CLAIMS

1. A method of altering a differentiation status of a cell, the method comprising:

modulating the expression of one or more differentiation factors with a nuclease-deactivated Cas9 (dCas9) fusion protein, the dCas9 fusion protein comprising dCas9 and an effector comprising a transcriptional regulator,

wherein the differentiation factors are selected from the group consisting of Pax6, Mitf, Otx2 and combinations thereof; and

wherein the cell produced from the method expresses premelanosome marker 17 (PMEL17) and Pax6.

2. The method of claim 1, the method further comprising:

providing a guide RNA (gRNA) in the cell, wherein the gRNA is capable of guiding the dCas9 fusion protein to a target site that is/that is in proximity of a promoter region of the one or more differentiation factors to allow the dCas9 fusion protein to modulate the expression of the one or more differentiation factors, optionally

wherein the target site that is/that is in proximity of the promoter region is within -300 base pairs (bp) to +5 bp window of the promoter region, optionally

the method further comprising:

providing an activator module comprising a RNA-binding protein capable of binding to the gRNA, optionally wherein the RNA-binding protein comprises MS2 coat protein (MCP), optionally

wherein the activator module further comprises one or more transcriptional activators, optionally the transcriptional activator is selected from the group consisting of VP64, p65, HSF1, Rta and combinations thereof, optionally

wherein the activator module comprises p65 and/or HSF1, optionally

wherein the dCas9 fusion protein comprises VP64 and optionally, p65 and/or Rta, optionally

the method further comprising expressing the dCas9 fusion protein, optionally a dCas9-VP64 fusion protein and/or a dCas9-VP64-p65-Rta (dCas9-VPR) fusion protein, prior to the modulating step, optionally

wherein the method comprises modulating the expression of one or more differentiation factors with a CRISPR/dCas9 synergistic activation mediators (CRISPR/dCas9-SAM) complex/ dCas9 ribonucleoprotein complex (e.g. a complex comprising the dCas9 fusion protein)/ dCas9-VP64/ dCas9-VPR/ dCas9-VP64 and MS2-P65-HSF1, optionally

wherein the one or more differentiation factors comprises transcription factors.

3. The method of any of the preceding claims, wherein the cell is a stem cell, a progenitor cell or a precursor cell, optionally the cell comprises one that is selected from the group consisting

of: embryonic stem cell (e.g. hESC3), adult stem cell, induced pluripotent stem cell (iPSC), mesenchymal stem cell (MSC), and/or, human embryonic kidney cell (HEK293).

4. The method of any of the preceding claims, wherein the method is a method of differentiating a cell, optionally

wherein the one or more differentiation factors influence an expression of a neuroprogenitor gene and/or a retinal pigment epithelium (RPE)-associated gene, optionally the RPE-associated gene comprises a gene associated with a mature RPE/RPE specific mature gene, a gene associated with pigmentation/RPE specific pigmentation gene or early eye field gene.

5. The method of any of the preceding claims, wherein the expression of PMEL17 in the produced cell is at least 50%.

6. The method of claims 1 to 4, wherein the cell is a neuroprogenitor cell.

7. The method of any of claims 1 to 3, wherein the method is a method of maintaining and/or expanding a cell, optionally maintaining and/or expanding a haematopoietic stem cells.

8. The method of claim 7, wherein the one or more differentiation factors is selected from the group consisting of erythropoietin (EPO), stem cell factor (SCF), thrombopoietin (TPO), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), and combinations thereof.

9. The method of any of the preceding claims, wherein the method is free of modulating the expression of a transcription activator selected from the group consisting of: cMyc, Klf4, Nrl, Crx, Rax, LHX2, SIX3, SOX9, GLIS3, FOXD1, ZNF92, C11orf9 and combinations thereof directly via the dCas9 fusion protein, and/or

wherein the method is free of the use of a gRNA specific to a target site that is/that is in proximity of a promoter region of: cMyc, Klf4, Nrl, Crx, Rax, LHX2, SIX3, SOX9, GLIS3, FOXD1, ZNF92, C11orf9 and combinations thereof, and/or

wherein the method is free of exogenous growth factor, free of inducible system, and/or is free of whole exogenous nucleic acid, optionally

wherein modulating the expression of one or more differentiation factors comprises an endogenous activation of the one or more differentiation factors.

10. A cell comprising a dCas9 fusion protein that is configured to modulate the expression of one or more differentiation factors selected from the group consisting of PAX6, MITF, OTX2 and

combinations thereof, the dCas9 fusion protein comprising dCas9 and an effector, or progenies thereof, optionally

wherein the cell comprises a guide RNA (gRNA) capable of guiding the dCas9 fusion protein to a target site that is/that is in proximity of the promoter region of the one or more differentiation factors to allow the dCas9 fusion protein to modulate the expression of the one or more differentiation factors, optionally for use in treating an eye disease/disorder, optionally wherein the eye disease/disorder is selected from the group consisting of macular degeneration, acute macular degeneration (AMD), atrophic age-related macular degeneration (atrophic AMD), dry age-related macular degeneration (Dry-type AMD), retinitis pigmentosa (RP), Stargardt's disease, and myopia,

11. A cell having a second differentiation status (or its progenies thereof) that was differentiated from a cell having a first differentiation status, wherein the cell having the first differentiation status comprises a dCas9 fusion protein that is configured to modulate the expression of one or more differentiation factors selected from the group consisting of PAX6, MITF, OTX2 and combinations thereof, the dCas9 fusion protein comprising dCas9 and an effector, optionally

wherein the cell having the second differentiation status is devoid of a dCas9 fusion protein or a CRISPR/dCas9-SAM complex, optionally for use in treating an eye disease/disorder, optionally wherein the eye disease/disorder is selected from the group consisting of macular degeneration, acute macular degeneration (AMD), atrophic age-related macular degeneration (atrophic AMD), dry age-related macular degeneration (Dry-type AMD), retinitis pigmentosa (RP), Stargardt's disease, and myopia.

12. A guide RNA (gRNA) to a target site that is or that is in proximity of the promoter region of one or more differentiation factors to modulate the expression of the one or more differentiation factors selected from the group consisting of PAX6, MITF, OTX2 and combinations thereof, wherein the gRNA is configured to guide a fusion protein selected from the group consisting of dCas9 fusion protein, CRISPR/dCas9 synergistic activation mediators (CRISPR/dCas9-SAM) complex, dCas9 ribonucleoprotein complex, dCas9-VP64, dCas9-VPR, dCas9-VP64, and MS2-P65-HSF1.

13. The gRNA of claim 12, wherein at least a portion of the guide RNA is capable of binding to the target site/target genomic locus that is in an about -300 base pairs (bp) to about +5 bp window of the promoter region of one or more differentiation factors, selected from the group consisting of PAX6, MITF, OTX2, EPO, SCF, TPO, GM-CSF, G-CSF, and combinations thereof.

14. The gRNA of claim 12 or 13, wherein the gRNA has at least about 80% identity with a sequence selected the group consisting of SEQ ID NO: 1 (AATGTGTGTGTGCCGGCGCC),

SEQ ID NO: 2 (GCCAGCACACCTATGCTGAT), SEQ ID NO: 3 (GCTTCGCTAATGGGCCAGTG), SEQ ID NO: 4 (ACAATAAAATGGGCTGTCAG), SEQ ID NO: 5 (GAGTGAGAGATAAAGAGTGT), SEQ ID NO: 6 (CGGGCCGAACTACAGATCCC), SEQ ID NO: 7 (CCAAACAGGAGTTGCACTAG), SEQ ID NO: 8 (AGCTGTAGTTTTCGTGGGAG), SEQ ID NO: 9 (GCGGGGGAGAGGCAACGTGG), SEQ ID NO: 10 (CTGTACCCTTGAAGCAAGTG), SEQ ID NO: 11 (GAACATTCTGGTAATGTCGG), SEQ ID NO: 12 (GCGTCAAAAAGTTGCCAGAG), SEQ ID NO: 13 (AACAGGCCGCTGCTGCACGG), SEQ ID NO: 14 (GATTGACACATCTAAGCCAG), SEQ ID NO: 15 (TAAAAACACACAACAGGGGG), SEQ ID NO: 76 (GGGGTGGCCCAGGGACTCTG), SEQ ID NO: 77 (TGTGCGTGAGGGGTCGCCAG), SEQ ID NO: 78 (GCCCCTGCTCTGACCCCGGG), SEQ ID NO: 79 (GGAGAGGCTGTGTGCGTGAG), SEQ ID NO: 80 (GAACTGTATAAAAGCGCCGG), SEQ ID NO: 81 (CCTAATCTGCCAAACTTCTG), SEQ ID NO: 82 (GAGGCGTGTCCGGAGCAGGC), SEQ ID NO: 83 (GGTAGGCGAGAAGCAGGCAA), SEQ ID NO: 84 (TCCTTCCCTTCCGGAGCCCG), SEQ ID NO: 85 (GAGCCACCAGACACTGGTGA), SEQ ID NO: 86 (CCCTATCCAAATCTTCTCCG), SEQ ID NO: 87 (ACTTCTGCCCAATCAGAGAA), SEQ ID NO: 88 (AAGAGAAGGCGTCACTTCCG), SEQ ID NO: 89 (AGCAGGTCATACGCCTGCCT), SEQ ID NO: 90 (AAGAGCTCTTAAATACACAG), SEQ ID NO: 91 (GTGACCACAAAATGCCAGGG), SEQ ID NO: 92 (CGGGGGAACTACCTGAACTG), SEQ ID NO: 93 (GGCCCTTATCAGCCACACAT), SEQ ID NO: 94 (AGGCTCACCGTTCCCATGTG), SEQ ID NO: 95 (GTGTCCAAGACAATGCAGGG), SEQ ID NO: 96 (GGGCAAGGCGACGTCAAAGG), SEQ ID NO: 97 (GCGAAAGTTTTGTGAAATTG), SEQ ID NO: 98 (GGGGGGCAAGGCGACGTCAA), and SEQ ID NO: 99 (CACCAAATTTGCATAAATCC).

15. The gRNA of any of claims 12 to 14, wherein the gRNA has about 15 bp to about 25 bp, optionally wherein the gRNA is a single/short gRNA (sgRNA).

16. A set of gRNA comprising at least two of the gRNA of any of claims 10 to 14, wherein the gRNA is selected from the group consisting of: a gRNA that is specific to a target site that is/that is in proximity of the promoter region of PAX6, a gRNA that is specific to a target site that is/that is in proximity of the promoter region of MITF and a gRNA that is specific to a target site that is/that is in proximity of the promoter region of OTX2.

17. An oligonucleotide/primer for cloning a gRNA of any of claims 12 to 16, the oligonucleotide/primer having at least about 80% with a sequence selected from Table 2 below:

Table 2

Name	Sequence	SEQ ID NO.
Pax6_1_Fwd	CACCGACAATAAAATGGGCTGTCAG	16
Pax6_1_Rev	AAACCTGACAGCCCATTTTATTGTC	17
Pax6_2_Fwd	CACCGGAGTGAGAGATAAAGAGTGT	18
Pax6_2_Rev	AAACACACTCTTTATCTCTCACTCC	19
Pax6_3_Fwd	CACCGGCCAGCACACCTATGCTGAT	20
Pax6_3_Rev	AAACATCAGCATAGGTGTGCTGGCC	21
Pax6_4_Fwd	CACCGAATGTGTGTGTGCCGGCGCC	22
Pax6_4_Rev	AAACGGCGCCGGCACACACACATTC	23
Pax6_5_Fwd	CACCGGCTTCGCTAATGGGCCAGTG	24
Pax6_5_Rev	AAACCACTGGCCCATTAGCGAAGCC	25
MITF_1_Fwd	CACCGCGGGCCGAACTACAGATCCC	26
MITF_1_Rev	AAACGGGATCTGTAGTTCGGCCCGC	27
MITF_2_Fwd	CACCGCCAAACAGGAGTTGCACTAG	28
MITF_2_Rev	AAACCTAGTGCAACTCCTGTTTGGC	29
MITF_3_Fwd	CACCGGCGGGGGAGAGGCAACGTGG	30
MITF_3_Rev	AAACCCACGTTGCCTCTCCCCCGCC	31
MITF_4_Fwd	CACCGAGCTGTAGTTTTCGTGGGAG	32
MITF_4_Rev	AAACCTCCCACGAAAACTACAGCTC	33
MITF_5_Fwd	CACCGCTGTACCCTTGAAGCAAGTG	34
MITF_5_Rev	AAACCACTTGCTTCAAGGGTACAGC	35
OTX2_1_Fwd	CACCGGCGTCAAAAAGTTGCCAGAG	36
OTX2_1_Rev	AAACCTCTGGCAACTTTTTGACGCC	37
OTX2_2_Fwd	CACCGGAACATTCTGGTAATGTCGG	38
OTX2_2_Rev	AAACCCGACATTACCAGAATGTTCC	39

OTX2_3_Fwd	CACCGTAAAAACACACAACAGGGGG	40
OTX2_3_Rev	AAACCCCCCTGTTGTGTGTTTTTAC	41
OTX2_4_Fwd	CACCGAACAGGCCGCTGCTGCACGG	42
OTX2_4_Rev	AAACCCGTGCAGCAGCGGCCTGTTC	43
OTX2_5_Fwd	CACCGGATTGACACATCTAAGCCAG	44
OTX2_5_Rev	AAACCTGGCTTAGATGTGTCAATCC	45

18. A composition comprising:

a dCas9 fusion protein, the dCas9 fusion protein comprising dCas9 and an effector;

a gRNA, optionally a sgRNA, wherein the gRNA is capable of guiding the dCas9 fusion protein to a target site that is/that is in proximity of the promoter region of one or more differentiation factors selected from the group consisting of PAX6, MITF, OTX2 and combinations thereof to allow the dCas9 fusion protein to modulate the expression of the one or more differentiation factors; and

optionally an activator module comprising a RNA-binding protein capable of binding to the gRNA, further optionally wherein the RNA-binding protein comprises MS2 coat protein (MCP).

19. A kit comprising reagents for altering a differentiation status of a cell, the kit comprising:

a nucleic acid transcribing a gRNA, optionally a sgRNA, that is capable of guiding a dCas9 fusion protein to a target site that is/that is in proximity of the promoter region of the one or more differentiation factors to allow the dCas9 fusion protein to modulate the expression of the one or more differentiation factors selected from the group consisting of PAX6, MITF, OTX2 and combinations thereof.

20. The kit of claim 19, the kit further comprising one or more of the following:

a) a second or further nucleic acid transcribing a second or further gRNA, optionally a sgRNA, that is capable of guiding a dCas9 fusion protein to a target site that is/that is in proximity of the promoter region of the one or more differentiation factors to allow the dCas9 fusion protein to modulate the expression of the one or more differentiation factors;

b) a nucleic acid encoding a dCas9 fusion protein, optionally a dCas9-VP64 fusion protein and/or a dCas9-VPR fusion protein;

c) a nucleic acid encoding an activator module comprising a RNA-binding protein capable of binding to the gRNA, optionally wherein the RNA-binding protein comprises MS2 coat protein (MCP);

d) a fusion protein selected from the group consisting of CRISPR/dCas9 synergistic activation mediators (CRISPR/dCas9-SAM) complex, dCas9 ribonucleoprotein complex, dCas9-VP64, dCas9-VPR, dCas9-VP64, and MS2-P65-HSF1;

e) one or more oligonucleotide/primer having at least about 80% identity with a sequence selected from Table 2;

f) a viral vector, a virus packaging plasmid and/or a virus expression vector;

g) one or more probes, capture agents, dyes, labels, nucleotides, salts, buffering agents, various additives, PCR enhancers and combinations thereof; and

h) instructions for use.