector according to an aspect of the present disclosure.

[0033] **Figure 18** provides a CLYBL TALEN **R** vector map and sequence of a recombinant vector according to an aspect of the present disclosure.

[0034] **Figure 19** provides a CLYBL hOPM vector map and sequence of a recombinant vector according to an aspect of the present disclosure.

[0035] **Figure 20** provides a AAVS1 TALEN L vector map and sequence of a recombinant vector according to an aspect of the present disclosure.

[0036] **Figure 21** provides a AAVS1 TALEN **R** vector map and sequence of a recombinant vector according to an aspect of the present disclosure.

[0037] **Figure 22** provides a **AAVS** hOPM vector map and sequence of a recombinant vector according to an aspect of the present disclosure.

[0038] **Figure 23** provides a schematic of the hOPM construct.

[0039] **Figure 24** provides images comparing results for RPE generation using the Leach et al. protocol and Zhu et al. protocol (left and middle, repsectively), and the methods described herein (right).

[0040] **Figure** 25 provides a schematic illustrating a timeline for RPE generation according to an aspect of the present disclosure as well as images showing the stage of RPE development at different time points.

[0041] **Figure 26** provides schematics showing experimental set-up and results of differentiation condition experiments for a method of RPE differentiation according to an aspect of the present disclosure.

[0042] **Figure 27** provides a schematic illustrating variations in optimized conditions between cell lines.

[0043] **Figure 28** provides a schematic illustrating a timeline for variations in optimized conditions between cell lines according to an aspect of the present disclosure as well as images showing the stage of RPE development at different time points.

[0044] **Figure 29** provides images showing the stage of RPE development at 60+ days for wild-type B (WTB) (left) and wild-type C (WTC) cell lines (right - scale bar = 50 pm).

[0045] **Figure 30** provides images showing the stage of RPE development at 60+ days for Best Disease Patient 6 (BTP6) and Best Disease Patient 7 (BTP7) cell lines. Scale bar = 50 pm.

[0046] **Figure 31** provides images showing the stage of RPE development at 60+ days for Best Disease Patient 8 (BTP8) and Best Disease Patient 10 (BTP10) cell lines. Scale bar = 50 pm.

[0047] **Figure** 32 provides images showing the stage of RPE development that are stained with generic tight junction proteins occluding and phalloidan.

[0048] **Figure 33** provides images showing the stage of RPE development at day 60 for WTB Traffic Light (left - scale bar = 500 pm) and day 60+ for BTP8 (right - scale bar = 100 pm).

[0049] **Figure 34** provides images showing the stage of RPE development at day 45 for BTP10 (left - scale bar = 50 pm) and BTP6 (right - scale bar = 100 pm).

[0050] **Figure 35** provides images showing the stage of RPE development at day 60 for WTB and WTC.

[0051] **Figure 36** provides gene expression results using ddPCR according to an aspect of the present disclosure.

DEFINITIONS

[0052] As used herein, the terms “Retinal Pigment Epithelium” and “RPE” refer to a pigmented layer of hexagonal cells, present *in vivo* in mammals, just outside of the neurosensory retinal that is attached to the underlying choroid. These cells are densely packed with pigment granules, and shield the retinal from incoming light. The retinal pigment epithelium also serves as the limiting transport factor that maintains the retinal environment by supplying small molecules such as amino acid, ascorbic acid and D-glucose while remaining a tight barrier to choroidal blood home substances. In addition, the terms “Retinal Pigment Epithelium” and “RPE” are used herein to refer to a population of cells differentiated from iPSCs using the methods disclosed herein, wherein such cells exhibit one or more of pigmentation, epithelial morphology, and apical-basal polarity characteristic of naturally occurring RPE.

[0053] A “retinal pigment epithelial (RPE) cell”, e.g. a cell differentiated from an iPSC using the methods disclosed herein, can be recognized based on pigmentation, epithelial morphology, and apical-basal polarity. RPE cells express, both at the mRNA and protein level, one or more of the following: MERTK, PAX6, MITF, RPE65, CRALBP, PEDF, OCCLUDIN, EZRIN, CLAUDIN-10, Bestrophin (e.g., BEST1) and/or OTX2. In certain other embodiments, the RPE cells express, both at the mRNA and protein level, one or more of PAX6, MITF, and tyrosinase. RPE cells do not express (at any detectable level) the embryonic stem cell markers Oct-4, nanog, or Rex-l. Specifically, expression of these genes is approximately 100-1000 fold lower in RPE cells than in ES cells or iPSC cells, when assessed by quantitative RT-PCR. Differentiated RPE cells also can be visually recognized by their cobblestone morphology and the initial appearance of pigment. In addition, differentiated RPE cells have trans-epithelial resistance/TER, and trans-epithelial potential/TEP across the monolayer (TERM 00 ohms cm^2; TEP>2 mV), transport fluid and CCh from apical to basal side, and regulate a polarized secretion of cytokines.

[0054] As used herein, the term “embryonic stem cells” refers to embryonic cells derived from the inner cell mass of blastocysts or morulae, optionally that have been serially passaged as cell lines. The term includes cells isolated from one or more blastomeres of an embryo, preferably without destroying the remainder of the embryo. The term also includes cells produced by somatic cell nuclear transfer. “Human embryonic stem cells” (hES cells) includes embryonic cells derived from the inner cell mass of human blastocysts or morulae, optionally that have been serially passaged as cell lines. The hES cells may be derived from fertilization of an egg cell with sperm or DNA, nuclear transfer, parthenogenesis, or by means to generate hES cells with homozygosity in the HLA region. Human ES cells can be produced or derived from a zygote, blastomeres, or blastocyst-staged mammalian embryo produced by the fusion of a sperm and egg cell, nuclear transfer, parthenogenesis, or the reprogramming of chromatin and subsequent incorporation of the reprogrammed chromatin into a plasma membrane to produce an embryonic cell. Human embryonic stem cells include, but are not limited to, MA01, MA09, ACT-4, No. 3, Hl, H7, H9, H14 and ACT30 embryonic stem cells. Human embryonic stem cells, regardless of their source or the particular method used to produce them, can be identified based on (i)

the ability to differentiate into cells of all three germ layers, (ii) expression of at least Oct-4 and alkaline phosphatase, and (iii) ability to produce teratomas when transplanted into immunocompromised animals.

[0055] As used herein, the term “pluripotent stem cells” refers to stem cells that: (a) are capable of inducing teratomas when transplanted in immunodeficient (SCID) mice; (b) are capable of differentiating to cell types of all three germ layers (e.g., can differentiate to ectodermal, mesodermal, and endodermal cell types); and (c) express one or more markers of embryonic stem cells (e.g., express Oct 4, alkaline phosphatase, SSEA-3 surface antigen, SSEA-4 surface antigen, nanog, TRA-l-60, TRA-1-81, SOX2, REX1, etc.), but that cannot form an embryo and the extraembryonic membranes (are not totipotent).

[0056] Exemplary pluripotent stem cells include embryonic stem cells derived from the inner cell mass (ICM) of blastocyst stage embryos, as well as embryonic stem cells derived from one or more blastomeres of a cleavage stage or morula stage embryo (optionally without destroying the remainder of the embryo). These embryonic stem cells can be generated from embryonic material produced by fertilization or by asexual means, including somatic cell nuclear transfer (SCNT), parthenogenesis, and androgenesis. PSCs alone cannot develop into a fetal or adult animal when transplanted in utero because they lack the potential to contribute to all extraembryonic tissue (e.g., placenta in vivo or trophoblast in vitro).

[0057] Pluripotent stem cells also include “induced pluripotent stem cells (iPSCs)” generated by reprogramming a somatic cell by expressing or inducing expression of a combination of factors (herein referred to as reprogramming factors). iPSCs can be generated using fetal, postnatal, newborn, juvenile, or adult somatic cells. In certain embodiments, factors that can be used to reprogram somatic cells to pluripotent stem cells include, for example, Oct4 (sometimes referred to as Oct 3/4), Sox2, c-Myc, and Klf4, Nanog, and Lin28. In some embodiments, somatic cells are reprogrammed by expressing at least two reprogramming factors, at least three reprogramming factors, or four reprogramming factors to reprogram a somatic cell to a pluripotent stem cell. iPSCs are similar in properties to embryonic stem cells.

[0058] As used herein, the term “reprogramming factors” refers to one or more biologically active factors that act on a cell to alter transcription, thereby reprogramming a cell to multipotency or to pluripotency. Reprogramming factors may be provided to cells,

e.g. cells from an individual with a family history or genetic make-up of interest for an RPE related disease, e.g., age-related macular degeneration; individually or as a single composition, that is, as a premixed composition, of reprogramming factors. The factors may be provided at the same molar ratio or at different molar ratios. The factors may be provided once or multiple times in the course of culturing the cells. In some embodiments the reprogramming factor is a transcription factor, including without limitation, Oct3/4; Sox2; Klf4; c-Myc; Nanog; and Lin-28.

[0059] Somatic cells are contacted with reprogramming factors, as defined above, in a combination and quantity sufficient to reprogram the cell to pluripotency. Reprogramming factors may be provided to the somatic cells individually or as a single composition, that is, as a premixed composition, of reprogramming factors. In some embodiments the reprogramming factors are provided as a plurality of coding sequences on a vector.

[0060] Genes may be introduced into the somatic cells or the iPSCs derived therefrom for a variety of purposes, *e.g.* to replace genes having a loss of function mutation, provide marker genes, *etc.* Alternatively, vectors are introduced that express antisense mRNA or ribozymes, thereby blocking expression of an undesired gene. Other methods of gene therapy are the introduction of drug resistance genes to enable normal progenitor cells to have an advantage and be subject to selective pressure, for example the multiple drug resistance gene (MDR), or anti-apoptosis genes, such as bcl-2. Various techniques known in the art may be used to introduce nucleic acids into the target cells, *e.g.* electroporation, calcium precipitated DNA, fusion, transfection, lipofection, infection and the like, as discussed above. The particular manner in which the DNA is introduced is not critical to the practice of the invention.

[0061] As used herein, the term “differentiation” refers to the process whereby relatively unspecialized cells (such as embryonic stem cells or other stem cells) acquire specialized structural and/or functional features characteristic of mature cells. Similarly, “differentiate” refers to this process. Typically, during differentiation, cellular structure alters and tissue-specific proteins appear.

[0062] As used herein, the term “expand” refers to a process by which the number or amount of cells in a cell culture is increased due to cell division. Similarly, the terms “expansion” or “expanded” refers to this process. The terms “proliferate,” “proliferation” or “proliferated” may be used interchangeably with the words

"expand," "expansion", or "expanded." Typically, during an expansion phase, the cells do not differentiate to form mature cells, but divide to form more cells.

[0063] As used herein, the term "growth factor" refers to a substance that promotes cell growth, survival, and/or differentiation. Growth factors include molecules that function as growth stimulators (mitogens), factors that stimulate cell migration, factors that function as chemotactic agents or inhibit cell migration or invasion of tumor cells, factors that modulate differentiated functions of cells, factors involved in apoptosis, or factors that promote survival of cells without influencing growth and differentiation. Examples of growth factors are a fibroblast growth factor (such as FGF-2), epidermal growth factor (EGF), cilliary neurotrophic factor (CNTF), and nerve growth factor (NGF), and activin-A.

[0064] As used herein the terms "growth medium" and "expansion medium" refer to a synthetic set of culture conditions with the nutrients necessary to support the growth (cell proliferation/expansion) of a specific population of cells. In one embodiment, the cells are stem cells, such as iPSCs. Growth media generally include a carbon source, a nitrogen source and a buffer to maintain pH. In one embodiment, growth medium contains a minimal essential media, such as DMEM, supplemented with various nutrients to enhance stem cell growth. Additionally, the minimal essential media may be supplemented with additives such as horse, calf or fetal bovine serum.

[0065] A "host cell," as used herein, denotes an *in vivo* or *in vitro* cell (e.g., a eukaryotic cell cultured as a unicellular entity), which eukaryotic cell can be, or has been, used as recipients for a nucleic acid (e.g., an exogenous nucleic acid), and include the progeny of the original cell which has been genetically modified by the nucleic acid. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

[0066] As used herein, the term "isolated" with reference to a cell, refers to a cell that is in an environment different from that in which the cell naturally occurs, e.g., where the cell naturally occurs in a multicellular organism, and the cell is removed from the multicellular organism, the cell is "isolated." An isolated genetically modified host cell can be present in a mixed population of genetically modified host cells, or in a mixed population comprising genetically modified host cells and host cells that are not genetically modified. For example, an isolated genetically modified host cell can

be present in a mixed population of genetically modified host cells *in vitro,* or in a mixed *in vitro* population comprising genetically modified host cells and host cells that are not genetically modified.

[0067] As used herein, the term "transcription factor" refers to a protein that targets a specific DNA sequence(s) in order to promote or block transcription of genetic information, encoded in DNA, into RNA.

[0068] The term "genetic modification," as used herein, refers to a permanent or transient genetic change induced in a cell following introduction of a nucleic acid (i.e., exogenous nucleic acid) into the cell. Genetic change ("modification") can be accomplished by incorporation of exogenous nucleic acid into the genome of the host cell, or by transient or stable maintenance of the exogenous nucleic acid as an extrachromosomal element. Where the cell is a eukaryotic cell, a permanent genetic change can be achieved by introduction of exogenous nucleic acid into the genome of the cell. Suitable methods of genetic modification include viral infection, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, and the like.

[0069] As used herein, the term "exogenous nucleic acid" refers to a nucleic acid that is not normally or naturally found in and/or produced by a cell in nature, and/or that is introduced into the cell (e.g., by electroporation, transfection, infection, lipofection, or any other means of introducing a nucleic acid into a cell).

[0070] The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. "Oligonucleotide" generally refers to polynucleotides of between about 5 and about 100 nucleotides of single- or double-stranded DNA. However, for the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as oligomers or oligos and may be isolated from genes, or chemically synthesized by methods known in the art. It will be understood that when a nucleotide sequence is represented by a DNA sequence (i.e., A, T, G, C), this also includes an RNA sequence (i.e., A, U, G, C) in which "U" replaces "T."

[0071] A first nucleic acid sequence is "operably linked" with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence.

[0072] The terms "polypeptide," "peptide," and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like.

[0073] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST. See, e.g., Altschul *etal.* (1990), *J. Mol. Biol.* 215:403-10. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. *SeeMeth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See J. *Mol. Biol.* 48: 443-453 (1970).

[0074] Polypeptide described herein may include one or more conservative amino acid substitutions relative to a known amino acid sequence, e.g., a native amino acid

sequence. Conservative amino acid substitution tables providing functionally similar amino acids are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

[0075] A non-conservative amino acid substitution can result from changes in: (a) the structure of the amino acid backbone in the area of the substitution; (b) the charge or hydrophobicity of the amino acid; or (c) the bulk of an amino acid side chain. Substitutions generally expected to produce the greatest changes in protein properties are those in which: (a) a hydrophilic residue is substituted for (or by) a hydrophobic residue; (b) a proline is substituted for (or by) any other residue; (c) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine; or (d) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histadyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl.

[0076] Variant amino acid sequences may, for example, be 80, 90 or even 95 or 98% identical to the native amino acid sequence. Programs and algorithms for determining percentage identity can be found at the NCBI website.

[0077] As used herein, the term “cDNA” refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

[0078] As used herein the terms “encode” and “encoding” refer to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (for example, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA produced by that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is

identical to the mRNA sequence and is usually provided in sequence listings, and non-coding strand, used as the template for transcription, of a gene or cDNA can be referred to as encoding the protein or other product of that gene or cDNA. Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA may include introns.

[0079] As used herein, the term “recombinant”, refers to a nucleic acid or polypeptide molecule that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis of polypeptide or nucleic acid molecules, or by the artificial manipulation of isolated segments of nucleic acid molecules, such as by genetic engineering techniques.

[0080] As used herein, the terms “treatment,” “treating,” and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. “Treatment,” as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0081] The terms “individual,” “subject,” “host,” and “patient,” used interchangeably herein, refer to a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc.

[0082] A “therapeutically effective amount” or “efficacious amount” refers to the amount of a compound or a number of cells that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The “therapeutically effective amount” will vary depending on the compound or the cell, the disease and its severity and the age, weight, etc., of the subject to be treated.

[0083] "AAVS1" is an abbreviation for a site in the human genome that was defined as a preferred integration site of the adeno-associated virus. This "safe harbor" site is in the middle of PPP1R12C gene (see, e.g., DeKelver et al, *Genome Research* 2010).

[0084] The term "gene editing tool" is used herein to refer to one or more components of a gene editing system.

[0085] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0086] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0087] Unless defined otherwise, 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 invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. To the extent the instant disclosure conflicts with any material incorporated by reference herein, the instant disclosure shall control.

[0088] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an induced pluripotent stem cell" includes a plurality of such cells and reference to "the OTX2 polypeptide" includes

reference to one or more OTX2 polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0089] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0090] Methods of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs) are provided. The present disclosure also provides RPE generated using a subject method as well as methods of using RPE generated according to the disclosed methods. Also provided are recombinant vectors useful for the generation of RPE according to the disclosed methods.

METHODS OF GENERATING RETINAL PIGMENT EPITHELIUM

[0091] *In vitro* methods of generating RPE from iPSCs are provided. The methods generally involve: introducing into the iPSCs an exogenous nucleic acid including a nucleotide sequence encoding an orthodenticle homeobox 2 (OTX2) polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a paired box protein (PAX6) polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding a microphthalmia-associated transcription factor (MITF) polypeptide to provide genetically modified iPSCs. The genetically modified iPSCs are cultured under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and under conditions sufficient for differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.

[0092] In some embodiments, the methods specifically exclude the introduction into the iPSCs or genetically modified iPSCs of a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide, or an exogenous

nucleic acid comprising a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide. The present disclosure surprisingly demonstrates that RPE having desired characteristics as described herein can be generated via the introduction of a limited number of specific transcription factors into iPSCs, specifically via the introduction of OTX2, PAX6 and MITF and without the introduction of transcription factors other than OTX2, PAX6 and MITF. In some embodiments, in addition, or alternatively, the methods specifically exclude introducing into the iPSCs or genetically modified iPSCs an exogenous nucleic acid comprising a tyrosinase enhancer.

[0093] As illustrated generally in **FIG. 2,** in an aspect of the present disclosure, suitable iPSCs, e.g., genetically modified iPSCs as described herein, are cultured in a suitable growth medium prior to exposing the cells to differentiation conditions on Day 0 of the differentiation protocol. A suitable grown medium may be, for example, a feeder free maintenance medium for human ES and iPSCs. Exemplary media includes mTeSR™ media available from Stemcell Technologies, e.g., Catalog #85850, Catalog #85851, or Catalog #85852. The growth medium may be supplemented with a selective Rho-associated kinase (ROCK) inhibitor, e.g., Y-27632. See, e.g., Lai et al. *Cell Reprogram.* 2010 Dec; 12(6): 641-653. iPSCs may be seeded at a suitable time prior to introduction of a suitable differentiation medium, e.g., at Day -2 of the differentiation protocol, at a density allowing the culture to grow to confluence at Day 0. For example, prior to the introduction of a suitable differentiation medium, e.g., a neural induction medium, iPSCs may be plated at a density of from about 35,000 cells/cm^2 growth area to about 55,000 cells/cm^2 of growth area, e.g., from about 40,000 cells/cm^2 growth area to about 50,000 cells/cm^2 growth area, such as about 44,000 cells/cm^2 growth area. Depending on the growth rate of the cell line, the plating density, e.g., at Day -2, may be adjusted such that the culture grows to confluence at Day 0.

[0094] A suitable differentiation medium, e.g., a neural induction medium, is then added to the culture (e.g., on Day 0 of the differentiation protocol). Suitable neural induction media are described in the examples section of the present disclosure as well as in, e.g., Yan et al. *Stem Cells Transl Med.* 2013 Nov; 2(11): 862-870; and Chambers, S.M. et al. (2009) *Nat. Biotechnol.* 27:275-280. The cells may be cultured in a suitable neural induction medium for a period of time prior to culturing the

genetically modified iPSCs under conditions sufficient for expression of an OTX2 polypeptide, a PAX6 polypeptide, and an MITF polypeptide from exogenous nucleic acids in the genetically modified iPSCs. This period of time may range from about 1 to about 7 days, e.g., from about 2 to about 6 days, or about 3 to about 5 days, such as about 4 days. In some embodiments, expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from exogenous nucleic acids in the genetically modified iPSCs is induced at Day 4 of culture in a suitable neural induction medium. Where expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from exogenous nucleic acids in the genetically modified iPSCs is under the control of a Tet-inducible promoter, expression may be induced via the addition of tetracycline or a derivative or analogue thereof, e.g., doxycycline, to the neural induction medium.

[0095] Following induction of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from exogenous nucleic acids in the genetically modified iPSCs, the cells may be cultured (e.g., starting on about Day 5 to about Day 7, e.g., Day 6) in a suitable maintenance medium, e.g., in the presence of tetracycline or a derivative or analogue thereof, e.g., doxycycline, to maintain expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from exogenous nucleic acids in the differentiating cells. Suitable maintenance media for differentiating RPE cells are described in the examples section (RPE maintenance medium) of the present disclosure as well as in, e.g., Fronk et al. *J Tissue Eng.* 2016 Jan-Dec; 7:2041731416650838.

[0096] After pigment is first observed, e.g., about Day 23 to about Day 27, such as Day 24 to Day 26, or Day 25, the culture medium may be changed to a suitable chemically defined, serum-free hematopoietic cell medium, e.g., X-vivo™ 10 available from Lonza Group AG under catalog numbers: 04-380Q, 04-743Q, BE04-743Q, and BE02-055Q, e.g., in the presence of tetracycline or a derivative or analogue thereof, e.g., doxycycline, to maintain expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from exogenous nucleic acids in the differentiating cells.

[0097] After mature RPE morphology is observed, e.g., as evidenced by pigmentation and hexagonal shape, e.g., about Day 33 to about Day 37, such as Day 34 to Day 36, or Day 35, the medium may be changed to a suitable chemically defined, serum-free

hematopoietic cell medium, e.g., X-vivo™ 10 as described above, without the addition of tetracycline or a derivative or analogue thereof.

[0098] As discussed above and elsewhere herein, iPSCs may be cultured in a suitable differentiation medium, e.g., a neural induction medium, for a period of time prior to culturing the genetically modified iPSCs under conditions sufficient for expression of an OTX2 polypeptide, a PAX6 polypeptide, and an MITF polypeptide from exogenous nucleic acids in the genetically modified iPSCs. Delaying expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, e.g., by delaying induction of a Tet-inducible promoter operably linked to exogenous nucleic acids including nucleotide sequences encoding the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, for a period of time while the cells are exposed to a suitable differentiation medium has been found to provide for improved generation of RPE.

[0099] Accordingly, in some embodiments, a method as described herein, e.g., which includes a step of culturing genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, includes a step of culturing the genetically modified iPSCs under differentiation conditions, e.g., in a suitable differentiation medium, e.g., a neural induction medium, prior to culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, e.g., prior to induction of a Tet-inducible promoter operably linked to exogenous nucleic acids including nucleotide sequences encoding the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide. This prior culturing step may be for a suitable period of time, e.g., from about 1 to about 5 days, e.g., from about 2 to about 4 days, such as 3 days.

[00100] It has been found that introduction and subsequent expression of an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide, is sufficient to differentiate iPSCs into RPE cells and for the generation of RPE. Thus, an iPSC can be differentiated to become an RPE cell without the need for introducing any other transcription factor (or nucleic acid

encoding same) into the iPSC. Similarly, an iPSC can be differentiated to become an RPE cell without the need for introducing nucleic acids encoding other factors that may influence stem cell to RPE differentiation, or RPE function, or physiology, or pathology, into the iPSC. For example, in some embodiments, a subject method does not require and does not involve introducing into a iPSC a nucleic acid that encodes any one or more of the following: TFEC (NM_012252, NP_036384.1), LHX2 (NM_004789, NP_004780.3), VMD2 (NM_001 139443.1, NP_001 132915.1), CFTR (NM_000492.3, NP_000483.3), RPE65 (NM_000329.2, NP_000320.1), MFRP (NM_031433.3, NP_113621.1), CTRP5 (AF329841.1, AAK17965.1), CFH (NM_000186.3, NP_000177.2), C3 (K02765.1, AAA85332.1), C2B, APOE (NM_001302688.1, NP_001289617.1), APOB (NM_000384.2, NP_000375.2), mTOR (NM_004958.3, NP_004949.1), FOXO1 (NM_002015.3, NP_002006.2), AMPK (NM_006251.5, NP_006242.5), SIRT1-6 (NM_012238.4, NP_036370.2; AY030277.1, AAK51 133.1; NM_012239.5, NP_036371.1; AF083109.1, AAD40852.1; NM_012241.4, NP_036373.1; NM_016539.3, NP_057623.2), HTRP1 (NM_00 125 1845.1, NP_001238774.1), ABCA4 (NM_000350.2, NP_000341.2), TIMP3 (NM_000362.4, NP_000353.1), VEGFA (NM_001025366.2, NP_001 020537.2), CFI (NM_000204.4 , NP_000195.2), TLR3 (NM_003265.2, NP_003256. 1), TLR4 (NM_003266.3, NP_003257.1), APP (NM_000484.3, NP_000475.1), CD46 (NM_002389.4, NP_002380.3), BACE1 (NM_00 1207048.1, NP_001 193977.1), ELOVL4 (NM_022726.3, NP_073563.1), ADAM 10 (NM_001110.3, NP_001101.1), CD55 (NM_000574.4, NP_000565.1), CD59 (NM_00061 1.5, NP_000602.1), and ARMS2 (NM_001099667.2, NP_001 093 137.1). Furthermore, recombinant vectors according to the present disclosure may exclude a nucleic acid that encodes any one or more of the following: TFEC, LHX2, VMD2, CFTR, RPE65, MFRP, CTRP5, CFH, C3, C2B, APOE, APOB, mTOR, FOXO, AMPK, SIRT1-6, HTRP1, ABCA4, TIMP3, VEGFA, CFI, TLR3, TLR4, APP, CD46, BACE1, ELOVL4, ADAM 10, CD55, CD59, and ARMS2.

[00101] In some embodiments, a subject method does not involve forced expression of a polypeptide comprising an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 95%, 97%, or at least 99% amino acid sequence identity, or any other percent identity from at least 70% to 100%, to a TFEC, LHX2, VMD2, CFTR, RPE65, MFRP, CTRP5, CFH, C3,

C2B, APOE, APOB, mTOR, FOXO, AMPK, SIRT1-6, HTRP1, ABCA4, TIMP3, VEGFA, CFI, TLR3, TLR4, APP, CD46, BACE1, ELOVL4, ADAM 10, CD55, CD59, or ARMS2 polypeptide.

[00102] A subject method can involve forced expression of an OTX2 polypeptide, a PAX6 polypeptide, and an MITF polypeptide in an iPSC and/or a differentiating RPE cell. Forced expression of a polypeptide can include introducing an expression vector encoding polypeptides of interest into cells. In some embodiments, a subject method does not involve forced expression of any polypeptide other than an OTX2 polypeptide, a PAX6 polypeptide, and an MITF polypeptide in the iPSC and/or a differentiating RPE cell. Thus, e.g., in some embodiments, a subject method does not involved forced expression of any of a TFEC, LHX2, VMD2, CFTR, RPE65, MFRP, CTRP5, CFH, C3, C2B, APOE, APOB, mTOR, FOXO, AMPK, SIRT1-6, HTRP1, ABCA4, TIMP3, VEGFA, CFI, TLR3, TLR4, APP, CD46, BACE1, ELOVL4, ADAM 10, CD55, CD59, or ARMS 2 polypeptide.

[00103] An RPE cell differentiated from an iPSC using the methods disclosed herein, can be recognized based on pigmentation, epithelial morphology, and apical-basal polarity. RPE cells express, both at the mRNA and protein level, one or more of the following: Pax6, MITF, RPE65 (NM_000329.2, NP_000320.l), CRALBP (NM_000326.4, NP_0003l7.l), PEDF (NM_00 1329903.1, NP_00l3 16832.1), Bestrophin (e.g., BEST1 (NM_001 139443.1, NP_00l 132915.1) and/or Otx2. In certain other embodiments, the RPE cells express, both at the mRNA and protein level, one or more of Pax-6, MitF, and tyrosinase (NM_000372.4, NP_000363.l). RPE cells do not express (at any detectable level) the embryonic stem cell markers Oct-4, nanog, or Rex-l. Specifically, expression of these genes is approximately 100-1000 fold lower in RPE cells than in ES cells or iPSC cells, when assessed by quantitative RT-PCR. Differentiated RPE cells also can be visually recognized by their cobblestone morphology and the initial appearance of pigment. In addition, differentiated RPE cells have trans-epithelial resistance/TER, and trans-epithelial potential/TEP across the monolayer (TERM 00 ohms cm^2; TEP>2 mV), transport fluid and CCh from apical to basal side, and regulate a polarized secretion of cytokines.

[00104] Whether a subject RPE cell expresses particular markers and/or lacks expression of particular markers, can be readily determined using well-established

assays. For example, cell-specific markers and differentiation-specific markers can be detected using any suitable immunological technique, such as fluorescence-activated cell sorting (FACS) for cell-surface markers, immunohistochemistry (for example, of fixed cells or tissue sections) for intracellular or cell-surface markers, Western blot analysis of cellular extracts, and enzyme-linked immunoassay, for cellular extracts or products secreted into the medium. Expression of an antigen (a marker) by a cell is said to be “antibody-detectable” if a significantly detectable amount of antibody will bind to the antigen in a standard immunocytochemistry or flow cytometry assay, optionally after fixation of the cells, and optionally using a labeled secondary antibody or other conjugate (such as a biotin-avidin conjugate) to amplify labeling.

[00105] The expression of cell-specific and differentiation-specific gene products can also be detected at the mRNA level by Northern blot analysis, dot-blot hybridization analysis, or by a reverse transcription polymerase chain reaction (RT-PCR) using sequence-specific primers in standard amplification methods. Expression at the mRNA level is said to be “detectable” according to a standard assay if the performance of the assay on cell samples according to standard procedures in a typical controlled experiment results in clearly discemable hybridization or amplification product. Expression of cell-specific (e.g., RPE cell-specific) and differentiation-specific markers as detected at the protein or mRNA level is considered positive if the level is at least 2-fold, or more than 10- or 50-fold above that of a control cell, such as a pluripotent embryonic stem cell, a fibroblast, or other unrelated cell type.

OTX2 polypeptides

[00106] OTX2 (orthodenticle homeobox 2) polypeptides are known in the art, and any OTX2 polypeptide that retains OTX2 activity (or a nucleic acid encoding such polypeptide) is suitable for use. An OTX2 polypeptide that retains OTX2 activity is also referred to as a “biologically active OTX2 polypeptide.” A suitable OTX2 polypeptide that retains OTX2 activity is one that: (i) binds and activates the DOPAchrome tautomerase (DCT) gene (or homolog thereof) promoter in RPE cells; and/or (ii) binds and synergistically activates the BEST1 (or homolog thereof) promoter in combination with SOX9 (or homolog thereof) and MITF (or homolog

thereof). See, e.g., Takeda et al. *Biochem Biophys Res Commun.* 2003 Jan 24;300(4):908-l4; and Masuda and Esumi, *Journal of Biological Chemistry*. vol. 285, no. 35, pp. 26933-26944, August 27, 2010. OTX2 amino acid sequences can be found in, e.g., GenBank Accession Nos/NCBI Reference Sequences: AAD31385.1, NP_001257452.1, NP_001257453.1, NP_001257454.1, NP_068374.1, and NP_758840.l. In some embodiments, a suitable OTX2 polypeptide retains OTX2 activity and comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 95%, 97%, or at least 99% amino acid sequence identity, or any other percent identity from at least 70% to 100%, to the OTX2 amino acid sequence depicted in Figure 12 or to the amino acid sequence provided by one of AAD31385.1, NP_001257452.1, NP_00l257453.l, NP_00l257454.l, NP_068374.l, and NP_758840.l.

[00107] The OTX2 gene is conserved in chimpanzee, dog, cow, mouse, rat, and zebrafish. See, e.g., XP_00 1163043.2, XP_003435 187.1, NP_00l 180130.1, NP_001273410.1, XP_00625 1897.1, and NP_5 71326.1. OTX2 polypeptide-encoding nucleic acids can be found in, e.g., GenBank Accession Nos/NCBI Reference Sequences: NM_00l270523.l. NM_00l270524.l, NM_00 1270525.1, NM_02l728.3, NM_l 72337.2.

PAX6 polypeptides

[00108] PAX6 (paired box 6) polypeptides are known in the art, and any PAX6 polypeptide that retains PAX6 activity (or a nucleic acid encoding such polypeptide) is suitable for use. A PAX6 polypeptide that retains PAX6 activity is also referred to as a “biologically active PAX6 polypeptide.” A suitable PAX6 polypeptide that retains PAX6 activity is one that: (i) includes two DNA binding domains (a paired domain (PD) at the N-terminus and a paired-like homeodomain (HD), a glycine-rich region that links the two DNA binding domains, and a transactivation domain at the C-terminus; and (ii) binds the PAX6-specific binding site Tsp2Asl (GGCCAGTCACGCGTGTAATT (SEQ ID NO: 1)) and/or Tspl lBs (AATTAGCCAGGCATGGTAGCACA (SEQ ID NO:2)), and/or GCalBLs5 (TTAGTTCCAGGTCAG (SEQ ID NO:3)). See, e.g., Singh et al. *J Biol Chem.* 2000 Jun 9;275(23): 17306-13; and Zhou et al. *Genome Res.* 2002 Nov; 12(11): 1716-1722. PAX6 amino acid sequences can be found in, e.g., GenBank Accession

Nos/NCBI Reference Sequences: NP_000271.1, NP_001 121084.1, NP_001245391.1, NP_001245392.1, NP_001245393.1, NP_001245394.1, NP_001297087.1, NP_001297088.1, NP_001297089.1, NP_001297090.1, and NP_001595.2. In some embodiments, a suitable PAX6 polypeptide retains PAX6 activity and comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 95%, 97%, or at least 99% amino acid sequence identity, or any other percent identity from at least 70% to 100%, to the PAX6 amino acid sequence depicted in Figure 13 or to the amino acid sequence provided by one ofNP_000271.1, NP_001 121084.1, NP_001245391.1, NP_001245392.1, NP_001245393.1, NP_00 1245394.1, NP_001297087.1, NP_001297088.1, NP_001297089.1, NP_00 1297090.1, and NP_001595.2.

[00109] The PAX6 gene is conserved in chimpanzee, Rhesus monkey, cow, mouse, rat, chicken, zebrafish, fruit fly, mosquito, C.elegans, and frog. See, e.g., XP_003 9544 13.1, XP_001084865.2, NP_001035735.1, NP_001231 127.1, NP_037133.1, NP_990397.1, NP_00 1006763.1, NP_571379.1, NP_524638.3, XP_311087.5, and NP_001 024570.1. PAX6 polypeptide-encoding nucleic acids can be found in, e.g., GenBank Accession Nos/NCBI Reference Sequences: NM_000280.4, NM_001 127612.1, NM_001258462.1, NM_001258463.1, NM_00 1258464.1, NM_00 125 8465.1, NM_001310158.1, NM_001310159.1, NM_001310160.1, NM_001310161.1, and NM_001604.5.

MITF polypeptides

[00110] MITF (Microphthalmia-associated transcription factor, also known as melanogenesis associated transcription factor) polypeptides are known in the art, and any MITF polypeptide that retains MITF activity (or a nucleic acid encoding such polypeptide) is suitable for use. A MITF polypeptide that retains MITF activity is also referred to as a "biologically active MITF polypeptide." A suitable MITF polypeptide that retains MITF activity is one that: (i); is capable of forming both a homodimer and a heterodimer and (ii) binds consensus M-box (TCATGTG (SEQ ID NO:4)) and E-box (CACGTG (SEQ ID NO:5)) sequences. See, e.g., Pogenberg et al. *Genes Dev.* 2012 Dec 1; 26(23): 2647-2658; and George, et al. *Am J Hum Genet.* 2016 Dec 1; 99(6): 1388-1394. MITF amino acid sequences can be found in, e.g., GenBank Accession Nos/NCBI Reference Sequences: NP_000239.1,

NP_001 171896. 1, NP_00l 171 897.1, NP_001341533.1, NP_001341534.1, NP_001341535.1, NP_001341536.1, NP_001341537.1, NP_006713.1, NP_93780l.l, NP_937802.l, NP_937820.l, and NP_93782l.2. In some embodiments, a suitable MITF polypeptide retains MITF activity and comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 95%, 97%, or at least 99% amino acid sequence identity, or any other percent identity from at least 70% to 100%, to the MITF amino acid sequence depicted in Figure 14 or to the amino acid sequence provided by one ofNP_000239.l, NP_00l 171896.1 , NP_00l 171897. 1, NP_001341533.1, NP_001341534.1, NP_001341535.1, NP_001341536.1, NP_001341537.1 , NP_006713.1, NP_93780l.l, NP_937802.l, NP_937820.l, and NP_93782l.2.

[00111] The MITF gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, and frog. See, e.g., XP_00l 138876.1, XP_00 1086496. 1, NP_00 1003337. 1, NP_00 1001150.1, NP_00 1106669. 1, NP_001178018.1, NP_990360.1, NP_001093747.1, NP_571922.2. MITF polypeptide-encoding nucleic acids can be found in, e.g., NM_000248.3, NM_00 1184967.1, NM_00l 184968.1, NM_00l 354604.1, NM_00 1354605.1, NM_00 1354606.1, NM_00l354607.l, NM_00l 354608.1, NM_006722.2, NM_198158.2, NM_198159.2, NM_198177.2, and NM_198178.2.

Introduction of exogenous nucleic acids encoding OTX2. PAX6 and MITF polypeptides into iPSCs

[00112] In some embodiments, introduction of an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide into an iPSC is achieved by genetic modification of the iPSC with an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide. An exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide is also referred to herein as “an exogenous OTX2 nucleic acid.” An exogenous nucleic acid comprising a nucleotide sequence encoding a

PAX6 polypeptide is also referred to herein as "an exogenous PAX6 nucleic acid." An exogenous nucleic acid comprising a nucleotide sequence encoding a MITF polypeptide is also referred to herein as "an exogenous MITF nucleic acid."

[00113] The exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid can be a recombinant expression vector, where suitable vectors include, e.g., recombinant retroviruses, lentiviruses, and adenoviruses; retroviral expression vectors, lentiviral expression vectors, nucleic acid expression vectors, and plasmid expression vectors. In some cases, the exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid is integrated into the genome of a host iPSC cell and its progeny. In other cases, the exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid persists in an episomal state in the host iPSC cell and its progeny. In some cases, an endogenous, natural version of the OTX2-encoding nucleic acid, PAX6-encoding nucleic acid, and/or MITF-encoding nucleic acid may already exist in the cell but an additional "exogenous gene" is added to the host iPSC cell to increase OTX2, PAX6, and/or MITF polypeptide expression. In other cases, the exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid encodes an OTX2 polypeptide, PAX6 polypeptide, and/or MITF polypeptide, respectively, having an amino acid sequence that differs by one or more amino acids from an OTX2 polypeptide, PAX6 polypeptide, and/or MITF polypeptide encoded by an endogenous OTX2-encoding nucleic acid, endogenous PAX6-encoding nucleic acid, and/or endogenous MITF-encoding nucleic acid, respectively, within the host iPSC cell.

[00114] In some embodiments, an exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid is introduced into a single iPSC (e.g., a single iPSC host cell) *in vitro.* In other embodiments, an exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid is introduced into a population of iPSCs (e.g., a population of host iPSCs) *in vitro.*

[00115] Where a population of iPSCs is genetically modified with an exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid, the an exogenous OTX2 nucleic acid, exogenous PAX6 nucleic acid, and/or exogenous MITF nucleic acid can be introduced into greater than 20% of the total

population of cells somatic cells, e.g., 25%, 30%, 35%, 40%, 44%, 50%, 57%, 62%, 70%, 74%, 75%, 80%, 90%, or other percent of cells greater than 20%.

[00116] Examples of suitable mammalian expression vectors include, but are not limited to: recombinant viruses, nucleic acid vectors, such as plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, human artificial chromosomes, cDNA, cRNA, and polymerase chain reaction (PCR) product expression cassettes. Examples of suitable promoters for driving expression of an OTX2-encoding nucleotide sequence, PAX6-encoding nucleotide sequence, and/or MITF-encoding nucleotide sequence include, but are not limited to, retroviral long terminal repeat (LTR) elements; constitutive promoters such as CMV, HSV1-TK, SV40, EF-la, β-actin; phosphoglycerol kinase (PGK), and inducible promoters, such as those containing Tet-operator elements. In some cases, the mammalian expression vector encodes, in addition to an exogenous OTX2 polypeptide, PAX6 polypeptide and/or MITF polypeptide, a marker gene that facilitates identification or selection of cells that have been transfected or infected. Examples of marker genes include, but are not limited to, genes encoding fluorescent proteins, e.g., enhanced green fluorescent protein, DS-Red, yellow fluorescent protein, and cyanofluorescent protein; and genes encoding proteins conferring resistance to a selection agent, e.g., the neomycin resistance gene, and the blasticidin resistance gene.

[00117] Examples of recombinant viruses include, but are not limited, to retroviruses (including lentiviruses); adenoviruses; and adeno-associated viruses. An example of a suitable recombinant retrovirus is murine moloney leukemia virus (MMLV); however, other recombinant retroviruses may also be used, e.g., Avian Leukosis Virus, Bovine Leukemia Virus, Murine Leukemia Virus (MLV), Mink-Cell focus-inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis virus, Gibbon Abe Leukemia Virus, Mason Pfizer Monkey Virus, or Rous Sarcoma Virus, see, e.g., U.S. Pat. No. 6,333,195.

[00118] In other cases, the recombinant retrovirus is a lentivirus (e.g., Human Immunodeficiency Virus-l (HIV-l); Simian Immunodeficiency Virus (SIV); or Feline Immunodeficiency Virus (FIV)), See, e.g., Johnston et al, (1999), Journal of Virology, 73(6):499l-5000 (FIV); Negre D et al, (2002), Current Topics in Microbiology and Immunology, 261:53-74 (SIV); Naldini et al, (1996), Science, 272:263-267 (HIV).

[00119] The recombinant retrovirus may comprise a viral polypeptide (e.g., retroviral env) to aid entry into the target cell. Such viral polypeptides are well-established in the art, see, e.g., U.S. Pat. No. 5,449,614, the disclosure of which is incorporated by reference herein. The viral polypeptide may be an amphotropic viral polypeptide, e.g., amphotropic env, which aids entry into cells derived from multiple species, including cells outside of the original host species. The viral polypeptide may be a xenotropic viral polypeptide that aids entry into cells outside of the original host species. In some embodiments, the viral polypeptide is an ecotropic viral polypeptide, e.g., ecotropic env, which aids entry into cells of the original host species.

[00120] Examples of viral polypeptides capable of aiding entry of retroviruses into cells include but are not limited to: MMLV amphotropic env, MMLV ecotropic env, MMLV xenotropic env, vesicular stomatitis virus-g protein (VSV-g), HIV-l env, Gibbon Ape Leukemia Virus (GALV) env, RD114, FeLV-C, FeLV-B, MLV 10A1 env gene, and variants thereof, including chimeras. See e.g., Yee et al, (1994), Methods Cell Biol., Pt A:99-ll2 (VSV-G); U.S. Pat. No. 5,449,614. In some cases, the viral polypeptide is genetically modified to promote expression or enhanced binding to a receptor.

[00121] In general, a recombinant virus is produced by introducing a viral DNA or RNA construct into a producer cell. In some cases, the producer cell does not express exogenous genes. In other cases, the producer cell is a “packaging cell” comprising one or more exogenous genes, e.g., genes encoding one or more gag, pol, or env polypeptides and/or one or more retroviral gag, pol, or env polypeptides. The retroviral packaging cell may comprise a gene encoding a viral polypeptide, e.g., VSV-g that aids entry into target cells. In some cases, the packaging cell comprises genes encoding one or more lentiviral proteins, e.g., gag, pol, env, vpr, vpu, vpx, vif, tat, rev, or nef. In some cases, the packaging cell comprises genes encoding adenovirus proteins such as E1A or E1B or other adenoviral proteins. For example, proteins supplied by packaging cells may be retrovirus-derived proteins such as gag, pol, and env; lentivirus-derived proteins such as gag, pol, env, vpr, vpu, vpx, vif, tat, rev, and nef; and adenovirus-derived proteins such as E1A and E1B. In many examples, the packaging cells supply proteins derived from a virus that differs from the virus from which the viral vector derives.

[00122] Packaging cell lines include but are not limited to any easily-transfectable cell line. Packaging cell lines can be based on 293T cells, NIH3T3, COS or HeLa cell lines. Packaging cells are often used to package virus vector plasmids deficient in at least one gene encoding a protein required for virus packaging. Any cells that can supply a protein or polypeptide lacking from the proteins encoded by such virus vector plasmid may be used as packaging cells. Examples of packaging cell lines include but are not limited to: Platinum-E (Plat-E); Platinum-A (Plat-A); BOSC 23 (ATCC CRL 11554); and Bing (ATCC CRL 11270), see, e.g., Morita et al, (2000), Gene Therapy, 7:1063-1066; Onishi et al., (1996), Experimental Hematology, 24:324-329; and U.S. Pat. No. 6,995,009, the disclosure of which is incorporated by reference herein. Commercial packaging lines are also useful, e.g., Ampho-Pak 293 cell line, Eco-Pak 2-293 cell line, RetroPack PT67 cell line, and Retro-X Universal Packaging System (all available from Clontech).

[00123] The retroviral construct may be derived from a range of retroviruses, e.g., MMLV, HIV-l, SIV, FIV, or other retrovirus described herein. The retroviral construct may encode all viral polypeptides necessary for more than one cycle of replication of a specific virus. In some cases, the efficiency of viral entry is improved by the addition of other factors or other viral polypeptides. In other cases, the viral polypeptides encoded by the retroviral construct do not support more than one cycle of replication, e.g., U.S. Pat. No. 6,872,528. In such circumstances, the addition of other factors or other viral polypeptides can help facilitate viral entry. In an exemplary embodiment, the recombinant retrovirus is HIV-l virus comprising a VSV-g polypeptide but not comprising a HIV-l env polypeptide.

[00124] The retroviral construct may comprise: a promoter, a multi-cloning site, and/or a resistance gene. Examples of promoters include but are not limited to CMV, SV40, EFla, β-actin; retroviral LTR promoters, and inducible promoters. The retroviral construct may also comprise a packaging signal (e.g., a packaging signal derived from the MFG vector; a psi packaging signal). Examples of some retroviral constructs known in the art include but are not limited to: pMX, pBabeX or derivatives thereof. See e.g., Onishi et al. (1996) *Experimental Hematology* 24:324-329. In some cases, the retroviral construct is a self-inactivating lentiviral vector (SIN) vector, see, e.g., Miyoshi et al. (1998) *J. Virol.* 72(10):8150-8157. In some cases, the retroviral construct is LL-CG, LS-CG, CL-CG, CS-CG, CLG or MFG.

Miyoshi et al. (1998) *J. Virol.* 72(10):8150-8157; Onishi et al. (1996) *Experimental Hematology* 24:324-329; Riviere et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:6733-6737. Virus vector plasmids (or constructs), include: pMXs, pMxs-IB, pMXs-puro, pMXs-neo (pMXs-IB is a vector carrying the blasticidin-resistant gene instead of the puromycin-resistant gene of pMXs-puro) Kimatura et al. (2003) *Experimental Hematology* 31: 1007-1014; MFG Riviere et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:6733-6737; pBabePuro; Morgenstem et al. (1990) *Nucleic Acids Research* 18:3587-3596; LL-CG, CL-CG, CS-CG, CLG Miyoshi et al. (1998) J. *Virol.* 72:8150-8157 and the like as the retrovirus system; and pAdexl Kanegae et al. (1995) *Nucleic Acids Research* 23:3816-3821 and the like as the adenovirus system. In exemplary embodiments, the retroviral construct comprises blasticidin (e.g., pMXs-IB), puromycin (e.g., pMXs-puro, pBabePuro); or neomycin (e.g., pMXs-neo). See, e.g., Morgenstem et al. (1990) *Nucleic Acids Research* 18:3587-3596.

[00125] Methods of producing recombinant viruses from packaging cells and their uses are well established; see, e.g., U.S. Pat. Nos. 5,834,256; 6,910,434; 5,591,624; 5,817,491; 7,070,994; and 6,995,009, the disclosures of each of which are incorporated by reference herein. Many methods begin with the introduction of a viral construct into a packaging cell line. The viral construct may be introduced by any method known in the art, including but not limited to: a calcium phosphate method, a lipofection method (Felgner et al. (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84:7413-7417), an electroporation method, microinjection, Fugene transfection, and the like, and any method described herein.

[00126] A nucleic acid construct can be introduced into a cell using a variety of well-known techniques, such as non-viral based transfection of the cell. In an exemplary aspect the construct is incorporated into a vector and introduced into a host cell. Introduction into the cell may be performed by any non-viral based transfection known in the art, such as, but not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion. Other methods of transfection include transfection reagents such as Lipofectamine™, Dojindo Hilymax™, Fugene™, jetPEI™, Effectene™, and DreamFect™.

[00127] Introduction of exogenous nucleic acids encoding OTX2, PAX6 and MITF polypeptides into iPSCs may be accomplished using a suitable gene editing system (e.g. a site specific gene editing system such as a programmable gene editing system) as described herein. A gene editing system as described herein may make use of a "targeted nuclease". By a "targeted nuclease" it is meant a nuclease that cleaves a specific DNA sequence to produce a double strand break at that sequence. Of particular interest in the context of the present disclosure is the use of a TALEN system, which includes a transcription activator-like effector (TALE) protein fused to a nuclease (e.g., Fokl nuclease) - TALEN, or suitable vector and/or vectors encoding same, for targeted integration of a nucleic acid sequence encoding an OTX2 polypeptide, a nucleic acid sequence encoding a PAX6 polypeptide, and/or a nucleic acid sequence encoding a MITF polypeptide. In such embodiments, a recombinant vector according to the present disclosure may serve as a donor vector for use with a suitable TALEN system designed to introduce a nucleic acid sequence encoding an OTX2 polypeptide, a nucleic acid sequence encoding aPAX6 polypeptide, and/or a nucleic acid sequence encoding a MITF polypeptide, e.g., an OTX2-T2A-PAX6-T2A-MITF encoding nucleic acid, into the genome of an iPSC. Such a donor vector may include an expression cassette including an inducible promoter, e.g., a Tet-0 promoter, driving expression of OTX2-T2A-PAX6-T2A-MITF. A suitable TALEN system may include, e.g., suitable left and right TALEN plasmids. In some embodiments, the TALEN system is specifically configured to integrate a nucleic acid sequence encoding an OTX2 polypeptide, a nucleic acid sequence encoding a PAX6 polypeptide, and/or a nucleic acid sequence encoding a MITF polypeptide, e.g., an OTX2-T2A-PAX6-T2A-MITF encoding nucleic acid, at the AAVS1 locus and/or the CLYBL locus of an iPSC genome. TALENs are described in greater detail in US Patent Application No. 2011/0145940, the disclosure of which is incorporated by reference herein. See also, Christian M. et al. *Nucleic Acids Res.* 2011 Jul;39(l2):e82. Epub 2011 Apr 14.

[00128] Another example of a targeted nuclease that finds use in the subject methods is a zinc finger nuclease or "ZFN", which may include, e.g., a designed zinc finger domain and/or a selected zinc finger domain. ZFNs are targeted nucleases comprising a nuclease fused to a zinc finger DNA binding domain (see, e.g., DeKelver et al, *Genome Research* 2010). By a "zinc finger DNA binding domain" or

"ZFBD" it is meant a polypeptide domain that binds DNA in a sequence-specific manner through one or more zinc fingers. A zinc finger is a domain of about 30 amino acids within the zinc finger binding domain whose structure is stabilized through coordination of a zinc ion. Examples of zinc fingers include C2H2 zinc fingers, C3H zinc fingers, and C4 zinc fingers. A "designed" zinc finger domain is a domain not occurring in nature whose design/composition results principally from rational criteria, e.g., application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFP designs and binding data. See, for example, U.S. Pat. Nos. 6,140,081; 6,453,242; and 6,534,261; see also WO 98/53058; WO 98/53059; WO 98/53060; and WO 03/016496. A "selected" zinc finger domain is a domain not found in nature whose production results primarily from an empirical process such as phage display, interaction trap or hybrid selection. ZFNs are described in greater detail in US Patent No. 7,888,121 and US Patent No. 7,972,854, the disclosures of which are incorporated herein by reference. The most recognized example of a ZFN in the art is a fusion of the Fokl nuclease with a zinc finger DNA binding domain.

[00129] Other non-limiting examples of targeted nucleases include naturally occurring and recombinant nucleases, e.g. CRISPR/Cas9, restriction endonucleases, meganucleases homing endonucleases, and the like. See, e.g., F. Oceguera-Yanez et al. *Methods.* 101 (2016) 43-55, which discusses the adaptation of TALEN and CRISPR/Cas9 nuclease technologies in order to introduce transgenes into the AAVS1 locus in iPSCs.

[00130] Typically, targeted nucleases are used in pairs, with one targeted nuclease specific for one sequence of an integration site and the second targeted nuclease specific for a second sequence of an integration site. In the methods presented herein, any targeted nuclease(s) that are specific for the integration site of interest and promote the cleavage of an integration site may be used. The targeted nuclease(s) may be stably expressed by the cells. Alternatively, the targeted nuclease(s) may be transiently expressed by the cells, e.g. it may be provided to the cells prior to, simultaneously with, or subsequent to contacting the cells with the donor vector. If transiently expressed by the cells, the targeted nuclease(s) may be provided to cells as DNA, e.g. plasmid or vector, as described herein, e.g., by using transfection, nucleofection, or the like. Alternatively, targeted nuclease(s) may be provided to

cells as mRNA encoding the targeted nuclease(s), e.g. using well-developed transfection techniques; see, e.g. Angel and Yanik (2010) *PLoS ONE* 5(7): el 1756; Beumer et al. (2008) *PNAS 105(50):* 19821-19826, and the commercially available TransMessenger® reagents from Qiagen, Stemfect™ RNA Transfection Kit from Stemgent, and TransIT®-mRNA Transfection Kit from Mirus Bio LLC. Alternatively, the targeted nuclease(s) may be provided to cells as a polypeptide. Such polypeptides may optionally be fused to a polypeptide domain that increases solubility of the product, and/or fused to a polypeptide permeant domain to promote uptake by the cell. The targeted nuclease(s) may be produced by eukaryotic cells or by prokaryotic cells, it may be further processed by unfolding, e.g. heat denaturation, DTT reduction, etc. and may be further refolded, using methods known in the art. It may be modified, e.g. by chemical derivatization or by molecular biology techniques and synthetic chemistry, e.g. so as to improve resistance to proteolytic degradation or to optimize solubility properties.

RECOMBINANT VECTORS AND HOST CELLS

[00131] In one aspect, the present disclosure provides a recombinant vector, e.g., a donor vector for use with a suitable gene editing system, including a promoter, a nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide operably linked to the promoter, a nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide operably linked to the promoter; and a nucleic acid including a nucleotide sequence encoding an MITF polypeptide operably linked to the promoter.

[00132] In another aspect, the present disclosure provides a recombinant vector, e.g., a donor vector for use with a suitable gene editing system, including an inducible promoter, e.g., aTet-inducible promoter, an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide, wherein one or more of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide are operably linked to the inducible promoter.

[00133] In another aspect, the present disclosure provides a recombinant vector, e.g., a donor vector for use with a suitable gene editing system, including an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide, wherein the recombinant vector is targeted to an AAVS1 locus or a CLYBL locus of an iPSC.

[00134] A variety of suitable promoters are known in the art, which may be used in connection with the recombinant vectors described herein, including for example, ubiquitous promoters (CMV, CAG, CBA, EFla) and tissue specific promoters (Synapsin, CamKIIa, GFAP, RPE, ALB, TBG, MBP, MCK, TNT, and aMHC). The promoter may be an inducible promoter, such as a Tet-inducible promoter, e.g., a PTRE3G or PTRE3GS promoter. See, e.g., Zhou et al. *Gene Ther. 2006* Oct;13(19):1382-90. Epub 2006 May 25; and Heinz et al. (2011) *Human Gene Ther.* 22(2): 166-176.

[00135] In some embodiments, the nucleotide sequence encoding an OTX2 polypeptide encodes a biologically active OTX2 polypeptide having an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 95%, 97%, or at least 99% amino acid sequence identity, or any other percent identity from at least 70% to 100%, to the OTX2 amino acid sequence depicted in Figure 12 or to the amino acid sequence provided by one of AAD31385.1, NP_001257452.1, NP_001257453.1, NP_001257454.1, NP_068374.1, and NP_758840.1.

[00136] In some embodiments, the nucleotide sequence encoding a PAX6 polypeptide encodes a biologically active PAX6 polypeptide having an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 95%, 97%, or at least 99% amino acid sequence identity, or any other percent identity from at least 70% to 100%, to the PAX6 amino acid sequence depicted in Figure 13 or to the amino acid sequence provided by one of NP_000271.1, NP_001121084.1, NP_001245391.1, NP_001245392.1, NP_001245393.1, NP_001245394.1, NP_001297087.1, NP_001297088.1, NP_001297089.1, NP_001297090.1, and NP_001595.2.

[00137] In some embodiments, the nucleotide sequence encoding a MITF polypeptide encodes a biologically active MITF polypeptide having an amino acid sequence

having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 95%, 97%, or at least 99% amino acid sequence identity, or any other percent identity from at least 70% to 100%, to the MITF amino acid sequence depicted in Figure 14 or to the amino acid sequence provided by one of NP_000239. 1, NP_00l 171 896.1 , NP_00l 171 897.1, NP_001341533.1, NP_001341534.1, NP_001341535.1, NP_001341536.1, NP_001341537.1 , NP_006713.1, NP_93780l.l, NP_937802.l, NP_937820.l, and NP_93782l.2.

[00138] The recombinant vector, e.g., a donor vector for use with a suitable gene editing system, may also express one or more reporter molecules, e.g., one or more fluorescent reporters, e.g., GFP, RFP, mCherry, CFP, YFP, tdTomato, etc.). In some embodiments, the vector does not include a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide. In addition, or alternatively, in some embodiments the recombinant vector does not include a tyrosinase enhancer.

[00139] Recombinant vectors according to the present disclosure may exclude a nucleic acid that encodes any one or more of the following: TFEC, LHX2, VMD2, CFTR, RPE65, MFRP, CTRP5, CFH, C3, C2B, APOE, APOB, mTOR, FOXO, AMPK, SIRT1-6, HTRP1, ABCA4, TIMP3, VEGFA, CFI, TLR3, TLR4, APP, CD46, BACE1, ELOVL4, ADAM 10, CD55, CD59, and ARMS2.

[00140] In some embodiments, the nucleotide sequence encoding an **OTX2** polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence. Ribosomal skipping sequences are known in the art, including, e.g., sequences encoding the T2A (EGRGSLLTCGDVEENPGP (SEQ ID NO:6), P2A (ATNFSLLKQAGDVEENPGP (SEQ ID NO: 7), E2A (QCTNYALLKLAGDVESNPGP (SEQ ID NO: 8)) and F2A (VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:9) peptides. (GSG) residues can be added to the 5' end of the peptide to improve cleavage efficiency. In some embodiments, the nucleotide sequence encoding an OTX2 polypeptide is position closer to the promoter, e.g., the Tet-inducible promoter, than the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding a PAX6

polypeptide is positioned closer to the promoter than the nucleotide sequence encoding a MITF polypeptide.

[00141] The above vectors may be used to genetically modify one or more host cells. Accordingly, the present disclosure provides a genetically modified host cell, e.g., a genetically modified iPSC, including an exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide. In some embodiments, the exogenous nucleic acid including a nucleotide sequence encoding an OTX2 polypeptide, the exogenous nucleic acid including a nucleotide sequence encoding a PAX6 polypeptide, and the exogenous nucleic acid including a nucleotide sequence encoding an MITF polypeptide are integrated into the genome of the genetically modified host cell, e.g., a genetically modified iPSC. In other embodiments, the exogenous nucleic acids are maintained in an episomal state. The host cell may also include one or more gene editing tools as described herein, e.g. a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and/or one or more nucleic acids encoding a ZFN.

[00142] In some embodiments, the genetically modified host cell does not include an exogenous nucleic acid(s) encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide. In addition, or alternatively, in some embodiments the genetically modified host cell does not include an exogenous tyrosinase enhancer.

[00143] In some embodiments, the genetically modified host cell does not include an exogenous nucleic acid(s) encoding one or more of the following: TFEC, LHX2, VMD2, CFTR, RPE65, MFRP, CTRP5, CFH, C3, C2B, APOE, APOB, mTOR, FOXO, AMPK, SIRT1-6, HTRP1, ABCA4, TIMP3, VEGFA, CFI, TLR3, TLR4, APP, CD46, BACE1, ELOVL4, ADAM10, CD55, CD59, and ARMS2.

Induced pluripotent stem cells (IPSCs)

[00144] iPSCs cells for use in the disclosed methods may be obtained from any suitable source, e.g., a commercial provider, or prepared from any suitable somatic cell of interest. For example, any cells other than germ cells of mammalian origin (such as, humans, non-human primates, (e.g., monkeys), pigs, rodents (e.g., mice or

rats), etc.) can be used as starting material for the production of iPSCs. Examples include keratinizing epithelial cells, mucosal epithelial cells, exocrine gland epithelial cells, endocrine cells, liver cells, epithelial cells, endothelial cells, fibroblasts, muscle cells, cells of the blood and the immune system, cells of the nervous system including nerve cells and glia cells, pigment cells, and progenitor cells, including hematopoietic stem cells, amongst others. There is no limitation on the degree of cell differentiation, the age of an animal from which cells are collected and the like; even undifferentiated progenitor cells (including somatic stem cells) and finally differentiated mature cells can be used as sources of somatic cells in connection with the preparation of iPSCs. In some embodiments, the somatic cell is itself a RPE cell such as a human RPE cell. The RPE cell can be an adult or a fetal RPE cell. Thus, in some embodiments, the somatic cell is a fetal human RPE cell, obtained from a human fetus of about week 9 to about week 38 of gestation, such as about week 9 to about week 16 of gestation, about week 17 to about week 25 of gestation, about 16 to about 19 weeks, or about week 26 to about week 38 of gestation.

[00145] In some embodiments, the somatic cell includes at least one allele encoding a mutation associated with an RPE-associated disease, e.g. a mutation in the RPE65 gene and/or the BEST1 gene.

[00146] The choice of mammalian individuals as a source of somatic cells is not particularly limited. Allogeneic cells can be used. Thus, in some embodiments, the iPSCs are not matched for MHC (e.g., HLA). In other embodiments, the somatic cells are obtained from an individual, for example to provide patient-specific RPE. This may be desirable, for example, where the RPE that is ultimately generated will be used to test specific active agents or specific genome editing tools for use in a particular patient or subgroup of patients.

[00147] Patient-specific RPE generated according to a method as described herein may also be transplanted into a patient, e.g., a patient from which the somatic cells used to prepare the iPSCs were derived, e.g., to treat a disease characterized by degeneration of RPE, e.g., age-related macular degeneration (AMD), macular dystrophies such as Stargardf s and Stargardf s-like disease, Best disease (vitelliform macular dystrophy), and adult vitelliform dystrophy or subtypes of retinitis pigmentosa. Such transplantation may take place following genomic modification of the RPE cells, e.g., using a genome editing tool as described herein.

[00148] Somatic cells isolated from a mammal can be pre-cultured using a medium known to be suitable for their cultivation according to the choice of cells before being subjected to the step of nuclear reprogramming. Specific non-limiting examples of such media include, but are not limited to, minimal essential medium (MEM) containing about 5 to 20% fetal calf serum (FCS), Dulbecco's modified Eagle medium (DMEM), RPMI 1640 medium, 199 medium, F12 medium, and the like. One of skill in the art can readily ascertain appropriate tissue culture conditions to propagate particular cell types from a mammal, such as a human. In some embodiments, to obtain completely xeno-free human iPSCs, the medium can exclude ingredients derived from non-human animals, such as FCS. Media comprising a basal medium supplemented with human-derived ingredients suitable for cultivation of various somatic cells (particularly, recombinant human proteins such as growth factors), non-essential amino acids, vitamins and the like are commercially available; those skilled in the art are able to choose an appropriate xeno-free medium according to the source of somatic cells. Somatic cells pre-cultured using a xeno-free medium are dissociated from the culture vessel using an appropriate xeno-free cell dissociation solution, and recovered, after which they are brought into contact with nuclear reprogramming substances.

[00149] Generally, cells are cultured at about 35 to 38° C, usually at 37° C, in about 4-6% CO2 generally at 5% CO2, unless specifically indicated otherwise below.

[00150] Somatic cells can be reprogrammed to produce induced pluripotent stem cells (iPSCs) using methods known to one of skill in the art. One of skill in the art can readily produce induced pluripotent stem cells, see for example, U.S. Patent Application Publication No. 20090246875, U.S. Patent Application Publication No. 20100210014; U.S. Patent Application Publication No. 20120276636; U.S. Pat. No. 8,058,065; U.S. Pat. No. 8,129,187; PCT Publication No. WO 2007/069666 Al, and U.S. Pat. No. 8,268,620, the disclosures of each of which are incorporated by reference herein. Generally, nuclear reprogramming factors are used to produce pluripotent stem cells from a somatic cell. In some embodiments, at least three, or at least four, of Klf4, c-Myc, Oct3/4, Sox2, Nanog, and Lin28 are utilized. In other embodiments, Oct3/4, Sox2, c-Myc and Klf4 is utilized.

[00151] The somatic cells are treated with a nuclear reprogramming substance, which is generally one or more factor(s) capable of inducing an iPSC from a somatic cell or

a nucleic acid that encodes these substances (including forms integrated in a vector). The nuclear reprogramming substances generally include at least Oct3/4, Klf4 and Sox2 or nucleic acids that encode these molecules. A functional inhibitor of p53, L-myc or a nucleic acid that encodes L-myc, and Lin28 or Lin28b or a nucleic acid that encodes Lin28 or Lin28b, can be utilized as additional nuclear reprogramming substances. Nanog can also be utilized for nuclear reprogramming. As disclosed in U.S. Patent Application Publication No. 20120196360, exemplary reprogramming factors for the production of iPSCs include (1) Oct3/4, Klf4, Sox2, L-Myc (Sox2 can be replaced with Soxl, Sox3, Soxl5, Soxl7 or Soxl8; Klf4 is replaceable with Klfl, Klf2 or Klf5); (2) Oct3/4, Klf4, Sox2, L-Myc, TERT, SV40 Large T antigen (SV40LT); (3) Oct3/4, Klf4, Sox2, L-Myc, TERT, human papilloma virus (HPV)l6 E6; (4) Oct3/4, Klf4, Sox2, L-Myc, TERT, HPV16 E7 (5) Oct3/4, Klf4, Sox2, L-Myc, TERT, HPV16 E6, HPV16 E7; (6) Oct3/4, Klf4, Sox2, L-Myc, TERT, Bmil; (7) Oct3/4, Klf4, Sox2, L-Myc, Lin28; (8) Oct3/4, Klf4, Sox2, L-Myc, Lin28, SV40LT; (9) Oct3/4, Klf4, Sox2, L-Myc, Lin28, TERT, SV40LT; (10) Oct3/4, Klf4, Sox2, L-Myc, SV40LT; (11) Oct3/4, Esrrb, Sox2, L-Myc (Esrrb is replaceable with Esrrg); (12) Oct3/4, Klf4, Sox2; (13) Oct3/4, Klf4, Sox2, TERT, SV40LT; (14) Oct3/4, Klf4, Sox2, TERT, HPV16 E6; (15) Oct3/4, Klf4, Sox2, TERT, HPV16 E7; (16) Oct3/4, Klf4, Sox2, TERT, HPV16 E6, HPV16 E7; (17) Oct3/4, Klf4, Sox2, TERT, Bmil; (18) Oct3/4, Klf4, Sox2, Lin28 (19) Oct3/4, Klf4, Sox2, Lin28, SV40LT; (20) Oct3/4, Klf4, Sox2, Lin28, TERT, SV40LT; (21) Oct3/4, Klf4, Sox2, SV40LT; or (22) Oct3/4, Esrrb, Sox2 (Esrrb is replaceable with Esrrg). In one non-limiting example, Oct3/4, Klf4, Sox2, and c-Myc are utilized. In other embodiments, Oct4, Nanog, and Sox2 are utilized, see for example, U.S. Pat. No. 7,682,828, the disclosure of which is incorporated by reference herein. These factors include, but are not limited to, Oct3/4, Klf4 and Sox2. In other examples, the factors include, but are not limited to Oct 3/4, Klf4 and Myc. In some non-limiting examples, Oct3/4, Klf4, c-Myc, and Sox2 are utilized. In other non-limiting examples, Oct3/4, Klf4, Sox2 and Sal 4 are utilized.

[00152] Mouse and human cDNA sequences of these nuclear reprogramming substances are available with reference to the NCBI accession numbers mentioned in WO 2007/069666, which is incorporated herein by reference. Methods for introducing one or more reprogramming substances, or nucleic acids encoding these

reprogramming substances, are known in the art, and disclosed for example, in U.S. Patent Application Publication No. 20120196360 and U.S. Pat. No. 8,071,369, the disclosures of each of which are incorporated by reference herein.

[00153] After being cultured with nuclear reprogramming substances, the cell can, for example, be cultured under conditions suitable for culturing ES cells. In the case of mouse cells, the culture is carried out with the addition of Leukemia Inhibitory Factor (LIF) as a differentiation suppression factor to an ordinary medium. In the case of human cells, it is desirable that basic fibroblast growth factor (bFGF) be added in place of LIF.

[00154] In some embodiments, the cell is cultured in the co-presence of mouse embryonic fibroblasts (MEFs) treated with radiation or an antibiotic to terminate the cell division, as feeder cells. Mouse embryonic fibroblasts in common use as feeders include the STO cell line (ATCC CRL-1503) and the like; for induction of an iPSC, useful cells can be generated by stably integrating the neomycin resistance gene and the LIF gene in the STO cell (SNL76/7 STO cell; ECACC 07032801) (McMahon, A. P. & Bradley, A. Cell 62, 1073-1085, 1990) and the like can be used. Mitomycin C-treated MEFs are commercially available from Millipore. Gamma-irradiated MEFs are commercially available from Global Stem Cells. Generally, somatic cells are transduced with reprogramming factors in the absence of MEFs. In some embodiments, about 7 to eight days after transduction, the cells are re-seeded onto MEFs. In some embodiments, the iPSC can be modified to express one or more exogenous nucleic acids.

Purification and Storage

[00155] An RPE cell obtained as described herein will in some embodiments be present in a mixed cell population comprising the RPE cell and other, non-RPE cells, where possible non-RPE cells include host iPSC cells that failed to differentiate into RPE cells.

[00156] In some embodiments, an RPE cell will be purified from a mixed cell population, e.g., separated from non-RPE cells that may be present in a mixed cell population comprising a subject RPE cell. For example, an RPE cell can be sorted based on phenotypic features characteristic of RPE cells. Sorting can involve contacting each cell with an antibody or ligand that binds to a marker characteristic

of an RPE cell, followed by separation of the specifically recognized cells from other cells in the population. One method is immunopanning, in which specific antibody is coupled to a solid surface. The cells are contacted with the surface, and cells not expressing the marker are washed away. The bound cells are then recovered by more vigorous elution. Variations of this are affinity chromatography and antibody-mediated magnetic cell sorting. In an exemplary sorting procedure, the cells are contacted with a specific primary antibody, and then captured with a secondary anti-immunoglobulin reagent bound to a magnetic bead. The adherent cells are then recovered by collecting the beads in a magnetic field.

[00157] For example, cells selected positively for expression of BEST1 can provide a population that is 60%, 70%, 80%, 90%, or greater than 90% (e.g., 95%, 98%, 99%, or greater than 99%), BEST1 positive. Similarly, cells selected positively for expression of RPE65 can provide a population that is 60%, 70%, 80%, 90%, or greater than 90% (e.g., 95%, 98%, 99%, or greater than 99%), RPE65 positive.

[00158] Separation of RPE cells from non-RPE cells present in a mixed cell population comprising RPE cells and non-RPE cells results in a purified RPE cell population, where at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or more than 99%, of the cells of the purified RPE cell population are RPE cells.

[00159] A subject RPE cell can be amplified, e.g., to obtain a purified RPE cell population comprising from about 10^3 to about 10^8 or more purified RPE cells, e.g., from about 10^3 purified RPE cells to about 10^4 purified induced RPE cells, from about 10^4 purified RPE cells to about 10^5 purified RPE cells, from about 10^5 purified RPE cells to about 10^6 purified RPE cells, from about 10^6 purified RPE cells to about 10^7 purified RPE cells, from about 10^7 purified RPE cells to about 10^8 purified RPE cells, or more than 10^8 purified RPE cells.

[00160] A subject RPE cell (or a population of purified RPE cells) can be stored. For example, a subject RPE cell, or a population of purified RPE cells, can be cryopreserved, using well-established methods. See for example, PCT Publication No. WO 2012/149484 A2, the disclosure of which is incorporated by reference herein. The cells can be cryopreserved with or without a substrate. In several embodiments, the storage temperature ranges from about -50° C. to about -60° C, about -600° C. to about -70° C, about -70° C to about -80° C, about -80° C to about -

90° C, about -90° C to about -100° C, and overlapping ranges thereof. In some embodiments, lower temperatures are used for the storage (e.g., maintenance) of the cryopreserved cells. In several embodiments, liquid nitrogen (or other similar liquid coolant) is used to store the cells. In further embodiments, the cells are stored for greater than about 6 hours. In additional embodiments, the cells are stored about 72 hours. In several embodiments, the cells are stored 48 hours to about one week. In yet other embodiments, the cells are stored for about 1, 2, 3, 4, 5, 6, 7, or 8 weeks. In further embodiments, the cells are stored for 1, 2, 3, 4, 5, 67, 8, 9, 10, 11 or 12 months. The cells can also be stored for longer times. The cells can be cryopreserved separately or on a substrate, such as any of the substrates disclosed herein.

[00161] A general method of cryopreserving cells is disclosed herein, that can be used for cryopreservation of any cell type, such as stem cells, including iPSCs, and differentiated cells, such as RPE cells. The method includes the use of alginate. "Alginate" (or "alginic acid" or "algin") refers to the anionic polysaccharide distributed widely in the cell walls of brown algae. Alginate forms water-soluble salts with alkali metals, such as sodium, potassium, lithium, magnesium, ammonium, and the substituted ammonium cations derived from lower amines, such as methyl amine, ethanol amine, diethanol amine, and triethanol amine. Alginate includes calcium alginate, sodium alginate, propylene-glycol alginate, and potassium alginate.

[00162] In some embodiments, cells, such as the disclosed RPE cells are contacted with an effective amount of alginate. The cells are contacted with alginate, and then exposed to divalent cations, such as Calcium, Barium, Copper, Zinc or Strontium) which results in cross-linking of the alginate polymers in the cell/liquid alginate suspension (see for example, U.S. Patent Application Publication No. 20120171295, the disclosure of which is incorporated by reference herein). In certain embodiments, the divalent cation used to cross-link the alginate in the cell/liquid alginate solution is calcium chloride (CaCh), barium chloride (BaCf), strontium chloride (SrCh), copper chloride (CuCh), or zinc chloride (ZnCh). In a specific embodiment, the divalent cation used to cross-link the alginate in the cell/liquid alginate solution is calcium chloride (CaCL). In certain embodiments, the solution of divalent cation comprises about 0.5%, about 0.75%, about 1.0%, about 1.25%, about 1.5%, about 1.75%, or about 2.0% divalent cation. In a specific embodiment, the solution of divalent cation comprises 1.5% divalent cation, e.g., CaCh.

[00163] In some embodiments, additional cryoprotectants can be used. For example, the cells can be cryopreserved in a cryopreservation solution comprising one or more cryoprotectants, such as DMSO, serum albumin, such as human or bovine serum albumin. In certain embodiments, the solution comprises about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 4%, about 5%, about 6%) about 7%, about 8%, about 9%, or about 10% DMSO. In other embodiments, the solution comprises about 1% to about 3%, about 2% to about 4%, about 3% to about 5%, about 4% to about 6%, about 5% to about 7%, about 6% to about 8%, about 7% to about 9%, or about 8% to about 10% DMSO or albumin. In a specific embodiment, the solution comprises 2,5% DMSO, In another specific embodiment, the solution comprises 10% DMSO.

[00164] Cells can be cryopreserved in small containers (e.g., ampoules); in bags suitable for cryopreservation; or in any other suitable container for cryopreservation. In some embodiments, cells are cryopreserved in commercially available cryopreservation medium. The cells can be cryopreserved in a cryopreservation solution comprising one or more solutions for use in storing cells. Cryopreservation solutions included CryoStor CS10® and HYPOTHERMOSOl® (BioLife Solutions, Bothell, Wash.)). In certain embodiments, the solution comprises about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, or about 70% HYPOTHERMOSOL®. In other embodiments, the solution comprises about 25% to about 50%, about 40% to about 60%, about 50% to about 60%, about 50% to about 70%, or about 60% to about 70% HYPOTHERMOSOL®. In a specific embodiment, the solution comprises 55% HYPOTHERMOSOL®. In another specific embodiment, the solution comprises 57.5% HYPOTHERMOSOL®. In additional embodiments, the cryopreservation solution can include one or more excipients, such as dextran, starch, glucose, lactose, sucrose, gelatin, silica gel, glycerol monostearate, sodium chloride, glycerol, propylene, and/or glycol. The cryopreservation solution can include media, such as the media disclosed herein. In additional embodiments, the medium can be phosphate buffered saline or Dulbecco's Modified Eagle's Medium (DMEM).

[00165] Cells may be cooled, for example, at about 1° C/minute during cryopreservation. In some embodiments, the cryopreservation temperature is about -80° C to about -180° C, or about -125° C to about -140° C. In some embodiments,

the cells are cooled to 4° C prior to cooling at about 1° C/minute. Cryopreserved cells can be transferred to vapor phase of liquid nitrogen prior to thawing for use. In some embodiments, for example, once the cells have reached about -80° C, they are transferred to a liquid nitrogen storage area. Cryopreservation can also be done using a controlled-rate freezer. Cryopreserved cells may be thawed, e.g., at a temperature of about 25° C to about 40° C, and typically at a temperature of about 37° C.

Compositions comprising RPE cells

[00166] The present disclosure provides a composition comprising a subject RPE cell (or a population of RPE cells, e.g., a population of purified RPE cells, e.g., such as a population of purified RPE cells in an RPE monolayer).

[00167] A subject composition can include various components in addition to the RPE cells. For example, a subject composition can include a subject RPE cell(s) and a culture medium. In some cases, the culture medium comprises one or more growth factors. In some embodiments, the culture medium is a serum-free culture medium. In some cases, the composition comprises RPE cells and a cryopreservative agent, e.g., a cryopreservation medium, e.g. as described previously herein.

[00168] A subject composition can include a subject RPE cell(s) and a matrix, e.g., a matrix component. Suitable matrix components include, e.g., collagen; gelatin; fibrin; fibrinogen; laminin; a glycosaminoglycan; elastin; hyaluronic acid; a proteoglycan; a glycan; poly(lactic acid); poly(vinyl alcohol); poly(vinyl pyrrobdone); poly(ethylene oxide); cellulose; a cellulose derivative; starch; a starch derivative; poly(caprolactone); poly(hydroxy butyric acid); mucin; and the like. In some embodiments, the matrix comprises one or more of collagen, gelatin, fibrin, fibrinogen, laminin, and elastin; and can further comprise a non-proteinaceous polymer, e.g., can further comprise one or more of poly(lactic acid), poly(vinyl alcohol), poly(vinyl pyrrobdone), poly(ethylene oxide), poly(caprolactone), poly(hydroxy butyric acid), cellulose, a cellulose derivative, starch, and a starch derivative. In some embodiments, the matrix comprises one or more of collagen, gelatin, fibrin, fibrinogen, laminin, and elastin; and can further comprise hyaluronic acid, a proteoglycan, a glycosaminoglycan, or a glycan. Where the matrix comprises collagen, the collagen can comprise type I collagen, type II collagen, type III collagen, type V collagen, type XI collagen, and combinations thereof.

[00169] The matrix can be a hydrogel. A suitable hydrogel is a polymer of two or more monomers, e.g., a homopolymer or a heteropolymer comprising multiple monomers. Suitable hydrogel monomers include the following: lactic acid, glycolic acid, acrylic acid, 1-hydroxy ethyl methacrylate (HEMA), ethyl methacrylate (EMA), propylene glycol methacrylate (PEMA), acrylamide (AAM), N-vinylpyrrolidone, methyl methacrylate (MMA), glycidyl methacrylate (GDMA), glycol methacrylate (GMA), ethylene glycol, fumaric acid, and the like. Common cross linking agents include tetraethylene glycol dimethacrylate (TEGDMA) and N,N'-methylenebisacrylamide. The hydrogel can be homopolymeric, or can comprise co-polymers of two or more of the aforementioned polymers. Exemplary hydrogels include, but are not limited to, a copolymer of poly(ethylene oxide) (PEO) and polypropylene oxide) (PPO); Pluronic™ F-127 (a difunctional block copolymer of PEO and PPO of the nominal formula EO100-PO65-EO100, where EO is ethylene oxide and PO is propylene oxide); poloxamer 407 (a tri-block copolymer consisting of a central block of polypropylene glycol) flanked by two hydrophilic blocks of polypthylene glycol)); a polypthylene oxide)-poly(propylene oxide)-poly(ethylene oxide) co-polymer with a nominal molecular weight of 12,500 Daltons and a PEO:PPO ratio of 2:1); a poly(/V-isopropylacrylamide)-base hydrogel (a PNIPAAm-based hydrogel); a PNIPAAm-acrylic acid co-polymer (PNIPAAm-co-AAc); poly(2-hydroxyethyl methacrylate); polypinyl pyrrolidone); and the like.

[00170] The cell density in a subject RPE cell composition can range from about 10^2 cells/mm^3 to about 10^9 cells/mm^3, e.g., from about 10^2 cells/mm^3 to about 10^4 cells/mm^3, from about 10^4 cells/mm^3 to about 10^6 cells/mm^3, from about 10^6 cells/mm^3 to about 10^7 cells/mm^3, from about 10^7 cells/mm^3 to about 10^8 cells/mm^3, or from about 10^8 cells/mm^3 to about 10^9 cells/mm^3.

[00171] The matrix can take any of a variety of forms, or can be relatively amorphous. For example, the matrix can be in the form of a sheet, a cylinder, a sphere, etc.

UTILITY

[00172] A subject RPE, RPE cell, or population of RPE cells is useful in various applications, which are also provided in the present disclosure.

Screening methods

[00173] Methods are provided herein for identifying an agent that alters the differentiation and/or proliferation of RPE cells. In some embodiments, methods are provided for identifying an agent that alters the proliferation of RPE cells. The methods include contacting an RPE cell with an effective amount of an agent of interest. In some embodiments, methods are provided for identifying an agent that increases differentiation of RPE.

[00174] In additional embodiments, methods are provided for identifying an agent that affects RPE cell survival, and/or changes the endogenous expression of genes in RPE cells. Therapeutic agents can be identified for the treatment of disease using these methods. In some embodiments, methods are provided for identifying an agent that affects the epithelial phenotype of RPE cells. The method includes contacting iPSCs, RPE or RPE cells with the agent of interest, and assaying the production and/or survival and/or phenotype of RPE cells. The agent can be introduced into any step of the methods disclosed herein.

[00175] Thus, in an aspect of the present disclosure, a screening method is provided which includes contacting RPE or an RPE cell or cells as described herein, or generated using a method as described herein, with a test agent, and assaying to determine the effect of the test agent on the RPE or RPE cell or cells, e.g., assaying to determine the effect of the test agent on differentiation and/or proliferation and/or production and/or survival and/or phenotype of the RPE or RPE cell or cells.

[00176] These methods can be used to identify agents that affect expression of genes, or to identify agents that affect survival of RPE cells. In some embodiments, the RPE cells are treated with a stressor, such as thapsigargin, A23187, DL-dithiotreitol, or 2-deoxy-D-glucose.

[00177] In connection with the methods provided herein, expression (RNA or protein) of one or more of the following genes may be assessed: MITF (GENBANK® Accession No. NM-000248), PAX6 (GENBANK® Accession No. NM-000280), LHX2 (GENBANK® Accession No. NM-004789), TFEC (GENBANK® Accession No. NM_012252), CDH1 (GENBANK® Accession No. NM-004360), CDH3 (GENBANK® Accession No. NM-001793), CLDN10 (GENBANK® Accession No. NM_182848), CLDN16 (GENBANK® Accession No. NM-006580), CLDN19 (GENBANK® Accession No. NM-148960), BEST1 (GENBANK® Accession No.

NM_004183), TIMP3 (GENBANK® Accession No. NM-000362), TRPM1 (GENBANK® Accession No. NM-002420), TRPM3 (GENBANK® Accession No. NM_020952), TTR (GENBANK® Accession No. NM-000371), VEGFA (GENBANK® Accession No. NM-003376), CSPG5 (GENBANK® Accession No. NM_006574), DCT (GENBANK® Accession No. NM-001922), TYRP1 (GENBANK® Accession No. NM-000550), TYR (GENBANK® Accession No. NM_000372), SILV (GENBANK® Accession No. NM-006928), SIL1 (GENBANK® Accession No. NM-022464), MLANA (NM-00551 1), RAB27A (GENBANK® Accession No. NM-l 83236), OCA2 (GENBANK® Accession No. NM_000275), GPR143 (GENBANK® Accession No. NM-000273), GPNMB (GENBANK® Accession No. NM-002510), MY06 (GENBANK® Accession No. NM_004999), MYRIP (GENBANK® Accession No. NM-015460), RPE65 (GENBANK® Accession No. NM-000329), RBP1 (GENBANK® Accession No. NM_002899), RBP4 (GENBANK® Accession No. NM-006744), RDH5 (GENBANK® Accession No. NM-002905), RDH11 (GENBANK® Accession No. NM_016026), RLBP1 (GENBANK® Accession No. NM-000326), MERTK (GENBANK® Accession No. NM-006343), ALDH1A3 (GENBANK® Accession No. NM_000693), FBLN1 (GENBANK® Accession No. NM-001996), SLC16A1 (GENBANK® Accession No. NM-003051), KCNV2 (GENBANK® Accession No. NM_133497), KCNJ13 (GENBANK® Accession No. NM-002242), and CFTR (GENBANK® Accession No. NM_000492). Increased expression of the one or more mRNAs or proteins following exposure to the agent, as compared to iPSC or RPE cell not contacted with the agent, indicates that the agent affects RPE cell differentiation, survival and/or proliferation, specifically that it increases RPE cell differentiation, survival and/or proliferation. Decreased expression of the one or more mRNAs or proteins following exposure to the agent, as compared to iPSC or RPE cell not contacted with the agent, indicates that the agent affects RPE cell differentiation, survival and/or proliferation, specifically that it decreases RPE cell differentiation, survival and/or proliferation. Additional screening methods are described in U.S. Patent Application Publication No. 20150368713. The disclosure of which is incorporated by reference herein.

[00178] Assessment of the activity of candidate pharmaceutical compounds generally involves combining a subject RPE, a subject RPE cell, or a subject population of

RPE cells with the candidate compound, either alone or in combination with other drugs. The investigator determines any change in the morphology, marker phenotype, or functional activity of the cells that is attributable to the compound (compared with untreated cells or cells treated with an inert compound), and then correlates the effect of the compound with the observed change.

[00179] Cytotoxicity can be determined in the first instance by the effect on cell viability, survival, morphology, and the expression of certain markers and receptors. Effects of a drug on chromosomal DNA can be determined by measuring DNA synthesis or repair. ¾-thymidine or BrdU incorporation, especially at unscheduled times in the cell cycle, or above the level required for cell replication, is consistent with a drug effect. Unwanted effects can also include unusual rates of sister chromatid exchange, determined by metaphase spread. The reader is referred to A. Vickers (pp 375-410 in “In vitro Methods in Pharmaceutical Research,” Academic Press, 1997) for further elaboration.

[00180] Effect of cell function can be assessed using any standard assay to observe phenotype or activity of RPE, an RPE cell, or population of RPE cells, such as assaying for epithelial integrity of RPE cells by measuring trans epithelial resistance (TER) and/or assaying RPE cells for the ability to phagocytose rod cell OS at a rate similar to immortalized RPE cells. See, e.g., Kevany and Palczewski, Physiology (Bethesda). 2010 Feb; 25(1): 8-15.

[00181] A subject screening method generally includes appropriate controls, e.g., a control sample that lacks the test agent. Generally, a plurality of assay mixtures is run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

[00182] A variety of other reagents may be included in the screening assay. These include reagents such as salts, neutral proteins, e.g. albumin, detergents, etc that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc. may be used. The components of the assay mixture are added in any order that provides for the requisite binding or other activity. Incubations are performed at any suitable temperature, typically between 4°C and 40°C. Incubation periods are selected for

optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically, between 0.1 and 1 hour will be sufficient.

[00183] As used herein, the term "determining" refers to both quantitative and qualitative determinations and as such, the term "determining" is used interchangeably herein with "assaying," "measuring," and the like.

[00184] The terms "candidate agent," "test agent," "agent", "substance" and "compound" are used interchangeably herein. Candidate agents encompass numerous chemical classes, typically synthetic, semi-synthetic, or naturally occurring inorganic or organic molecules. Candidate agents include those found in large libraries of synthetic or natural compounds. For example, synthetic compound libraries are commercially available from Maybridge Chemical Co. (Trevillet, Cornwall, UK), ComGenex (South San Francisco, CA), and MicroSource (New Milford, CT). A rare chemical library is available from Aldrich (Milwaukee, Wis.) and can also be used. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available from Pan Labs (Bothell, WA) or are readily producible.

[00185] Candidate agents may be small organic or inorganic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents may comprise functional groups necessary for structural interaction with proteins, e.g., hydrogen bonding, and may include at least an amine, carbonyl, hydroxyl or carboxyl group, and may contain at least two of the functional chemical groups. The candidate agents may comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[00186] A test agent can be a small molecule. The test molecules may be individual small molecules of choice or in some cases, the small molecule test agents to be screened come from a combinatorial library, i.e., a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical "building blocks." For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks called amino acids in every possible way for a

given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks. Indeed, theoretically, the systematic, combinatorial mixing of 100 interchangeable chemical building blocks results in the synthesis of 100 million tetrameric compounds or 10 billion pentameric compounds. See, e.g., Gallop et al, (1994), J. Med. Chern, 37(9), 1233-1251. Preparation and screening of combinatorial chemical libraries are well known in the art. Combinatorial chemical libraries include, but are not limited to: diversomers such as hydantoins, benzodiazepines, and dipeptides, as described in, e.g., Hobbs et al, (1993), Proc. Natl. Acad. Sci. U.S.A., 90:6909-6913; analogous organic syntheses of small compound libraries, as described in Chen et al, (1994), J. Amer. Chem. Soc., 116:2661-2662; Oligocarbamates, as described in Cho, et al, (1993), Science, 261:1303-1305; peptidyl phosphonates, as described in Campbell et al, (1994), J. Org. Chem., 59: 658-660; and small organic molecule libraries containing, e.g., thiazobdinones and metathiazanones (U.S. Pat. No. 5,549,974), pyrrolidines (U.S. Pat. Nos. 5,525,735 and 5,519,134), benzodiazepines (U.S. Pat. No. 5,288,514).

[00187] Numerous combinatorial libraries are commercially available from, e.g., ComGenex (Princeton, N.J.); Asinex (Moscow, Russia); Tripos, Inc. (St. Louis, Mo.); ChemStar, Ltd. (Moscow, Russia); 3D Pharmaceuticals (Exton, Pa.); and Martek Biosciences (Columbia, MD).

[00188] In some embodiments, a test agent is a gene editing system or a component thereof, as described in greater detail below.

[00189] A subject RPE, RPE cell, or population of RPE cells which finds use in the disclosed screening methods may be an RPE, RPE cell, or population of RPE, which was differentiated using a method as described herein from an iPSC derived from a cell from an individual with a family history or genetic make-up of interest for an RPE related disease, e.g., age-related macular degeneration.

Screening methods using gene editing systems and/or tools

[00190] In some embodiments, a subject RPE, RPE cell, or population of RPE cells (e.g., an RPE, an RPE cell, or a population of RPE cells prepared according to a method as described herein) finds use in methods designed to screen one or more gene editing tools (i.e., a component of a gene editing system, e.g., a site specific

gene editing system such as a programmable gene editing system). In some embodiments, a subject RPE, RPE cell, or population of RPE cells is a patient-derived RPE, RPE cell, or population of RPE cells, e.g., which include a genetic defect, such as a mutant allele (dominant negative) of the BEST1 gene.

[00191] A subject RPE, RPE cell, or population of RPE cells may be contacted with (i) a CRISPR/Cas guide RNA, (ii) a DNA encoding a CRISPR/Cas guide RNA, (iii) a DNA and/or RNA encoding a programmable gene editing protein such as a zinc finger protein (ZFP) (e.g., a zinc finger nuclease - ZFN), a transcription activator-like effector (TALE) protein (e.g., fused to a nuclease - TALEN), a DNA-guided polypeptide such as *Natronobacterium gregoryi* Argonaute (NgAgo), and/or a CRISPR/Cas RNA-guided polypeptide (e.g., Cas9, CasX, CasY, Cpfl, and the like); (iv) a DNA donor template; (v) a nucleic acid molecule (DNA, RNA) encoding a site-specific recombinase (e.g., Cre recombinase, Dre recombinase, Flp recombinase, KD recombinase, B2 recombinase, B3 recombinase, R recombinase, Hin recombinase, Tre recombinase, PhiC3l integrase, Bxbl integrase, R4 integrase, lambda integrase, HK022 integrase, HP1 integrase, and the like); (vi) a DNA encoding a resolvase and/or invertase (e.g., Gin, Hin, γδ3, Tn3, Sin, Beta, and the like); and (vii) a transposon and/or a DNA derived from a transposon (e.g., bacterial transposons such as Tn3, Tn5, Tn7, Tn9, TnlO, Tn903, Tnl68l, and the like; eukaryotic transposons such as Tcl/mariner super family transposons, PiggyBac superfamily transposons, hAT superfamily transposons, PiggyBac, Sleeping Beauty, Frog Prince, Minos, Himarl, and the like). In some cases a gene editing tool includes a protein, e.g., a gene editing protein such as a ZFP (e.g., ZFN), a TALE (e.g., TALEN), a DNA-guided polypeptide such as *Natronobacterium gregoryi* Argonaute (NgAgo), a CRISPR/Cas RNA-guided polypeptide (e.g., Cas9, CasX, CasY, Cpfl, and the like), a site-specific recombinase (e.g., Cre recombinase, Dre recombinase, Flp recombinase, KD recombinase, B2 recombinase, B3 recombinase, R recombinase, Hin recombinase, Tre recombinase, PhiC3l integrase, Bxbl integrase, R4 integrase, lambda integrase, HK022 integrase, HP1 integrase, and the like), a resolvase / invertase (e.g., Gin, Hin, γδ3, Tn3, Sin, Beta, and the like); and/or a transposase (e.g., atransposase related to transposons such as bacterial transposons such as Tn3, Tn5, Tn7, Tn9, TnlO, Tn903, Tnl68l, and the like; or eukaryotic transposons such as Tcl/mariner super family transposons, PiggyBac superfamily

transposons, hAT superfamily transposons, PiggyBac, Sleeping Beauty, Frog Prince, Minos, Himarl, and the like). In some cases, the gene editing tool includes a nucleic acid and a protein, and in some such cases the gene editing tool includes a ribonucleoprotein complex (RNP).

[00192] Depending on the nature of the system and the desired outcome, a gene editing system (e.g. a site-specific gene editing system such as a programmable gene editing system) can include a single component (e.g., a ZFP, a ZFN, a TALE, a TALEN, a site-specific recombinase, a resolvase / integrase, a transpose, a transposon, and the like) or can include multiple components. In some cases a gene editing system includes at least two components. For example, in some cases a gene editing system (e.g. a programmable gene editing system) includes (i) a donor template nucleic acid; and (ii) a gene editing protein (e.g., a programmable gene editing protein such as a ZFP, a ZFN, a TALE, a TALEN, a DNA-guided polypeptide such as *Natronobacterium gregoryi* Argonaute (NgAgo), a CRISPR/Cas RNA-guided polypeptide such as Cas9, CasX, CasY, or Cpfl, and the like), or a nucleic acid molecule encoding the gene editing protein (e.g., DNA or RNA such as a plasmid or mRNA). As another example, in some cases a gene editing system (e.g. a programmable gene editing system) includes (i) a CRISPR/Cas guide RNA, or a DNA encoding the CRISPR/Cas guide RNA; and (ii) a CRISPR/CAS RNA-guided polypeptide (e.g., Cas9, CasX, CasY, Cpfl, and the like), or a nucleic acid molecule encoding the RNA-guided polypeptide (e.g., DNA or RNA such as a plasmid or mRNA). As another example, in some cases a gene editing system (e.g. a programmable gene editing system) includes (i) an NgAgo-like guide DNA; and (ii) a DNA-guided polypeptide (e.g., NgAgo), or a nucleic acid molecule encoding the DNA-guided polypeptide (e.g., DNA or RNA such as a plasmid or mRNA). In some cases a gene editing system (e.g. a programmable gene editing system) includes at least three components: (i) a donor DNA template; (ii) a CRISPR/Cas guide RNA, or a DNA encoding the CRISPR/Cas guide RNA; and (iii) a CRISPR/Cas RNA-guided polypeptide (e.g., Cas9, CasX, CasY, or Cpfl), or a nucleic acid molecule encoding the RNA-guided polypeptide (e.g., DNA or RNA such as a plasmid or mRNA). In some cases a gene editing system (e.g. a programmable gene editing system) includes at least three components: (i) a donor DNA template; (ii) an NgAgo-like guide DNA, or a DNA encoding the NgAgo-like guide DNA; and (iii) a DNA-guided polypeptide

(e.g., NgAgo), or a nucleic acid molecule encoding the DNA-guided polypeptide (e.g., DNA or RNA such as a plasmid or mRNA).

[00193] In some cases, a subject RPE, RPE cell or population of RPE cells is contacted with a gene editing system and in some cases a subject RPE, RPE cell or population of RPE cells is contacted with one or more components of a gene editing system (i.e., one or more gene editing tools). For example, a target cell might already include one of the components of a gene editing system and the user need only add the remaining components. In such a case the a subject RPE, RPE cell or population of RPE cells does not necessarily need to be contacted with all of the components of a given gene editing system.

[00194] As an illustrative example, a target RPE cell might already include a gene editing protein (e.g., a ZFP, a TALE, a DNA-guided polypeptide (e.g., NgAgo), a CRISPR/Cas RNA-guided polypeptide such Cas9, CasX, CasY, Cpfl, and the like, a site-specific recombinase such as Cre recombinase, Dre recombinase, Flp recombinase, KD recombinase, B2 recombinase, B3 recombinase, R recombinase, Hin recombinase, Tre recombinase, PhiC3l integrase, Bxbl integrase, R4 integrase, lambda integrase, HK022 integrase, HP1 integrase, and the like, a resolvase/invertase such as Gin, Hin, γδ3, Tn3, Sin, Beta, and the like, a transposase, etc.) and/or a DNA or RNA encoding the protein, and therefore the RPE cell can be contacted with one or more of: (i) a donor template; and (ii) a CRISPR/Cas guide RNA, or a DNA encoding the CRISPR/Cas guide RNA; or an NgAgo-like guide DNA. Likewise, the target RPE cell cell may already include a CRISPR/Cas guide RNA and/or a DNA encoding the guide RNA or an NgAgo-like guide DNA, and the RPE cell can be contacted with one or more of: (i) a donor template; and (ii) a CRISPR/Cas RNA-guided polypeptide (e.g., Cas9, CasX, CasY, Cpfl, and the like), or a nucleic acid molecule encoding the RNA-guided polypeptide (e.g., DNA or RNA such as a plasmid or mRNA); or a DNA-guided polypeptide (e.g., NgAgo), or a nucleic acid molecule encoding the DNA-guided polypeptide.

[00195] As would be understood by one of ordinary skill in the art, a gene editing system need not be a system that ‘edits’ a nucleic acid. For example, it is well recognized that a gene editing system can be used to modify target nucleic acids (e.g., DNA and/or RNA) in a variety of ways without creating a double strand break (DSB) in the target DNA. For example, in some cases a double stranded target DNA

is nicked (one strand is cleaved), and in some cases (e.g., in some cases where the gene editing protein is devoid of nuclease activity, e.g., a CRISPR/Cas RNA-guided polypeptide may harbor mutations in the catalytic nuclease domains), the target nucleic acid is not cleaved at all. For example, in some cases a CRISPR/Cas protein (e.g., Cas9, CasX, CasY, Cpfl) with or without nuclease activity, is fused to a heterologous protein domain. The heterologous protein domain can provide an activity to the fusion protein such as (i) a DNA-modifying activity (e.g., nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity), (ii) a transcription modulation activity (e.g., fusion to a transcriptional repressor or activator), or (iii) an activity that modifies a protein (e.g., a histone) that is associated with target DNA (e.g., methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity or demyristoylation activity). As such, a gene editing system can be used in applications that modify a target nucleic acid in way that do not cleave the target nucleic acid, and can also be used in applications that modulate transcription from a target DNA.

[00196] For additional information related to programmable gene editing tools (e.g., CRISPR/Cas RNa-guided proteins such as Cas9, CasX, CasY, and Cpfl, Zinc finger proteins such as Zinc finger nucleases, TALE proteins such as TALENs, CRISPR/Cas guide RNAs, and the like) refer to, for example, Dreier, et al, (2001) *J Biol Chem* 276:29466-78; Dreier, et al, (2000) *J Mol Biol* 303:489-502; Liu, et al, (2002) *J Biol Chem* 277:3850-6); Dreier, et al., (2005) *J Biol Chem* 280:35588-97; Jamieson, et al, (2003) *Nature Rev Drug Discov* 2:361-8; Durai, et al, (2005) *Nucleic Acids Res* 33:5978-90; Segal, (2002) *Methods* 26:76-83; Porteus and Carroll, (2005) *Nat Biotechnol* 23:967-73; Pabo, et al, (2001) *Ann Rev Biochem* 70:313-40; Wolfe, et al, (2000) *Ann Rev Biophys Biomol Struct* 29: 183-212; Segal and Barbas, (2001) *Curr Opin Biotechnol* 12:632-7; Segal, et al, (2003) *Biochemistry* 42:2137-

48; Beerli and Barbas, (2002) *Nat Biotechnol* 20:135-41; Carroll, et al., (2006) *Nature Protocols* 1:1329; Ordiz, et al., (2002) *Proc Natl Acad Sci USA* 99:13290-5; Guan, et al., (2002) *Proc Natl Acad Sci USA* 99:13296-301; Sanjana et al., *Nature Protocols,* 7:171-192 (2012); Zetsche et al, *Cell.* 2015 Oct 22; 163(3): 759-71; Makarova et al, *Nat Rev Microbiol.* 2015 Nov;l3(ll):722-36; Shmakov et al.,*Mol Cell.* 2015 Nov 5;60(3):385-97; Jinek et al, *Science.* 2012 Aug 17;337(6096):816-21; Chylinski et al, RNA Biol. 2013 May;l0(5):726-37; Ma et al, *Biomed Res Int.* 20l3;20l3:270805; Hou et al, *Proc Natl Acad Sci USA.* 2013 Sep 24;l 10(39): 15644-9; Jinek et al, Elife. 20l3;2:e0047l; Pattanayak et al., *Nat Biotechnol.* 2013 Sep;3l(9):839-43; Qi et al, *Cell.* 2013 Feb 28;l52(5):ll73-83; Wang et al, *Cell.* 2013 May 9;153(4):910-8; Auer et. al, *Genome Res.* 2013 Oct 31; Chen et. al, *Nucleic Acids Res.* 2013 Nov l;4l(20):el9; Cheng et. al, *Cell Res.* 2013 Oct;23(lO): 1163-71; Cho et. al, *Genetics.* 2013 Nov;l95(3): 1177-80; DiCarlo et al, *Nucleic Acids Res.* 2013 Apr;4l(7):4336-43; Dickinson et. al., *Mat Methods.* 2013 Oct; 10(10): 1028-34; Ebina et. al, *Sci Rep.* 20l3;3:25l0; Fujii et. al, *Nucleic Acids Res.* 2013 Nov l;4l(20):el87; Hu et. al, *Cell Res.* 2013 Nov;23(ll): 1322-5; Jiang et. al, *Nucleic Acids Res.* 2013 Nov l;4l(20):el88; Larson et. al, *Nat Protoc.* 2013 Nov;8(ll):2l80-96; Mali et. at., *Nat Methods.* 2013 Oct;lO(lO):957-63; Nakayama et. al, *Genesis.* 2013 Dec;5l(l2):835-43; Ran et. al, *Nat Protoc.* 2013 Nov;8(ll):228 1-308; Ran et. al., *Cell.* 2013 Sep 12;154(6): 1380-9; Upadhyay et. al, *G3* (Bethesda). 2013 Dec 9;3(l2):2233-8; Walsh et. al, *Proc Natl Acad Sci U SA.* 2013 Sep 24; 110(39): 15514-5; Xie et. al, *Mol Plant.* 2013 Oct 9; Yang et. al, *Cell.* 2013 Sep 12;154(6): 1370-9; Briner et al, *Mol Cell.* 2014 Oct 23;56(2):333-9; Burstein et al, *Nature.* 2016 Dec 22 - Epub ahead of print; Gao et al, *Nat Biotechnol.* 2016 Jul 34(7):768-73; as well as international patent application publication Nos. W02002099084; WOOO/42219; WO02/42459; W02003062455; W003/080809; W005/014791; W005/084190; W008/021207; W009/042186; WO09/054985; and W010/065123; U.S. patent application publication Nos. 20030059767, 20030108880, 20140068797; 20140170753; 20140179006; 20140179770; 20140186843; 20140186919; 20140186958; 20140189896; 20140227787; 20140234972; 20140242664; 20140242699; 20140242700; 20140242702; 20140248702; 20140256046; 20140273037; 20140273226; 20140273230; 20140273231; 20140273232; 20140273233; 20140273234;

20140273235; 20140287938; 20140295556; 20140295557; 20140298547; 20140304853; 20140309487; 20140310828; 20140310830; 20140315985; 20140335063; 20140335620; 20140342456; 20140342457; 20140342458; 20140349400; 20140349405; 20140356867; 20140356956; 20140356958; 20140356959; 20140357523; 20140357530; 20140364333; 20140377868; 20150166983; and 20160208243; and U.S. Patent Nos. 6,140,466; 6,511,808; 6,453,242 8,685,737; 8,906,616; 8,895,308; 8,889,418; 8,889,356; 8,871,445; 8,865,406; 8,795,965; 8,771,945; and 8,697,359; all of which are hereby incorporated by reference in their entirety.

Methods of Treatment Using RPE

[00197] In some embodiments, a subject RPE, RPE cell, or population of RPE cells (e.g., an RPE, an RPE cell, or a population of RPE cells prepared according to a method as described herein) finds use in methods of treating a disease characterized by degeneration of RPE, e.g., age-related macular degeneration (AMD), macular dystrophies such as Stargardf s and Stargardf s-like disease, Best disease (vitelliform macular dystrophy), and adult vitelliform dystrophy or subtypes of retinitis pigmentosa. Such treatment may take the form of transplantation, e.g., subretinal transplantation, of RPE prepared according to a method as described herein. Such transplantation may take place following genomic modification of the RPE cells, e.g., using a genome editing tool as described herein.

[00198] In some embodiments, a subject RPE, RPE cell, or population of RPE cells is a patient-derived RPE, RPE cell, or population of RPE cells, e.g., which include a genetic defect, such as a mutant allele (dominant negative) of the BEST1 gene.

[00199] Patient-specific RPE generated according to a method as described herein may be transplanted, e.g., via subretinal transplantation, into a patient, e.g., a patient from which the somatic cells used to prepare the iPSCs were derived, e.g., to treat a disease characterized by degeneration of RPE as described above.

EXAMPLES OF NON-LIMITING ASPECTS OF THE DISCLOSURE

[00200] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting,

numbered aspects of the disclosure are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

1. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

   introducing into the iPSCs an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs; and

   culturing the genetically modified iPSCs under conditions sufficient for

   expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

   differentiation of the genetically modified iPSCs into retinal pigment epithelial cells,

   wherein the method does not comprise introducing into the iPSCs or genetically modified iPSCs a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide, or an exogenous nucleic acid comprising a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide; thereby generating RPE.
2. The method of 1, wherein the exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, the exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and the exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide are comprised by a recombinant vector.
3. The method of 2, wherein the recombinant vector is a viral vector.
4. The method of 2, wherein the recombinant vector is a donor vector for a gene editing system.

5. The method of 4, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.
6. The method of 5, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.
7. The method of any one of 2-6, wherein the recombinant vector does not comprise a tyrosinase enhancer.
8. The method of any one of 1-7, wherein iPSCs are human iPSCs.
9. The method of any one of 1-7, wherein iPSCs are rodent iPSCs.
10. The method of any one of 1-7, wherein the iPSCs are non-human primate iPSCs.
11. The method of any one of 1-10, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.
12. The method of any one of 1-1 1, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.
13. The method of any one of 1-12, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.
14. The method of any one of 2-13, wherein the vector comprises a Tet-inducible promoter.
15. The method of 14, wherein the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide comprises contacting the genetically modified iPSCs with tetracycline or a derivative or analogue thereof to induce expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide.
16. The method of 15, wherein the tetracycline or a derivative or analogue thereof is doxycycline.
17. The method of any one of 1-16, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.

18. The method of any one of 2-17, wherein the vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs.
19. The method of any one of 2-18, wherein the vector is targeted to a plurality of integration sites.
20. The method of any one of 2-19, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.
21. The method of 20, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.
22. The method of any one of 2-21, wherein the recombinant vector comprises a promoter and each of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding a MITF polypeptide is operably linked to the promoter.
23. The method of any one of 2-22, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.
24. The method of any one of 1-23, wherein the method does not comprise introducing into the iPSCs or genetically modified iPSCs an exogenous nucleic acid comprising a tyrosinase enhancer.
25. The method of any one of 2-24, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.
26. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

    introducing into the iPSCs an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid

comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs; and

culturing the genetically modified iPSCs under conditions sufficient for

expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

differentiation of the genetically modified iPSCs into retinal pigment epithelial cells,

wherein the method does not comprise introducing into the iPSCs or genetically modified iPSCs an exogenous nucleic acid comprising a tyrosinase enhancer; thereby generating RPE.

27. The method of 26, wherein the exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, the exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and the exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide are comprised by a recombinant vector.
28. The method of 27, wherein the recombinant vector is a viral vector.
29. The method of 27, wherein the recombinant vector is a donor vector for a gene editing system.
30. The method of 29, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.
31. The method of 30, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.
32. The method of any one of 26-29, wherein iPSCs are human iPSCs.
33. The method of any one of 26-29, wherein iPSCs are rodent iPSCs.
34. The method of any one of 26-29, wherein the iPSCs are non-human primate iPSCs.
35. The method of any one of 26-34, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.
36. The method of any one of 26-35, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

37. The method of any one of 26-36, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.
38. The method of any one of 27-37, wherein the vector comprises a Tet-inducible promoter.
39. The method of 38, wherein the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide comprises contacting the genetically modified iPSCs with tetracycline or a derivative or analogue thereof to induce expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide.
40. The method of 39, wherein the tetracycline or a derivative or analogue thereof is doxycycline.
41. The method of any one of 26-40, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.
42. The method of any one of 27-41, wherein the vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs.
43. The method of any one of 27-41, wherein the vector is targeted to a plurality of integration sites.
44. The method of any one of 27-43, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.
45. The method of 44, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.
46. The method of any one of 27-45, wherein the recombinant vector comprises a promoter and each of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding a MITF polypeptide is operably linked to the promoter.
47. The method of any one of 27-46, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for

expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.

48. The method of any one of 27-47, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.
49. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

    introducing into the iPSCs a recombinant vector comprising a Tet-inducible promoter, an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein one or more of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide are operably linked to the Tet-inducible promoter; and

    culturing the genetically modified iPSCs under conditions sufficient for

        expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

        differentiation of the genetically modified iPSCs into retinal pigment epithelial cells; thereby generating RPE.

50. The method of 49, wherein the recombinant vector is a viral vector.
51. The method of 49, wherein the recombinant vector is a donor vector for a gene editing system.
52. The method of 51, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.
53. The method of 52, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.
54. The method of any one of 49-51, wherein iPSCs are human iPSCs.

55. The method of any one of 49-51, wherein iPSCs are rodent iPSCs.
56. The method of any one of 49-51, wherein the iPSCs are non-human primate iPSCs.
57. The method of any one of 49-56, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.
58. The method of any one of 49-57, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.
59. The method of any one of 49-58, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.
60. The method of any one of 49-59, wherein the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide comprises contacting the genetically modified iPSCs with tetracycline or a derivative or analogue thereof to induce expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide.
61. The method of 60, wherein the tetracycline or a derivative or analogue thereof is doxycycline.
62. The method of any one of 49-61, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.
63. The method of any one of 49-62, wherein the vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs.
64. The method of any one of 49-62, wherein the vector is targeted to a plurality of integration sites.
65. The method of any one of 49-64, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.
66. The method of 65, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

67. The method of any one of 49-66, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.
68. The method of any one of 49-67, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.
69. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

    introducing into the iPSCs a recombinant vector comprising an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein the recombinant vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs; and

    culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells; thereby generating RPE.
70. The method of 69, wherein the recombinant vector is a viral vector.
71. The method of 69, wherein the recombinant vector is a donor vector for a gene editing system.
72. The method of 71, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.
73. The method of 72, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.

74. The method of any one of 69-71, wherein iPSCs are human iPSCs.
75. The method of any one of 69-71, wherein iPSCs are rodent iPSCs.
76. The method of any one of 69-71, wherein the iPSCs are non-human primate iPSCs.
77. The method of any one of 69-76, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.
78. The method of any one of 69-77, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.
79. The method of any one of 69-78, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.
80. The method of any one of 69-79, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.
81. The method of any one of 69-80, wherein the vector is targeted to a plurality of integration sites.
82. The method of any one of 69-81, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.
83. The method of 82, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.
84. The method of any one of 69-83, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.
85. The method of any one of 69-84, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the

PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.

86. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

    introducing into the iPSCs a recombinant vector comprising a promoter, an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein each of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide is operably linked to the promoter; and

    culturing the genetically modified iPSCs under conditions sufficient for

        expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

        differentiation of the genetically modified iPSCs into retinal pigment epithelial cells; thereby generating RPE.

87. The method of 86, wherein the recombinant vector is a viral vector.
88. The method of 86, wherein the recombinant vector is a donor vector for a gene editing system.
89. The method of 88, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.
90. The method of 89, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.
91. The method of any one of 86-88, wherein iPSCs are human iPSCs.
92. The method of any one of 86-88, wherein iPSCs are rodent iPSCs.
93. The method of any one of 86-88, wherein the iPSCs are non-human primate iPSCs.
94. The method of any one of 86-93, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

95. The method of any one of 86-94, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.
96. The method of any one of 86-95, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.
97. The method of any one of 86-96, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.
98. The method of any one of 86-97, wherein the vector is targeted to a plurality of integration sites.
99. The method of any one of 86-98, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.
100. The method of 99, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.
101. The method of any one of 86-100, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.
102. The method of any one of 86-101, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.
103. The method of any one of 1-102, comprising culturing the genetically modified iPSCs for a period of time under differentiation conditions prior to culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs.

104. The method of 103, wherein the period of time is from about 2 to about 4 days.

105. The method of 104, wherein the differentiation conditions comprise culturing the genetically modified iPSCs in a differentiation medium.

106. The method of 105, wherein the differentiation medium is a neural induction medium.

107. A retinal pigment epithelium (RPE) generated by the method of any one of 1-106.

108. A composition comprising the retinal pigment epithelium (RPE) of 107.

109. The composition of 108, wherein the composition comprises a liquid culture medium.

110. The composition of 108, wherein the composition comprises a cryopreservative agent.

111. A screening method, the method comprising:
contacting the retinal pigment epithelium (RPE) of 107 with a test agent; and
assaying to determine the effect of the test agent on the RPE.

112. The screening method of 111, wherein the method is a multiplexed screening method.

113. The screening method of 111 or 112, wherein the assaying comprises assaying for modulation of retinal pigment epithelial cell proliferation and/or modulation of retinal pigment epithelial cell differentiation.

114. The screening method of 113, wherein the modulation is increased retinal pigment epithelial cell proliferation and/or increased retinal pigment epithelial cell differentiation relative to retinal pigment epithelial cell proliferation and/or retinal pigment epithelial cell differentiation in the absence of the test agent.

115. The screening method of 113, wherein the modulation is decreased retinal pigment epithelial cell proliferation and/or decreased retinal pigment epithelial cell differentiation relative to retinal pigment epithelial cell proliferation and/or retinal pigment epithelial cell differentiation in the absence of the test agent.

116. The screening method of 111, wherein the test agent is a gene editing tool.

117. A kit comprising the retinal pigment epithelium (RPE) of 107 or the composition of one of 108-1 10 and a liquid culture medium.

118. The kit of 117 comprising one or more of fetal bovine serum, an epithelial cell growth supplement, and an antibiotic.

119. A recombinant vector comprising:

a promoter;

a nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide operably linked to the promoter;

a nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide operably linked to the promoter; and

a nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide operably linked to the promoter.

120. The recombinant vector of 119, wherein the vector does not comprise a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

121. The recombinant vector of 119 or 120, wherein the recombinant vector does not comprise a tyrosinase enhancer.

122. The recombinant vector of any one of 119-121, wherein the recombinant vector is a viral vector.

123. The recombinant vector of 122, wherein the recombinant vector is a donor vector for a gene editing system.

124. The recombinant vector of 123, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

125. The recombinant vector of any one of 119-124, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

126. The recombinant vector of any one of 119-125, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

127. The recombinant vector of any one of 119-126, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

128. The recombinant vector of any one of 119-127, wherein the promoter is a Tet-inducible promoter.

129. The recombinant vector of any one of 119-128, wherein the vector is targeted to an AAVS1 locus, or ortholog thereof, or a CLYBL locus, or ortholog thereof, of a mammalian cell.

130. The recombinant vector of 129, wherein the mammalian cell is selected from a human cell, a rodent cell, and a non-human primate cell.

131. The recombinant vector of any one of 119-130, wherein the vector is targeted to a plurality of integration sites.

132. The recombinant vector of any one of 119-131, wherein the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

133. The recombinant vector of 132, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

134. A recombinant vector comprising:

an inducible promoter;

an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide;

an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide; and

an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide, wherein one or more of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide are operably linked to the inducible promoter.

135. The recombinant vector of 134, wherein the vector does not comprise a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

136. The recombinant vector of 134 or 135, wherein the recombinant vector does not comprise a tyrosinase enhancer.

137. The recombinant vector of any one of 134-136, wherein the recombinant vector is a viral vector.

138. The recombinant vector of 137, wherein the recombinant vector is a donor vector for a gene editing system.

139. The recombinant vector of 138, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

140. The recombinant vector of any one of 134-139, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

141. The recombinant vector of any one of 134-140, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

142. The recombinant vector of any one of 134-141, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

143. The recombinant vector of any one of 134-142, wherein the inducible promoter is a Tet-inducible promoter.

144. The recombinant vector of any one of 134-143, wherein the vector is targeted to an AAVS1 locus, or ortholog thereof, or a CLYBL locus, or ortholog thereof, of a mammalian cell.

145. The recombinant vector of 144, wherein the mammalian cell is selected from a human cell, a rodent cell, and a non-human primate cell.

146. The recombinant vector of any one of 134-145, wherein the vector is targeted to a plurality of integration sites.

147. The recombinant vector of any one of 134-146, wherein the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

148. The recombinant vector of 147, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

149. A recombinant vector comprising:

an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide;

an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide; and

an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide, wherein the vector is targeted to an AAVS1 locus, or ortholog thereof, or a CLYBL locus, or ortholog thereof, of a mammalian cell.

150. The recombinant vector of 149, wherein the vector does not comprise a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

151. The recombinant vector of 149 or 150, wherein the recombinant vector does not comprise a tyrosinase enhancer.

152. The recombinant vector of any one of 149-151, wherein the recombinant vector is a viral vector.

153. The recombinant vector of 152, wherein the recombinant vector is a donor vector for a gene editing system.

154. The recombinant vector of 153, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

155. The recombinant vector of any one of 149-154, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

156. The recombinant vector of any one of 149-155, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

157. The recombinant vector of any one of 149-156, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

158. The recombinant vector of any one of 149-157, wherein the vector is targeted to a plurality of integration sites.

159. The recombinant vector of any one of 149-158, wherein the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

160. The recombinant vector of 159, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

161. An isolated host cell comprising the recombinant vector of any one of 119-160.

162. An isolated genetically modified host cell comprising an exogenous OTX2 nucleic acid, an exogenous PAX6 nucleic acid, and an exogenous MITF nucleic acid integrated into the genome of the host cell.

163. The host cell of 162, wherein the host cell is an iPSC.

164. The host cell of 162, wherein the host cell is an RPE cell.

165. The host cell of any one of 162-164, comprising a gene editing tool.

166. The host cell of 165, wherein the gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

167. The host cell of any one of 162-166, wherein the host cell does not comprise an exogenous nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

168. A method of administering a retinal pigment epithelium (RPE), the method comprising transplanting into a subject in need thereof a RPE generated by the method of any one of 1-106 or administering a therapeutically effective amount of a composition of 108 to a subject in need thereof.

169. The method of 168, wherein the transplanting comprises subretinal transplantation.

170. The method of 168 or 169, wherein the iPSCs are derived from cells of the subject.

171. A method of treating a disease characterized by degeneration of retinal pigment epithelium (RPE), the method comprising transplanting into a subject in need thereof a RPE generated by the method of any one of 1-106 or administering a therapeutically effective amount of a composition of 108 to a subject in need thereof.

172. The method of 171, wherein the transplanting comprises subretinal transplantation.

173. The method of 171 or 172, wherein the iPSCs are derived from cells of the subject.

174. The method of any one of claims 171-173, wherein the disease is selected from age-related macular degeneration (AMD), a macular dystrophy (e.g., Stargardfs or Stargardt's-like disease), Best disease (vitelliform macular dystrophy), and adult vitelliform dystrophy or subtypes of retinitis pigmentosa.

175. The method of any one of claims 1-106, wherein the culturing comprises culturing the genetically modified iPSCs in the presence of dual SMAD inhibitors.

176. The method of 175, wherein the dual SMAD inhibitors are LDN-193189 and SB-43 1542.

EXAMPLES

[00201] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m, intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Materials and Methods

Reagents and cell culture

[00202] Neural Induction (NI) medium comprised the following:

- 98mL DMEM F12
- 98mL Neurobasal
- l.5mL glutamax
- lmL N2 supplement
- 2 mL B27 + insulin supplement
- 200pl 55mM b-mercaptoethanol

[00203] RPE maintenance medium comprised the following:

- 389mL Knockout DMEM
- 5mL glutamax
- 5mL NEAA
- 909 µl 55mM β-mercaptoethanol
- 100mL Knockout serum replacement
- 2 mL 2.5M Nicotinamide

[00204] The RPE differentiation protocol using the AAVS hOPM cell line comprised the following:

[00205] iPSC culture conditions were maintained until start of the differentiation protocol.

- Day -2: WTB6. 13 iPSCs were seeded from 2 wells of >70% confluent 6 well plate into 12 wells of 12-well plate on mTeSR™ (feeder free maintenance medium for human ES and iPS cells - Stemcell Technologies - Catalog #85850, Catalog #85851, Catalog #85852) + Ri (Y-27632, ROCK inhibitor) (roughly 44,000 cells/cm ^{2} growth area).
- Day -1: Cells were fed with mTeSR™.
- Day 0: NI (neural induction) medium was used, cells were >90% confluent.
- Day 2: NI medium used and warmed to room temperature.
- Day 4: NI + 2µL/mL doxycycline were used.
- Day 6: RPE maintenance medium + 2µL/mL doxycycline were used.
- Day 6+: Fed with RPE maintenance medium + 2µL/mL dox every Monday, Wednesday, and Friday until first pigment emerged.
- -Day 25: After first pigment was observed, medium was changed to X-vivo™ 10, containing L-glutamine, gentamicin, and phenol red, (Lonza Catalog # 04-380Q) (Chemically Defined, Serum-free Hematopoietic Cell Medium) + doxycycline and medium was changed every Monday, Wednesday, and Friday.
- -Day 35: After mature RPE morphology was observed, medium was changed with X-vivo™ 10 (no dox) when medium turned yellow.

Preparation and delivery of lentiviral constructs

[00206] For initial experiments, cDNAs for the transcription factors human OTX1 (Figure 11), human OTX2 (Figure 12), human MITF (Figure 15), human PAX2 (Figure 13), and human PAX6 (Figure 14) were cloned individually into the lentiviral vector (pl372), with the EF-la promoter driving TF(OTXl, OTX2, MITF, PAX2 or PAX6)-T2A-GFP expression. OTX1, OTX2, MITF, PAX2, and PAX6 were expressed in separate viruses that were co-transfected in human iPSCs.

Cloning of lentiviral expression vectors

[00207] Plasmid pl372 was digested using Nhel and EcoRI. Using infusion cloning, each of the transcription factors attached to a T2A-GFP was cloned into the vector. Resulting plasmids were sequence verified before use.

Lentiviral packaging

[00208] Packaging and harvest of virus was performed generally as described below.

A. Day 0-1: Seeding the Cells

- The day prior to packaging the virus, HEK293T cells were seeded in an appropriate format. Either 7.5e6 was plated for a l5cm dish or 2.6e6 for a lOcm dish. HEK cell media comprised of DMEM, high glucose, no glutamine 11960069; 10% FBS; 1% NEAA; 1% glutamax.

B. Day 1: Transfection

- For each well/plate, a 1.5 mL tube (Tube 1) was prepared by adding: a. 1300 µL OptiMEM and 48µL Mirus Reagent for a l5cm dish. b. 470µL of OptiMEM and 18µL Mirus Reagent for a lOcm dish. Incubate at RT for 5 minutes.
- In another l.5mL tube (Tube 2), a total of 17 µL (l5cm) or 6.3 µL (lOcm) of DNA was added together:

	dR8.91	*pMD 2G*	*gRNA*
15 cm	8µg	1µg	8µg
10 cm	3µg	360ng	3µg

- Tube 1 and Tube 2 were mixed together. The mix was left at room temperature for 30 minutes.
- After incubation, 1300 µL (l5cm) or 470 µL (lOcm) of media was removed from the cells and the mixture was added dropwise.

- The plate was rocked gently and left at 37°C overnight.

C. Day 2: Checkup and media replacement

- Cells were checked on a fluorescence microscope for transfection based on selected reporter, e.g., mCherry. The presence of syncytia (multinucleated, globular cells) confirmed lentiviral production.

D. Day 4: Harvest Virus

- Viral soup was placed in either a 15 ml or 50 ml tube.
- The soup was filtered through a Milipore Millex-HP 0.45 µM filter (hydrophobic PES) by adding the soup to a syringe with the filter attached.
- The virus was aliquoted out into cryotubes for storage.
- Viral supernatant was stored at -80°C until use.

E. Transduction

- Cells were passaged at least a few hours before transduction.
- Virus was slowly added in a dropwise manner to the cells.
- Cells were incubated overnight.
- The cells were kept incubated, and the media was changed as normal.

Induction and transfection of hIPSC

- Day -2-Day 0: iPSCs were grown on GFR Matrigel and mTeSR until confluence on 12 well plate.
- Day 0-7: Neural induction medium was added and changed every other day.
- Day 8: Cells were split into RPE maintenance media and 5µM ROCKi in 1:3 surface area ratio split into 12 well plates.
- Day 9: Media was changed to RPE maintenance media.
  Day 10: 250µl of each virus supernatant was added to each well of 12 well plate and the plate was left for 5 days.
- Day 15: Media was changed to RPE maintenance media.
- Day 17+: Media was changed every other day.

Preparation and deliveryfor hOPM-AAVS and hOPM-CLYBL vectors

TALENS used for knock in

[00209] AAVS: addgene ID 89813, 89814

CLYBL: addgene ID 62196, 62197

Donor Vector Construction

[00210] cDNAs for each of the transcription factors human OTX1, human PAX6, and human MITF were cloned into a single poly-cistronic vector under the control of a Tet-0 -inducible promoter, with the coding sequence for each transcription factor separated by a T2A (ribosomal skipping sequence) (Tet-0 promoter driving OTX2-T2A-PAX6-T2A-MITF-STOP*) to provide for co-expression of human OTX1, human PAX6, and human MITF polypeptides. Two different vectors were prepared, one targeted to the AAVS1 genomic locus and the other targeted to the CLYBL genomic locus. The vector map for the AAVS 1-targeted vector (hOPM-AAVS) is provided in FIG. 1.

[00211] Individual cDNA fragments were amplified from cDNA expression vectors from Genscript for OTX2, PAX6, and MITF using primers designed for Infusion cloning for the CLYBL and AAVS donor vectors. The cDNA sequences for OTX2, PAX6, and MITF are provided in FIGs. 12, 14, and 15, respectively.

[00212] After self-cleaving peptide activity, protein fragments were as follows:

[00213] OTX2:MMSYLKQPPYAVNGLSLTTSGMDLLHPSVGYPGPWASCPAAT PRKQRRERTTFTRAQLDVLEALFAKTRYPDIFMREEVALKINLPESRVQVWF KNRRAKCRQQQQQQNGGQNKVRPAKKKTSPAREVSSESGTSGQFTPPSST SVPTIASSSAPVSIWSPASISPLSDPLSTSSSCMQRSYPMTYTQASGYSQGYAG STSYFGGMDCGSYLTPMHHQLPGPGATLSPMGTNAVTSHLNQSPASLSTQG YGASSLGFNSTTDCLDYKDQTASWKLNFNADCLDYKDQTSSWKFQVLGSG *EGRGSLLTCGDVEENPG* (SEQ ID NO:20).

[00214] PAX6:PMQNSHSGVNQLGGVFVNGRPLPDSTRQKIVELAHSGARPCDI SRILQTHADAKVQVLDNQNVSNGCVSKILGRYYETGSIRPRAIGGSKPRVATP EVVSKIAQYKRECPSIFAWEIRDRLLSEGVCTNDNIPSVSSINRVLRNLASEKQ QMGADGMYDKLRMLNGQTGSWGTRPGWYPGTSVPGQPTQDGCQQQEGG GENTNSISSNGEDSDEAQMRLQLKRKLQRNRTSFTQEQIEALEKEFERTHYPD VFARERLAAKIDLPEARIQVWFSNRRAKWRREEKLRNQRRQASNTPSHIPISS SFSTSVYQPIPQPTTPVSSFTSGSMLGRTDTALTNTYSALPPMPSFTMANNLP MQPPVPSQTSSYSCMLPTSPSVNGRSYDTYTPPHMQTHMNSQPMGTSGTTST GLISPGVSVPVQVPGSEPDMSQYWPRLQ *GSGEGRGSLL TCGD VEENPG* (SEQ ID NO:2l).

[00215] MITF:PMQSESGIVPDFEVGEEFHEEPKTYYELKSQPLKSSSSAEHPGAS KPPISSSSMTSRILLRQQLMREQMQEQERREQQQKLQ AAQFMQQRVPV SQTP AINVSVPTTLPSATQVPMEVLKVQTHLENPTKYHIQQAQRQQVKQYLSTTLA NKHANQVLSLPCPNQPGDHVMPPVPGSSAPNSPMAMLTLNSNCEKEGFYKF EEQNRAESECPGMNTHSRASCMQMDDVIDDIISLESSYNEEILGLMDPALQM ANTLPVSGNLIDLYGNQGLPPPGLTISNSCPANLPNIKRELTESEARALAKERQ KKDNHNLIERRRRFNINDRIKELGTLIPKSNDPDMRWNKGTILKASVDYIRKL QREQQRAKELENRQKKLEHANRHLLLRIQELEMQARAHGLSLIPSTGLCSPD LVNRIIKQEPVLENCSQDLLQHHADLTCTTTLDLTDGTITFNNNLGTGTEANQ AYSVPTKMGSKLEDILMDDTLSPVGVTDPLLSSVSPGASKTSSRRSSMSMEE TEHTC* (SEQ ID NO: 19).

[00216] In the above sequences, italicized amino acids indicate residual amino acids left by the T2A self-cleaving peptide following cleavage.

[00217] CLYBL hOPM and AAVS hOPM plasmids were sequence verified after construction.

General vector information:

[00218] Selection cassette was flanked by LoxP sites, which allowed for Cre treatment to remove selection markers. Positive selection marker comprised EF-la mCherry; gene trapping with puromycin selection (puromycin resistance only if properly integrated into target locus).

Cell Line Generation

[00219] iPSCs were maintained so that they were actively dividing. One million well-singularized cells were collected in a pellet. Nucleofection was performed using Lonza 4D nucleofector using P3 solution and supplemented, pulse code EN138.

[00220] Reaction volume comprised as follows:

1. 1 million cells
2. 100ul nucleofection solution
3. 2 µg TALEN R (AAVS or CLYBL)
4. 2 µg TALEN L (AAVS or CLYBL)
5. 4 µg donor vector (AAVS hOPM or CLYBL hOPM)

[00221] Cells were left in nucleofection solution for 5 minutes after pulse. Cells were recovered using 500pl StemFit media+lOuM Rock inhibitor (Ri). Cells were plated in serial dilution in GFR-Matrigel coated 6 well plate (highest dilution = 2/7 total cell

count). Cells were fed with StemFit+Ri every other day until the most confluent well was -90% confluent (cells were not removed from Ri) (-3-7 days). Puromycin selection was performed at 0.5 µL/ml puromycin in StemFit+lOpM Ri. Media changed every other day until no mCherry -negative cells remain. Cells were cultured on StemFit (+Ri) until colonies were big enough to passage or pick for characterization.

RPE Matrigel Dome Protocol

[00222] The protocol was adapted from: Zhu Y., Carido M., Meinhardt A., Kurth T., Karl M.O., Ader M., Tanaka E.M. (2013), Three-Dimensional Neuroepithelial Culture from Human Embryonic Stem Cells and Its Use for Quantitative Conversion to Retinal Pigment Epithelium, *PLoS ONE,* 8(1), art. no. e54552, for comparison with the methods of the present disclosure.

[00223] The protocol required mixing iPSCs into Matrigel suspension, followed by manual dissection of pigmented clumps pigmented cells. One well of iPSC at approximately 80% confluency from a 6-well plate was used. A 300pl aliquot of pure GFR-Matrigel was thawed out on ice.

Day 0

[00224] Media was removed from iPSCs and washed with 2ml of **1** x PBS (-Mg/-Ca). Cells were treated with lml of Accutase for lmin and the iPSC colonies were lifted from the well using a cell scraper. 4ml of 1 x PBS was added to the well, the mix was transferred to a l5ml falcon tube and the colonies were broken up with gentle trituration. The cells were pelleted by centrifugation at 800rpm for 3 min. The majority of the upper solution was aspirated, leaving a couple of pl and the pellet was gently flicked to disperse. 300 µΐ of cold Matrigel was added to the cells, mixed by stirring and 50 µΐ of the cell suspension was quickly transferred to a 6 well plate (a dome of Matrigel resulted). The remaining cell suspension was repeated so that a number of discrete domes (up to 4) was formed per well of a 6 well plate. The plate was incubated at 37°C for 30min. 2ml of Neural Differentiation medium was added with 1x ROCKi per well.

Dav 1-4

[00225] The media was changed daily, and the ROCKi was omitted.

Dav 5

[00226] The media was removed from the wells, washed with PBS and 4ml 1x PBS/ well was added. The edge of a cell scraper was used to cut each dome into 4 pieces. Using a pipette, the pieces of Matrigel and PBS were transferred into a l5ml falcon tube. The Matrigel was allowed to settle at the bottom of the tube before the PBS was carefully removed. 5ml of Coming Cell Recovery Solution was added to the tube and the tube was incubated at 4°C for 40min to release the Neuroepithelial cysts from the Matrigel. The spheres were centrifuged at 400rpm for lmin and the cell recovery solution was carefully removed. 2ml of TryplE solution was added to the falcon tube and the tube was incubated at 37°C for 10 min, and triturated to make a single cell suspension. lOml of Neural induction media was added to the cells and the mix was centrifuged at 800rpm for 3 min. The cells were resuspended in 2ml of NI medium with 1 x ROCKi and cells were counted. 200,000cells/cm ^{2} were plated out in Neural induction medium + lx ROCKi.

Day 6

[00227] Medium was replaced with HESC-bFGF.

Dav 7

[00228] Medium was replaced with HESC-bFGF + lOOng/ml Activin A

Dav 9-23

[00229] Medium was replaced every other day.

Neural Induction Media

[00230] Neural Induction Media comprised the following: 195.5ml of Neurobasal medium, 195.5ml of DMEM, 4ml B27 supplement, 2ml N2 supplement, 400 µΐ β-ME, and 2.5ml glutamax.

RPE 14-day differentiation protocol

[00231] Protocol was adapted from Buchholz et al. *Stem Cells Transl Med.* 2013 May;2(5):384-93. doi: l0.5966/sctm.20l2-0l63. Epub 2013 Apr 18; and Leach et al. *Invest Ophthalmol Vis Sci.* 2015 Jan 20;56(2): 1002-13. doi: l0.H67/iovs.l4-l5835, for comparison with the methods of the present disclosure.

Media Components

Retinal Differentiation Media (RDM)

[00232] RDM comprised of the following: DMEM/F12 (with Glutamax supplement; Invitrogen #10565018); IX N2 Supplement (#17502048, Invitrogen); IX B27 Supplement; and IX NEAA.

[00233] *Growthfactors and other reagents (stock concentrations)*

- Nicotinamide (Sigma#N0636-l00G) had a working concentration of 10 mM.
- Recombinant mouse noggin (R&D Systems #l967-NG-25) was reconstituted to l00 µL/mL and had a working concentration of lOng/ml.
- Recombinant human DKK-l (R&D Systems #5439-DK-0l0) was reconstituted to lOOµL/mL and had a working concentration of lOng/ml.
- Recombinant human IGF-l (R&D Systems #291- Gl-200) was reconstituted to lOOµL/mL and had a working concentration of lOng/ml.
- bFGF (ThermoFisher #13256029 1OµL) was reconstituted to 1OµL/mL and had a working concentration of 5ng/ml.
- Recombinant human/mouse/rat Activin A (Peprotech, #120-14E, 1OµL; stable at -80°C for lyr) and the working concentration was reconstituted to lOOµL/mL.
- SU5402 FGF inhibitor (Santa Cruz Biotechnology, #sc-204308, lmg) was reconstituted to lOmM and had a working concentration of 10 pM.
- Vasoactive intestinal peptide (VIP) described in the original Buchholz et al. paper was omitted.

Substratefor 14-day Differentiation

[00234] The substrate comprised of Matrigel, non-growth factor reduced (GF), Phenol Red-Free, (Coming, product #356237,10 mL).

[00235] The stock was made in -150-200 µL aliquots of concentrated Matrigel and stored at -20°C. For the working concentration, 1 aliquot was diluted into 12-12.5 mL serum-free media (just DMEM/F12). 0.5 mL/well was plated in a l2-well plate, and was aspirated right before seeding.

Substratefor RPE enrichment

[00236] Cells enriched on day 14 were plated onto growth factor reduced (GFR), Matrigel w/ Phenol Red (Coming). The protein concentration varied from lot-to-lot, so bigger or smaller aliquots were created based on which lot.

Other reagents utilized:

- IX Versene (EDTA) - day 0 only
- IX PBS (no calcium, no magnesium) - day 0 & day 14
- TryµLE - day 14 only. (ordered Express enzyme 1x w Phenol Red (lOOml) 12605010
- XVIVO 10 (Lonza) or other RPE supporting media - day 14 only
- Y27632, stock:l0mM, working: IOµM, (Stem cell core) - day 14 only

Day -1: Plates were prepared

[00237] Matrigel was thawed on ice at 4°C overnight. From 9 mg/mL stock, 200µL aliquots were made. To make the working concentration, the 200µL aliquot was added to l2ml DMEM/F12 to give 150 µL/mL final concentration.

[00238] Day 0: Day of stem cell passage for differentiation.

- Plates were coated with diluted Matrigel using DMEM/F12, “Thin Coating Method” using cooled pipets, the Matrigel aliquot was mixed to homogeneity and diluted into 12-12.5mL DMEM/F12. 0.5mL/well was plated in a l2-well plate and the plate was incubated at room temperature for one hour. Any excess was aspirated off right before seeding cells.
- RDM media was made by adding NOGGIN (50 ng/ml), DKK1 (10 ng/ml), IGF1 (10 ng/ml), and nicotinamide (10 mM).
- hiPS cells were passaged 1:4 (into l2-well) depending on confluence. For every 6 well of cells to passage, 4 x l2-wells were plated for differentiation. If another plate format was used, the density was optimized. Cells were -50% confluent on day 1.
- Differentiating colonies in iPSC colonies were removed. Media was aspirated off and washed once with PBS. PBS was aspirated.
- The EDTA passage method was started, following protocol (Beers et al., Nat Protoc 2012):
    1. Each well was rinsed 2-3X with lmL Versene (EDTA). Versene was gently aspirated so colonies were not disturbed or accidentally lifted from the plate.
    2. After 2-3 washes, lmL/well Versene was added, and incubated at room temperature in the hood without disturbance for 3-5 minutes. The length of time Versene was left on determined the size of the

dissociated stem cell clumps; longer incubation yielded smaller clumps and shorter incubation yielded bigger clumps.

3. After 3-5 minutes, the Versene was gently aspirated off.
4. Immediately, lmL retinal differentiation medium (RDM) was pipetted into each 6-well being passed for differentiation.
5. Using a cell scraper, colonies were gently scraped off well and pipetted up and down a few times (~3X) to evenly distribute dissociated clumps.
6. The colonies were transferred to a 50mL conical tube. Enough RDM media was added to re-plate into the number of wells/plates needed. lmL/l2-well (2mL/6-well) was re-plated. Too much pipetting up and down was avoided to prevent further dissociation of stem cell clumps (50mL conical tube was swirled to evenly mix cells).

- Once cells were plated for differentiation, plate was quickly rocked back and forth to evenly disperse cells in the wells and placed in incubator. Time was noted. Cells were fed consistently at this time or close to it for the remainder of the protocol whenever the media composition changed (day 2, day 4, day 6 & day 8).

Day 1

[00239] Media was changed. Media was the same as Day 0 (RDM media + NOGGIN 50 ng/ml; DKK1 10 ng/ml; IGF1 10 ng/ml; nicotinamide lOmM). Media was changed 24 hours post-seed because of the amount of cell death that could occur as a result of passaging.

Day 2

[00240] Change media was changed to RDM media + NOGGIN (50 ng/ml); DKK1 (10 ng/ml); IGF1 (10 ng/ml); nicotinamide (lOmM) + bFGF (5 ng/mL).

Dav 6

[00241] Media was changed to include 10 μM SU5402 and 100 μL/mL Activin A. If the CHIR protocol was not performed, the media was the same from now until Day 14.

Dav 8

[00242] Media was changed to contain SU5402, Activin A, 3pM CHIR99021.

Dav 10

[00243] Media was changed.

Dav 12

[00244] Media was changed.

[00245] Dav 14: RPE Enrichment

- IX PBS, lmL RDM (no GFs added) per number of wells enriched, TryµLE, XVIVO 10 or any RPE media used to enrich cells into, was warmed up. An aliquot of Y27632 (or however much needed to use it at 10µM or 1µL/mL media) was thawed. Y27632 was added to the media when changed for the first 4-7 days after enrichment to p.0.
- Old media was aspirated from wells to be enriched, lmL RDM (no GFs) was added per well.
- In the dissecting scope, all non-RPE clusters of cells were manually dissected out.
- Once all contaminating cell types were removed, RDM + unwanted cells were aspirated and wells were washed twice with lmL PBS, dissociated, spun, strained, and counted.
- The cells were re-plated on GFR-Matrigel-coated plates in RPE medium containing Y27632 at 1 x 10^5 cells/cm^2.
- Cells were checked the day after enrichment to p.0 and media was changed 48 hours after enrichment to p.0. Y27632 was left on cells until days 4-7.
- Cells were left to mature in culture for 4-5 weeks before passing to p.1. Subsequent passages to p.l and p.2 did not require use of Y27632. Cells were passaged at day 30.

Plasmids

[00246] Figures 17-22 depict vector maps of the plasmids used: Figure 17 depicts the CLYBL TALEN L plasmid and sequence (SEQ ID NO:30); Figure 18 depicts the CLYBL TALEN R plasmid and sequence (SEQ ID NO:3l); Figure 19 depicts the CLYBL hOPM plasmid and sequence (SEQ ID NO:32); Figure 20 depicts the AAVS1 TALEN L plasmid and sequence (SEQ ID NO:33); Figure 21 depicts the AAVS1 TALEN R plasmid and sequence (SEQ ID NO:34); and Figure 22 depicts the AAVS hOPM plasmid and sequence (SEQ ID NO:35).

[00247] Figure 23 depicts a schematic of the hOPM construct. hOPM was inserted into a safe harbor locus (either CLYBL or AAVS1). When doxycycline was added, hOPM was transcribed. These transcription factors stayed on in mature RPE without doxycycline induction. This method required only media changes and yielded entire monolayers of RPE as opposed to small colonies that needed to be dissected.

Nucleofection of Induced Pluripotent Stem Cells

[00248] Wild type and patient derived induced pluripotent stem cells were maintained in StemFit Basic02 (Ajinomoto, AS-BolR) with 5.8nM bFGF (Peprotech, 100-18B) in 6 well plates until the cells reached 70 percent confluency. Cells were then dissociated with Accutase (StemCell Technologies, 07920) and counted. A nucleofection solution consisting of 1 μL of the left and right TALENs as well as 2μL of the hOPM plasmid and P3 supplement was prepared according to the instructions of the P3 Primary Cell 4D-Nucleofector X Kit L (Lonza, V4XP-3024). 1,000,000 cells were resuspended in this nucleofection solution then transferred to a cuvette provided by the kit. The cuvette was placed inside the Lonza 4D Nucleofector Core Unit and zapped with a pulse code EN138. Cells were allowed to remain in the cuvette for 5 minutes post nucleofection then resuspended in StemFit Basic02 (Ajinomoto, AS-BolR) with 10μM rock inhibitor (Selleckchem, S1049) and seeded into 3 wells of a 6 well plate. Cells were allowed to grow to 70 percent confluency then were subject to antibiotic selection using 0.25μL/ml puromycin (Sigma Aldrich, P8833) in StemFit (Ajinomoto, AS-BolR) with 10μM rock inhibitor (Selleckchem, S1049). Media with puromycin was exchanged every other day until cells in anon-nucleofection control well all died. Cells were allowed to recover in StemFit Basic02 (Ajinomoto, AS-BolR) with 10μM rock inhibitor (Selleckchem, S1049) until colonies were a suitable size for picking. Colonies were picked and genotyped using junction PCR for the hOPM plasmid. Primer sequences are listed in *Table 1* below. Clones that were junction positive for both the 5' and 3' junction were expanded in culture and frozen down to use for RPE differentiation.

[00249] **Table 1: Primer list for genotyping clones for the hOPM construct**

Primer Name	**Primer Sequence (5' → 3')**	**SEQ ID NO.**
CLYBL_5'_Junction_F	CACATGTCAAGCAGGAACCC	SEQ ID NO:22

CLYBL_5'_Junction_R	GTGGGCTTGTACTCGGTCAT	SEQ ID NO:23
CLYBL_3'_Junction_F	CACCAGCAACCTGACGTTTT	SEQ ID NO:24
CLYBL_3'_Junction_R	CGGGGGTTATCATTGGCGTA	SEQ ID NO:25
AAVS1_5'_Junction_F	CCTGAGTCCGGACCACTTTG	SEQ ID NO:26
AAVS1_5'_Junction_R	CTGCCAGATCTCTCGAGGCC	SEQ ID NO:27
AAVS1_3'_Junction_F	TGTGGGGTGGAGATATCAGC	SEQ ID NO:28
AAVS1_3'_Junction_R	GCCTGGTAGACAGGGCTGG	SEQ ID NO:29

Cell Culture

Induced Pluripotent Stem Cell Culture

[00250] All wild type and patient derived induced pluripotent stem cells were maintained in StemFit Basic02 (Ajinomoto, AS-BolR) with 5.8nM bFGF (Peprotech, 100-18B). Cells were grown in 6 well plates coated with Matrigel (Coming, 356231) and re-plated once they reached 70% confluency, approximately every 4 days. Accutase (StemCell Technologies, 07920) was used to dissociate cells and they were re-plated at about 9,000 cells/cm^2. The media was changed to fresh media every 2 days.

Differentiation into Retinal Pigment Epithelium

[00251] Induced pluripotent stem cells were dissociated with Accutase (StemCell Technologies, 07920) and passaged into a 96-well plate coated with Matrigel (Coming, 356231). Cells were seeded at a density of roughly 48,000 cells per cm^2 and maintained in StemFit Basic02 (Ajinomoto, AS-BolR) with lOuM ROCK inhibitor (Selleckchem, S1049) for 24 hours. The media was then changed to StemFit Basic02 (Ajinomoto, AS-BolR) and the cells were allowed to grow to 90 to 100 percent confluency. On Day 0, cells were washed with PBS (Life Technologies, 14190-144) then treated with the optimization condition for Day 0 listed in *Table 2* below. For Days 0 - 4 the following 96 optimization conditions were carried out with a change in media occurring every other day. (NI = Neural Induction, RPEMM = RPE Maintenance Media, Dox = Doxycycline, LSB = LDN-193189 + SB-43 1542)

Table 2: RPE differentiation 96 well plate optimization

Day 0	1	2	3	4	5	6	7	8	9	10	11	12
LSB 0.4x	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, Dox	NI	NI
LSB 0.2x	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, Dox	NI	NI
LSB 0.1x	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, Dox	NI	NI
LSB 0.05x	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, LSB, Dox	NI, LSB	NI, LSB	NI, Dox	NI	NI
LSB 0.4x	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, Dox	RPEM M	RPEM M
LSB 0.2x	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, Dox	RPEM M	RPEM M
LSB 0.1x	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, Dox	RPEM M	RPEM M
LSB 0.05x	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB	RPEM M, Dox	RPEM M	RPEM M

Table 2 (Cont.)

Day 2	1	2	3	4	5	6	7	8	9	10	11	12
LSB 0.4x	NI, Dox	NI, Dox	NI	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB
LSB 0.2x	NI, Dox	NI, Dox	NI	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB
LSB 0.1x	NI, Dox	NI, Dox	NI	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB
LSB 0.05x	NI, Dox	NI, Dox	NI	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB	NI, LSB, Dox	NI, LSB, Dox	NI, LSB
LSB 0.4x	RPEM M, Dox	RPEM M, Dox	RPEM M	RPEM M,LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB
LSB 0.2x	RPEM M, Dox	RPEM M, Dox	RPEM M	RPEM M,LSB, Dox	RPEM M, LSB,	RPEM M, LSB	RPEM M, LSB,	RPEM M, LSB,	RPEM M, LSB	RPEM M, LSB,	RPEM M, LSB,	RPEM M, LSB

					Dox		Dox	Dox		Dox	Dox	
LSB 0.1x	RPEM M, Dox	RPEM M, Dox	RPEM M	RPEM M,LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB
LSB 0.05x	RPEM M, Dox	RPEM M, Dox	RPEM M	RPEM M,LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB

Table 2 (Cont.)

Day 4	1	2	3	4	5	6	7	8	9	10	11	12
LSB 0.4x	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox
LSB 0.2x	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox
LSB 0.1x	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox
LSB 0.05x	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox	NI, LSB, Dox
LSB 0.4x	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox
LSB 0.2x	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox
LSB 0.1x	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox
LSB 0.05x	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox	RPEM M, LSB, Dox

[00252] Induction Media comprised of 49% DMEM F12 (ThermoFisher Scientific 11330032), 49% Neurobasal (Life Technologies, 21103049), 0.625% Glutamax (Life Technologies, 35050-061), 0.5% N2 supplement (Life Technologies, 17502048), 1% B27 supplement with insulin (Life Technologies, 17504-044), 0.1% 55mM B-mercaptoethanol (Life Technologies, 21985-023). The 1x concentration of LDN-193189 (StemCell Technologies, 72146) and SB-431542 (StemCell Technologies,

72232) used were lOOnM and IOμM respectively. At Day 6, the media for the whole plate was changed to RPE Maintenance Media with 2μM doxycycline (Sigma Aldrich, 1226003). RPE Maintenance Media comprised of Knockout DMEM (Life Technologies, 10829-018), 20% Knockout Serum Replacement (ThermoFisher Scientific, 10828028), 1% Glutamax (Life Technologies, 35050-061), 1% NEAA (Life Technologies, 11140-050), 0.18% 55mM B-mercaptoethanol (Life Technologies, 21985-023), and 0.4% 2.5M Nicotinamide (Sigma Aldrich, N0636-100G). The cells were fed every Monday, Wednesday, and Friday in this media until Day 30. On Day 30, the media was changed to Pig RPE Maintenance Media with 2μM doxycycline (Sigma Aldrich, 1226003). Pig RPE Maintenance Media comprised of DMEM (Coming, 10-013-CV), 1% NEAA (Life Technologies, 11140-050), 1% FBS (ATCC, 30-2020), and 0.4% of THT supplement, which was prepared using 99ml Earle's Balanced Salt Solution (Life Technologies, 14155-063), l2.5g taurine (Sigma Aldrich, 107-35-7), lmg hydrocortisone (Sigma Aldrich, 50237), 0.65 μL liothyronine (Sigma Aldrich, 1368008). The cells were fed with this media every Monday and Thursday until Day 60 when the cells were taken off doxycycline induction. Cells past Day 60 continued to be maintained with Pig RPE Maintenance Media with biweekly feedings. The best wells from this 96 well plate optimization were then expanded into a whole plate format to validate the optimization protocol.

Gene Expression Analysis Using ddPCR

[00253] WTB6. 13 AAVS hOPM C4 cell pellets were collected at different points in the differentiation starting with day zero as induced pluripotent stem cells and including iPSC-RPE cells at week 6, week 8, and week 12 of the differentiation. Total RNA was isolated using the DirectZol Miniprep Plus Kit (Zymo Research, R2060). cDNA was synthesized from RNA using the Superscript IV First Strand Synthesis System (ThermoFisher Scientific, 18091050). The primers and probes used are shown in *Table 3*. The reaction was prepared using 10μL of 2x ddPCR Supermix for Probes (Bio-Rad, 186-3010), 9μL of 100 ng/μL DNA and 1μL of 10 μM TaqMan probe. Droplets were generated by the Bio-Rad QX100 Droplet Generator (Bio-Rad, 186-3001) from 20μL reaction mixtures and 70μL Droplet Generator Oil (Bio-Rad, 186-3005) to result in about 35μL of product. The PCR plate was sealed using the PX1 PCR Plate Sealer (Bio-Rad, 181-4000), a C1000 Touch Thermal Cycler (Bio-

Rad, 185-1196) was used for the ddPCR reaction, and the QX100 Droplet Reader (Bio-Rad, 186-4003) for the analysis of the droplets. The threshold was set on Quantasoft Software (Bio-Rad, 186-401 1) and the data were normalized to the housekeeping gene, GAPDH, and graphed on excel with respect to the iPSC concentration.

Table 3: ddPCR Probes

Probe	Assay ID
RLBP1	ThermoFisher (Hs_00165632)
SERPINF1	ThermoFisher (Hs_01106937_m1)
BEST1	ThermoFisher (Hs_00188249_m1)
MITF	ThermoFisher (Hs_01117294_m1)
RPE65	ThermoFisher (Hs_01071462_m1)

Example 1: Accelerated RPE Formation by Forced Transcription Factor Induction

[00254] To accelerate RPE formation five retinal enriched transcription factors (OTX1, OTX2, MITF, PAX2, and PAX6) were initially tested using individual lentiviral vectors. Five weeks of transcription factor overexpression in human iPSCs resulted in the generation of RPE as shown in Figure 2. Based on these preliminary positive results an attempt was made to co-express transcription factors in a single vector to provide more uniform expression. It was tested whether OTX1 and PAX2 could be eliminated, while maintaining RPE generation, since they have redundant roles with the remaining three factors. By reducing the number of transcription factors to three, it was possible to combine the three factors (OTX2, PAX6, MITF (OMP)) in a single Tet-controlled poly-cistronic vector, where each factor is separated by a T2A (ribosomal skipping sequence), allowing each transcription factor to be physically separated (where the factors closer to the to the promoter are more highly translated). This single vector was targeted to a single genomic locus (AAVS1), allowing uniform expression in iPSCs and differentiation of iPSCs to a monolayer RPE. See, e.g., Figures 4-8. This vector is referred to herein as hOPM-AAVS. In an attempt to achieve higher levels of expression, another version of the vector was prepared that targets the CLYBL locus (hOPM-CLYBL). The CLYBL

locus has been shown to have higher levels of expression with other transgenes. In addition, it is contemplated that expression may be increased by combining multiple integration sites. In addition, by combining higher transcription factor dosing, with different doxycycline dosing, the optimal timing and extent of transcription factor over-expression can be determined.

[00255] An advantage of this system (hOPM-AAVS or hOPM-CLYBL) is that the Tet system keeps the transcription factors (OTX2, PAX6 and MITF) off in the pluripotent state, allowing the cell line to be propagated prior to differentiation. This allows for unlimited production of source material without the need for repeated introduction of transcription factors via viral delivery.

[00256] An additional advantage of this system (hOPM-AAVS or hOPM-CLYBL) is that the timing of OPM expression can be controlled. For example, in the context of the above protocol, when hOPM was induced in the iPSC stage prior to neural induction, the cells peeled off the plate and died off (data not shown). By varying the timing of OPM induction it was found that better results were obtained when OPM expression was induced several days after chemical differentiation towards neurons was begun. This system allows one to precisely control the timing of induction to provide for an optimized system. The inducible system also allows for the expression of OPM to be turned off after RPE is formed, allowing the RPE to be studied without artificial expression of OPM.

[00257] The quality of the OPM-RPE appears to be superior to the published, chemical induction methods (see, e.g., Zhu et al. (2013) PLoS ONE, 8(1), art. no. e54552) in that the RPE produced using the current method is more uniform, and has markers of maturity, including pigment, cuboidal shape, and expression of the maturity marker BEST1. See, e.g., Figure 9, which provides a comparison showing a pigmented colony at day 26 of differentiation using the Matrigel dome protocol described in Zhu et al. (2013) PLoS ONE, 8(1), art. no. e54552) (left) relative to a pigmented monolayer of RPE cells produced at day 30 of the transcription factor induction protocol described herein using the hOPM-AAVS vector. BEST1 cDNA was detected at the exon 6/7 junction at 6 weeks, 8 weeks, 10 weeks, and 12 weeks of differentiation using the hOPM-AAVS vector in the presence of doxycycline induction as described herein. In contrast, in the absence of doxycycline induction, but otherwise using the same conditions, BEST1 cDNA was not detected until 10

weeks using the hOPM-AAVS vector. No BEST1 cDNA was detected at 8 weeks at the exon 6/7 junction in the absence of vector, either in the presence or absence of doxycycline. Remarkably the OPM-RPE is significantly easier to make (requires fewer media changes, and there is no need to re-plate cells) and does not require the expensive recombinant hormones used in the chemical induction methods. The OPM-RPE is more uniform than that provided by other available methods (even methods taking twice as long) which form RPE in patches, that are at varying degrees of maturity. See, e.g., Figure 10, which provides a comparison showing pigmented colonies at day 59 of differentiation using the protocol described in Figure 10 (top) relative to a pigmented monolayer of RPE cells produced at day 56 of the transcription factor induction protocol described herein using the hOPM-AAVS vector (bottom). Initial examination of the RPE produced by the current method suggests that it is more mature and of higher quality than that produced by chemical induction methods that take twice as long. For instance, expression of the maturity marker gene (BEST1) and the production of pigment were observed, whereas such characteristics have not been observed using some chemical induction methods.

Example 2: Differentiation into Retinal Pigment Epithelium

[00258] Figure 24 depicts a comparison of the Leach et al. and Zhu et al. protocols described herein with a method of the present disclosure, which required only media changes and yielded entire monolayers of RPE as opposed to small colonies that needed to be dissected.

[00259] iPSCs were seeded, grown to 100% confluency in 96 well plate format, and conditioned for optimization with Dual Smad inhibition (Figure 26). RPE cells were grown to maturity (around 60 days) and best wells were picked to expand. Condition(s) that worked the best were expanded. If the chosen condition made RPE efficiently, optimized differentiation for a cell line was considered complete.

[00260] In vivo, RPE are derived from the neurectodermal lineage. LSB, (LDN + SB) are small molecule dual-smad inhibitors used to specify the neurectodermal state and are more directed than use of NI.

[00261] Optimized conditions varied between cell lines as shown in Figure 27. Conditions were varied between cell lines at the beginning of the differentiation as shown in Figure 28. From days 0-6, the following components were varied: Basal

media, Doxycycline timing, LSB concentration, LSB timing, and LSB duration. Some of the cell lines preferred the "Neural Induction" medium from Days 0-6 while others preferred "RPE Maintenance Media." The timing of Doxycycline addition also varied between cell lines with some lines preferring Doxycycline induction beginning on Day 0, while others preferred it on Day 2 or Day 4. The concentration of LSB that these cell lines preferred varied with one cell line differentiating well without LSB and others differentiating well with O.lx and 0.05x concentrations of LSB. Timing and duration of LSB also varied. Most lines with LSB in their differentiation protocol preferred it at Day 0 yet the preferred length of LSB treatment varied. Some cell lines performed well with LSB treatment until Day 6 and other cell lines performed better with LSB treatment terminated on Day 2 or Day 4. At Day 6 and on, all of the cell lines received the same condition: LSB treatment was terminated, Dox induction was present, and RPE Maintenance Media was used until Day 30. The basal media switched at Day 30 to "Pig RPE Maintenance Media". Images were all taken at 20x at Day 60+ and the same images are shown in larger format in Figures 29-3 1.

[00262] Figure 29 depicts images of RPE development at 60+ days for WTB and WTC cell lines, showing the dark pigmentation and hexagonal or cobblestone morphology that are characteristic of RPE.

[00263] Figure 30 depicts images of RPE development at 60+ days for BTP6 and BTP7 cell lines, showing the dark pigmentation and hexagonal or cobblestone morphology that are characteristic of RPE.

[00264] Figure 3 1 depicts images of RPE development at 60+ days for BTP8 and BTP10 cell lines, showing the dark pigmentation and hexagonal or cobblestone morphology that are characteristic of RPE.

[00265] Figure 32 depicts images showing the stage of RPE development that are stained with generic tight junction proteins occludin and phalloidin. RPE formed a monolayer with strong tight junctions between cells.

[00266] Figures 33-35 depict images showing mature induced pluripotent stem cell (iPS)-derived RPE. The image showing RPE development at day 60 for WTB Traffic Light was taken at 5x magnification (Figure 33, left). The monolayer of RPE was shown forming a fluid-filled dome, indicating that the RPE was carrying out the function of transporting fluid. The image showing RPE development at day 60+ for

BTP8 was taken at 20x magnification and shows the characteristic hexagonal or cobblestone shape and dark pigmentation of RPE (Figure 33, right).

[00267] Figure 34 depicts images showing RPE development at -day 45 for BTP10 (left) and BTP6 (right). These images were taken at lOx magnification and show RPE characteristic hexagonal shape as well as pigmentation beginning to develop. Although some cells did not have pigmentation yet, they had the correct hexagonal morphology and would begin to develop pigment in the coming weeks.

[00268] Figure 35 depicts images showing RPE development at day -60 for WTB (left) and WTC (right). This image was taken at 40x magnification and shows the dark pigmentation and hexagonal shape of these RPE.

Example 3: Gene Expression Analysis Using ddPCR

[00269] Figure 36 depicts gene expression results using ddPCR for RPE specific genes. The probes used included SERPINF, BEST1, MITF RLBP1, and RPE65. SERPINF refers to "PEDF" or "Pigment Epithelium Derived Factor," and is secreted apically from RPE cells, antiangiogenic. BEST1 encodes bestrophinl which is a calcium activated chloride channel localized to the basolateral membrane of RPE. MITF encodes a transcription factor that is responsible for turning on RPE specific genes and pigmentation genes. RLBP1 encodes a protein that is a ligand to 1l-cis retinal; a component of the visual cycle. RPE65 is also a component of the visual cycle, and is an isomerohydrolase that regenerates visual pigment.

[00270] RPE specific gene expression data in WTB AAVS hOPM C4 is shown as a function of timepoint mRNA/iPSC over age. The RPE specific genes depict exponential fold increase from iPSCs.

[00271] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

    introducing into the iPSCs an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs; and

    culturing the genetically modified iPSCs under conditions sufficient for

    expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

    differentiation of the genetically modified iPSCs into retinal pigment epithelial cells,

    wherein the method does not comprise introducing into the iPSCs or genetically modified iPSCs a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide, or an exogenous nucleic acid comprising a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide; thereby generating RPE.

2. The method of claim 1, wherein the exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, the exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and the exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide are comprised by a recombinant vector.

3. The method of claim 2, wherein the recombinant vector is a viral vector.

4. The method of claim 2, wherein the recombinant vector is a donor vector for a gene editing system.

5. The method of claim 4, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

6. The method of claim 5, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.

7. The method of any one of claims 2-6, wherein the recombinant vector does not comprise a tyrosinase enhancer.

8. The method of any one of claims 1-7, wherein iPSCs are human iPSCs.

9. The method of any one of claims 1-7, wherein iPSCs are rodent iPSCs.

10. The method of any one of claims 1-7, wherein the iPSCs are non-human primate iPSCs.

11. The method of any one of claims 1-10, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

12. The method of any one of claims 1-1 1, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

13. The method of any one of claims 1-12, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

14. The method of any one of claims 2-13, wherein the vector comprises a Tet-inducible promoter.

15. The method of claim 14, wherein the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide comprises contacting the genetically modified iPSCs with

tetracycline or a derivative or analogue thereof to induce expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide.

16. The method of claim 15, wherein the tetracycline or a derivative or analogue thereof is doxycycline.

17. The method of any one of claims 1-16, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.

18. The method of any one of claims 2-17, wherein the vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs.

19. The method of any one of claims 2-18, wherein the vector is targeted to a plurality of integration sites.

20. The method of any one of claims 2-19, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

21. The method of claim 20, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

22. The method of any one of claims 2-21, wherein the recombinant vector comprises a promoter and each of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding a MITF polypeptide is operably linked to the promoter.

23. The method of any one of claims 2-22, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF

polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.

24. The method of any one of claims 1-23, wherein the method does not comprise introducing into the iPSCs or genetically modified iPSCs an exogenous nucleic acid comprising a tyrosinase enhancer.

25. The method of any one of claims 2-24, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.

26. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

introducing into the iPSCs an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs; and

culturing the genetically modified iPSCs under conditions sufficient for

expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

differentiation of the genetically modified iPSCs into retinal pigment epithelial cells,

wherein the method does not comprise introducing into the iPSCs or genetically modified iPSCs an exogenous nucleic acid comprising a tyrosinase enhancer; thereby generating RPE.

27. The method of claim 26, wherein the exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, the exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and the exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide are comprised by a recombinant vector.

28. The method of claim 27, wherein the recombinant vector is a viral vector.

29. The method of claim 27, wherein the recombinant vector is a donor vector for a gene editing system.

30. The method of claim 29, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

31. The method of claim 30, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.

32. The method of any one of claims 26-29, wherein iPSCs are human iPSCs.

33. The method of any one of claims 26-29, wherein iPSCs are rodent iPSCs.

34. The method of any one of claims 26-29, wherein the iPSCs are non-human primate iPSCs.

35. The method of any one of claims 26-34, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

36. The method of any one of claims 26-35, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

37. The method of any one of claims 26-36, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

38. The method of any one of claims 27-37, wherein the vector comprises a Tet-inducible promoter.

39. The method of claim 38, wherein the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide,

and the MITF polypeptide comprises contacting the genetically modified iPSCs with tetracycline or a derivative or analogue thereof to induce expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide.

40. The method of claim 39, wherein the tetracycline or a derivative or analogue thereof is doxycycline.

41. The method of any one of claims 26-40, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.

42. The method of any one of claims 27-41, wherein the vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs.

43. The method of any one of claims 27-41, wherein the vector is targeted to a plurality of integration sites.

44. The method of any one of claims 27-43, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

45. The method of claim 44, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

46. The method of any one of claims 27-45, wherein the recombinant vector comprises a promoter and each of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding a MITF polypeptide is operably linked to the promoter.

47. The method of any one of claims 27-46, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the

MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.

48. The method of any one of claims 27-47, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.

49. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

introducing into the iPSCs a recombinant vector comprising a Tet-inducible promoter, an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein one or more of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide are operably linked to the Tet-inducible promoter; and

culturing the genetically modified iPSCs under conditions sufficient for

expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

differentiation of the genetically modified iPSCs into retinal pigment epithelial cells; thereby generating RPE.

50. The method of claim 49, wherein the recombinant vector is a viral vector.

51. The method of claim 49, wherein the recombinant vector is a donor vector for a gene editing system.

52. The method of claim 51, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

53. The method of claim 52, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.

54. The method of any one of claims 49-51, wherein iPSCs are human iPSCs.

55. The method of any one of claims 49-51, wherein iPSCs are rodent iPSCs.

56. The method of any one of claims 49-51, wherein the iPSCs are non-human primate iPSCs.

57. The method of any one of claims 49-56, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

58. The method of any one of claims 49-57, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

59. The method of any one of claims 49-58, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

60. The method of any one of claims 49-59, wherein the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide comprises contacting the genetically modified iPSCs with tetracycline or a derivative or analogue thereof to induce expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide.

61. The method of claim 60, wherein the tetracycline or a derivative or analogue thereof is doxycycline.

62. The method of any one of claims 49-61, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.

63. The method of any one of claims 49-62, wherein the vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs.

64. The method of any one of claims 49-62, wherein the vector is targeted to a plurality of integration sites.

65. The method of any one of claims 49-64, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

66. The method of claim 65, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

67. The method of any one of claims 49-66, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.

68. The method of any one of claims 49-67, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.

69. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

introducing into the iPSCs a recombinant vector comprising an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein the

recombinant vector is targeted to an AAVS1 locus or a CLYBL locus of the iPSCs; and

culturing the genetically modified iPSCs under conditions sufficient for

expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

differentiation of the genetically modified iPSCs into retinal pigment epithelial cells; thereby generating RPE.

70. The method of claim 69, wherein the recombinant vector is a viral vector.

71. The method of claim 69, wherein the recombinant vector is a donor vector for a gene editing system.

72. The method of claim 71, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

73. The method of claim 72, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.

74. The method of any one of claims 69-71, wherein iPSCs are human iPSCs.

75. The method of any one of claims 69-71, wherein iPSCs are rodent iPSCs.

76. The method of any one of claims 69-71, wherein the iPSCs are non-human primate iPSCs.

77. The method of any one of claims 69-76, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

78. The method of any one of claims 69-77, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

79. The method of any one of claims 69-78, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

80. The method of any one of claims 69-79, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.

81. The method of any one of claims 69-80, wherein the vector is targeted to a plurality of integration sites.

82. The method of any one of claims 69-81, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

83. The method of claim 82, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

84. The method of any one of claims 69-83, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.

85. The method of any one of claims 69-84, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.

86. A method of generating retinal pigment epithelium (RPE) from induced pluripotent stem cells (iPSCs), the method comprising:

introducing into the iPSCs a recombinant vector comprising a promoter, an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide, an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide, and an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide to provide genetically modified iPSCs, wherein each of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide is operably linked to the promoter; and

culturing the genetically modified iPSCs under conditions sufficient for

expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs, and

differentiation of the genetically modified iPSCs into retinal pigment epithelial cells; thereby generating RPE.

87. The method of claim 86, wherein the recombinant vector is a viral vector.

88. The method of claim 86, wherein the recombinant vector is a donor vector for a gene editing system.

89. The method of claim 88, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

90. The method of claim 89, comprising introducing into the iPSCs or genetically modified iPSCs one or more nucleic acids encoding a TALEN or a nucleic acid encoding a ZFN.

91. The method of any one of claims 86-88, wherein iPSCs are human iPSCs.

92. The method of any one of claims 86-88, wherein iPSCs are rodent iPSCs.

93. The method of any one of claims 86-88, wherein the iPSCs are non-human primate iPSCs.

94. The method of any one of claims 86-93, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

95. The method of any one of claims 86-94, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

96. The method of any one of claims 86-95, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

97. The method of any one of claims 86-96, comprising exposing the RPE to conditions sufficient for termination of expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide from the exogenous nucleic acids.

98. The method of any one of claims 86-97, wherein the vector is targeted to a plurality of integration sites.

99. The method of any one of claims 86-98, wherein, in the recombinant vector, the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

100. The method of claim 99, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

101. The method of any one of claims 86-100, comprising culturing the genetically modified iPSCs under conditions sufficient for replication of the genetically modified iPSCs prior to the culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide, and differentiation of the genetically modified iPSCs into retinal pigment epithelial cells.

102. The method of any one of claims 86-101, wherein the vector is configured such that expression level of the OTX2 polypeptide is greater than expression level of the PAX6 polypeptide, and expression level of the PAX6 polypeptide is greater than expression level of the MITF polypeptide.

103. The method of any one of claims 1-102, comprising culturing the genetically modified iPSCs for a period of time under differentiation conditions prior to culturing the genetically modified iPSCs under conditions sufficient for expression of the OTX2 polypeptide, the PAX6 polypeptide, and the MITF polypeptide in the genetically modified iPSCs.

104. The method of claim 103, wherein the period of time is from about 2 to about 4 days.

105. The method of claim 104, wherein the differentiation conditions comprise culturing the genetically modified iPSCs in a differentiation medium.

106. The method of claim 105, wherein the differentiation medium is a neural induction medium.

107. A retinal pigment epithelium (RPE) generated by the method of any one of claims 1-106.

108. A composition comprising the retinal pigment epithelium (RPE) of claim 107.

109. The composition of claim 108, wherein the composition comprises a liquid culture medium.

110. The composition of claim 108, wherein the composition comprises a cryopreservative agent.

111. A screening method, the method comprising:

contacting the retinal pigment epithelium (RPE) of claim 107 with a test agent; and

assaying to determine the effect of the test agent on the RPE.

112. The screening method of claim 111, wherein the method is a multiplexed screening method.

113. The screening method of claim 111 or 112, wherein the assaying comprises assaying for modulation of retinal pigment epithelial cell proliferation and/or modulation of retinal pigment epithelial cell differentiation.

114. The screening method of claim 113, wherein the modulation is increased retinal pigment epithelial cell proliferation and/or increased retinal pigment epithelial cell differentiation relative to retinal pigment epithelial cell proliferation and/or retinal pigment epithelial cell differentiation in the absence of the test agent.

115. The screening method of claim 113, wherein the modulation is decreased retinal pigment epithelial cell proliferation and/or decreased retinal pigment epithelial cell differentiation relative to retinal pigment epithelial cell proliferation and/or retinal pigment epithelial cell differentiation in the absence of the test agent.

116. The screening method of claim 111, wherein the test agent is a gene editing tool.

117. A kit comprising the retinal pigment epithelium (RPE) of claim 107 or the composition of one of claims 108-110 and a liquid culture medium.

118. The kit of claim 117 comprising one or more of fetal bovine serum, an epithelial cell growth supplement, and an antibiotic.

119. A recombinant vector comprising:

a promoter;

a nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide operably linked to the promoter;

a nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide operably linked to the promoter; and

a nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide operably linked to the promoter.

120. The recombinant vector of claim 119, wherein the vector does not comprise a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

121. The recombinant vector of claim 119 or 120, wherein the recombinant vector does not comprise a tyrosinase enhancer.

122. The recombinant vector of any one of claims 119-121, wherein the recombinant vector is a viral vector.

123. The recombinant vector of claim 122, wherein the recombinant vector is a donor vector for a gene editing system.

124. The recombinant vector of claim 123, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

125. The recombinant vector of any one of claims 119-124, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

126. The recombinant vector of any one of claims 119-125, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

127. The recombinant vector of any one of claims 119-126, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

128. The recombinant vector of any one of claims 119-127, wherein the promoter is aTet-inducible promoter.

129. The recombinant vector of any one of claims 119-128, wherein the vector is targeted to an AAVS1 locus, or ortholog thereof, or a CLYBL locus, or ortholog thereof, of a mammalian cell.

130. The recombinant vector of claim 129, wherein the mammalian cell is selected from a human cell, a rodent cell, and a non-human primate cell.

131. The recombinant vector of any one of claims 119-130, wherein the vector is targeted to a plurality of integration sites.

132. The recombinant vector of any one of claims 119-131, wherein the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

133. The recombinant vector of claim 132, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

134. A recombinant vector comprising:

an inducible promoter;

an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide;

an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide; and

an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide, wherein one or more of the nucleotide sequence encoding an OTX2 polypeptide, the nucleotide sequence encoding a PAX6 polypeptide, and the nucleotide sequence encoding an MITF polypeptide are operably linked to the inducible promoter.

135. The recombinant vector of claim 134, wherein the vector does not comprise a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

136. The recombinant vector of claim 134 or 135, wherein the recombinant vector does not comprise a tyrosinase enhancer.

137. The recombinant vector of any one of claims 134-136, wherein the recombinant vector is a viral vector.

138. The recombinant vector of claim 137, wherein the recombinant vector is a donor vector for a gene editing system.

139. The recombinant vector of claim 138, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

140. The recombinant vector of any one of claims 134-139, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

141. The recombinant vector of any one of claims 134-140, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

142. The recombinant vector of any one of claims 134-141, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

143. The recombinant vector of any one of claims 134-142, wherein the inducible promoter is a Tet-inducible promoter.

144. The recombinant vector of any one of claims 134-143, wherein the vector is targeted to an AAVS1 locus, or ortholog thereof, or a CLYBL locus, or ortholog thereof, of a mammalian cell.

145. The recombinant vector of claim 144, wherein the mammalian cell is selected from a human cell, a rodent cell, and a non-human primate cell.

146. The recombinant vector of any one of claims 134-145, wherein the vector is targeted to a plurality of integration sites.

147. The recombinant vector of any one of claims 134-146, wherein the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

148. The recombinant vector of claim 147, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

149. A recombinant vector comprising:

an exogenous nucleic acid comprising a nucleotide sequence encoding an OTX2 polypeptide;

an exogenous nucleic acid comprising a nucleotide sequence encoding a PAX6 polypeptide; and

an exogenous nucleic acid comprising a nucleotide sequence encoding an MITF polypeptide, wherein the vector is targeted to an AAVS1 locus, or ortholog thereof, or a CLYBL locus, or ortholog thereof, of a mammalian cell.

150. The recombinant vector of claim 149, wherein the vector does not comprise a nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

151. The recombinant vector of claim 149 or 150, wherein the recombinant vector does not comprise a tyrosinase enhancer.

152. The recombinant vector of any one of claims 149-151, wherein the recombinant vector is a viral vector.

153. The recombinant vector of claim 152, wherein the recombinant vector is a donor vector for a gene editing system.

154. The recombinant vector of claim 153, wherein the gene editing system comprises a gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

155. The recombinant vector of any one of claims 149-154, wherein the OTX2 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 13.

156. The recombinant vector of any one of claims 149-155, wherein the PAX6 polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 17.

157. The recombinant vector of any one of claims 149-156, wherein the MITF polypeptide comprises an amino acid sequence having at least about 80% amino acid identity to the amino acid sequence set forth in SEQ ID NO: 19.

158. The recombinant vector of any one of claims 149-157, wherein the vector is targeted to a plurality of integration sites.

159. The recombinant vector of any one of claims 149-158, wherein the nucleotide sequence encoding an OTX2 polypeptide is separated from the nucleotide sequence encoding a PAX6 polypeptide by a first ribosomal skipping sequence, and the nucleotide sequence encoding a PAX6 polypeptide is separated from the nucleotide sequence encoding a MITF polypeptide by a second ribosomal skipping sequence.

160. The recombinant vector of claim 159, wherein the first and/or second ribosomal skipping sequence encodes a 2A peptide selected from T2A, P2A, E2A, and F2A.

161. An isolated host cell comprising the recombinant vector of any one of claims 119-160.

162. An isolated genetically modified host cell comprising an exogenous OTX2 nucleic acid, an exogenous PAX6 nucleic acid, and an exogenous MITF nucleic acid integrated into the genome of the host cell.

163. The host cell of claim 162, wherein the host cell is an iPSC.

164. The host cell of claim 162, wherein the host cell is an RPE cell.

165. The host cell of any one of claims 162-164, comprising a gene editing tool.

166. The host cell of claim 165, wherein the gene editing tool selected from a TALEN, a ZFN, one or more nucleic acids encoding a TALEN, and one or more nucleic acids encoding a ZFN.

167. The host cell of any one of claims 162-166, wherein the host cell does not comprise an exogenous nucleotide sequence encoding a transcription factor other than an OTX2 polypeptide, a PAX6 polypeptide, and/or an MITF polypeptide.

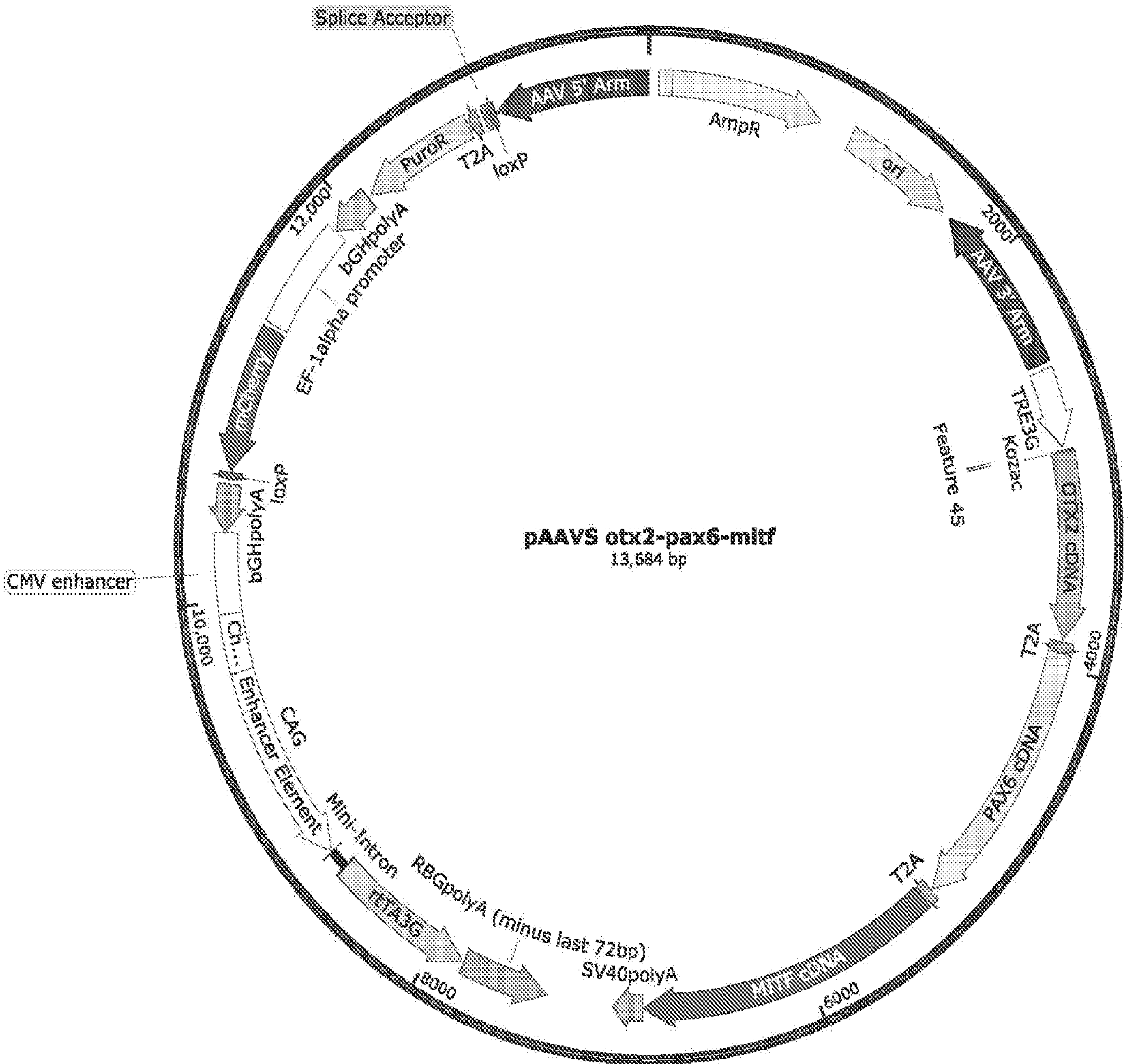
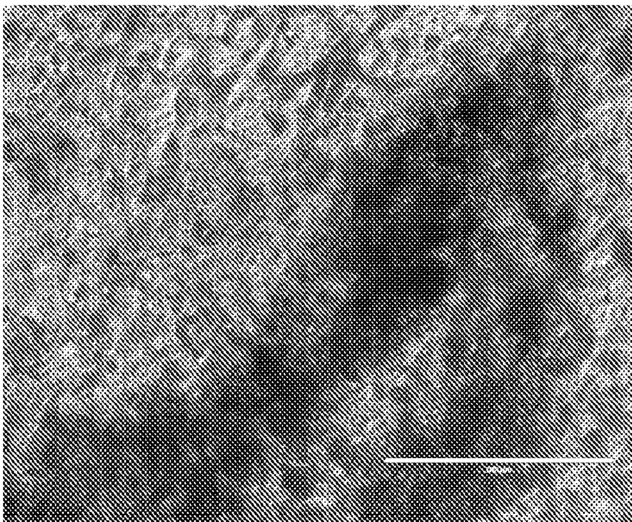
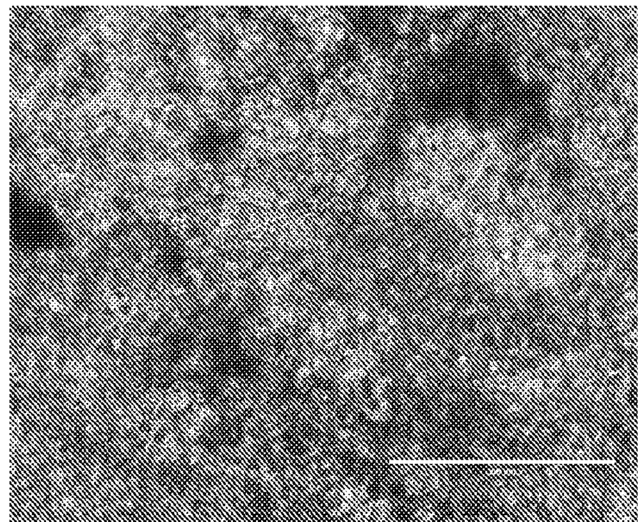

Splice Acceptor
AAV 5' Arm
AmpR
ori
2000
AAV 3' Arm
TRE3G
Kozac
Feature 45
OTX2 cDNA
T2A
4000
PAX6 cDNA
T2A
6000
SV40polyA
RBGpolyA (minus last 72bp)
8000
rtTA3G
Mini-Intron
Enhancer Element
CAG
Ch...
10,000
CMV enhancer
bGHpolyA
loxP
EF-1alpha promoter
bGHpolyA
12,000
PuroR
T2A
loxP
pAAVS otx2-pax6-mitf
13,684 bp

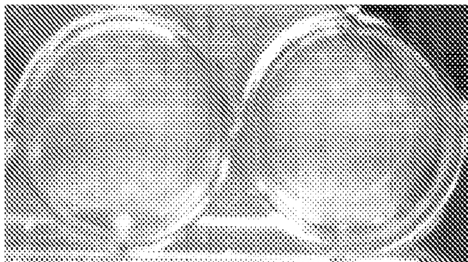
Figure 2
TF Overexpression for RPE (5 weeks)

No virus (control)

Virus treated (hOTX1, hOTX2, hPAX2, hPAX6, hMITF)







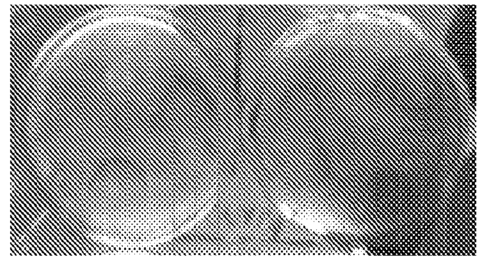


Figure 3

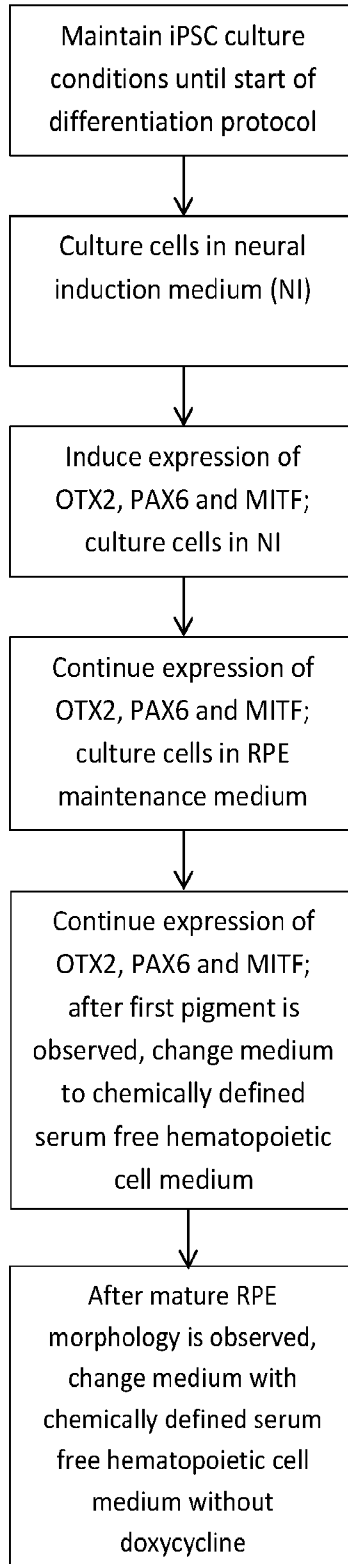


Figure 4

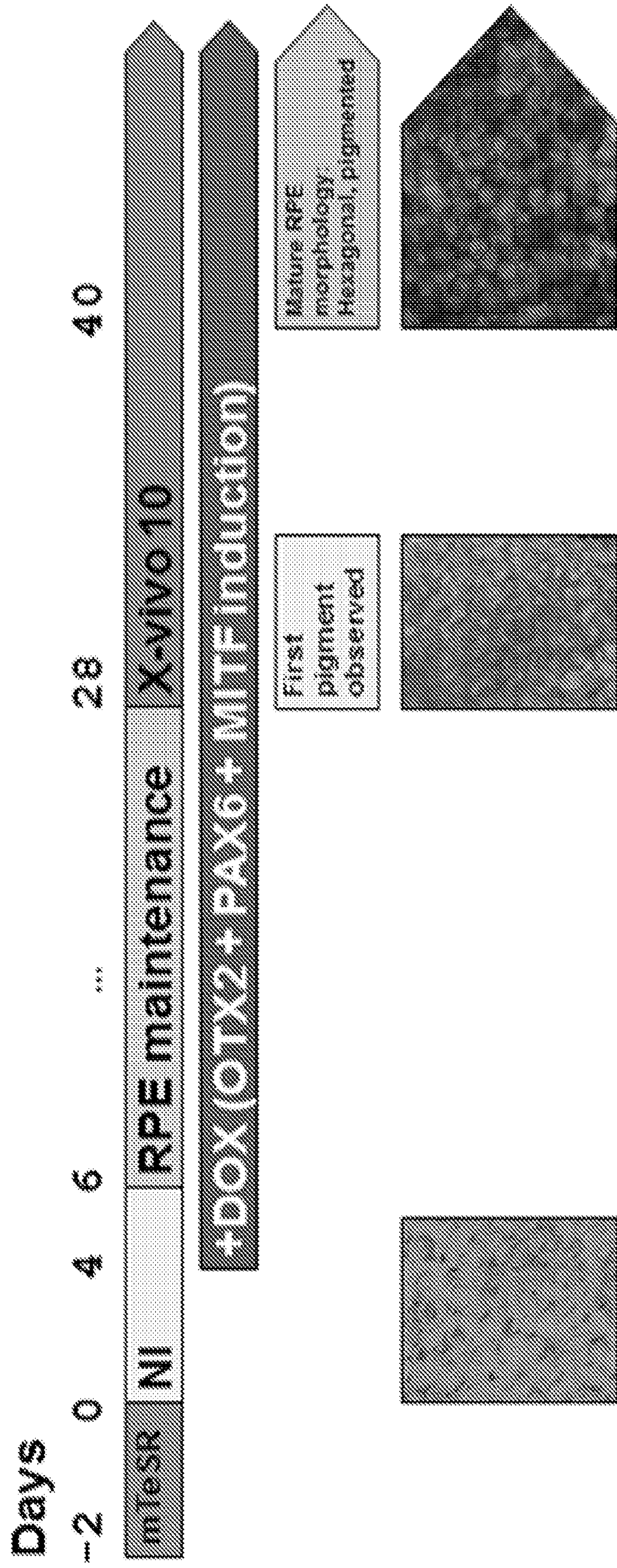
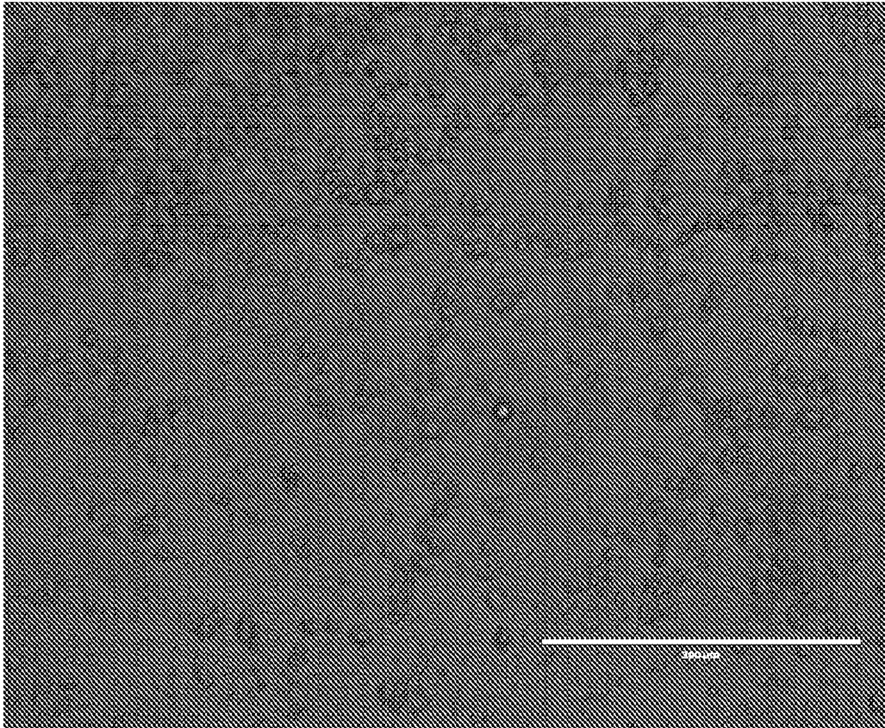


Figure 5
WTB6.13 hOPM RPE
-DOX (30 days)



+DOX (30 days)

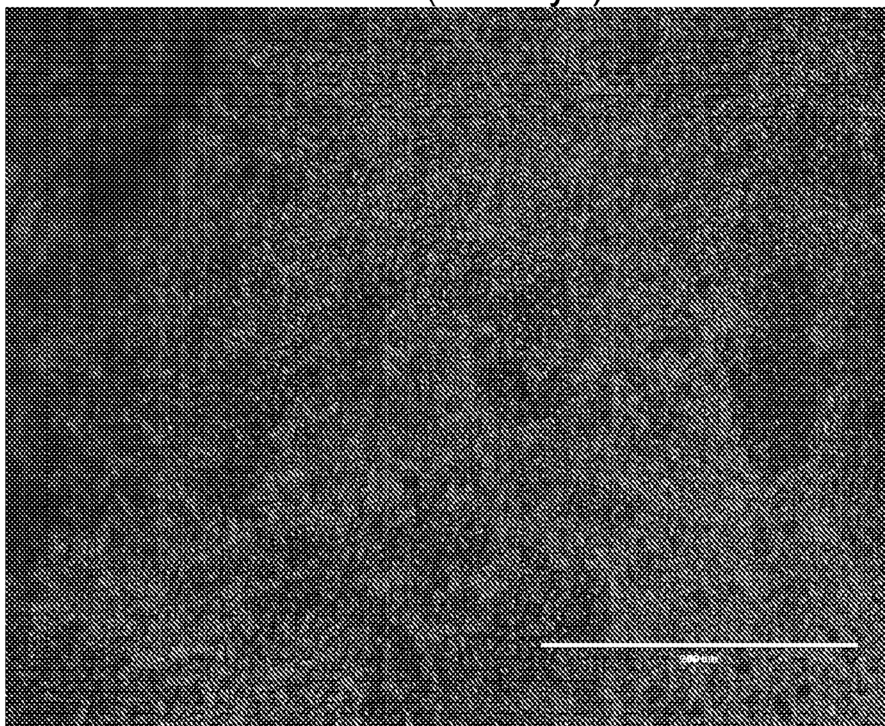
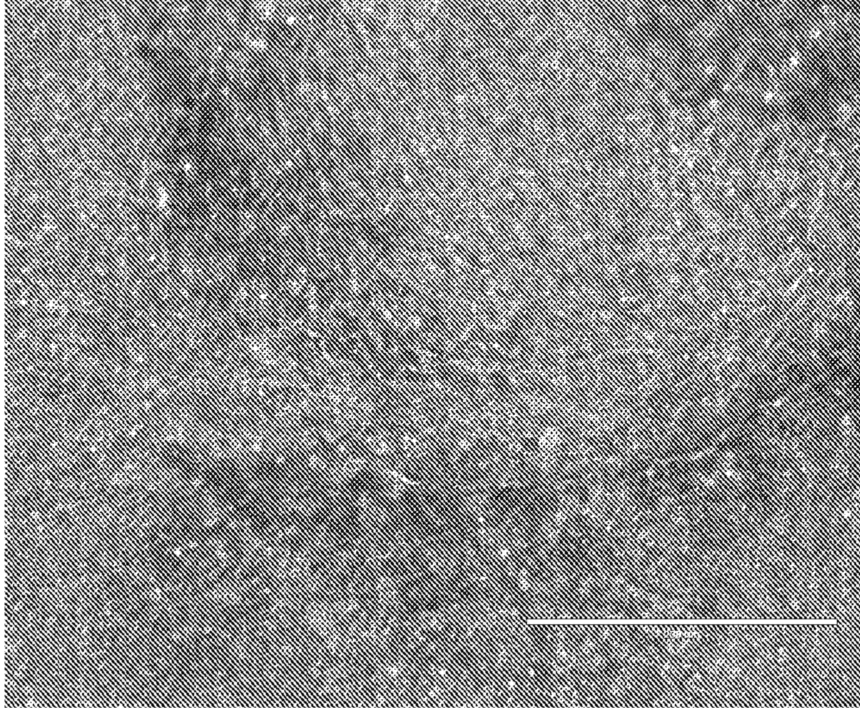


Figure 6
WTB6.13 hOPM RPE
-DOX (5 weeks)



WTB6.13 hOPM RPE
+DOX (5 weeks)

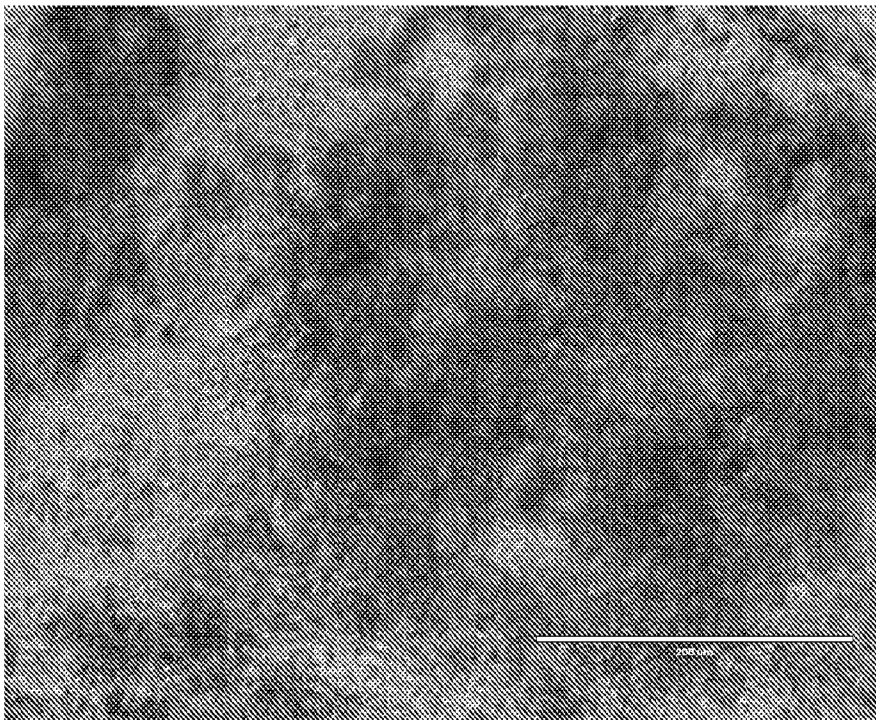
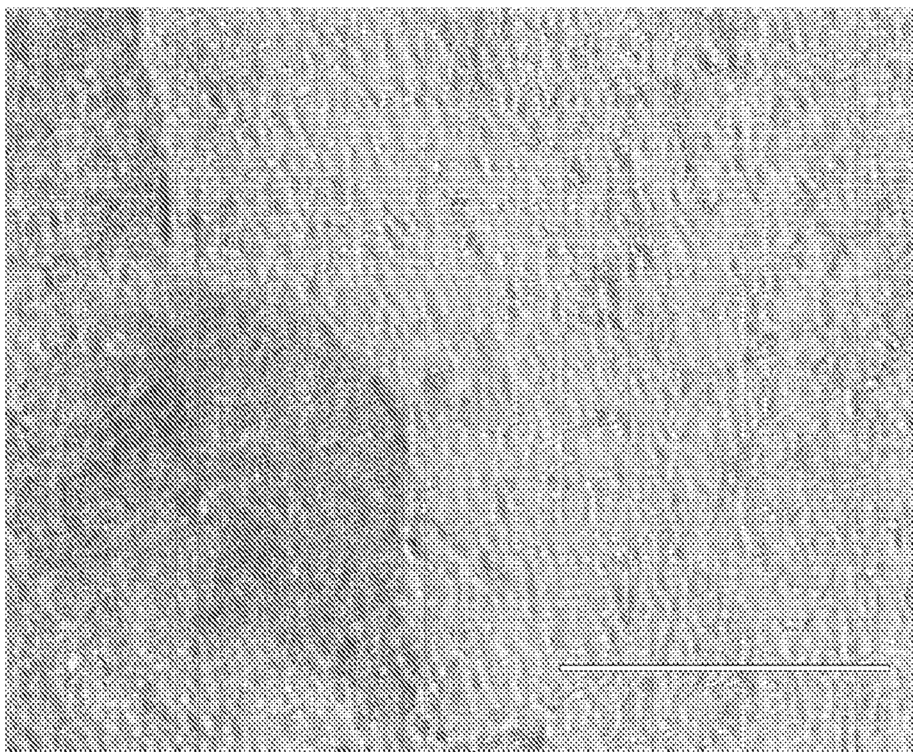


Figure 7
WTB6.13 hOPM RPE
-DOX (56 days)



+DOX (56 days)

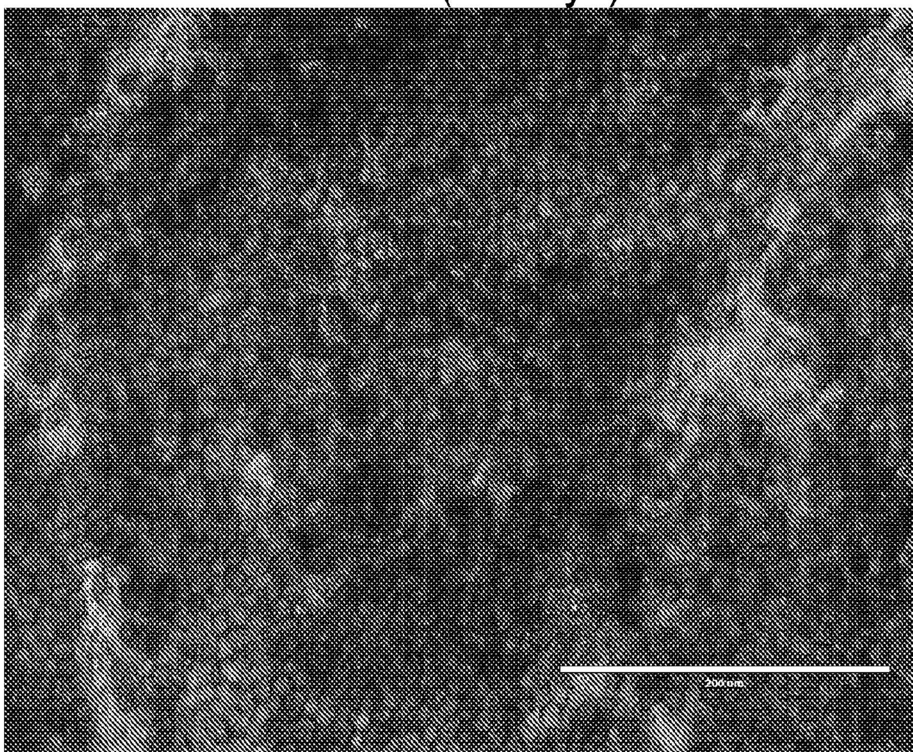
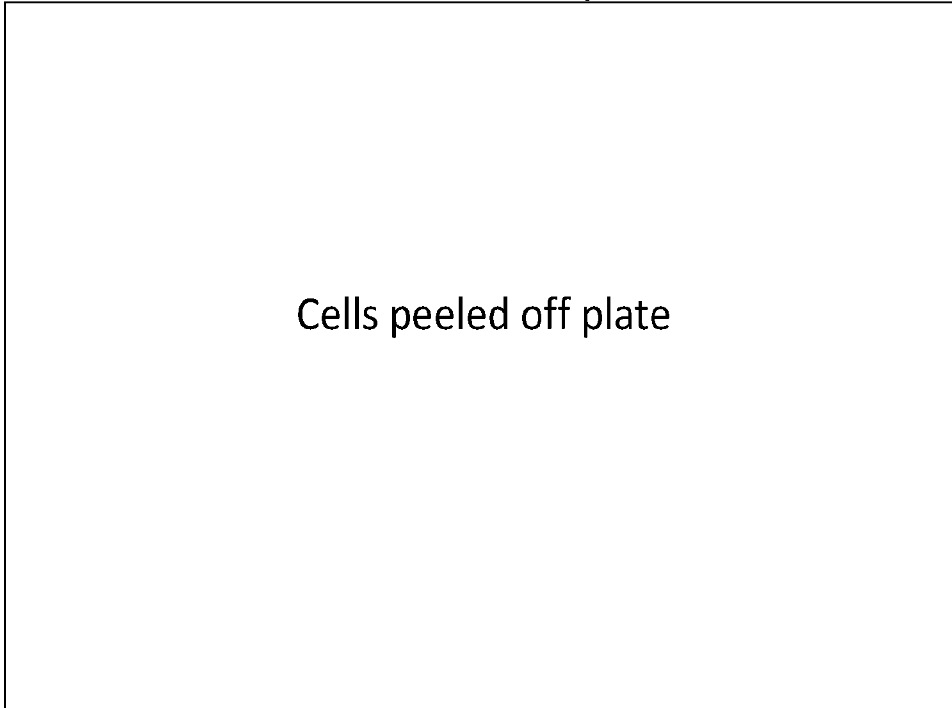


Figure 8

WTB6.13 hOPM RPE
-DOX (85 days)



+DOX (85 days)

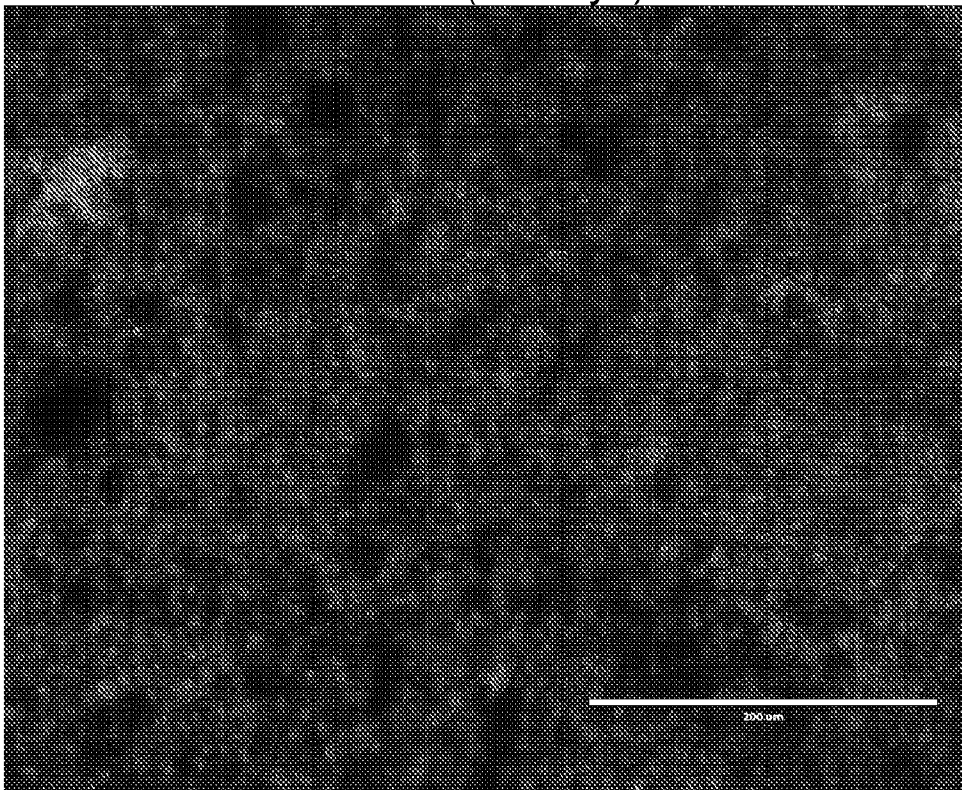
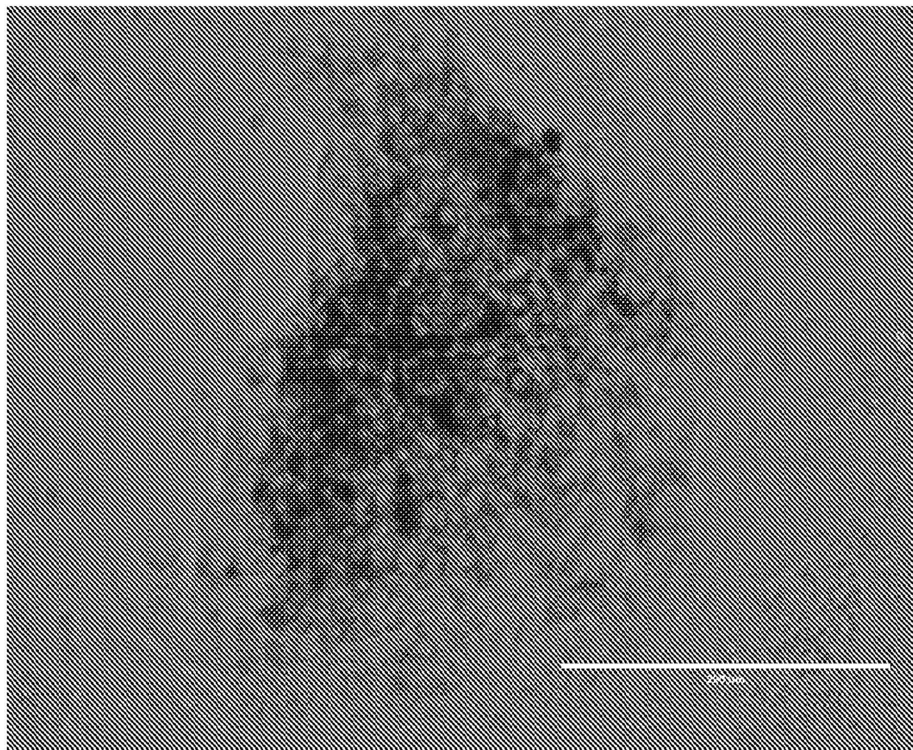


Figure 9

RPE Matrigel Dome Protocol – pigmented colony at day 26



hOPM TF induction – pigmented monolayer at day 30

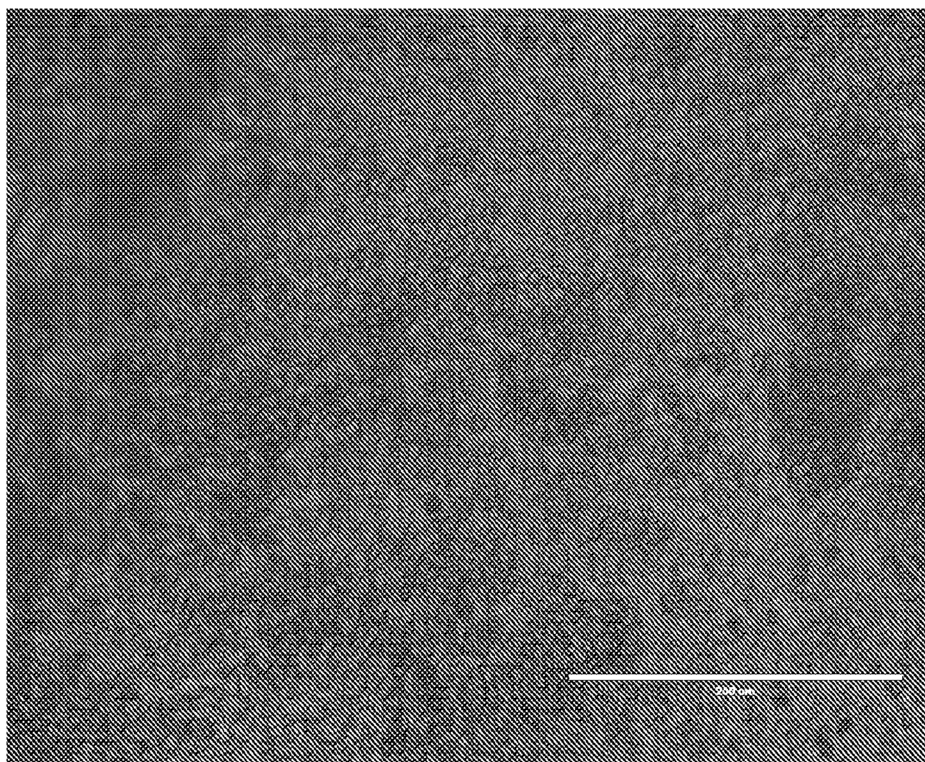
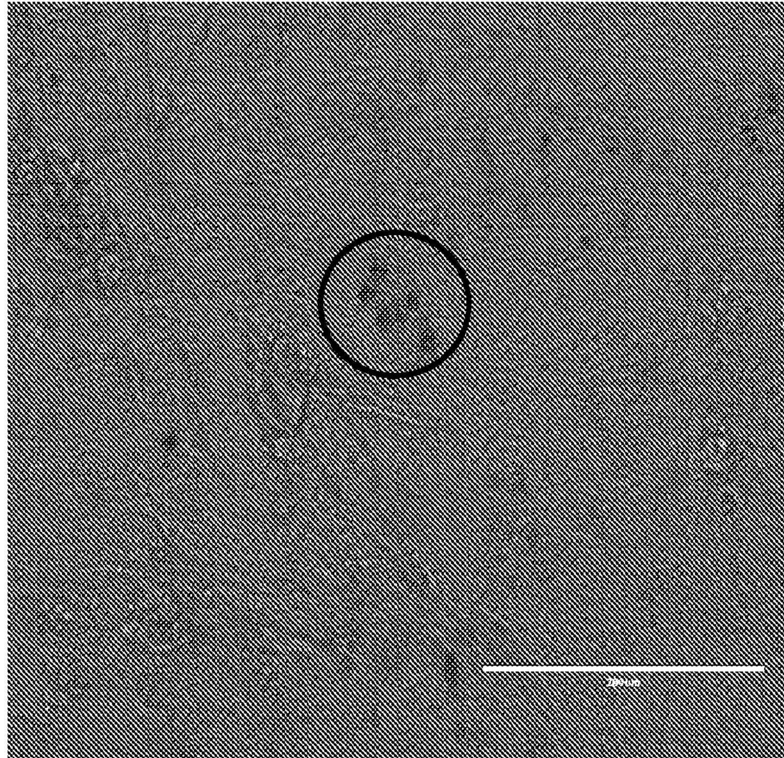


Figure 10

Protocol derived from Buchholz et al. & Leach et al. – pigmented cells at day 59



hOPM TF induction – pigmented monolayer at day 56

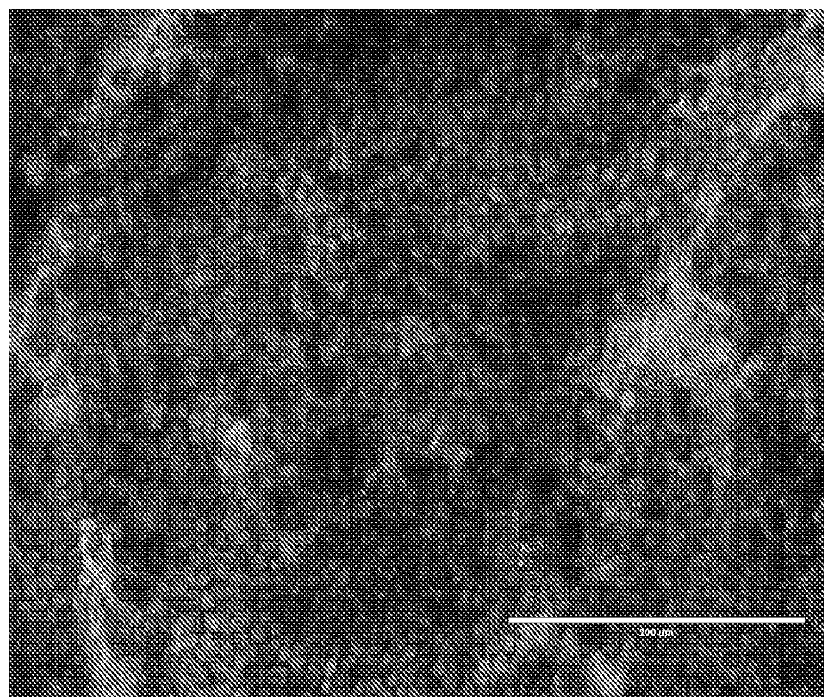


Figure 11

Human Homeobox protein (OTX1), transcript variant 1 cDNA coding sequence (NCBI Reference Sequence: NM_014562.3)

```
1     atgatgtctt acctcaaaca acccccatac ggcatgaacg ggctgggcct ggccgggccc
61    gccatggacc tcctgcaccc atccgtgggc tatccggcca ctccgcggaa gcagcggcgg
121   gagcgcacca ccttcacgcg ttcacagctg gacgtgctcg aggcgctctt cgccaagact
181   cgctaccctg acatcttcat gcgggaggag gtggcgctca agatcaacct gccggagtct
241   agagtccagg tctggttcaa gaaccgccgc gccaaatgcc gccagcagca gcagagcggg
301   agcggaacca agagccgccc agccaagaag aagtcctctc cagtgcggga gagctcgggc
361   tccgaaagca gtggccaatt cacgccgcca gctgtgtcca gctctgcctc gtcctctagc
421   tcggcgtcca gctcttccgc caacccagcg gctgcagcgg ctgcgggact aggtgggaac
481   ccggtggcgg ccgcgtcgtc gctgagtaca ccagctgcct catctatctg gagcccggcc
541   tccatctcgc caggctcagc gcccgcgtcc gtgtcggtgc cggagccatt ggccgcgcct
601   agcaacacct cgtgtatgca gcgctccgta gctgcaggcg ccgccaccgc agcagcctct
661   tatcccatgt cctacggcca gggcggcagc tacggccaag gctaccctac gccctcctct
721   tcctactttg gcggcgtgga ctgcagctca tacctagcgc ccatgcactc acatcaccac
781   ccgcaccagc tcagccccat ggcaccctcc tccatggcgg gccaccatca tcaccaccca
841   catgcgcacc acccgttgag ccagtcctca ggccaccacc accaccatca ccaccaccac
901   caccaaggct acggtggctc tgggcttgcc ttcaactctg ccgactgctt ggattacaag
961   gagcctggcg ccgctgctgc ttcctccgcc tggaaactca acttcaactc ccccgactgt
1021  ctggactata aggaccaagc ctcatggcgg ttccaggtct tgtga
(SEQ ID NO:10)
```

Human Homeobox protein (OTX1) amino acid sequence (NCBI Reference Sequence: NP_055377.1)

```
1   mmsylkqppy gmnglglagp amdllhpsvg ypatprkqrr erttftrsql dvlealfakt
61  rypdifmree valkinlpes rvqvwfknrr akcrqqqqsg sgtksrpakk ksspvressg
121 sessgqftpp avsssassss sassssanpa aaaaaglggn pvaaasslst paassiwspa
181 sispgsapas vsvpeplaap sntscmqrsv aagaataaas ypmsygqggs ygqgyptpss
241 syfggvdcss ylapmhshhh phqlspmaps smaghhhhhp hahhplsqss ghhhhhhhhh
301 hqgyggsgla fnsadcldyk epgaaaassa wklnfnspdc ldykdqaswr fqvl
(SEQ ID NO:11)
```

Figure 12

Human Homeobox protein (OTX2), transcript variant 5 cDNA coding sequence (NCBI Reference Sequence: NM_001270525.1)

```
1    atgatgtctt atcttaagca accgccttac gcagtcaatg ggctgagtct gaccacttcg
61   ggtatggact tgctgcaccc ctccgtgggc tacccggggc cctgggcttc ttgtcccgca
121  gccacccccc ggaaacagcg ccgggagagg acgacgttca ctcgggcgca gctagatgtg
181  ctggaagcac tgtttgccaa gacccggtac ccagacatct tcatgcgaga ggaggtggca
241  ctgaaaatca acttgcccga gtcgagggtg caggtatggt ttaagaatcg aagagctaag
301  tgccgccaac aacagcaaca acagcagaat ggaggtcaaa acaaagtgag acctgccaaa
361  aagaagacat ctccagctcg ggaagtgagt tcagagagtg gaacaagtgg ccaattcact
421  ccccccctcta gcacctcagt cccgaccatt gccagcagca gtgctcctgt gtctatctgg
481  agcccagctt ccatctcccc actgtcagat cccttgtcca cctcctcttc ctgcatgcag
541  aggtcctatc ccatgaccta tactcaggct tcaggttata gtcaaggata tgctggctca
601  acttcctact ttgggggcat ggactgtgga tcatatttga cccctatgca tcaccagctt
661  cccggaccag ggccacact cagtcccatg ggtaccaatg cagtcaccag ccatctcaat
721  cagtccccag cttctctttc cacccaggga tatggagctt caagcttggg ttttaactca
781  accactgatt gcttggatta taaggaccaa actgcctcct ggaagcttaa cttcaatgct
841  gactgcttgg attataaaga tcagacatcc tcgtggaaat tccaggtttt gtga
(SEQ ID NO:12)
```

Human Homeobox protein (OTX2) amino acid sequence (NCBI Reference Sequence: NP_001257454.1)

```
1   mmsylkqppy avnglsltts gmdllhpsvg ypgpwascpa atprkqrrer ttftraqldv
61  lealfaktry pdifmreeva lkinlpesrv qvwfknrrak crqqqqqqqn ggqnkvrpak
121 kktsparevs sesgtsgqft ppsstsvpti asssapvsiw spasisplsd plstssscmq
181 rsypmtytqa sgysqgyags tsyfggmdcg syltpmhhql pgpgatlspm gtnavtshln
241 qspaslstqg ygasslgfns ttdcldykdq taswklnfna dcldykdqts swkfqvl
(SEQ ID NO:13)
```

Figure 13

Human paired box protein (PAX2), transcript variant e cDNA coding sequence (NCBI Reference Sequence: NM_003990.3)

```
1     ATGGATATGC ACTGCAAAGC AGACCCCTTC TCCGCGATGC ACCCAGGGCA CGGGGGTGTG
61    AACCAGCTCG GGGGGGTGTT TGTGAACGGC CGGCCCCTAC CCGACGTGGT GAGGCAGCGC
121   ATCGTGGAGC TGGCCCACCA GGGTGTGCGG CCCTGTGACA TCTCCCGGCA GCTGCGGGTC
181   AGCCACGGCT GTGTCAGCAA AATCCTGGGC AGGTACTACG AGACCGGCAG CATCAAGCCG
241   GGTGTGATCG GTGGCTCCAA GCCCAAAGTG GCGACGCCCA AAGTGGTGGA CAAGATTGCT
301   GAATACAAAC GACAGAACCC GACTATGTTC GCCTGGGAGA TTCGAGACCG GCTCCTGGCC
361   GAGGGCATCT GTGACAATGA CACAGTGCCC AGCGTCTCTT CCATCAACAG AATCATCCGG
421   ACCAAAGTTC AGCAGCCTTT CCACCCAACG CCGGATGGGG CTGGGACAGG AGTGACCGCC
481   CCTGGCCACA CCATTGTTCC CAGCACGGCC TCCCCTCCTG TTTCCAGCGC CTCCAATGAC
541   CCAGTGGGAT CCTACTCCAT CAATGGGATC CTGGGGATTC CTCGCTCCAA TGGTGAGAAG
601   AGGAAACGTG ATGAAGTTGA GGTATACACT GATCCTGCCC ACATTAGAGG AGGTGGAGGT
661   TTGCATCTGG TCTGGACTTT AAGAGATGTG TCTGAGGGCT CAGTCCCCAA TGGAGATTCC
721   CAGAGTGGTG TGGACAGTTT GCGGAAGCAC TTGCGAGCTG ACACCTTCAC CCAGCAGCAG
781   CTGGAAGCTT TGGATCGGGT CTTTGAGCGT CCTTCCTACC CTGACGTCTT CCAGGCATCA
841   GAGCACATCA AATCAGAACA GGGGAACGAG TACTCCCTCC CAGCCCTGAC CCCTGGGCTT
901   GATGAAGTCA AGTCGAGTCT ATCTGCATCC ACCAACCCTG AGCTGGGCAG CAACGTGTCA
961   GGCACACAGA CATACCCAGT TGTGACTGGT CGTGACATGG CGAGCACCAC TCTGCCTGGT
1021  TACCCCCCTC ACGTGCCCCC CACTGGCCAG GGAAGCTACC CCACCTCCAC CCTGGCAGGA
1081  ATGGTGCCTG GGAGCGAGTT CTCCGGCAAC CCGTACAGCC ACCCCCAGTA CACGGCCTAC
1141  AACGAGGCTT GGAGATTCAG CAACCCCGCC TTACTAATGC CGCCCCCCGG TCCGCCCCTG
1201  CCGCTGCTGC CGCTGCCTAT GACCGCCACT AGTTACCGCG GGGACCACAT CAAGCTTCAG
1261  GCCGACAGCT TCGGCCTCCA CATCGTCCCC GTCTGA
(SEQ ID NO:14)
```

Human Paired box protein (PAX2) amino acid sequence isoform e (NCBI Reference Sequence: NP_003981.2)

```
1     MDMHCKADPF SAMHPGHGGV NQLGGVFVNG RPLPDVVRQR IVELAHQGVR PCDISRQLRV
61    SHGCVSKILG RYYETGSIKP GVIGGSKPKV ATPKVVDKIA EYKRQNPTMF AWEIRDRLLA
121   EGICDNDTVP SVSSINRIIR TKVQQPFHPT PDGAGTGVTA PGHTIVPSTA SPPVSSASND
181   PVGSYSINGI LGIPRSNGEK RKRDEVEVYT DPAHIRGGGG LHLVWTLRDV SEGSVPNGDS
241   QSGVDSLRKH LRADTFTQQQ LEALDRVFER PSYPDVFQAS EHIKSEQGNE YSLPALTPGL
301   DEVKSSLSAS TNPELGSNVS GTQTYPVVTG RDMASTTLPG YPPHVPPTGQ GSYPTSTLAG
361   MVPGSEFSGN PYSHPQYTAY NEAWRFSNPA LLMPPPGPPL PLLPLPMTAT SYRGDHIKLQ
421   ADSFGLHIVP V
(SEQ ID NO:15)
```

Figure 14

Human Paired box protein (PAX6) transcript variant 4 cDNA coding sequence (NCBI Reference Sequence: NM_001258462.1)

```
1       atgcagaaca gtcacagcgg agtgaatcag ctcggtggtg tctttgtcaa cgggcggcca
61      ctgccggact ccacccggca gaagattgta gagctagctc acagcggggc ccggccgtgc
121     gacatttccc gaattctgca gacccatgca gatgcaaaag tccaagtgct ggacaatcaa
181     aacgtgtcca acggatgtgt gagtaaaatt ctgggcaggt attacgagac tggctccatc
241     agacccaggg caatcggtgg tagtaaaccg agagtagcga ctccagaagt tgtaagcaaa
301     atagcccagt ataagcggga gtgcccgtcc atctttgctt gggaaatccg agacagatta
361     ctgtccgagg gggtctgtac caacgataac ataccaagcg tgtcatcaat aaacagagtt
421     cttcgcaacc tggctagcga aaagcaacag atgggcgcag acggcatgta tgataaacta
481     aggatgttga acgggcagac cggaagctgg ggcacccgcc ctggttggta tccggggact
541     tcggtgccag ggcaacctac gcaagatggc tgccagcaac aggaaggagg gggagagaat
601     accaactcca tcagttccaa cggagaagat tcagatgagg ctcaaatgcg acttcagctg
661     aagcggaagc tgcaaagaaa tagaacatcc tttacccaag agcaaattga ggccctggag
721     aaagagtttg agagaaccca ttatccagat gtgtttgccc gagaaagact agcagccaaa
781     atagatctac ctgaagcaag aatacaggta tggttttcta atcgaagggc caaatggaga
841     agagaagaaa aactgaggaa tcagagaaga caggccagca acacacctag tcatattcct
901     atcagcagta gtttcagcac cagtgtctac caaccaattc cacaacccac cacaccggtt
961     tcctccttca catctggctc catgttgggc cgaacagaca cagccctcac aaacacctac
1021    agcgctctgc cgcctatgcc cagcttcacc atggcaaata acctgcctat gcaaccccca
1081    gtccccagcc agacctcctc atactcctgc atgctgccca ccagcccttc ggtgaatggg
1141    cggagttatg atacctacac ccccccacat atgcagacac acatgaacag tcagccaatg
1201    ggcacctcgg gcaccacttc aacaggactc atttcccctg gtgtgtcagt tccagttcaa
1261    gttcccggaa gtgaacctga tatgtctcaa tactggccaa gattacagta a
(SEQ ID NO:16)
```

Human Paired box protein (PAX6) amino acid sequence (NCBI Reference Sequence: NP_001245391.1)

```
1   mqnshsgvnq lggvfvngrp lpdstrqkiv elahsgarpc disrilqtha dakvqvldnq
61  nvsngcvski lgryyetgsi rpraiggskp rvatpevvsk iaqykrecps ifaweirdrl
121 lsegvctndn ipsvssinrv lrnlasekqq mgadgmydkl rmlngqtgsw gtrpgwypgt
181 svpgqptqdg cqqqegggen tnsissnged sdeaqmrlql krklqrnrts ftqeqieale
241 keferthypd vfarerlaak idlpeariqv wfsnrrakwr reeklrnqrr qasntpship
301 isssfstsvy qpipqpttpv ssftsgsmlg rtdtaltnty salppmpsft mannlpmqpp
361 vpsqtssysc mlptspsvng rsydtytpph mqthmnsqpm gtsgttstgl ispgvsvpvq
421 vpgsepdmsq ywprlq
(SEQ ID NO:17)
```

Figure 15

Human melanogenesis associated transcription factor (MITF) transcript variant 1 cDNA coding sequence (NCBI Reference Sequence NM_198159.2)

```
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121     cctggggcct ccaagcctcc gataagctcc tccagtatga catcacgcat cttgctacgc
181     cagcaactca tgcgtgagca gatgcaggag caggagcgca gggagcagca gcagaagctg
241     caggcggccc agttcatgca acagagagtg cccgtgagtc agacaccagc cataaacgtc
301     agtgtgccca ccaccccttcc ctctgccacg caggtgccga tggaagtcct taaggtgcag
361     acccacctcg aaaaccccac caagtaccac atacagcaag cccaacggca gcaggtaaag
421     cagtaccttt ctaccacttt agcaaataaa catgccaacc aagtcctgag cttgccatgt
481     ccaaaccagc ctggcgatca tgtcatgcca ccggtgccgg ggagcagcgc acccaacagc
541     cccatggcta tgcttacgct taactccaac tgtgaaaaag agggatttta taagtttgaa
601     gagcaaaaca gggcagagag cgagtgccca ggcatgaaca cacattcacg agcgtcctgt
661     atgcagatgg atgatgtaat cgatgacatc attagcctag aatcaagtta taatgaggaa
721     atcttgggct tgatggatcc tgctttgcaa atggcaaata cgttgcctgt ctcgggaaac
781     ttgattgatc tttatggaaa ccaaggtctg cccccaccag gcctcaccat cagcaactcc
841     tgtccagcca accttcccaa cataaaaagg gagctcacag agtctgaagc aagagcactg
901     gccaaagaga ggcagaaaaa ggacaatcac aacctgattg aacgaagaag aagatttaac
961     ataaatgacc gcattaaaga actaggtact ttgattccca agtcaaatga tccagacatg
1021    cgctggaaca agggaaccat cttaaaagca tccgtggact atatccgaaa gttgcaacga
1081    gaacagcaac gcgcaaaaga acttgaaaac cgacagaaga aactggagca cgccaaccgg
1141    catttgttgc tcagaataca ggaacttgaa atgcaggctc gagctcatgg actttccctt
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1261    gttcttgaga actgcagcca agacctcctt cagcatcatg cagacctaac ctgtacaaca
1321    actctcgatc tcacggatgg caccatcacc ttcaacaaca acctcggaac tgggactgag
1381    gccaaccaag cctatagtgt ccccacaaaa atgggatcca aactggaaga catcctgatg
1441    gacgacaccc tttctcccgt cggtgtcact gatccactcc tttcctcagt gtcccccgga
1501    gcttccaaaa caagcagccg gaggagcagt atgagcatgg aagagacgga gcacacttgt
1561    tag
(SEQ ID NO:18)
```

Human melanogenesis associated transcription factor (MITF) isoform 1 (NCBI Reference Sequence: NP_937802.1)

```
1   mqsesgivpd fevgeefhee pktyyelksq plkssssaeh pgaskppiss ssmtsrillr
61  qqlmreqmqe qerreqqqkl qaaqfmqqrv pvsqtpainv svpttlpsat qvpmevlkvq
121 thlenptkyh iqqaqrqqvk qylsttlank hanqvlslpc pnqpgdhvmp pvpgssapns
181 pmamltlnsn cekegfykfe eqnraesecp gmnthsrasc mqmddviddi islessynee
241 ilglmdpalq mantlpvsgn lidlygnqgl pppgltisns cpanlpnikr eltesearal
301 akerqkkdnh nlierrrrfn indrikelgt lipksndpdm rwnkgtilka svdyirklqr
361 eqqrakelen rqkklehanr hlllriqele mqarahglsl ipstglcspd lvnriikqep
421 vlencsqdll qhhadltctt tldltdgtit fnnnlgtgte anqaysvptk mgskledilm
481 ddtlspvgvt dpllssvspg asktssrrss msmeetehtc
(SEQ ID NO:19)
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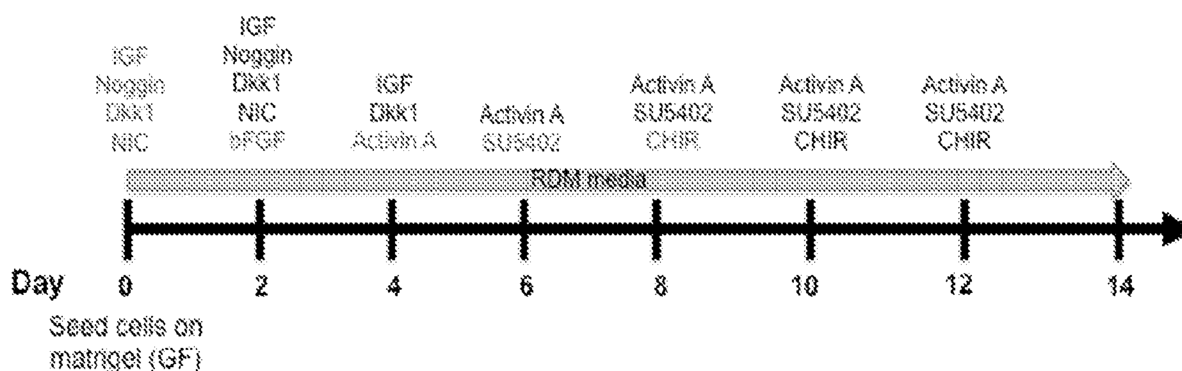
Figure 16

RPE differentiation – 14 day protocol



Retinal differentiation medium (RDM): DMEM/F12, 1xB27, 1xN2, 1xNEAA

In red – 1st exposure of reagent to cells

Day 1: wells should be 30-50% confluent

Adapted from Leach et al 2015, Buchholz et. al. 2013

Figure 17

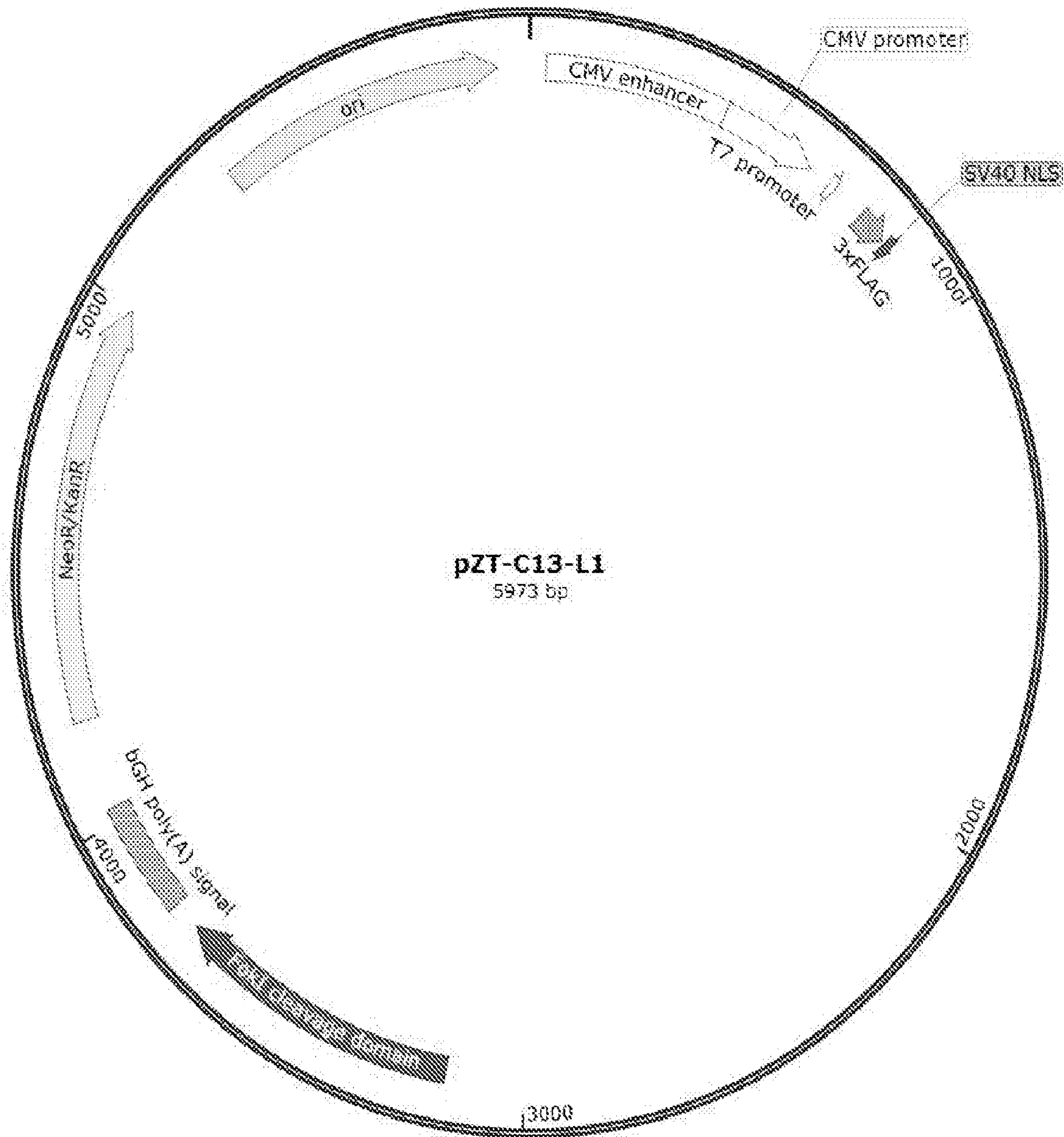


Figure 17 cont.

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```

Figure 17 cont.

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Figure 18

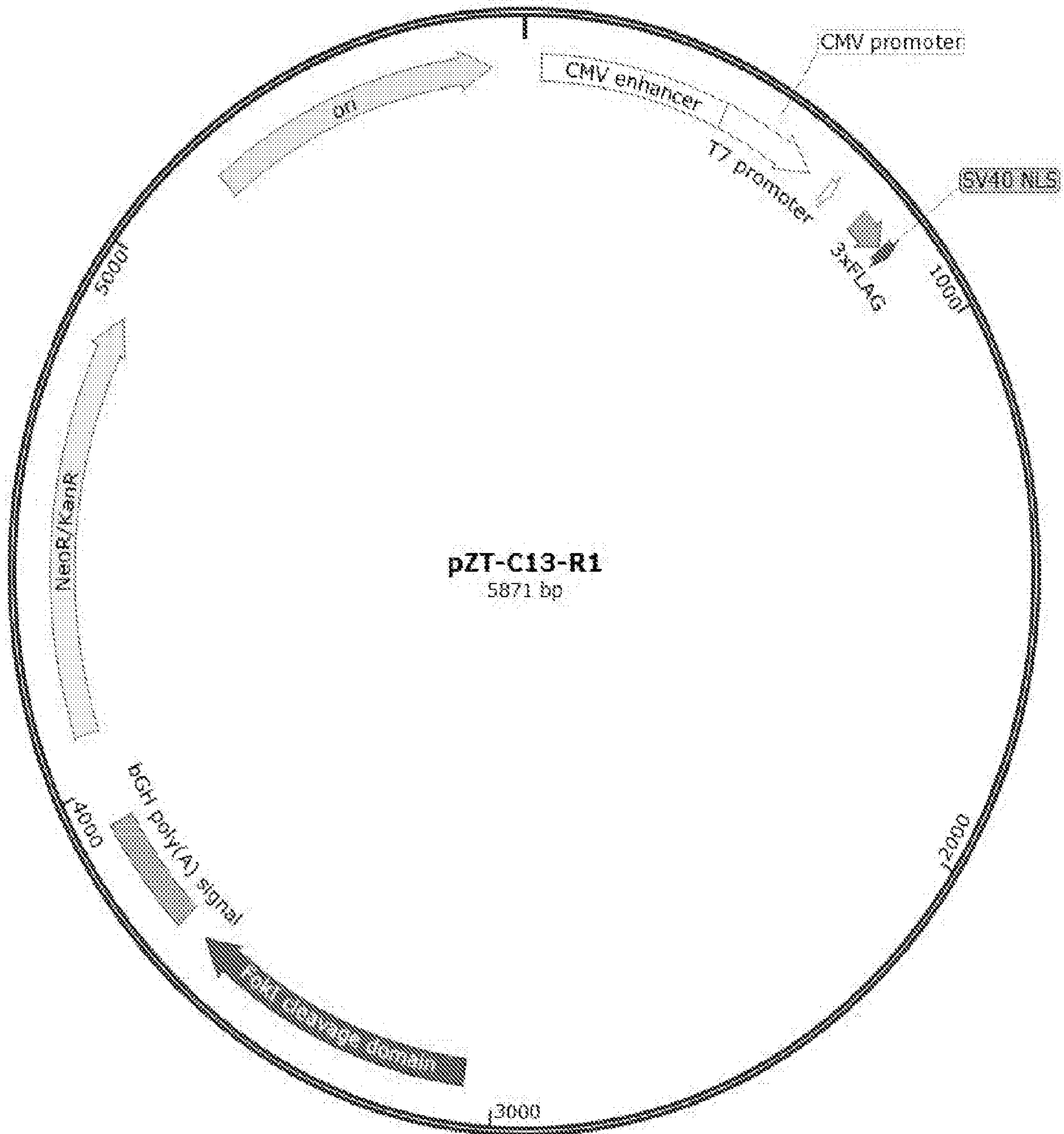

Figure 18 cont.

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Figure 18 cont.

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Figure 19

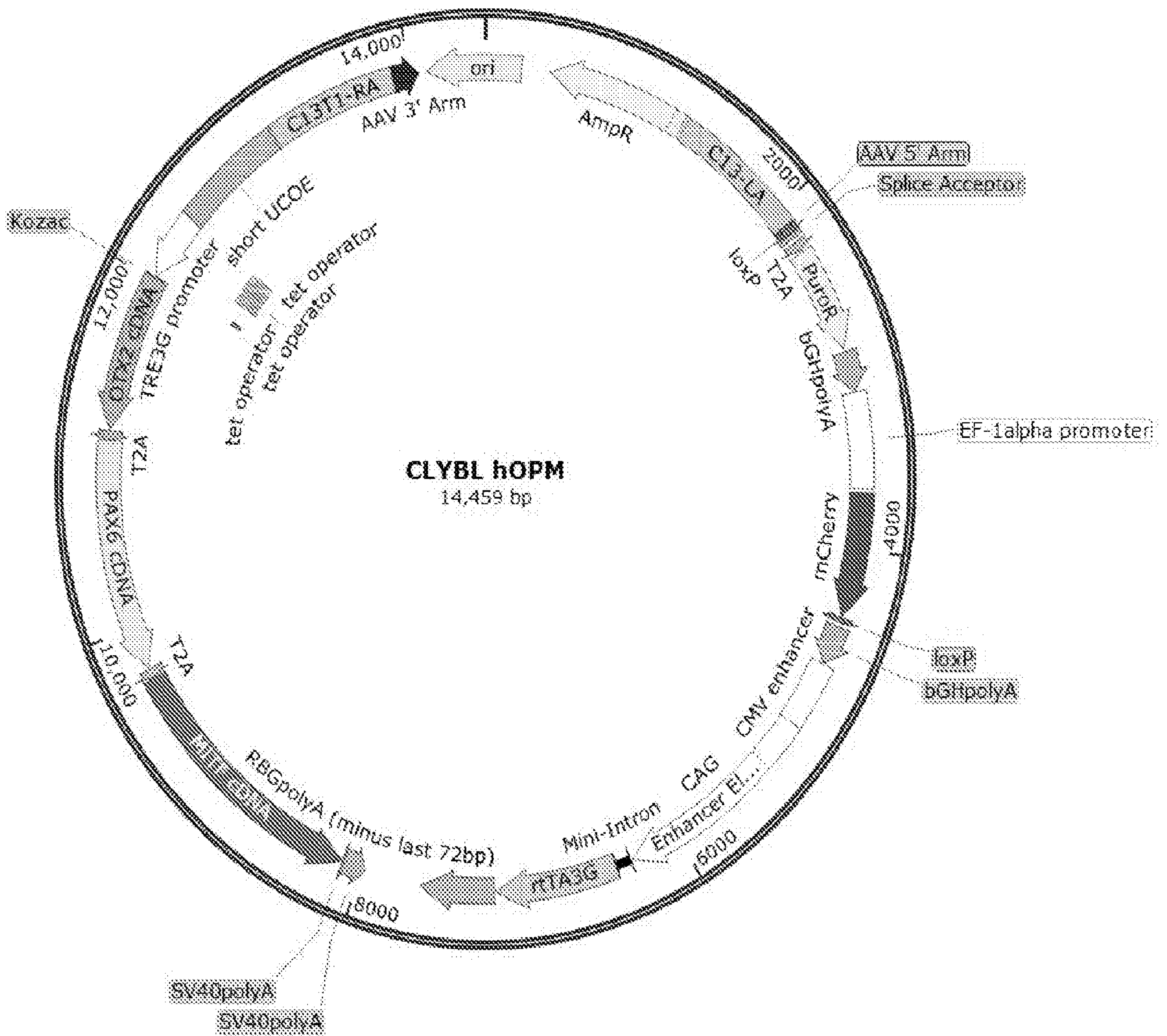

Figure 19 cont.

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Figure 19 cont.

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Figure 19 cont.

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Figure 19 cont.

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Figure 19 cont.

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Figure 20

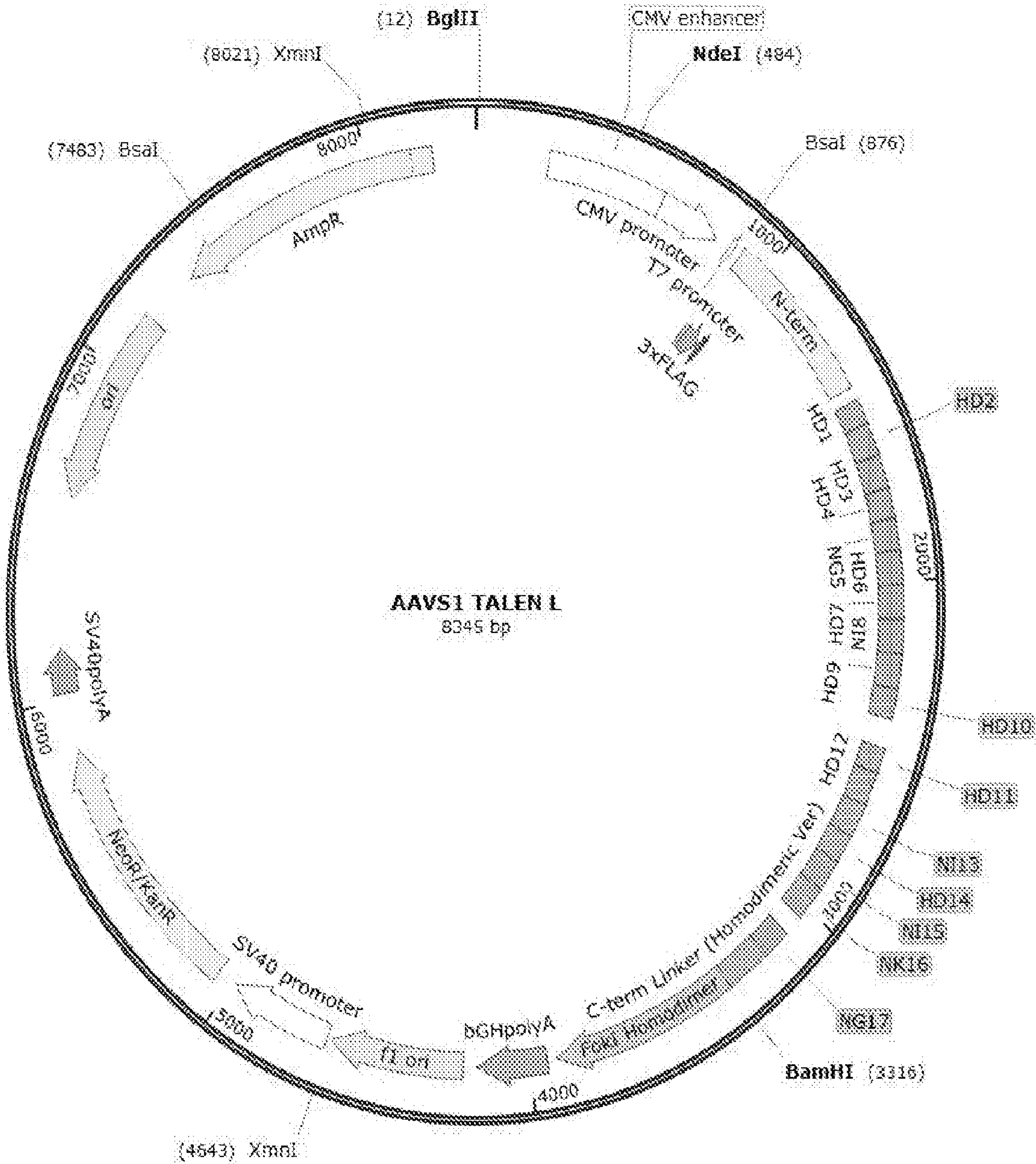

Figure 20 cont.

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Figure 20 cont.

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Figure 20 cont.

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Figure 21

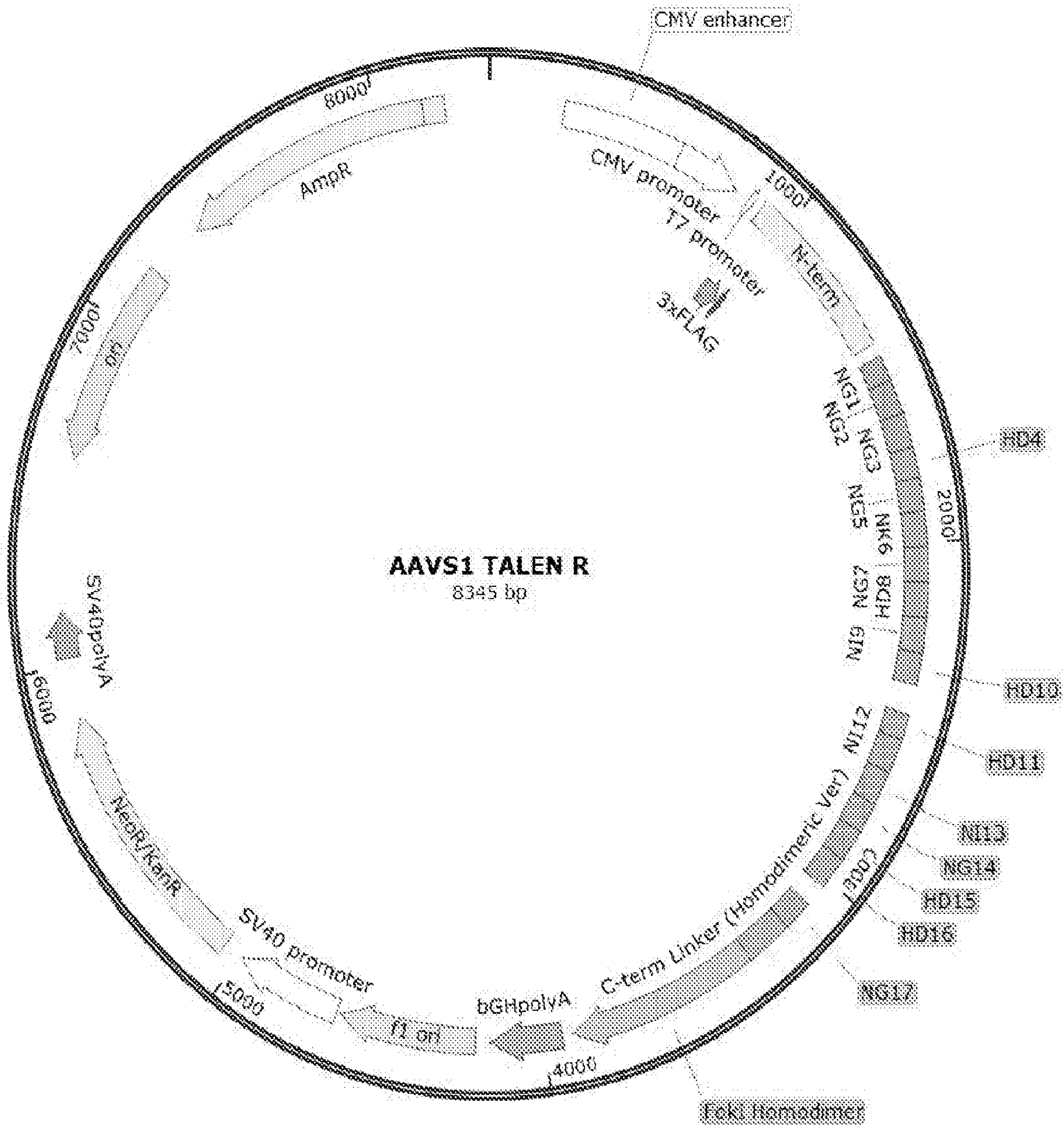

Figure 21 cont.

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Figure 21 cont.

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Figure 21 cont.

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Figure 22

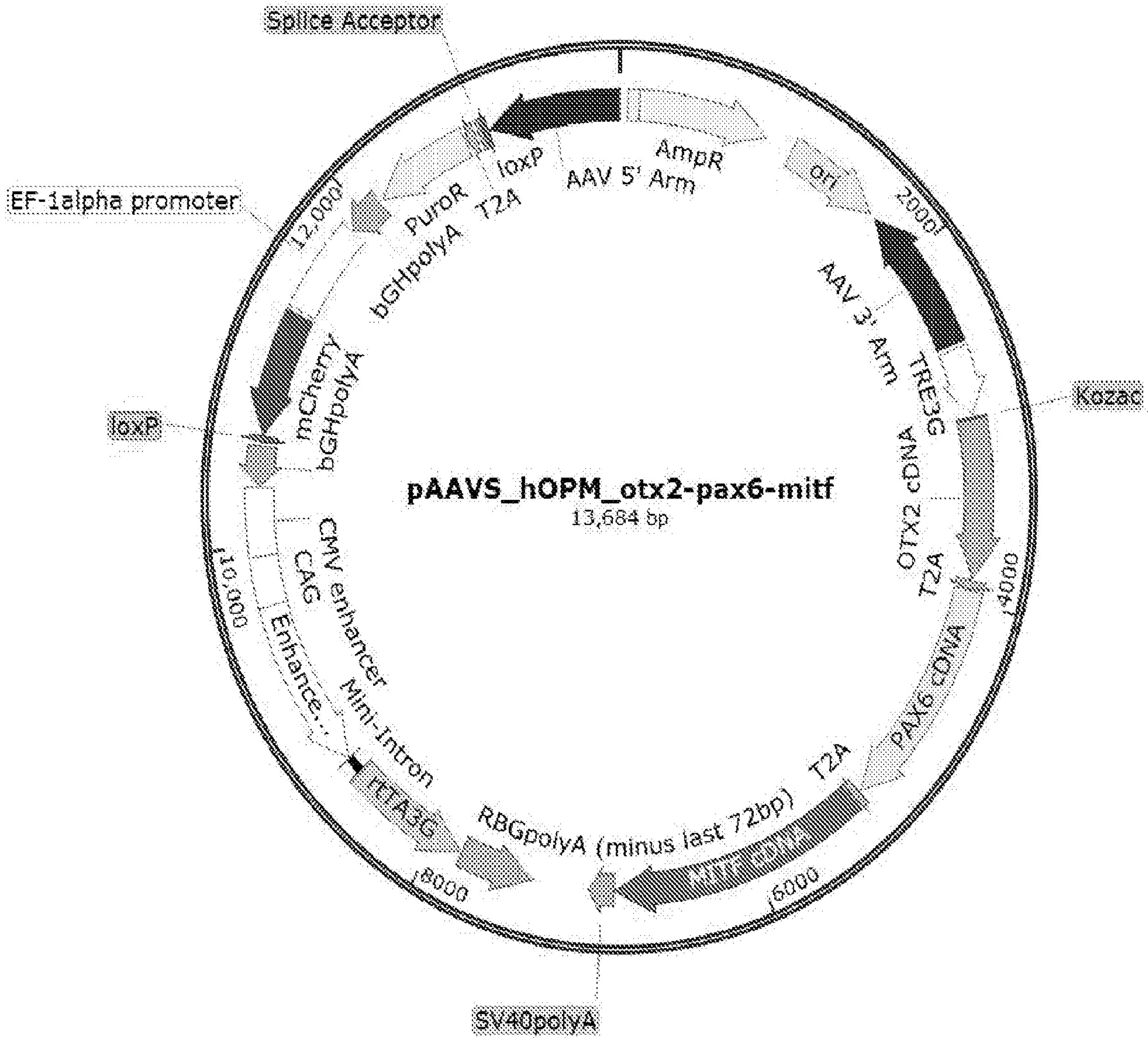

Figure 22 cont.

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Figure 22 cont.

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Figure 22 cont.

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Figure 22 cont.

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Figure 22 cont.

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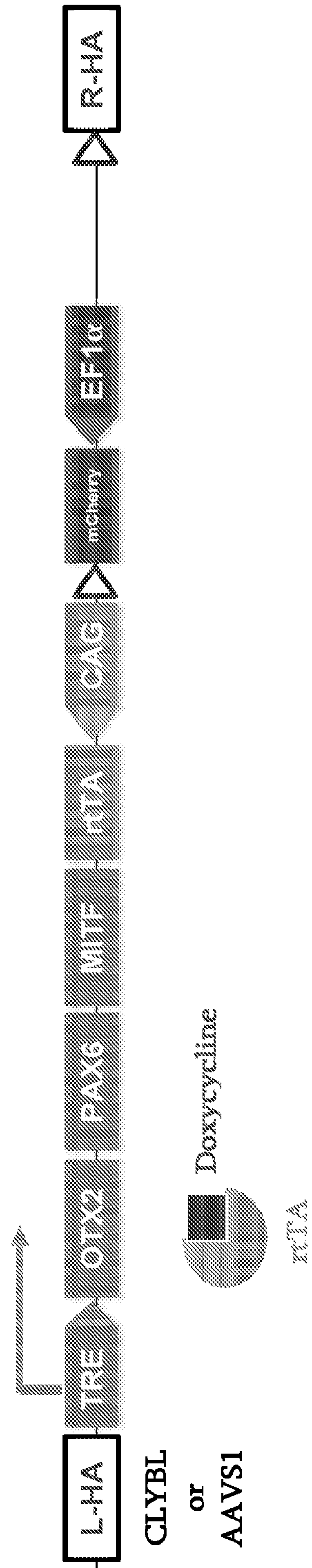
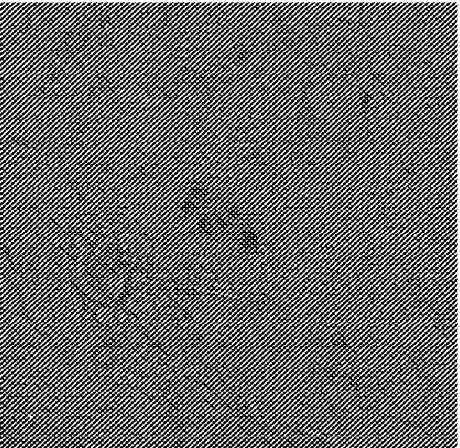


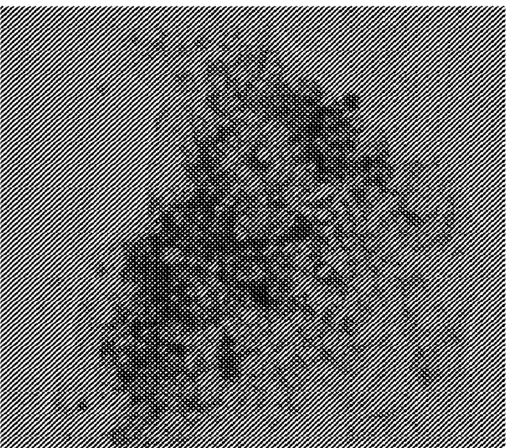
Figure 23

Figure 24

Leach et al. protocol:
Pigment at Day 59



Zhu et al. protocol:
Pigment at Day 26



Protocol of the
present disclosure:
Pigment at Day 30

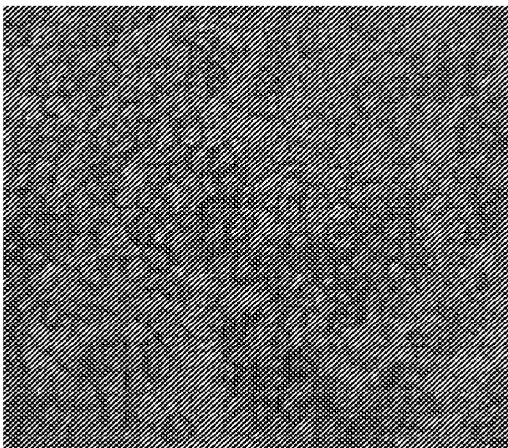


Figure 25

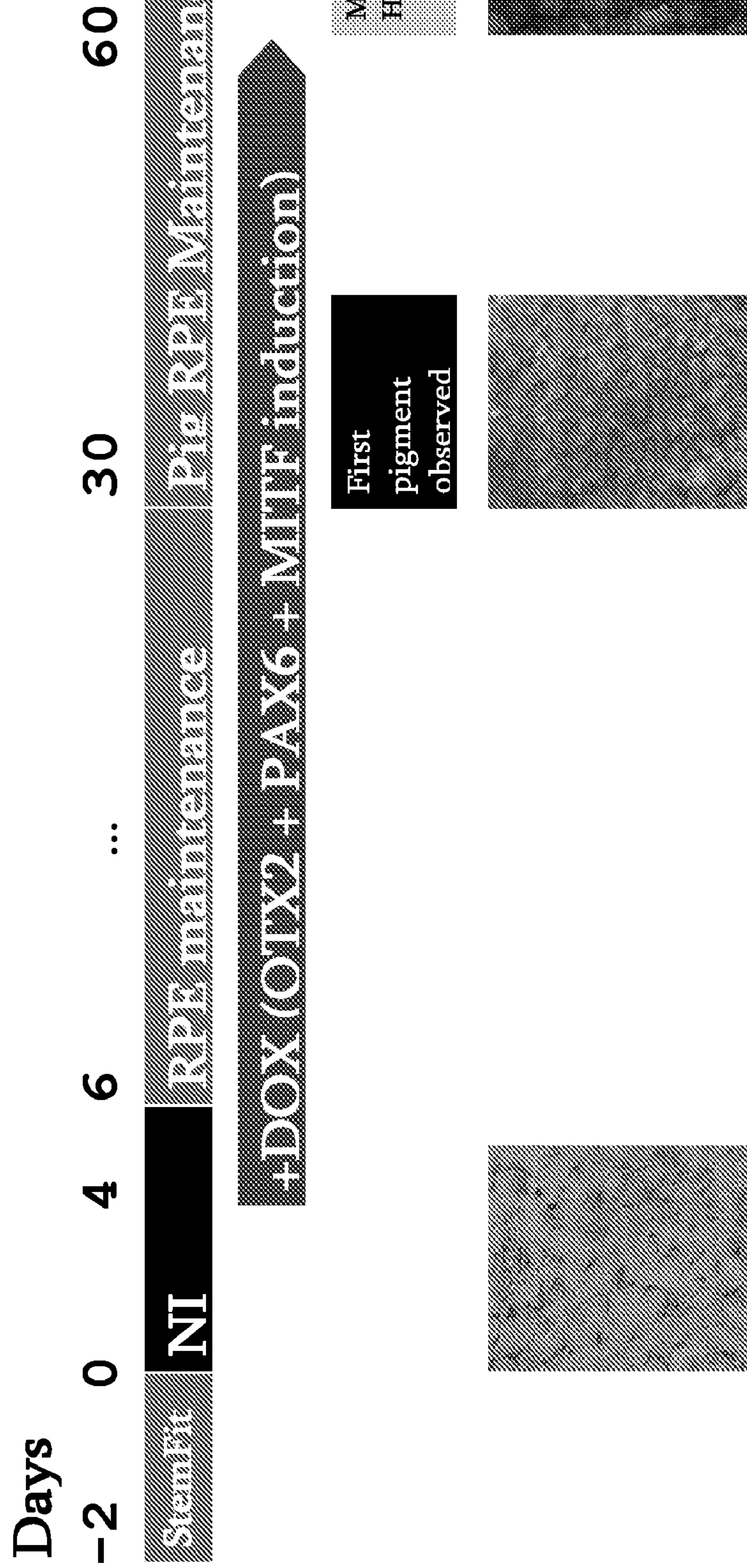
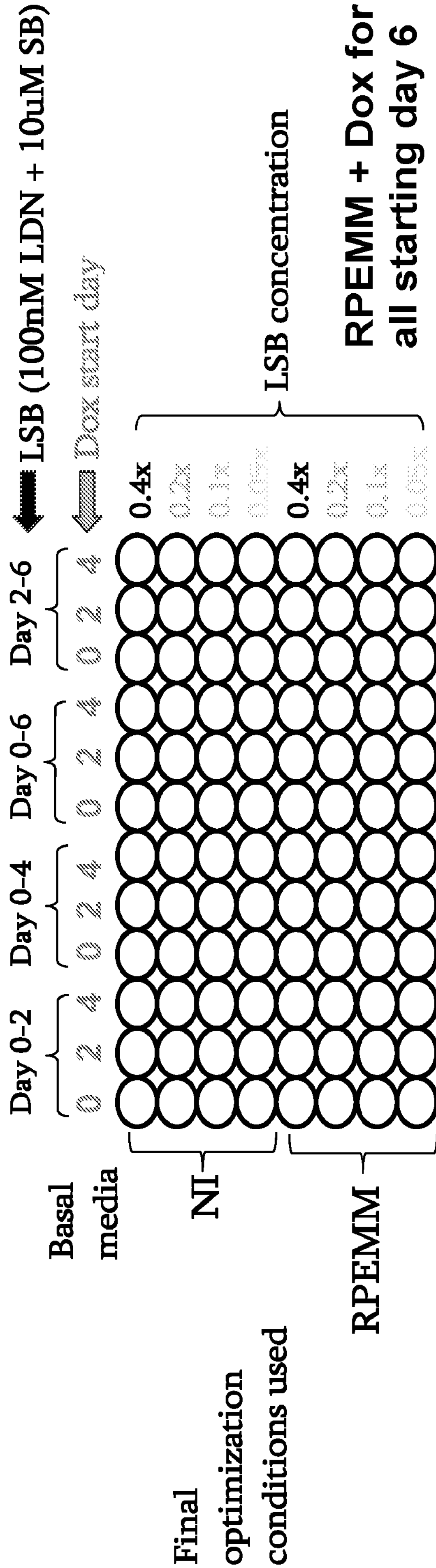


Figure 26



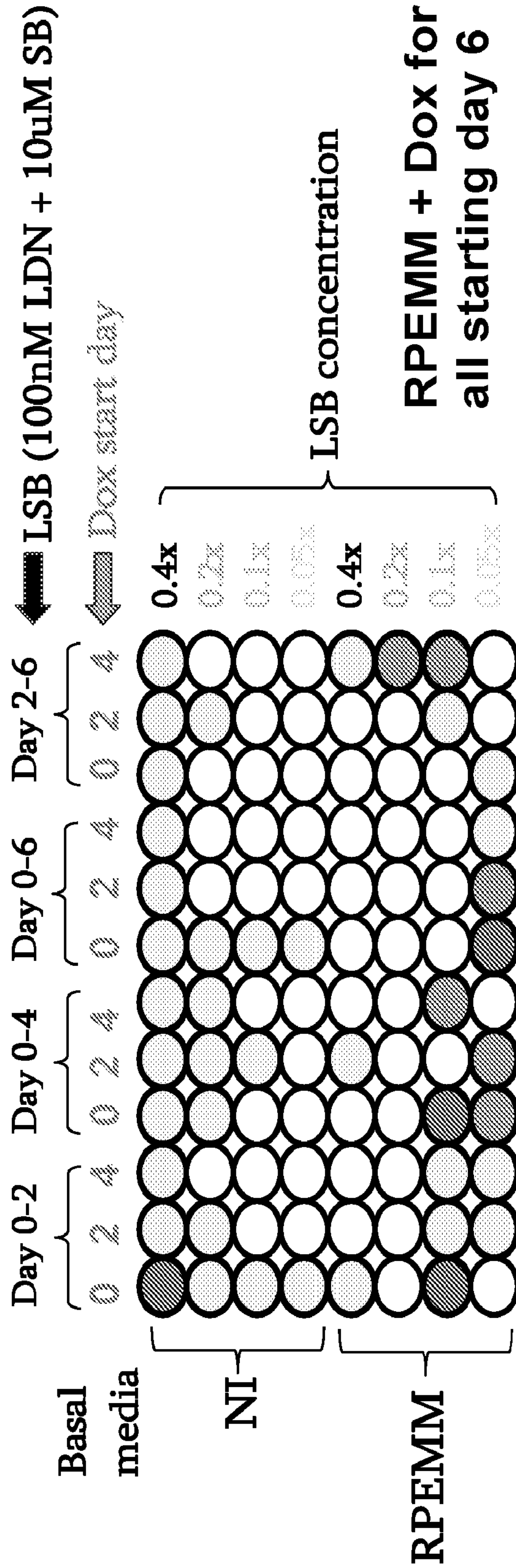
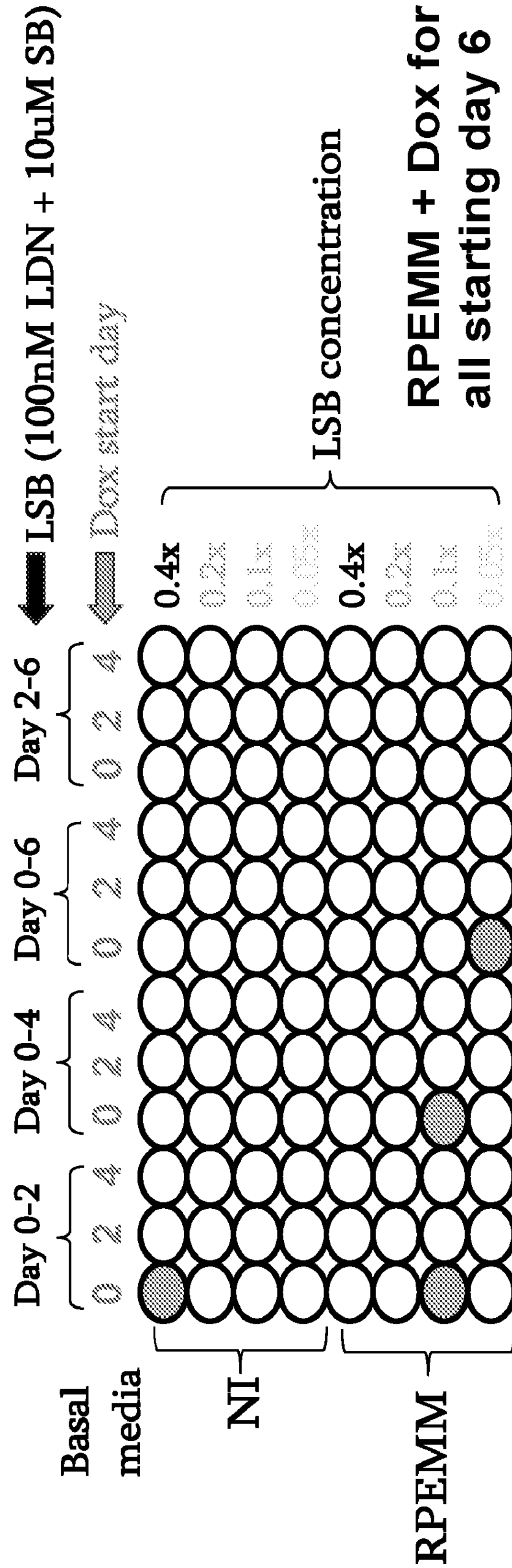


Figure 26 cont.

Figure 26 cont.



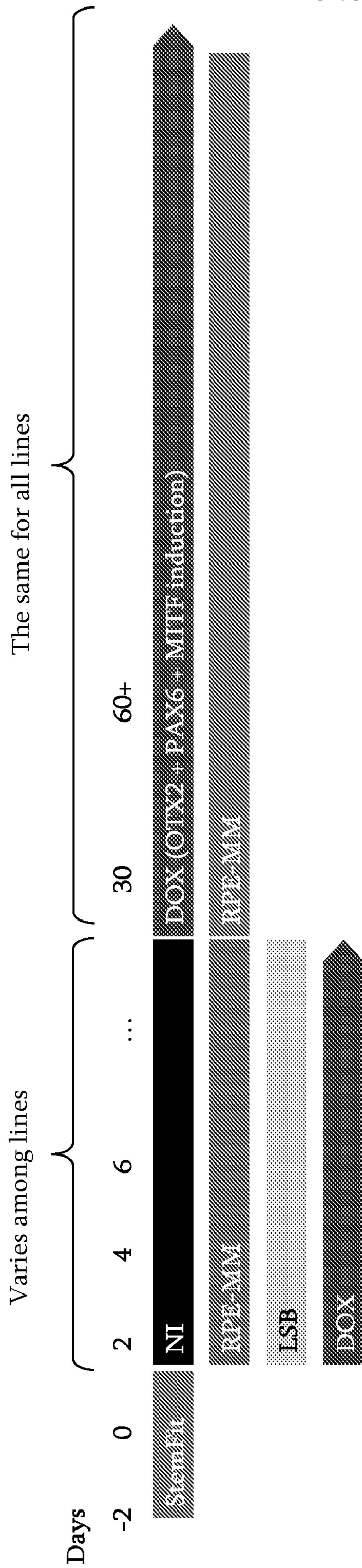


Figure 27

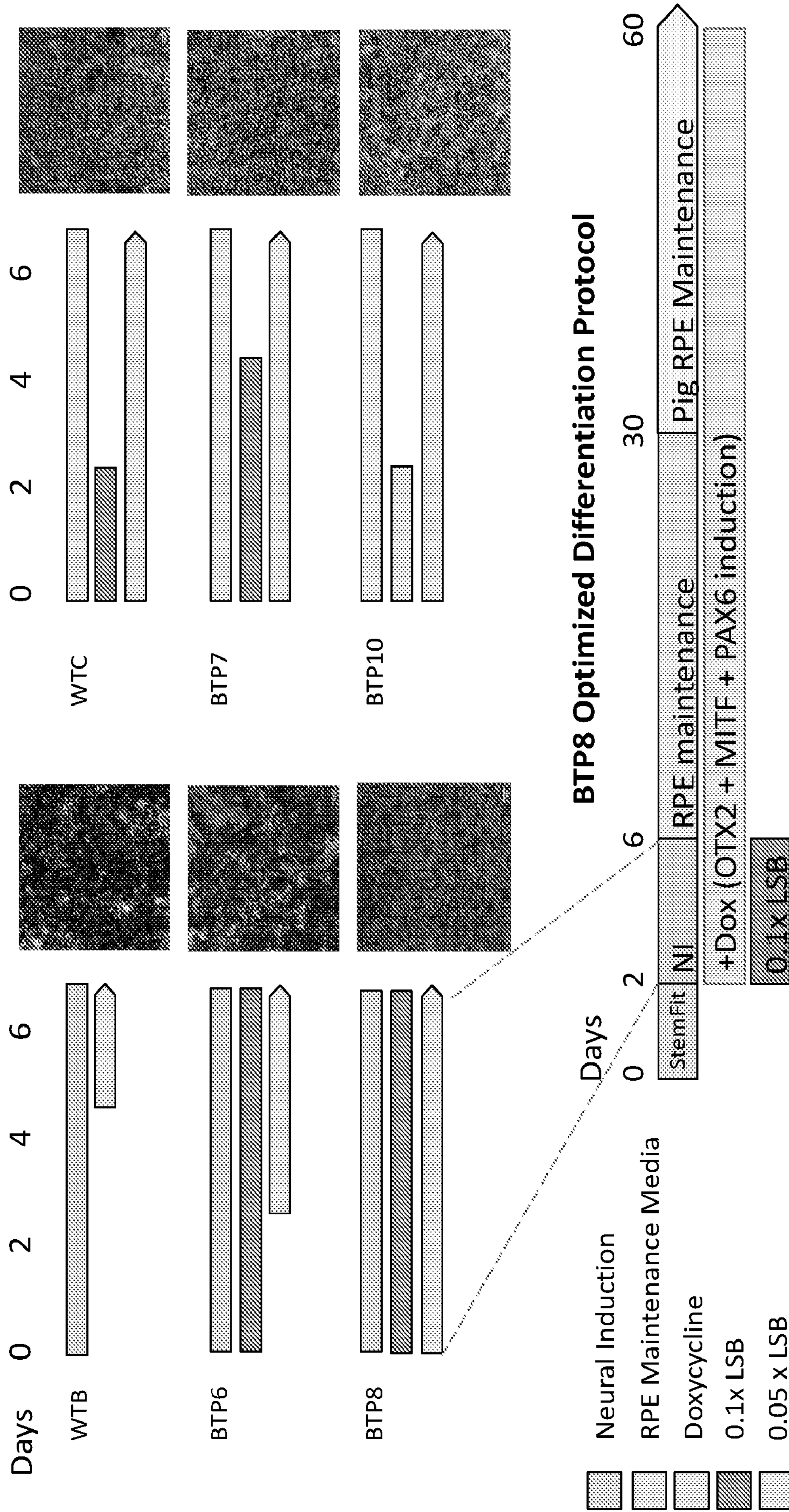
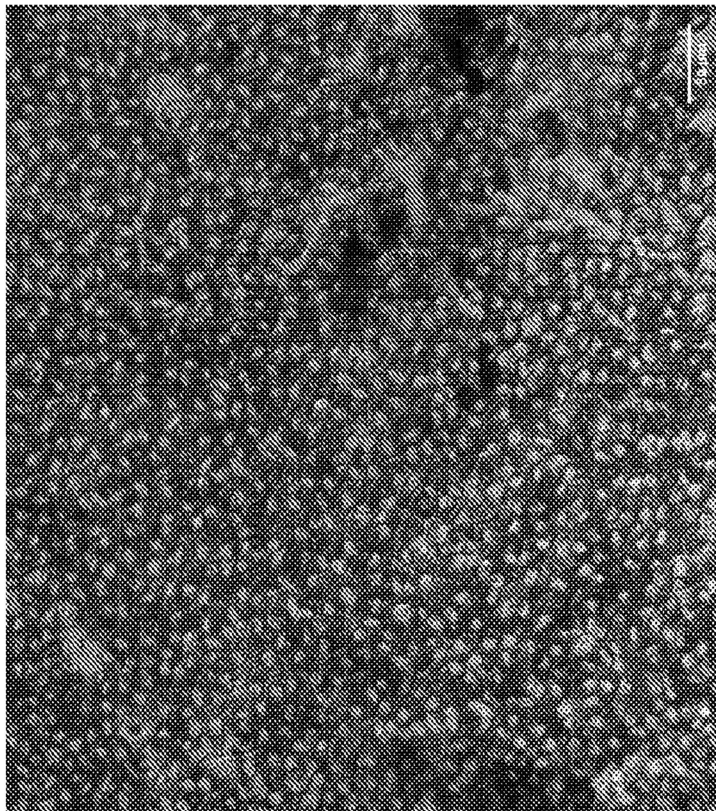
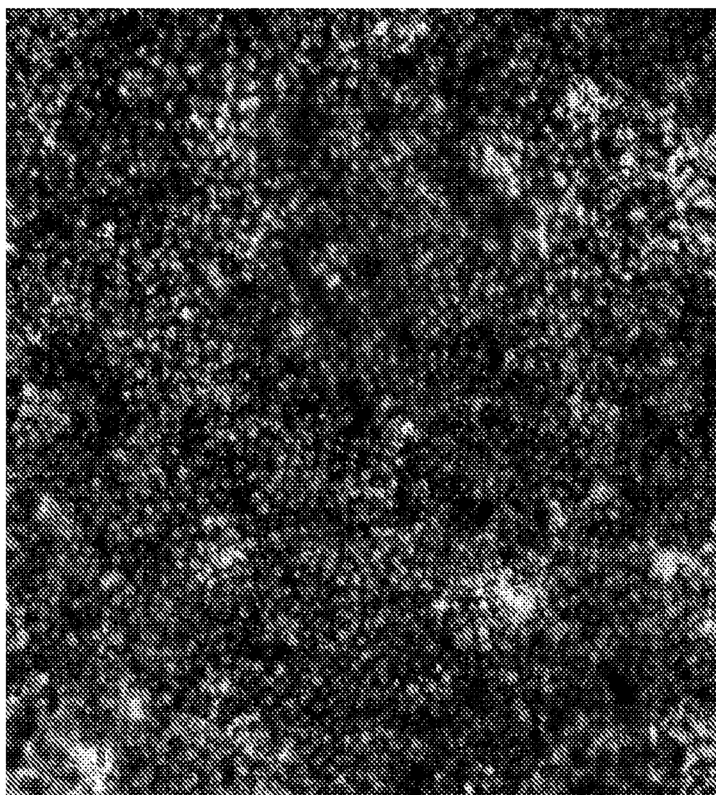


Figure 28

Figure 29

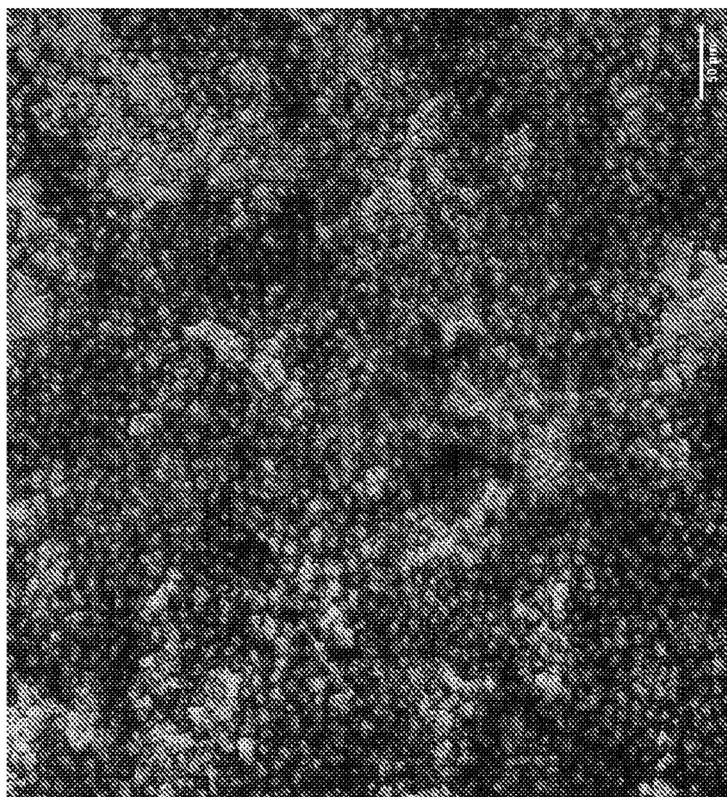


WTC day 60+

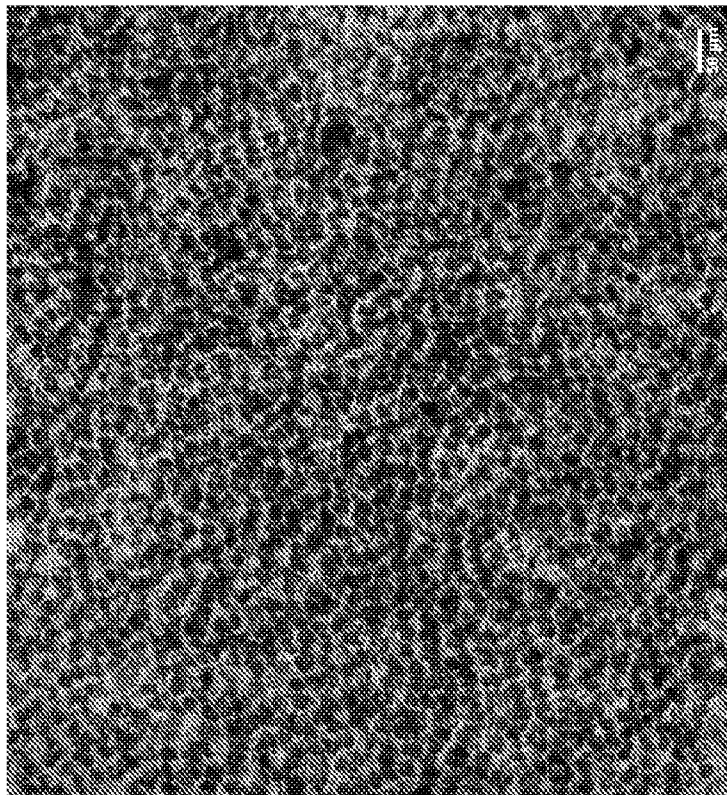


WTB day 60+

Figure 30

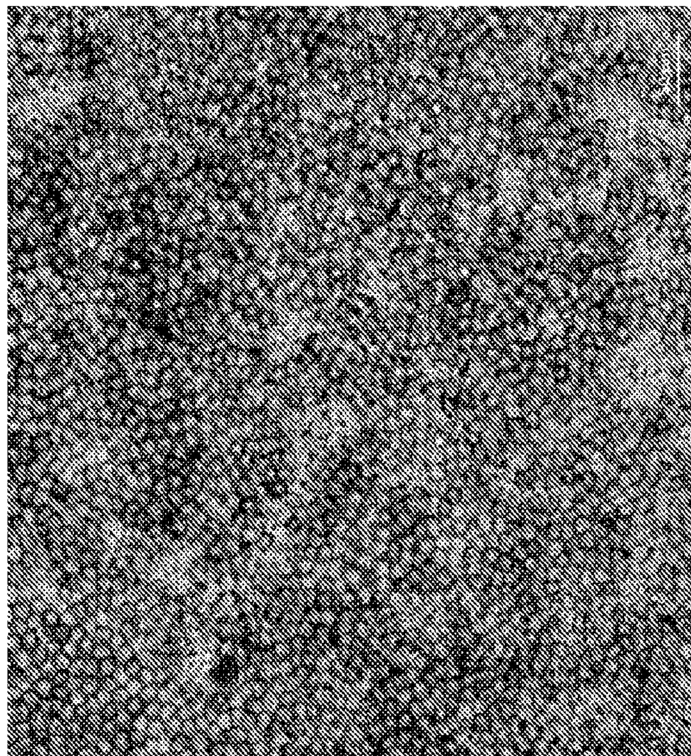


BTP6 day 60+

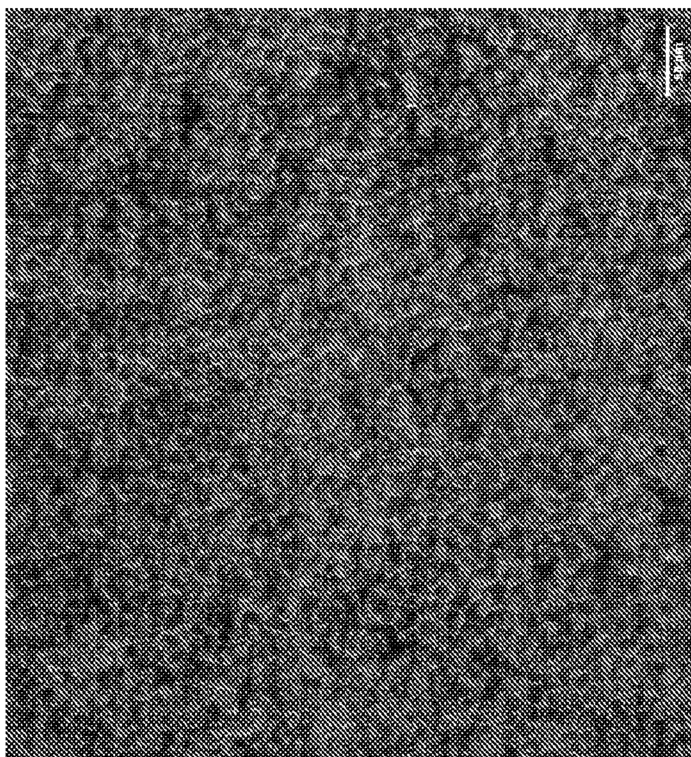


BTP7 day 60+

Figure 31

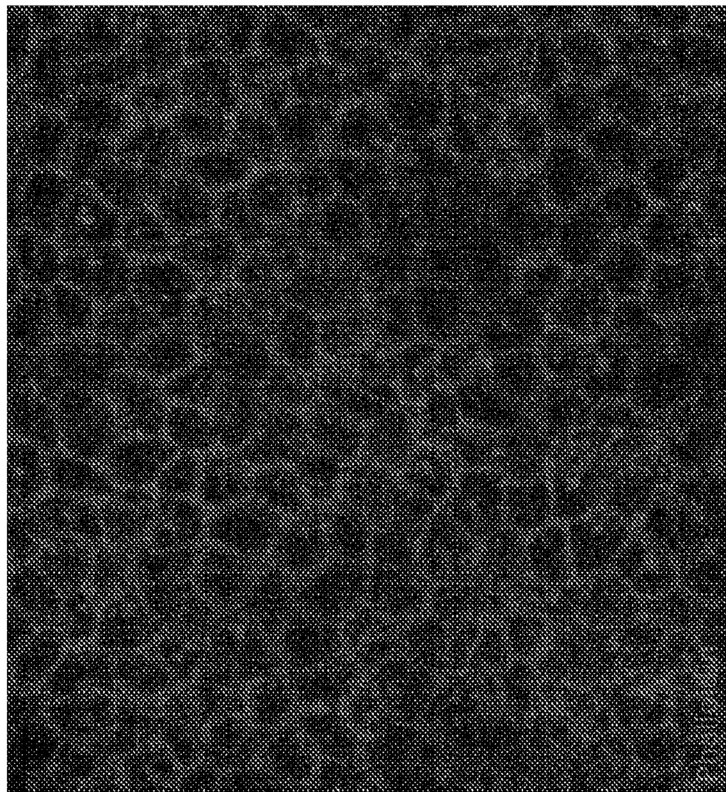


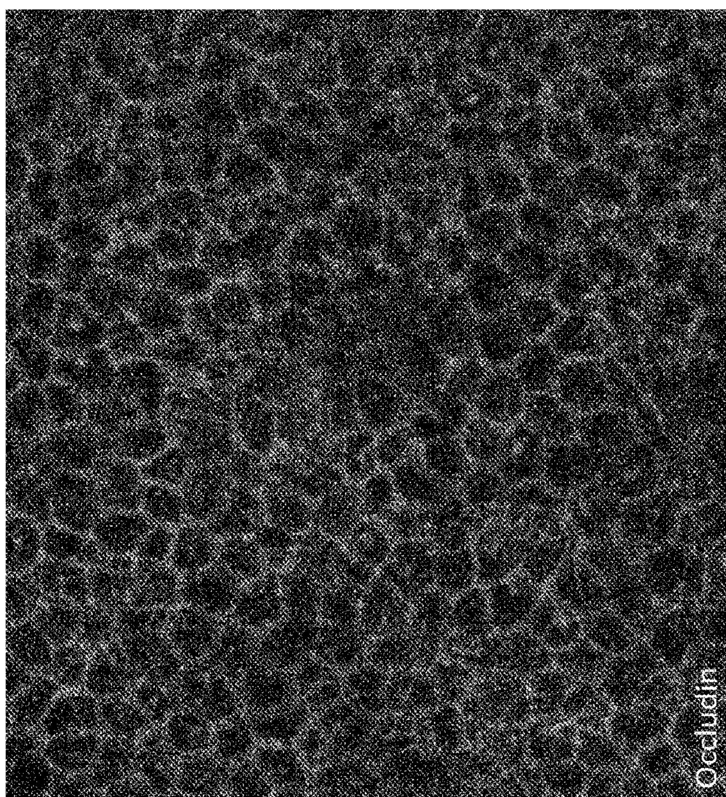
BTP10 day 60+



BTP8 day 60+

Figure 32





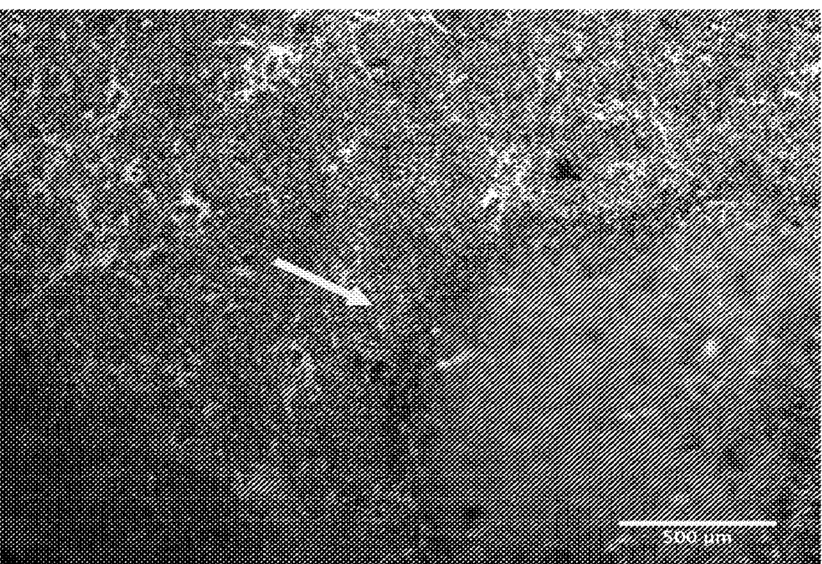
WTB Day 60+

Figure 33

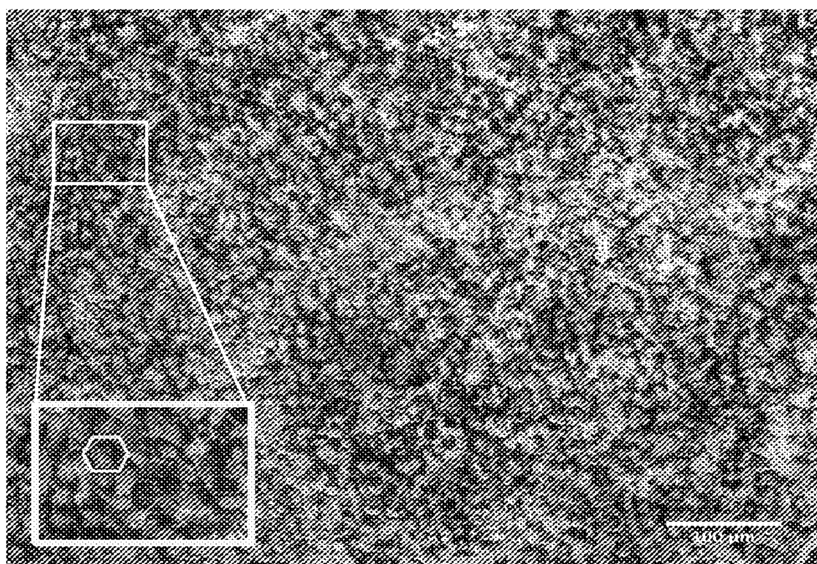
Mature iPS-derived RPE

Correct hexagonal morphology
Pigment starting around day 30

Tightly held monolayers
Fluid domes



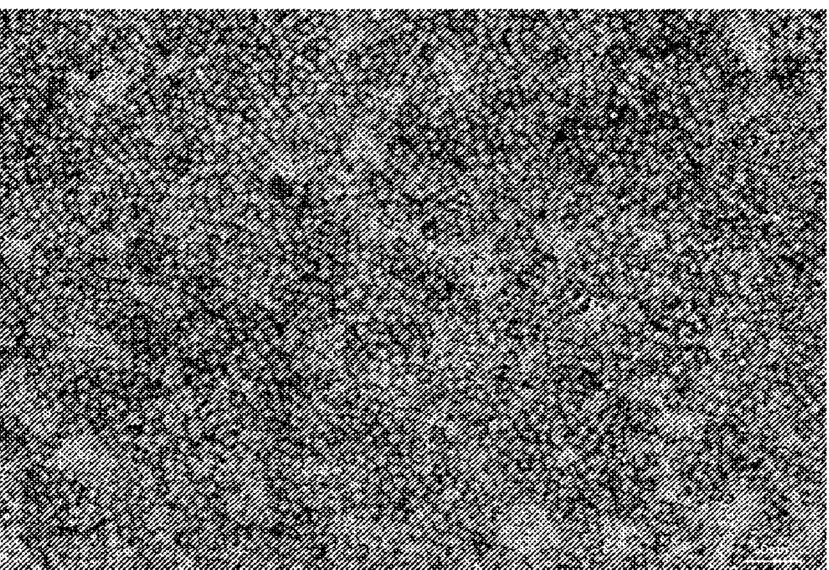
WTB Traffic Light ~ day 60



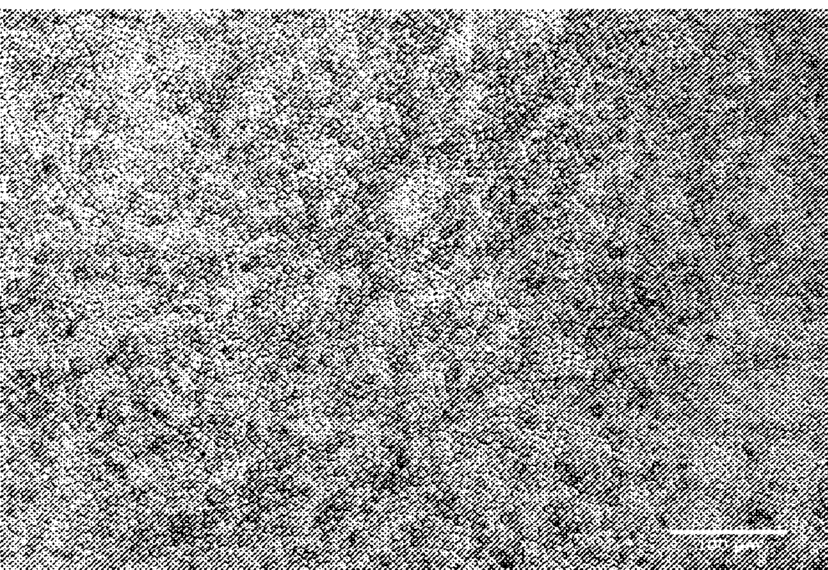
BTP8 on day 60+

Figure 34

Mature iPS-derived RPE



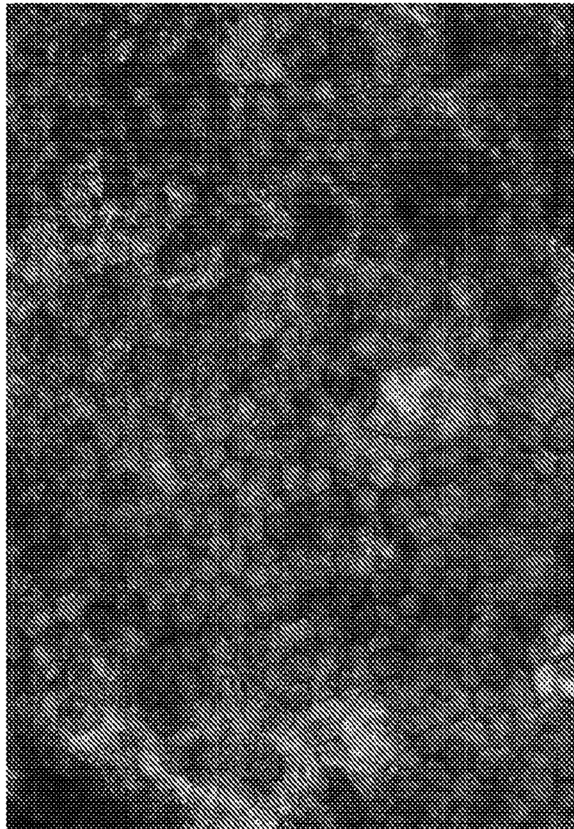
BTP10 ~ day 45



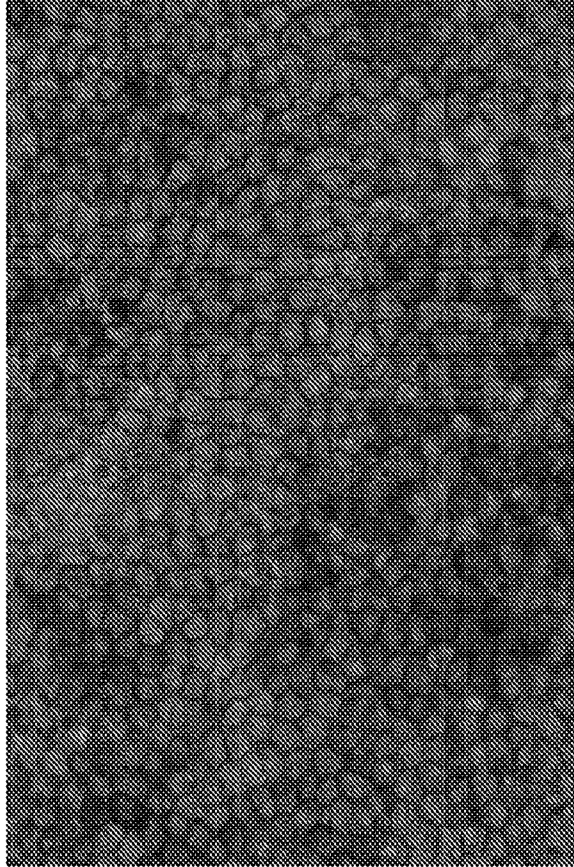
BTP6 ~ day 45

Figure 35

Mature iPS-derived RPE



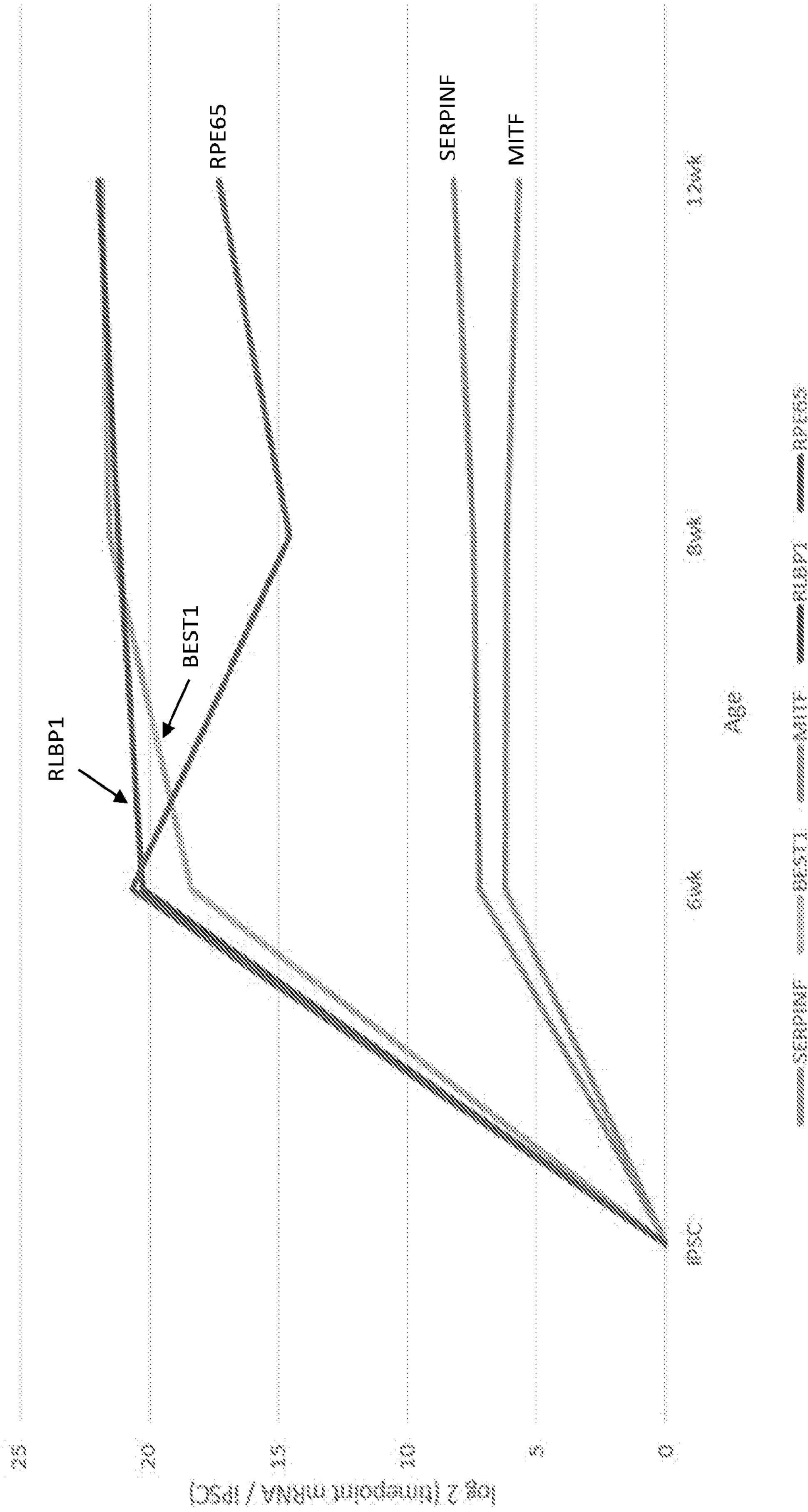
WTB ~ day 60



WTC ~ day 60

Figure 36

RPE Specific Gene Expression WTB AAVS hOPM C4



INTERNATIONAL SEARCH REPORT

Intematjonar application No.
PCT/US201 8/061 100

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/12; C12N 5/00; C12N 15/63; C12N 15/85; C12Q 1/68 (2019.01)

CPC - A61K 35/545; C12N 5/0621; C12N 5/0696; C12N 2501/60; C12N 2506/00; C12N 2506/45; C12N 2510/00 (2019.01)

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)

See Search History document

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

USPC - 435/6; 435/325; 435/377; 435/455; 435/320.1 ; 514/44R (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2015/077498 A1 (THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL) 28 May 2015 (28.05.2015) entire document	119, 120, 134, 135, 162 ------ 49-56, 86-93, 121, 136, 149-151
X -- Y	US 2015/0368713 A1 (THE UNITED STATES OF AMERICAN, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 24 December 2015 (24.12.2015) entire document	1-3, 7, 26-28, 32 ------ 4-6, 29-31 , 33, 34, 49-56, 69-76, 86-93
Y	US 2015/0265652 A1 (CELLULAR DYNAMICS INTERNATIONAL, INC.) 24 September 2015 (24.09.2015) entire document	4-6, 29-31 , 51-53, 69-76, 88-90, 149-151, 162-166
Y	US 2014/0093486 A1 (TAIPEI VETERANS GENERAL HOSPITAL) 03 April 2014 (03.04.2014) entire document	33, 55, 75, 92
Y	US 2014/0349401 A1 (ALLELE BIOTECHNOLOGY & PHARMACEUTICALS, INC.) 27 November 2014 (27.1 1.2014) entire document	34, 56, 76, 93
Y	US 2017/0067017 A1 (CELLULAR DYNAMICS INTERNATIONAL, INC.) 09 March 2017 (09.03.2017) entire document	121 , 136, 151 , 162-166
A	WO 2017/1 36805 A1 (GEORGETOWN UNIVERSITY) 10 August 2017 (10.08.2017) entire document	1-7, 26-34, 49-56, 69-76, 86-93, 119-121 , 134-136, 149-151 , 162-166

Ü Further documents are listed in the continuation of Box C. □ 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 tne invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2019	07 FEB 2019

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/061100

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:
      - ☒ in the form of an Annex C/ST.25 text file.
      - ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/061100

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-25, 35-48, 57-68, 77-85, 94-118, 122-133, 137-148, 152-161, 167
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/061100

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2017/0002319 A1 (WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH) 05 January 2017 (05.01.2017) entire document	1-7, 26-34, 49-56, 69-76, 86-93, 119-121, 134-136, 149-151, 162-166