

CLAIMS

1. An isolated nucleic acid, comprising:
 - (i) a promoter that is a hybrid promoter comprising a first nucleotide sequence comprising at least portion of a cone-specific promoter and a second nucleotide sequence comprising at least a portion of a rod specific promoter, where the first and second nucleotide sequences are operably linked and function as a single promoter for expression in cone photoreceptors, or a cone specific promoter, or an active variant, fragment or truncation of either of the foregoing;
 - (ii) a nucleotide sequence encoding a depolarizing optogenetic protein and optionally a reporter molecule; and
 - (iii) a Woodchuck Hepatitis Virus Posttranscriptional Regulatory element (WPRE);wherein the promoter, the nucleotide sequence encoding a depolarizing optogenetic protein and optionally a reporter molecule and the WPRE are operably linked.
2. The isolated nucleic acid of claim 1, wherein the promoter is a hybrid promoter comprising SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12 or SEQ ID NO: 11 or active variant, fragment or truncation of any of the foregoing.
3. The isolated nucleic acid of claim 1, wherein the promoter is a hybrid promoter and the first nucleotide sequence comprises at least about 150 consecutive nucleotides of SEQ ID NO:2 and the second nucleotide sequence comprises at least about 264 consecutive nucleotides of SEQ ID NO:10, or an active variant, fragment or truncation of any of the foregoing.
4. The isolated nucleic acid of claim 3, wherein the first nucleotide sequence is selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and a sequence that contains at least about 150 continuous nucleotides from the 3' terminus of SEQ ID NO:2, and the second nucleic acid comprises SEQ ID NO:54, or an active variant of any of the foregoing.

5. The isolated nucleic acid of claim 4, wherein the second nucleotide comprises SEQ ID NO:10 or an active variant, fragment or truncation thereof.

6. The isolated nucleic acid of claim 1, wherein the promoter is a cone specific promoter comprising at least about 150 consecutive nucleotides of SEQ ID NO:2 or an active variant thereof.

7. The isolated nucleic acid of claim 6, wherein the promoter is selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and a sequence that contains at least about 150 continuous nucleotides from the 3' terminus of SEQ ID NO:2, or an active variant thereof.

8. The isolated nucleic acid of any one of the preceding claims, wherein the hybrid promoter or cone specific promoter comprises one to about ten copies of a sequence selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, a sequence that contains at least about 150 continuous nucleotides from the 3' terminus of SEQ ID NO:2, an active variant of any of the foregoing, and combinations thereof.

9. The isolated nucleic acid of any one of the preceding claims, wherein the hybrid promoter comprises one to about ten copies of SEQ ID NO:54, or an active variant thereof.

10. The isolated nucleic acid of any one of the preceding claims, wherein the WPRE encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO: 3 (e.g., SEQ ID NO:8) or a nucleotide sequence having at least 70% identity to SEQ ID NO: 86.

11. The isolated nucleic acid of any one of the preceding claims, further comprising a nucleotide sequence encoding a polyadenylation signal (PolyA) that is 3' of the nucleotide sequence encoding the WPRE; wherein the nucleotide sequence encoding the PolyA and the nucleotide sequence encoding the WPRE are operably linked.

12. The isolated nucleic acid of any one of the preceding claims, further comprising a nucleotide sequence encoding an AAV inverted terminal repeat sequence (ITR).

13. The isolated nucleic acid of claim 12, comprising a first AAV ITR that is 5' of the promoter and a second AAV ITR that is 3' of the WPRE and preferably 3' of the PolyA signal.

14. The isolated nucleic acid of any one of the preceding claims, wherein the depolarizing optogenetic protein is a light responsive polypeptide.

15. The isolated nucleic acid of claim 14, wherein the light responsive polypeptide is a light-gated ion channel polypeptide.

16. The isolated nucleic acid of claim 15, wherein the light-gated ion channel polypeptide is a channelrhodopsin or a functional variant thereof.

17. The isolated nucleic acid of claim 16, wherein the channelrhodopsin is a ReaChR polypeptide or a functional variant thereof.

18. The isolated nucleic acid of claim any one of claim 17, wherein the isolated nucleic acid comprises a nucleotide sequence encoding ReaCHR or a functional variant thereof.

19. The isolated nucleic acid of claim 18, wherein the nucleotide sequence encoding ReaChR or a functional variant thereof is selected from the group consisting of SEQ ID NO:16, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 69, SEQ ID NO: 73 or an amino acid sequence with at least about 70% identity to any of the foregoing.

20. The isolated nucleic acid of claim 16, wherein the channelrhodopsin or functional variant thereof is engineered to enhance membrane localization when expressed in human cone photoreceptors.

21. The isolated nucleic acid of claim 20, wherein the engineered channelrhodopsin comprises SEQ ID NO:57 or a functional variant thereof.

22. The isolated nucleic acid of any one of the preceding claims, wherein the isolated nucleic acid does not encode the optional reporter molecule.

23. The isolated nucleic acid of any one of claims 1-9, wherein the promoter comprises:

- two or more first nucleotide sequences;
- two or more second nucleotide sequences;
- two or more cone-specific promoters; or
- two or more thereof.

24. The nucleic acid of any one of the preceding claims, further comprising an intron.

25. The nucleic acid of any one of the preceding claims, comprising a nucleotide sequence comprising SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 35, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 60, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83, or a sequence that has at least about 70% identity to any of the foregoing.

26. The nucleic acid of any one of claims 1-24, comprising a nucleotide sequence comprising SEQ ID NO: 60, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, or SEQ ID NO: 83.

27. The nucleic acid of any one of the preceding claims, wherein the active variant has at least about 70% identity to the corresponding reference sequence.

28. The nucleic acid of claim 27, wherein the active variant contains one or more of codon optimization, CpG-reduction, alternative start site removal, repeat removal, hairpin removal, unwanted splice donor and acceptor site removal, ITR terminal resolution site deletion, stuffer sequence addition, or miRNA addition.

29. A viral particle comprising the nucleic acid of any one of the preceding claims.
30. A host cell comprising the nucleic acid of any one of claims 1-28 or the viral particle of claim 28.
31. An AAV vector, comprising:
- (i) a nucleic acid of any one of claims 1-28; and
 - (ii) an AAV capsid.
32. The AAV vector of claim 31, wherein the AAV capsid is an AAV5 capsid, a PHP.eB capsid, an NHP26 capsid, an AAV8 capsid, an AAV8-BP2 capsid, an AAV9 capsid, or a PHP.B capsid.
33. The AAV vector of claim 32, wherein the AAV capsid is an AAV5 capsid, and AAV8 capsid or an AAV9 capsid.
34. A host cell comprising the AAV vector of any one of claims 31-33.
35. A pharmaceutical composition comprising:
- (i) a nucleic acid of any one of claims 1-28, a viral particle of claims 29, an AAV vector of any one of claims 31-33, or a host cell of claim 30 or 34; and
 - (ii) a pharmaceutically acceptable excipient.
36. Use of the nucleic acid of any one of claims 1-28, the viral particle of claim 29, the AAV vector of any one of claims 31-33, the host cell of claim 30 or 34, or the pharmaceutical composition of claim 35 in the manufacture of a medicament for treating retinal disease, restoring vision, restoring sensitivity to light in a human cone photoreceptor cell, or treating retinal degeneration in a subject in need thereof.
37. The use of claim 36, wherein the subject in need thereof has or is at risk of developing retinitis pigmentosa, rod-cone dystrophy, Leber's congenital amaurosis, Usher's syndrome, Bardet-Biedl Syndrome, Best disease, retinoschisis, Stargardt disease, untreated retinal detachment, pattern dystrophy, cone-rod dystrophy, achromatopsia, ocular albinism,

enhanced S cone syndrome, diabetic retinopathy, age-related macular degeneration, retinopathy of prematurity, sickle cell retinopathy, Congenital Stationary Night Blindness, Choroideremia, a tapetoretinal degeneration retinal vein occlusion or geographic atrophy.

38. The use of claim 37, wherein the subject has or is at risk of developing retinitis pigmentosa.

39. The use of claim 37, wherein the subject has or is at risk of developing geographic atrophy.

40. The use of any one of claims 36-39, wherein the medicament is to be administered by subretinal injection.

41. The use of any one of claims 36-39, wherein the medicament is to be administered before or after initiation of photoreceptor loss.

42. The use of any one of claims 36-41, wherein the depolarizing optogenetic protein is expressed in a cell membrane of a human cone cell.

43. The use of any one of claims 36-42, wherein the depolarizing optogenetic protein is capable of mediating a depolarizing current that depolarizes a human cone cell when exposed to light.

44. The use of claim 43, wherein depolarization of the human cone cell induces light-driven ganglion cell spiking that is comparable to light-driven ganglion cell spiking in a functional human cone cell.

45. The use of claim 44, wherein the light-driven ganglion cell spiking is assessed using a multi-electrode array.

46. The use of any one of claims 36-45, wherein the nucleic acid is capable of restoring light sensitivity when introduced into a human cone cell, wherein restoration of light sensitivity occurs when (i) the depolarizing optogenetic protein is expressed in a cell membrane of the human cone cell, (ii) the depolarizing optogenetic protein is capable of

mediating a depolarizing current that depolarizes a human cone cell when exposed to light, and (iii) depolarization of the human cone cell induces light-driven ganglion cell spiking.

47. The nucleic acid of any one of claims 1-28, the viral particle of claim 29, the AAV vector of any one of claims 31-33, the host cell of claim 30 or 34, or the pharmaceutical composition of claim 35 for use in therapy.