

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

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2013/134295 A1

(43) International Publication Date

12 September 2013 (12.09.2013)

WIPO | PCT

(51) International Patent Classification:

C07K 14/705 (2006.01)

(21) International Application Number:

PCT/US2013/029171

(22) International Filing Date:

5 March 2013 (05.03.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/606,663 5 March 2012 (05.03.2012) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,

DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

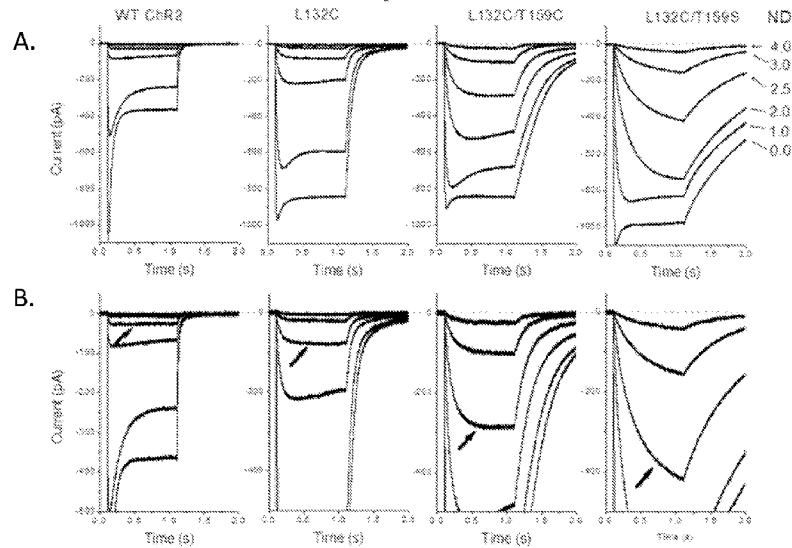
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE,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

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(54) Title: IDENTIFICATION OF CHANNELRHODOPSIN-2 (CHOP2) MUTATIONS AND METHODS OF USE

Figure 1



(57) Abstract: The invention provides compositions and kits including at least one nucleic acid or polypeptide molecule encoding for a mutant ChR2 protein. Methods of the invention include administering a composition comprising a mutant ChR2 to a subject to preserve, improve, or restore phototransduction. Preferably, the compositions and methods of the invention are provided to a subject having impaired vision, thereby restoring vision to normal levels.

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IDENTIFICATION OF CHANNELOPSIN-2 (Chop2) MUTATIONS AND METHODS OF USE

RELATED APPLICATIONS

[01] This application claims priority to and benefit of U.S. Provisional Application No. 61/606,663 filed March 5, 2012, the contents of which are incorporated herein in its entirety.

GOVERNMENT SUPPORT

[02] This invention was made with U.S. Government support under the National Institutes of Health/National Eye Institute grant NIH EY 17130. The Government has certain rights in the invention.

FIELD OF THE INVENTION

[03] This invention relates generally to the field of molecular biology. Mutations in the Channelopsin-2 (Chop2) gene are identified. Compositions comprising a mutant Chop2 gene are used in therapeutic methods to improve and restore vision loss.

BACKGROUND OF THE INVENTION

[04] The retina is composed of photoreceptors (or photoreceptor cells, rods and cones). Photoreceptors are highly specialized neurons that are responsible for phototransduction, or the conversion of light (in the form of electromagnetic radiation) into electrical and chemical signals that propagate a cascade of events within the visual system, ultimately generating a representation of our world.

[05] Photoreceptor loss or degeneration severely compromises, if not completely inhibits, phototransduction of visual information within the retina. Loss of photoreceptor cells and/or loss of a photoreceptor cell function are the primary causes of diminished visual acuity, diminished light sensitivity, and blindness. There is a long-felt need in the art for compositions and method that restore photosensitivity of the retina of a subject experiencing vision loss.

SUMMARY OF THE INVENTION

[06] The invention provides a solution for the long-felt need for a method of restoring and/or increasing the light sensitivity of photoreceptor cells by expression of advantageous mutations, and/or combinations thereof, of the Channelopsin-2 (Chop2) gene, and subsequently providing methods for Channelopsin-2 (Chop2)-based gene therapy.

[07] Channelopsin-2 (Chop2)-based gene therapy offers a superior strategy for restoring retinal photosensitivity after photoreceptor degeneration. The protein product of the Chop2 gene, when bound to the light-isomerizable chromophore all-*trans*-retinal, forms a functional light-gated channel, called channelrhodopsin-2 (ChR2). Native ChR2 shows low light sensitivity. Recently, two mutant ChR2s, L132C and T159C, were reported to markedly increase their light sensitivity (Kleinlogel et al. (2011) *Nat Neurosci.* 14:513-8; Berndt et al. (2011) *Proc Natl Acad Sci USA.* 108:7595-600; Prigge et al. (2012) *J Biol Chem.* 287(38)3104:12; the contents of each of which are incorporated herein in their entireties). The properties of these two ChR2 mutants (*i.e.*, L132C and T159C) were examined and compared with a number of double mutants at these two sites to identify suitable candidates for therapeutic methods. Compositions comprising one or more of these mutations are provided to a subject in need thereof for the purpose of restoring vision. Specifically, desired mutations in the Chop2 gene are introduced to a cell and/or integrated into the genomic DNA of a cell to improve or restore vision. Desired mutations in the Chop2 gene that are introduced to a cell to improve or restore vision may also remain episomal, not having integrated into the genomic DNA.

[08] Mutations at the L132 or T159 amino acid positions of Chop2 (and therefore, the resulting ChR2) markedly lower the threshold light intensity that is required to elicit the ChR2-mediated photocurrent. Double mutants at the amino acid positions L132 and T159 further increase the photocurrent at low light intensities, exceeding that of either of the corresponding single mutations. Retinal ganglion cells expressing the double mutants at the L132 and T159 positions can respond to light intensities that fall within the range of normal outdoor lighting conditions but should still maintain adequate, and high temporal resolution that are suitable for restoring useful vision.

Thus, mutant Chop2 protein of the present invention that form mutant ChR2s having improved light sensitivity are used alone or in combination to restore or improve vision.

[09] Specifically, the invention provides an isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L). In certain embodiments of the isolated polypeptide molecule, the amino acid at position 132 is cysteine (C) or alanine (A). When the amino acid at position 132 is cysteine (C), the polypeptide molecule may comprise or consist of SEQ ID NO: 13. When the amino acid at position 132 is alanine (A), the polypeptide molecule may comprise or consist of SEQ ID NO: 20.

[10] The invention provides an isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 159 of SEQ ID NO: 26 is not a threonine (T). In certain embodiments of the isolated polypeptide molecule, the amino acid at position 159 is cysteine (C), serine (S), or alanine (A). When the amino acid at position 159 is cysteine (C), the polypeptide molecule may comprise or consist of SEQ ID NO: 14. When the amino acid at position 159 is serine (S), the polypeptide molecule may comprise or consist of SEQ ID NO: 17. When the amino acid at position 159 is alanine (A), the polypeptide molecule may comprise or consist of SEQ ID NO: 23.

[11] The invention provides isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T). In certain embodiments of the isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), the amino acid at position 132 is cysteine (C), and the amino acid at position 159 is cysteine (C). In a preferred embodiment of this isolated polypeptide molecule, the polypeptide molecule comprises or consists of SEQ ID NO: 16. The invention provides an isolated nucleic acid molecule that encodes for the isolated polypeptide comprising or consisting of SEQ ID NO: 16. Preferably, the isolated nucleic acid molecule that encodes for the isolated polypeptide comprising or consisting of SEQ ID NO: 16, is a nucleic acid

molecule that comprises or consists of SEQ ID NO: 15.

[12] In certain embodiments of the isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), the amino acid at position 132 is cysteine (C) and the amino acid at position 159 is serine(S). The isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), may comprise or consist of SEQ ID NO: 19. Alternatively, or in addition, the isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), wherein the amino acid at position 132 is cysteine (C) and wherein the amino acid at position 159 is serine(S) may comprise or consist of SEQ ID NO: 19. The invention provides an isolated nucleic acid molecule that encodes for the isolated polypeptide that comprises or consists of SEQ ID NO: 19. Preferably, the nucleic acid molecule comprises or consists of SEQ ID NO: 18.

[13] In certain embodiments of the isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), the amino acid at position 132 is alanine (A) and the amino acid at position 159 is cysteine (C). The isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T) may comprise or consist of SEQ ID NO: 22. Alternatively, or in addition, the isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), wherein the amino acid at position 132 is alanine (A) and wherein the amino acid at position 159 is cysteine (C) may comprise or consist of SEQ ID NO: 22. The invention provides an isolated nucleic acid molecule that encodes for the isolated polypeptide that comprises or consists of SEQ ID NO: 22. Preferably, this nucleic acid molecule comprises or consists of SEQ ID NO: 21.

[14] In certain embodiments of the isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), the amino acid at position 132 is cysteine (C) and the amino acid at position 159 is alanine (A). The isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T) may comprise or consist of SEQ ID NO: 25. Alternatively, or in addition, the isolated polypeptide molecule comprising or consisting of SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T), wherein the amino acid at position 132 is cysteine (C) and wherein the amino acid at position 159 is alanine (A) may comprise or consist of SEQ ID NO: 25. The invention provides an isolated nucleic acid molecule that encodes for the isolated polypeptide that comprises or consists of SEQ ID NO: 25. Preferably, this nucleic acid molecule comprises or consists of SEQ ID NO: 24.

[15] The invention provides any one of the isolated polypeptide molecules described herein, wherein the polypeptide molecule encodes for a mutant Chop2 protein that forms a mutant ChR2, which elicits a current in response to a threshold intensity of light that is lower than the threshold of a wild type ChR2 protein. Moreover, the current conducts cations. Exemplary cations include, but are not limited to, H⁺, Na⁺, K⁺, and Ca²⁺ ions. The ChR2 wild type and mutant proteins described herein non-specifically conduct cations. Consequently, the current conducts one or more of the following: H⁺, Na⁺, K⁺, and Ca²⁺ ions.

[16] The invention provides any one of the isolated polypeptide molecules described herein further comprising a pharmaceutically acceptable carrier. The invention also provides a composition comprising at least one isolated polynucleotide molecule described herein. The composition may further comprise a pharmaceutically-acceptable carrier.

[17] The invention provides an isolated nucleic acid molecule that encodes for any of the isolated polypeptides described herein. Moreover, the isolated nucleic acid molecule may further include a pharmaceutically acceptable carrier. The invention

also provides a composition comprising at least one isolated nucleic acid molecule described herein. The composition may further comprise a pharmaceutically-acceptable carrier.

[18] The invention provides a cell, wherein the cell has been contacted with or comprises an isolated polypeptide molecule of the invention. Moreover, the invention provides a cell, wherein the cell has been contacted with or comprises an isolated nucleic acid molecule that encodes for an isolated polypeptide molecule of the invention. The invention provides, a composition comprising, consisting essentially of, or consisting of a cell that comprises an isolated polypeptide molecule of the invention or a nucleic acid molecule that encodes for an isolated polypeptide molecule of the invention. Cells of the invention may be contacted with the isolated polypeptide or an isolated nucleic acid encoding the polypeptide *in vitro*, *ex vivo*, *in vivo*, or *in situ*. In certain embodiments of the invention, the cell is a photoreceptor; a horizontal cell; a bipolar cell; an amacrine cell, and, especially, an AII amacrine cell; or a retinal ganglion cell, including a photosensitive retinal ganglion cell. Preferably, the cell is a retinal ganglion cell, a photosensitive retinal ganglion cell, a bipolar cell, an ON-type bipolar cell, a rod bipolar cell, or an AII amacrine cell. In certain aspects of the invention, the cell is a photoreceptor, a bipolar cell, a rod bipolar cell, an ON-type cone bipolar cell, a retinal ganglion cell, a photosensitive retinal ganglion cell, a horizontal cell, an amacrine cell, or an AII amacrine cell.

[19] The invention provides a method of improving or restoring vision, comprising administering to a subject any one of the compositions described herein. The invention further provides a prophylactic method of preserving vision, comprising administering to a subject any one of the compositions described herein.

[20] The methods described herein may also be applied to those subjects who are healthy, blind (in part or in total), and/or those subjects with retinal degeneration (characterized by a loss of rod and/or cone photoreceptor cells), but may be dependent upon the activity of photosensitive retinal ganglion cells for a determination of ambient light levels. For example, the methods described herein can be used to preserve, improve, or restore the activity of a photosensitive retinal ganglion cell that mediates the transduction of light information for synchronizing circadian rhythms to

the 24-hour light/dark cycle, pupillary control and reflexes, and photic regulation of melatonin release.

[21] In certain embodiments of the methods of the invention, the subject may have normal vision or impaired vision. Alternatively, or in addition, the subject may be at risk for developing an ocular disease that leads to impairment of vision. For example, the subject may have a family history of, ocular disease, including, macular degeneration and retinitis pigmentosa. The subject may be at risk for incurring an eye injury that causes damage to photosensitive cells in the retina. The subject may have a genetic marker or genetic/congenital condition that results in impaired vision, low vision, legal blindness, partial blindness, or complete blindness. Subjects may have a refractive defect that results in myopia (near-sightedness) or hyperopia (far-sightedness).

[22] Compositions of methods of the invention may be administered to a subject either systemically or locally. A preferred route of local administration is intravitreal injection.

[23] Other features and advantages of the invention will be apparent from and are encompassed by the following detailed description and claims.

BRIEF DESCRIPTION OF THE FIGURES

[24] **Figure 1** shows representative recordings of the light-evoked currents from wild-type (WT) ChR2, L132C, L132C/T159C, and L132C/T159S mutants in HEK cells for comparison of their light sensitivity (A). The light stimuli (photons/cm².s at 460 nm) were generated by a xenon arc lamp and attenuated by neutral density filters: ND4.0 (2.8x10¹⁴), ND3.0 (1.4x10¹⁵), ND2.5 (4.8x10¹⁵); ND2.0 (1.6x10¹⁶), ND1.0 (1.3x10¹⁷), ND0 (1.2x10¹⁸). (B) The same current traces are shown at a different current scale. The traces pointed by arrows are evoked by the same light intensity (ND2.5).

[25] **Figure 2** shows representative recordings of the light-evoked currents from wild-type (WT) ChR2, T159C, L132C, L132C/T159C, and L132C/T159S mutants to a 10 ms light pulse (1.2x10¹⁸ photons/cm²/s at 460 nm) in HEK cells for comparison of their deactivation time course (decay time course after light off).

[26] **Figure 3** shows representative multichannel array recordings of WT ChR2, L132C, L132C/T159C, and L132C/T159S mediated spiking activities from retinal ganglion cells in retinal whole-mounts for comparison of their light sensitivity. Light stimuli (photons/cm²/s) was generated by a 473 nm blue laser and attenuated by neutral density filters: ND0 (6.3×10^{16}), ND1.0 (7.4×10^{15}), ND1.5 (2.7×10^{15}), ND2.0 (7.3×10^{14}), ND2.5 (3.2×10^{14}), ND3.0 (8.5×10^{13}), ND3.5 (3.8×10^{13}), and ND4.0 (9.5×10^{12}).

[27] **Figure 4** shows representative multichannel array recordings of WT ChR2, L132C, L132C/T159C, and L132C/T159S mediated spiking activities from retinal ganglion cells in retinal whole-mounts for comparison of their temporal dynamics. In each panel, the raster plots of 10 consecutive light-elicited spikes originated from a single neuron (top) and the averaged spike rate histograms (bottom) are shown. Light pulses at different frequency was generated by a 473 nm blue laser with intensities about one log unit above the threshold intensity of each mutant. Recordings of WT ChR2 and L132C are shown in (A), and recordings of L132C/T159C and L132C/T159S are shown in (B).

DETAILED DESCRIPTION

Visual System

[28] The central nervous system mediates vision (also referred to herein as sight) through specialized cells and unique methods of signal transduction present in the visual system. The principle responsibility of the visual system is to transform light, in the form of electromagnetic radiation, into a representation or image of the surrounding world. In addition to the “visual” function of this system, the visual system also regulates the pupillary light reflex (PLR), circadian photoentrainment to periodic light/dark cycles, and release of the hormone melatonin.

[29] The cells of the retina are the first cells of the visual or nervous system to encounter light (electromagnetic radiation of varying wavelengths and intensities). Photons travel through the cornea, pupil, and lens before reaching the retina. The retina has a unique structure because the photoreceptor cells that directly absorb photons are located in the outer layer of the retina. Photons that traverse the lens first encounter an inner layer of retinal ganglion cells (a minority of which are

photosensitive through the expression of the opsin, melanopsin) and an intermediate layer of bipolar cells before reaching the outer layer of photoreceptor cells (also known as rods and cones). Rod photoreceptors operate in dim illumination condition (scotopic vision) while cone photoreceptors operate in bright illumination conditions (photopic vision) responsible for color vision. Cone photoreceptors synapse directly onto ON- and OFF-type cone bipolar cells, which in turn, synapse directly onto ON- and OFF-type retinal ganglion cells. Rod photoreceptors synapse to rod bipolar cells (a unique type of bipolar cells, which is ON-type), which synapse to AII amacrine cells. The AII amacrine cells then relay the visual signals to ON-type cone bipolar cells through gap junction and to OFF-type cone bipolar cells as well as OFF ganglion cells through inhibitory glycinergic synapses. Retinal ganglion cells are responsible for relating visual information to neurons of the brain.

Phototransduction

[30] Within the retina, photoreceptor cells absorb photon particles and transform the raw data of light frequency and wavelength into chemical and subsequently electrical signals that propagate this initial information throughout the visual and nervous systems. Specifically, an opsin protein located on the surface of a photoreceptor (rod, cone, and/or photosensitive retinal ganglion cell) absorbs a photon and initiates an intracellular signaling cascade, which results in the hyperpolarization of the photoreceptor. In the dark, the opsin proteins absorb no photons, the photoreceptors are depolarized. The visual signals of photoreceptors then relay through bipolar cells, amacrine cells, and ganglion cells to the high visual centers in the brain. Specifically, when rod and cone photoreceptors are depolarized (in the dark), they cause the depolarization of rod bipolar cells and ON-type cone bipolar cells, but the hyperpolarization of OFF-type cone bipolar cells, which in turn cause the depolarization of AII amacrine cells and the increase of the spiking of ON-type retinal ganglion cells and the decrease of the spiking of OFF-type retinal ganglion cells. The opposite happens (to rod, ON- and OFF-bipolar cells, AII amacrine and ON- and OFF-ganglion cells), when rod and cone photoreceptors are hyperpolarized (in response to light).

[31] Light information is processed and refined significantly by the actions of

photoreceptors, bipolar cells, horizontal cells, amacrine cells, and retinal ganglion cells. To add to the complexity of this system, photoreceptors are found in three main varieties, including rods, cones (of which three types respond most strongly to distinct wavelengths of light), and photosensitive retinal ganglion cells. Thus, a first layer of information processing occurs at the level of the photoreceptors which respond differentially to certain wavelengths and intensities of light. Bipolar cells of the retina receive information from both photoreceptor cells and horizontal cells. Horizontal cells of the retina receive information from multiple photoreceptor cells, and, therefore, integrate information between cell types and across distances in the retina. Bipolar cells further integrate information directly from photoreceptor cells and horizontal cells by producing mainly graded potentials to retinal ganglion cells, although some recent studies indicate that some bipolar cells can generate action potentials. Cone bipolar cells synapse on retinal ganglion cells and amacrine cells while rod bipolar cells synapse only to AII amacrine cells. Similar to horizontal cells, most amacrine cells integrate information laterally within the retina. Unlike horizontal cells, most amacrine cells are inhibitory (GABAergic) interneurons. Amacrine cells are also more specialized than horizontal cells, because each amacrine cell specifically synapses on a particular type of bipolar cell (one of the ten varieties of bipolar cell). Particularly, the AII amacrine cell is a critical relay neuron in the rod pathway (under scotopic vision when cone photoreceptors do not respond). The AII amacrine cells receive synaptic inputs from rod bipolar cells and then piggy-back the signals to cone pathway through ON- and OFF-cone bipolar cells to ON- and OFF-ganglion cells as described above. Therefore, expression of Chop2, and the resulting formation of ChR2, in rod bipolar cells or AII amacrine cells can create both ON and OFF responses in retinal ganglion cells. Furthermore, retinal ganglion cells integrate information from bipolar cells and from amacrine cells. Although retinal ganglion cells vary significantly with respect to size, connectivity, and responses to visual stimulation (*e.g.* visual fields), all retinal ganglion cells extend a long axon into the brain. Except for a minute portion of the retinal ganglion cells that transduce non-visual information regarding the pupillary light reflex and circadian entrainment, the totality of axons extending from the retinal ganglion cells form the optic nerve, optic

chiasm, and optic tract of the central nervous system. Consequently, a significant amount of information processing occurs in the retina itself.

[32] Photoreceptor cells express endogenous opsin proteins, such as rhodopsin. The mutant Chop2 proteins of the invention may be expressed in any cell type, and form functional ChR2 channels. Preferably, the cell is a retinal cell. Exemplary cells, include, but are not limited to, photoreceptor cells (e.g., rods, cones, and photosensitive retinal ganglion cells), horizontal cells, bipolar cells, amacrine cells, and retinal ganglion cells.

Channelopsin-2 (Chop2)

[33] Channelopsin-2 (Chop2) was first isolated from the green algae, *Chlamydomonas reinhardtii*. Channelopsin-2 is a seven transmembrane domain protein that becomes photo-switchable (light sensitive) when bound to the chromophore all-*trans*-retinal. Chop2, when linked to a retinal molecule via Schiff base linkage forms a light-gated, nonspecific, inwardly rectifying, cation channel, called Channelrhodopsin-2 (Chop2 retinalidene, abbreviated ChR2).

[34] As referred to herein, “channelopsin-2” or “Chop2” refers to the gene that encodes channelopsin-2, which then forms Channelrhodopsin-2 (ChR2) once bound to retinal. Gene constructs of the present invention refer primarily to channelopsin-2 (i.e., without the retinal), and all Chop2 variants disclosed herein form functional channelrhodopsin-2 variants. The methods disclosed herein may include delivering Chop2 to cells without exogenous retinal. It is understood that upon expression of Chop2 in cells (i.e., retinal neurons), endogenously available retinal binds to the wild-type Chop2 or the Chop2 mutants of the present invention to form functional light-gated channels, WT ChR2 or mutant ChR2. As such, Chop2 proteins, as referred to herein, can also be synonymous with ChR2.

[35] As used herein, “channelrhodopsin-2” or “ChR2” refers to the retinal-bound functional light-sensitive channel. In one embodiment, the bound retinal may be provided exogenously. In a preferred embodiment, the bound retinal is provided from endogenous levels available in the cell. The present invention also encompasses the functional channelrhodopsin-2 channels formed by the polypeptides and polynucleotides encoding the Chop2 mutants described herein.

[36] Upon illumination by the preferred dose of light radiation, ChR2 opens the pore of the channel, through which H^+ , Na^+ , K^+ , and/or Ca^{2+} ions flow into the cell from the extracellular space. Activation of the ChR2 channel typically causes a depolarization of the cell expressing the channel. Depolarized cells produce graded potentials and or action potentials to carry information from the Chop2/ChR2-expressing cell to other cells of the retina or brain.

[37] The wild type form of ChR2 or mutant ChR2s with high temporal resolution have become a central focus of neuroscience research. When expressed in a mammalian neuron, ChR2 mediates light-controlled depolarization of *in vitro* or *ex vivo* cultures. Wild type ChR2s or mutant ChR2s with high temporal resolution (the latter usually display low light sensitivity) presents several challenges that must be addressed to enable their use for the purpose of vision restoration. For the purpose of vision restoration, the ChR2 with high light sensitivity rather than high temporal resolution is desired.

[38] Wild type ChR2 proteins require illumination from high blue light intensities for full activation (i.e. 10^{18} - 10^{19} photons s^{-1} cm^{-2} at a wavelength of 480 nm). Continuous illumination of this type can damage cells.

[39] The kinetics of the wild type ChR2 protein is suboptimal for maximizing channel efficacy. Efficacy can be increased by modifying one or more amino acids of the wild type ChR2 protein either to prolong the open state of the channel or increase the unit conductance of the channel, or both. The single-channel conductance of wild-type ChR2 is small. Thus, neuronal activation *in vivo* would either require high expression of the wild type channel or very intense activation with the preferred wavelength of blue-light. A simpler solution may be found by altering the channel conductance or to prolong the channel open time. Either one of these mechanisms and, in particular, the combination of these mechanisms, enable lower and safer light intensities to be used to achieve the same level of cellular depolarization.

[40] For example, mutant ChR2 proteins of the invention achieve greater light sensitivity through the prolongation of the channel open state. Consequently, each mutant ChR2 channel conducts a greater photocurrent than a wild type ChR2 channel when activated by the same light intensities. Therefore, the mutant channels are

activated by light intensities that are lower than those required for activation of the wild type ChR2 channels. Quantitatively, detectable spiking activity of retinal ganglion cells expressing mutant ChR2 proteins can be elicited by a light intensity that is 1.5-2 log units lower than the light intensity required to elicit spiking activity from retinal ganglion cells expressing wild type ChR2. Thus, the light intensities required to activate the mutant ChR2 proteins are close to or fall within the range of normal outdoor lighting conditions.

[41] The following sequences provide non-limiting examples of wild type and mutant Chop2 proteins, and polynucleotides encoding said WT and mutant Chop2 proteins of the invention, and forming WT and mutant ChR2s of the invention.

[42] A wild type (WT) Chop2 of the invention may be encoded by the following *Chlamydomonas reinhardtii* chlamyopsin 4 light-gated ion channel (COP4) mRNA sequence (GenBank Accession No. XM_001701673, and SEQ ID NO: 1):

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1 gcagcaccat acttgacata tgcgcggcaag caagcattaa acatggatta tggaggcgcc
 61 ctgagtgccg ttggggcgca gctgttattt gtaacgaacc cagtagtcgt caatggctct
121 gtacttgcg ctgaggacca gtgttactgc gggggcttggg ttgagtcgctg tggcacaaac
181 ggtgcggccaaa cggcgctcgaa cgtgctgcaa tgggttgcgt ctggcttctc catcctactg
241 cttatgtttt acgcctacca aacatggaaag tcaacctggg gctggggagga gatctatgtg
301 tgcgcgtatcg agatggtcaa ggtgattctc gagttcttct tcgagttaa gaaccgcgtcc
361 atgctgtatc tagccacagg ccaccgcgtc cagtggttgc gttacccgaa gtggcttctc
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661 cgggtgtcgcc aggtgtgtac tggcatggct tggcttcttc tcgtatcatg ggttatgttc
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841 ctgcgcgtgc tgatccacga gcatatccctc atccacggcg acattcgcaa gaccacccaa
901 ttgaacatcg gtggcactga gattgagggtc gagacgttgg tggaggacga gggcgaggct
961 ggccgcgttca acaagggcac cggcaagtac gcctcccgcc agtccttcct ggtcatgcgc
1021 gacaagatga aggagaaggg cattgacgtc cgcccttc tggacaacag caaggagggt
1081 gagcaggaggc aggccgcagg ggctgcattt atgatgtatga acggcaatgg catgggtatg
1141 ggaatgggaa tgaacggcat gaacggaaatg ggccgtatga acgggatggc tggccggcc
1201 aagcccgcc tggagctcac tccgcagcta cagcccgcc gcgtcatccct ggcgggtgccc
1261 gacatcagca tgggtgactt ctccgcgag cagtttgc tgcgtatcggt gacgtacag
1321 ctgggtgcgg ccctgggcgc tgacaaacaca ctggcgctgg ttacgcaggc gcagaacctg
1381 ggccggcggtt acttttgtt gattccccc gagttccctgc ggcacccgtc tagcaccaggc
1441 atccctgagcc gcctgcgcgg cggggccag cgtgtggctc cggtccggctg ggccgcagctg
1501 gggccatgc gtgacgttgc cgttccgc aacctggacg gtcggctggaa gggcccttc
1561 ttccggacagg gcacatcgcc gggccacatc ttggccctgg tggccaaatg gcacgcaggatg
1621 cgcaagatgc agcagatgca gcagattggc atgatgaccc ggcgcattgaa cggcatgggc
1681 ggccgtatgg gcggccggcat gaacggcatg ggccggcgca acggcatgaa caacatgggc
1741 aacggcatgg gggccggcat gggcaacggc atggccggca atggcatgaa cggaaatgggt
1801 ggccggcaacgc gcatgaaacaa catggccggc aacggaaatgg cggcaacggg aatggggcc
1861 ggcattggcg gcaacggat gggtggttcc atgaaacggca tgagctccgg cgtgggtggcc
1921 aacgtgacgc cctccgcgc cggccggcatg ggccgcattt gtaacggcg catggctgcg
1981 ccccaactgc cccgcattt gggccggccgc ctgggttacca aaccgcctt caacgcggcc
2041 ccctcaccgc tcaagtcgc gctcggttcc gaggcaggca tggccaggat gggaggcatg
2101 ggccggatga gggaaatggg aggcattgggtt ggaatggggg gcatgggggg ccggccggcc
2161 gcccacgcgc aggtgtcgcc cggcaacgcg gaggcggaga tgctgcagaa tctcatgaa
2221 gagatcaatc gcctgaaagcg cggatggcc gataaaagg ctggaggccg gtactgcgt

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2281	acctgcgagc	tcgcgcgcct	gactcgctgt	acacacggct	caggagcacg	cgcgctggaa
2341	cttctcaacc	tgtgtgcaac	gtatctagag	cggcctgtgc	gcgaccgtcc	gtgagcattc
2401	cggcgcgatc	ttcccgccct	cgcaccgcaa	gttcccttcc	tggccctgt	gcgcctgacg
2461	catcgccgaa	acggaagggc	ggcttgatca	gtaaagcatt	gaagactgaa	gtcgtgcgac
2521	cgttagtgct	tggctctgca	cgtaaagtggg	cgctgcctcg	cttactacgc	attgccaag
2581	actgttccct	tttgggtggcc	gaggccctgg	tcccacatca	ttcatttgca	taacgtactg
2641	tttagttaca	tacggttgc	tttacatcgca	caattgcac	atgggcttag	agtccgtacg
2701	gcggctatgg	acgaagggtt	tatcgatgt	gatttaggaat	ctcggttggaa	aggcttcgag
2761	aaagttagatc	tcatctgtgg	cttctgttgg	ggtcatcaag	aagaacgacg	gtaaggccaa
2821	cgaggtaaaa	gtggcacgtc	tttgcacaca	acggggcccg	ggagagtggg	ggagtgcatg
2881	tgtgcggtcc	taacacgcga	gtgcaaaagcg	ggcttttctg	gagctgggtt	acggcttggc
2941	tcggcaactg	ctctgtgttt	taaccacagc	ttcggaaagtc	tgggtatgtt	ttgttggcag
3001	aaacatttgg	gtaaactttag	ggtgattcg	ctggagtcgg	acaacatggc	tgccgtccgt
3061	gtgcagggac	ggtaatcaat	gagctggac	tgtgtatgctc	accacacgtt	gcataaccct
3121	gcttacaaaa	acactttgtat	gtcgtggcca	aactatgcgt	gagcaaaagag	ttaaagaggc
3181	atgagtgcat	ggttgcggac	gtgcgcacaca	attgcatcaa	gtatttgac	ccttcaagcc
3241	aacaagtgcg	cgcgcggcaa	cttgataacaa	acgcggacgc	cagtggtggg	ggcgtgtaca
3301	gttttatgaa	gtctgcattat	tgcgatccgt	agtgttaggt	tgctgtgtac	gcccgcggcc
3361	tgtggccct	tatcatggaga	gttgggtgtct	tcacccacacg	ttttggccgg	ctgaagggtg
3421	tgctatgttt	tggtaaaaggc	ggggccctgaa	agaccgcac	cgtagaacc	tactgaaagg
3481	gtgtcagccc	ggggtaactg	gatgccttgg	gacatagcta	ttaatgttga	agtgaagccg
3541	tcaagccgag	tgccgtgcgc	cgtgtatca	ccaaggcccg	tccta	

[43] A wild type (WT) ChR2 of the invention may be encoded by the following Chlamydomonas reinhardtii chlamyopsin 4 light-gated ion channel (COP4) amino acid sequence (GenBank Accession No. XP_001701725, and SEQ ID NO: 2):

[44] A wild type (WT) Chop2 of the invention may be encoded by the following *Chlamydomonas reinhardtii* retinal binding protein (cop4) gene sequence (GenBank Accession No. AF461397, and SEQ ID NO: 3):

```
1 gcatctgtcg ccaaggcaagc attaaacatg gattatggag ggcgcctgag tggcggtgtgg  
61 cgcgagctgc tatttgcatac gaaccaggta gtcgtcaatg gctctgtact tggccctgag  
121 gaccagtgtt actgcgcggg ctggatttag tcgcgtggca caaacgggtc ccaaaccggcg  
181 tcgaacgtgc tgcaatggct tgctgctggc ttctccatcc tactgcttat gttttacgcc  
241 taccaaacat ggaagtcaac ctgcggctgg gaggagatct atgtgtgcgc tatcgagatg  
301 gtcaagggtga ttctcgagtt ctcttcgag ttaagaacc cgtccatgtc gtatctagcc  
361 acaggccacc gcgtccagtg gttgcgttac gcccaggtagt ttctcaccc cccggcttatt  
421 ctcattcacc tgtcaaacct gacgggcttg tccaaacact acagcaggcg caccatgggt  
481 ctgttgcgt ctgtatattgc cacaatgtg tggggggccca ttccggccat ggccacccgg  
541 tacgtcaagg tcataatcttc ctgcctgggt ctgtgtttagt gtgtcaacac gtttttcac  
601 gctgccaagg cttacatcgta gggtttaccac acgtgcggca agggccgggt tggccagggt  
661 tgactggca tggcttggct ctcttcgtt tcatgggtt tggccccat cctgttccatc  
721 ctggcccccg agggcttcggc cgtccgttggc gtgtacggct ccacccgtcg ccacaccatc  
781 attcacctga tgtcqaaqaa ctgtctgggt ctgtcgccact acatcacctgcg cgtqctqatc
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841 cacgagcata tcctcatcca cgccgacatt cgcaagacca ccaaattgaa cattgggtggc
901 actgagatg aggtcgagac gctgggtggag gacgaggccg aggctggcgc ggtcaacaag
961 ggcaccggca agtacgcctc cccgagatcc ttccctggta tgccgacaa gatgaaggag
1021 aaggccatg acgtcgccgc ctctctggac aacagcaagg aggtggagca ggagcaggcc
1081 gccagggtg ccatgatgat gatgaacggc aatggcatgg gtatggaaat gggaatgaac
1141 ggcataaaccg gaatggcggt tatgaacggg atggctggcg ggcggcaagcc cggcctggag
1201 ctcactccgc agctacagcc cggccgcgtc atcctgggg tgccggacat cagcatgggt
1261 gacttcttcc gcgagcgtt tgctcagcta tcggtgacgt acgagctggt gccggccctg
1321 ggcgctgaca acacactggc gctggttacg caggcgcaga acctggcgcc cgtggacttt
1381 gtgttgatc accccagtt cctgcgcgac cgctctagca ccagcattct gagccgcctg
1441 cgcggcgccg gccagcgtgt ggctgcgttc ggctggcgcc agctggggcc catgcgtgac
1501 ctgatcgagt ccgcaaacct ggacggctgg ctggaggggcc cctcgttcgg acagggcatc
1561 ctggccggcc acatcggttc cctgggtggcc aagatgcagc agatgcgca gatgcagcag
1621 atgcagcaga ttggcatgat gacccggcc atgaacggca tggggcgccg tattggcgcc
1681 ggcataaaccg gcatggcgcc cggcaacggc atgaacaacc tggggcaacggg catggcgcc
1741 ggcataaaccg acggcatggg cggcaatggc atgaacggaa tgggtggcgcc caacggcatg
1801 aacaacatgg gcgccaaacgg aatggccggc aacggaaatgg gcgccggcat gggccggcaac
1861 ggtatgggtg gtcctatgaa cggcatgagc tccggcgtgg tggcaaacgt gacgccttcc
1921 gccggccggcg gcatggcgcc catgatgaaac ggccgcattgg ctgcggccca gtcggccggc
1981 atgaacggcg gccgcctggg taccaccccg ctcttcacg ccgcggccctc accgctcagc
2041 tcgcagctcg gtgcggagggc aggcattggc agcatgggag gcatggcgcc aatgagcgga
2101 atggaggcgca tgggtgaaat gggggcgatg ggccggcccg ggcggcccac gacgcaggct
2161 gcgccggcgca acgcggaggc ggagatgctg cagaatctca tgaacgagat caatgcctg
2221 aagcgcgagc ttggcgagta a

```

[45] A wild type (WT) Chop2 of the invention may be encoded by the following Chlamydomonas reinhardtii retinal binding protein (cop4) amino acid sequence (GenBank Accession No. AAM15777, and SEQ ID NO: 4):

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1 mdyygalsav grellfvtnp vvvngsvlvp edqcycaqwi esrgtngaqt asnvlqwlaa
61 gfsilllmfy ayqtwkstcg weeiivcaine mvkvileffff efknpsmlyl atghrvqwlr
121 yaewlltcpv ilihlsnltg lsndysrrtm gllvsdigti vwgatsamat gyvkiffcl
181 glcygantff haakayiegy htvpkgrcrq vvtgmawlf vswgmpilf ilgpefgvli
241 svygstvght iidlmsknw gllghylrwl ihehilihgdrirkttklnig gteievetlv
301 edeaeagavn kgtgkyasre sflvrmrdkmk ekgidvrasl dnskeveeqq aaraammnn
361 gngmgmgnmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngm
421 lsvtyelvpa lgadntlalv tqaqnlggvd fvlihpeflr drsstsilr lrgaggrrvaa
481 fgwaqlgpmr dliesanldg wlegpsfqgg ilpahivalv akmqqmrkmq qmqqigmmmtg
541 gmnmgmgnmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngm
601 gngmgnmgnmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngm
661 plfnaapspl ssqlgaeagm gsmggmgnmngmngmngmngmngmngmngmngmngmngmngm
721 lqnlmneinr lkrelge

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[46] A wild type (WT) Chop2 of the invention may be encoded by the following Chlamydomonas reinhardtii sensory opsin B (CSOB) mRNA sequence (GenBank Accession No. AF508966, and SEQ ID NO: 5):

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1 ttgacatctg tcgccaagca agcattaaac atggattatg gaggcgccct gagtgccgtt
61 gggcgcgagc tgctatttgt aacgaaccca gtagtcgtca atggctctgt acttgtgcct
121 gaggaccagt gttactgcgc gggctggatt gagtcgcgtg gcacaaacgg tgcccaaaccg
181 gcgtcgaaacg tgctgcatacg qcttgctgt ggcttctcca tcctactgtat tatgtttac
241 gccttacaaa catggaagtcc aacctgcggc tggggaggaga tctatgtgtg cgctatcgag
301 atggtcaagg tgattctcgat gttcttcttc gagtttaaga acccgccat gctgtatcta
361 gcccacaggcc accgcgtccaa gttgggtgcgt tacgcgcgtt ggcttctcac ctgcccggc
421 attctcatcc acctgtcaaa cctgacggc ttgtccaaacg actacagcag ggcacccatg
481 ggtctgcctt gttctgat tggcacaatt gtgtggcgcc caacttccgc catggccacc
541 ggataacgtca aggtcatctt ctgtgcctt ggtctgtt atgggtctaa cacgttcttt

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601 caccgtgcacca aggccctacat cgagggttac cacaccgtgc cgaaggcccg gtgtcgccag
661 gtgggtgactg gcatggctt gctttcttc gtatcatggg gtatgttccc catctgttc
721 atccctggcc cggagggtt cggcgtctt agcgtgtac gctccaccgt cggccacacc
781 atcatgtacc tgatgtcgaa gaactgtctgg ggtctgtctg gccactacat gcgcgtgt
841 atccacgagc atatcctcat ccacggcgac attcgcagaaga ccaccaaatt gaacattgg
901 ggcactgaga ttgaggtcga gacgctgtt gaggacgagg ccgaggctgg cgcggtaaac
961 aaggggcaccg gcaagtacgc ctcccgag tccctcctgg tcatgcgcga caagatgaag
1021 gagaaggcga ttgacgtcg ggcctctctg gacaacagca aggagggttgc cggaggac
1081 gcccgcaggg ctgccatgt gatgtgaac gcataatggca tgggtatggg aatgggaatg
1141 aacggcatga acggaatggg cggtatgaac gggatggctg gccgcgc当地 gcccggctg
1201 gagctcactc cgcagctaca gccccggcgc gtcatcctgg cggtgc当地 catcagcatg
1261 gttgacttct tccgcgagca gtttgc当地 ctatcggtga cgtacgagct ggtgc当地
1321 ctggcgctg acaacacact ggcgtgtt acgcaggcgc agaacctggg cggcgtgg
1381 tttgtgtga ttcccccg gttcctgcgc gaccgtctca gcaccagcat cctgagccgc
1441 ctgcgc当地 cggccagcg tggcgtcg ttcggctggg cgcagctggg gccatgcgt
1501 gacctgatcg agtccgaaa cctggacgc tggcgtgggg gccc当地 cggacagg
1561 atccgtccgg cccacatgt tccctgtt gccaagatgc acaatgc当地
1621 cagatcgacg agattggcat gatgaccggc ggc当地 aacatgggg
1681 ggc当地 catga acggcatggg cggccgcaac ggc当地 aacaatgggg
1741 ggc当地 catgg gcaacggcat gggccgcaat ggc当地 aac
1801 atgaacaaca tggccgca cggaaatggcc ggcaacggaa tggccg
1861 aacggatggg gtggctccat gaacggcatg agctccggc tggcc
1921 tccgc当地 ccc gccgcatggg cggcatgtt aacggccgca tggct
1981 ggc当地 catgg gccgcatggt ggttaccaac cccgcttca
2041 agctcgacg tccgtgc当地 ggcaggcatg ggcc
2101 ggaatgggag gcatgggtt aatggggggc atggccggc
2161 gctgc当地 ccc gcaacggca ggc当地 gagatg
2221 ctgaagcg
2281 ggc当地 ccttca
2341 tggcaacgt
2401 cccgc当地
2461 ggaaggggcgg
2521 gcttc当地
2581 tggccg
2641 cgctt当地
2701 gaagggtt
2761 ttctgtgg
2821 ggc当地
2881 acacgc当地
2941 ctgtgtt
3001 aacttgggg
3061 taatcaat
3121 cactt
3181 gtt
3241 ggc当地
3301 ctgc当地
3361 acatgg
3421 ggt
3481 ggt
3541 ggc当地
3601 ggc当地
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[47] A wild type (WT) Chop2 of the invention may be encoded by the following Chlamydomonas reinhardtii sensory opsin B (CSOB) amino acid sequence (GenBank Accession No. AAM44040, and SEQ ID NO: 6):

1 mdyggalsav grellfvtnp vvvngsvlpv edqcycagwi esrgtngaqt asnvlqwlaa
61 gfsilllmfy ayqtwkstcg weeiylvcaie mvkvilefff efknpsmlyl atghrvqwlrl
121 yaewlltcpv ilihlsnltg lsndysrrtm gllvsdigti vwgatsamat gyvkviffcl
181 qlcygantff haakayieqy htvpkgrcrq vvtqmaulff vswgmpfplf ilqpeqfvql

241 svygstvght iidlmskncw gllghylrvl ihehilihg d rkttklnig gteievetlv
 301 edeaeagavn kgtgkyasre sflvmrdrkmk ekgidvrasl dnskeveqeq aaraammnn
 361 gngmgmgmgn gmngmgmgn gmaggakpgl eltpqlqpgr vilavpdism vdffreqfaq
 421 lsvtyelvpa lgadntlalv tqaqnlggvd fvlihpeflr drsstsilsr lrgagqravaa
 481 fgwaqlgpmr dliesanldg wlegpsfqeq ilpahivalv akmqqmrkmc qmqqigmmtg
 541 gmngmgggmg ggmngmgggn gmnnmgngmg ggmngmgggn gmngmgggng mnnmgngma
 601 gmngggmgmngmngm ssgvvanvtp saaggmgmngmngmngmngmngmngmngmngm
 661 plfnaapspl ssqqlgaaeagm gsmggmggms gmggmggmngmngmngmngmngmngm
 721 lqnlmneinr lkrelge

[48] A wild type (WT) Chop2 of the invention may be encoded by the following Chlamydomonas reinhardtii acop2 mRNA for archaeal-type opsin 2 nucleic acid sequence (GenBank Accession No. AB058891, and SEQ ID NO: 7):

1 catctgtcgc caagcaagca ttaaacatgg attatggagg cgcctgagt gccgttgggc
 61 gcgagctgtc attttaacg aacccagtag tcgtcaatgg ctctgtactt gtgcctgagg
 121 accagtgtta ctgcgcggc tggattgagt cgctgtggcac aaacgggtcc caaacggcg
 181 cgaacgtgct gcaatggctt octqctggct tctccatctt actgcttatg ttttacgcct
 241 accaaacatg gaagtcaacc tcggcgtggg aggagatcta tttgtgcgtc atcgagatgg
 301 tcaagggtat tctcgagttc ttcttcgagt ttaagaaccc gtccatgctg tatctagcca
 361 caggccaccc cggtcagtttgg ttgcgttacg ccgagttggct ttcacactgc ccggtcattc
 421 tcattcacct gtcacaaacatg acgggcttgc ccaacgacta cagcaggcgc accatgggc
 481 tgcttgcgtc tgatattggc acaattgtgt ggggcgcac ttccgcattt gccaccggat
 541 acgtcaaggt catcttccttc tgcctgggtc tttgttatgg tcttaacacg ttctttcacg
 601 ctgccaaggc ctacatcgag ggttaccaca ccgtggcggaa gggccgggtt cggcagggtt
 661 tgactggcat ggcttggctc ttcttcgtat catgggttat gttccccatc ctgttcatcc
 721 tcggccccca gggcttcggc tgcctgagcg ttgtacggctc caccgtcggc cacaccatca
 781 ttgacctgtat gtcgaaagaaac tgctgggtc tgctggccca ctacatcgcc gtgtctatcc
 841 acgagcatat cctcatccac ggccgacattc gcaagaccac caaattgaac attgggtggca
 901 ctgagatgtg ggtcgagacg ctgggtggagg acgaggccga ggctggcgcg gtcaacaagg
 961 gcacccggcaaa gtacccctcc cgcgagtcct tcctggcat gcgcgacaagg atgaaggaga
 1021 agggcatgtg cgtgcgcgccc tctctggaca acagaacaggaa ggtggagcag ggcaggccg
 1081 ccagggtcgc catgtatgtg atgaacaggca atggcatggg tatgggaatg ggaatgaacg
 1141 gcatgaacggg aatggggcggt atgaacgggg tggctggcgcc cggcaaggccc ggcctggagc
 1201 tcactccgca gtcacagccc ggccgcgtca tcctggcggt gccggacatc agcatgggt
 1261 acttcttcggc cgagcagttt gtcacgtat cggtgacgtt cggactgggtt ccggccctgg
 1321 ggcgtaccaa cacatggcg ctgggtacgc aggcgcagaa cctggggcgcc gtggactttt
 1381 tggtgattca ccccgagttc tcgcgcgacc gtcctagcac cagcatcctt agccgccttc
 1441 gcccggccggg ccagcggtgt gtcgtttcg gtcggcgca gtcggggccc atgcgtgacc
 1501 tgatcgagtc cgaaacatgg gacggctggc tggaggccc ctcgttcggaa cagggcatcc
 1561 tgccggccca catcggtgcc ctgggtggcca agatgcacca gatgcgcacat atgcagcaga
 1621 tgcagcagat tggcatgtat accggcgccca tgaacggcat gggccggcggt atggggcgcc
 1681 gcatgaacggg catggggcgccca ggcaacggca tgaacaacat gggcaacggc atggggcgcc
 1741 gcatggggcaaa cggcatgggc ggcacatggca tgaacggat ggggtggcgcc aacggcatg
 1801 acaacatggg cggcaacggca atggccggca acggaaatggg cggccggcatg ggcggcaacg
 1861 gtatgggtgg ctccatgtat ggcacatggat cccggctgggt ggcacacgtg acgcctccg
 1921 cccggccggcc catggggcgcc atgtatgtat ggcacatggc tggccccccatc tggccccgg
 1981 tgaacggcgcc cccgcgtgggtt accaaaccgc tcttcacacgc cgcgcctca cccgtcagct
 2041 cgcacgtccgg tggcgaggca ggcacatggca gcatggggagg catggggcgca atgagcgaa
 2101 tgggaggcat ggggtggatg gggggcatgg gcccggccgg cccggccacgc acgcacggct
 2161 cggggcgccaa cgcggaggcg gatgtctgc agaatctcat gacacgagatc aatgccttgc
 2221 agcgcgagct tggcgagttaa aaggctggag gcccgtactg cgtacactgc gacgtcgcc
 2281 gcctgactcg tcgtacacac ggctcaggag caccgcgcgc tgacttctc aacctgtgt
 2341 caacgtatct agacggccct gtcgcgcacc gtcgcgtgaccc attcgggtgc gatctcccg
 2401 ctttcgcacc gcaagttcccc ttccgtggccc tgctgcgcct gacgcac

[49] A wild type (WT) Chop2 of the invention may be encoded by the following Chlamydomonas reinhardtii acop2 mRNA for archaeal-type opsin 2 amino acid sequence (GenBank Accession No. BAB68567, and SEQ ID NO: 8):

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1 mdyggalsav grellfvtnp vvvngsvlvp edqcycaagwi esrgtngaqt asnvlqwlaa
61 gfsilllmfy ayqtwkstcg weeiyyvcaie mvkvilefff efknpsmlyl atghrvqwlr
121 yaewlltcpv ilihlsnltg lsndysrrtm gllvsdigti vwgatsamat gyvkviffcl
181 glcygantff haakayiegy htvpkgrcrq vvtgmawlff vswgmpfplf ilgpefgv
241 svygstvght iidlmsknkw gllghylrwl ihehilihgq irkttklnig gteievetlv
301 edeaeagavn kgtgkyasre sflvmrdrkmk ekgidvrasl dnskeveqeq aaraammnn
361 gngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngm
421 1sptyelvpa lgadntlalv tqaqnlggvfd fvlihpeflr drsstsilr lrgagqravaa
481 fgwaqlgpmr dliesanldg wlegpsfqqg ilpahivalv akmqqmrkmq qmqqigmmmtg
541 gmnmgmgmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngm
601 gngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngmngm
661 plfnaapspl ssqlgaeagm gsmgmgmngmngmngmngmngmngmngmngmngmngm
721 lqnlmneinr lkrelge

```

ChR2 mutants

[50] The present invention provides Chop2 mutants wherein one or more amino acids are mutated. In some embodiments, the Chop2 is the full-length polypeptide, such as SEQ ID NOs: 2, 4, 6, and 8, with at least one amino acid mutation. In some embodiments, the mutation is at amino acid 132 and/or amino acid 159. In some preferred embodiments, the amino acid at position 132 is mutated from a leucine to a cysteine or an alanine. In some preferred embodiments, the amino acid at position 159 is mutated from a threonine to an alanine, a cysteine, or a serine. In all embodiments, the Chop2 mutants form a functional ChR2 channel.

[51] The present invention also encompasses Chop2 proteins and nucleic acids that encode a biologically active fragment or a conservative amino acid substitution or other mutation variant of Chop2. Non-limiting examples of useful fragments include polypeptides encoding amino acids 1-315 of the wild-type Chop2, *i.e.*, SEQ ID NO: 26, wherein at least one amino acid is mutated or conservatively substituted, for example at amino acid positions 132 and/or 159. Smaller fragments of wild-type Chop2, wherein at least one amino acid is mutated or conservatively substituted (*i.e.*, at amino acid positions 132 and/or 159) may also be useful in the present invention. Accordingly, Chop2 polypeptides and nucleic acids of the present invention further include, but are not limited to, biologically active fragments encoding amino acids 1-315, 1-310, 1-300, 1-275, 1-250, 1-225, 1-200, 1-175, or 1-160 of the wild-type

Chop2, wherein at least one amino acid is mutated or conservatively substituted, for example at amino acid positions 132 and/or 159. In other embodiments, the Chop2 polypeptides and nucleic acids of the present invention can be up to, or about, 315 amino acids long, 310 amino acids long, 300 amino acids long, 275 amino acids long, 250 amino acids long, 225 amino acids long, 200 amino acids long, 175 amino acids long, or 160 amino acids long.

[52] A single mutant Chop2 of the invention may be encoded by the following Synthetic construct hVChR1-mKate-betaChR2(L132C) gene sequence (GenBank Accession No. JN836746, and SEQ ID NO: 9) with the following annotations, GFP sequence is in **bold**, L132C Chop2 sequence is underlined::

```

1 atggattacc ctgtggcccg qtccctgatt gtaagatacc ccaccgatct gggcaatgga
61 accgtgtca tgcccgagg acaatgctac tgcgaggggt ggctgaggag ccggggcact
121 agtatacgaaa aaaccatcgc tatcacccctc cagtggttag tttcgctct gtccgtagcc
181 tgtctcggt ggtatgcata ccaagcctgg agggctaccc ttgggtggga ggaagtatac
241 gtggccctga tcgagatgtat gaagtccatc atcgaggctt tccatgagtt cgactcccc
301 gccacactct ggctcagcag ttggaatggc gtagtgttga tgagatatgg agagtggctg
361 ctgacctgtc ccgtccgtct cattcatctg tccaaatctga cccggctgaa agatgactac
421 tccaaagagaa caatgggact gctgtttagt gacgtgggtt gtatttgtt gggagccacc
481 tccgcccattgt gcactggatg gaccaagatc ctcttttcc tgatttccct ctccatggg
541 atgtatacat acttccacgc cgctaaagggt tatattgagg cttccacac tgcacccaa
601 ggcacatgtt gggagctgtg cgggtttagt gcatggaccc tctttgttgc ctggggatg
661 ttccccgtgc tggccatccctt cggcacttagt ggattttggcc acattatgtcc ttacgggtcc
721 gcaattggac actccatccctt gatctgtt gccaagaata tgggggggt gctggaaat
781 tatctgcggg taaagatcca cgacatatc ctgtgtatg gcatatcag aaagaagcag
841 aaaatccca ttgtggaca gaaatggag gtggagacac tggtagcaga ggaggaggac
901 gggaccgcgg tcgcacat ggtgttcaag ggcgaagac tgattaagga gaacatgcac
961 atgaagctgt acatggaggg caccgtgaac aaccacact tcaagtgcac atccggggc
1021 gaaggcaagc cctacgggg cacccagacc atgagaatca aggtggtcga ggcggccct
1081 ctccccatcg ccttcgacat cctggctacc agtttcatgt acggcagcaa aacccatccatc
1141 aaccacaccc agggcatccc cgacttctt aaggacttct tccctgagggg cttcacatgg
1201 gagagatca ccacatcga agacgggggg gtgtgttgc ctacccagga caccagccct
1261 caggacggct gcctcatctt caacgttcaag atcagagggg tgaacttccc atccaaacggc
1321 cctgtatgc agaagaaaac actcggtgg gaggcttca ccgagatgt gtacccctgt
1381 gacggcgcc tggaaaggcag agccgacat gcccctgaagc tcgtggccgg gggcccacctg
1441 atctgcaact tgaagaccac atacagatcc aagaaaccc ctaagaacct caagatgccc
1501 ggcgttact atgtggacag aagactggaa agaatcaagg agccgacaa agagacctac
1561 gtcgagcagc acgagggtggc tgtggccaga tactgcgacc tccttagcaa actggggcac
1621 aaacttaatt gcctgcagga gaagaagtca tgcagccagc gcatggccga attccggcaa
1681 tactgttgg accggacac tggcagat ctggggcgca ccccagcccg gtgggtgtgg
1741 atcagctgt actatgcagc tttctacgt gtcatgact ggcttccatc cttgcgtatc
1801 tatgtgtca tgacgaccat tgtatccatc accccccact accaggacca gttaaagtca
1861 ccgggggtaa ccttgagacc ggatgtgtat ggggaaagag ggctgcagat ttccatcacac
1921 atctctgaaa acagctctag acaggcccc atcaccggac gtccggagac ttagacattg
1981 ccacccgtgg actacggggg ggccctgagc gctgtggca gagaacttct gttcgtgaca
2041 aatccatcg tggtaacgg ctccgtactc gtacccgagg atcgtgcta ttgcgcagga
2101 tggatcgaga gcagaggcac aaacggcgc cagactgc ccaaacgtgt ccagtggtt
2161 gcccgcagct tttccattct cctgttcat ttttacgcct accagactt gaagtccaca
2221 tgtggctgg aggaaatcta cgtgtgtca atcgaaatgg tgaaggtt cttggatgtt
2281 ttcttcgaaat ttaaaaaccc aagcatgtt tacctggcta ctggccacag agtgcagtt
2341 ctgcggatgt ccgaatggct gctgacttgc ccagtgattt gcatccacct gtccaaacctg
2401 actgggcgtgt ctaacgatta cagtaggaga acaatggac tgctcgatc cgacatcgcc
2461 actatcgat ggggcgcaac tagtgccat gccactggat acgtgaaagt gatcttcttc

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2521 tgcctggac tctgctacgg agcaaacaca tttttcatg ccgcaaaagc atatatcgag
 2581 gggtatcata ccgtcccaa gggccgggt agacaagtgg tgactggcat ggcttggctg
 2641 ttcttcgtgt cctggggat qttccatc ctcttatcc tggccca gaggctcggg
 2701 gtgctgagtg tgtatggcag taccgttagga cacactatca ttgacctgat gagcaaaaac
 2761 tgctggggc tgctcgccca ctacctgaga gtactcatcc acgagcatat cctgattcat
 2821 ggcgatatcc gaaaaactac caagctaat atcggggca ccgagattga agtggagaca
 2881 ctcgtggagg acggggcga ggcggagca gtgaacaaag gcaactggcaa gtatgcctcc
 2941 agagaatct ttctgtat gcccggacaaa atgaaggaga aaggcattga tgtacggtgc
 3001 agtaatgcca aagccgtcga gactgatgt tag

[53] A single mutant ChR2 of the invention may be encoded by the following Synthetic construct hVChR1-mKate-betaChR2(L132C) amino acid sequence (GenBank Accession No. AER29839, and SEQ ID NO: 10) with the following annotations, GFP sequence is in **bold**, L132C Chop2 sequence is underlined:

1 mdypvarsli vryptdlngng tvcmprrgqcy cegwlrsrgt siektiaitl qvvvfalsva
 61 clgwyayqaw ratcweevy valiemmkki ieafhefdsp atlwlssng vvwmygewl
 121 ltcpvllihl snltglkddy skrtmgllvs dvgcivwgat samctgwtki lfflislyg
 181 mytyfhaavk yieafhtvpk gicrelrvrm awtfvawgm fpvlflgte qfghispygs
 241 aighsildli aknmwgvlgn ylrvihehi llygdirkkq kitiaqgome vetlvaeed
 301 gtavatmvsk **geelikenmh** **mklymegtvn** **nhhfkctseg** **egkpyegtqt** **mrikvveggp**
 361 **lpfafdilat** **sfmygsktfi** **nhtqgipdff** **kqsfpegftw** **ervttyedgg** **vltatqdtsl**
 421 **qdgccliynvk** **irgvnfpsng** **pvmqkktlgw** **eastemlypa** **dgglegradm** **alklvvggħl**
 481 **icnlkttysr** **kkpaknlkmp** **gvyyvdrrle** **rikeadkety** **veqhevavar** **ycdlpsklgh**
 541 klnclqekks csqrmaefrq ycwnpdtqqm lgrtparww islyyaafyv vmtglfalci
 601 yvlmqtidpy tpdyqdqlks pgvtlrvpdv gerglqisyn isenssrqaq itgrpetetl
 661 ppvdyygals avgrellfvt npvvvngsrl vpedqycag wiesrgtnga qtasnvlqwl
 721 aagfsillim fyayqtwkst cgweeiyvca iemvkvilef ffefknpmsl ylatghrvqw
 781 lryaewlltc pvcihlsnl tglndysrr tmglvdsdig tivwgatsam atgyvkviff
 841 clglcygant ffhaakayie gyhtvpkgrc rqvvtgmawl ffvswgmfpf lfilgpefġ
 901 vlsvygstvg htiidlmskn cwgllghylr vlihehilih gdirkttkl iggteievet
 961 lvedeaeaga vnkgtgkyas resflvmrdk mkekgidvrc snakavetdv

[54] A single mutant Chop2 of the invention may be encoded by the following Synthetic construct hVChR1-mKate-betaChR2(L132C) gene sequence (GenBank Accession No. JN836745, and SEQ ID NO: 11) with the following annotations, GFP sequence is in **bold**, L132C Chop2 sequence is underlined:

1 atggattacc ctgtggcccg qtcctgatt gtaagatacc ccaccgatct gggcaatgg
 61 accgtgtca tgccctgagg acaatgtac tgcgggggt ggctgaggag ccggggcaact
 121 agtatcgaaa aaaccatcgcc tatcacccctc cagtgggtag tttcgctct gtccgtagcc
 181 tgtctcggt ggtatgcata ccaagctgg agggctacct gtgggtggga ggaagtatac
 241 gtggccctga tcgagatgtat gaagtccatc atcgaggctt tccatgagtt cgactcccc
 301 gcccacactc ggctcagcag tggaatggc gtagtgtgaa tgagatatgg agagtggctg
 361 ctgacctgtc ccgtccctgct cattcatctg tccaaatctga cccggctgaa agatgactac
 421 tccaaagagaa caatggact gctggtagt gacgtgggt gtattgtgtg gggagccacc
 481 tccgccccatgt gcactggatg gaccaagatc ctcttttcc tggatccctt ctccatgg
 541 atgtatacat acttccacgc cgctaaagggt tatattgagg cttccacac tggatctaaa
 601 ggcacatgtc gggagctcggt ggggtgtatg gcatgacact tctttgtggc ctggggatg
 661 ttccccgtgc tggatccctt cggactgtggat gggatggcc acattagtc ttacgggtcc
 721 gcaattggac actccatctt ggtatctgatt gccaagaata tgggggggt gctggaaat
 781 tatctcgccgg taaagatcca cgagcatatc ctgtgtatg gcatgatcag aaagaagcag
 841 aaaatccacca ttgtggaca gaaatggag gtggagacac tggatcaga ggaggaggac
 901 gggaccgcgg tcgccccatgt ggtatctgat ggcgaagagc **tgattaagga** **gaacatgcac**

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961 atgaagctgt acatggaggg caccgtgaac aaccaccact tcaagtgcac atccgagggc
1021 gaaggcaagc cctacgaggg caccaggacc atgagaatca aggtggctga gggcggccct
1081 ctcccctcg ccttcgacat cctggctacc agcttcatgt acggcagcaa aaccttcatc
1141 aaccacaccc agggatccc cgacttctt aagcgtctt ccctgaggg cttcacatgg
1201 gagagagtc acacatacga agacggggc gtgctgaccg ctacccagga caccagcctc
1261 caggacggct gcctcatcta caacgtcaag atcagagggg tgaacttccc atccaacggc
1321 cctgtatgc agaagaaaac actcggtgg gaggcctcca cccgatgtctt gtaaaaaacgt
1381 gacggcgccc tggaaaggcag agccgacatg gccctgaagc tcgtggcg gggccacctg
1441 atctgcaact tgaagaccat acatagatcc aagaacccct ctaagaacact caagatgecc
1501 ggcgtctact atgtggacag aagactggaa agaataaagg agggcggacaa agagacccatc
1561 gtcgagcagc acgagggtggc tggccaga tactgcgacc tccttagcaa actggggcac
1621 aaacttaatt gcctgcagga gaagaagtc tgccggccagc gcatggccga attccggcaa
1681 tactgttgg accccggacac tggggcagatg ctggggccga ccccagcccg gtgggtgtgg
1741 atcagcctgt actatgcagc ttctacgtg gtcatgactg ggctttgc ctgtgcac
1801 tatgtgtca tgccagaccat tgatccctac accccggact accaggacca gttaaagtca
1861 cgggggttaa ccttggagacc ggtatgtgtat ggggaaagag ggctgcagat ttccctacaac
1921 atctctgaaa acagctctag acaggcccgat acaccggac gtcggagac tgagacattg
1981 ccacccggggg actacggggg ggcctgagc gctgtggc gagaactcct gttcgtgaca
2041 aatccatcg tggtaacgg ctccgtactc gtacccggg atcagtgcata ttgcqcgagga
2101 tggatcgaga gcagaggcac aaacggcgca cagactgcata ccaacgtgct ccagtgggtt
2161 gccgcaggtt ttccattct cctgtcatg ttacgcctt accagactt gaaatccaca
2221 tggctggggg agggaaatcta cgtgtgtca atcgaatgg tgaagggtat cctggagttt
2281 ttcttcgaat ttaaaaaccc aagcatgctg tacctggcta ctggccacag agtgcagttt
2341 ctgcggatcg ccgaatggct gctgacttgc ccagtgttcc tcatccacatc gtccaaacctg
2401 actgggtgtt ctaacgatta cagtaggaga acaatgggac tgctgtatc cgacatcgcc
2461 actatcgat gggcgcaac tagtgcattt ggcactggat acgtgaaagt gatcttcttc
2521 tgcctggac tctgcgtacgg agcaaaacaca tttttcatg ccgcaaaagc atatatcgag
2581 gggatcata ccgtccaaa gggccgggtt agacaagtgg tgactggcat ggcttggctg
2641 ttcttcgtgt cctggggat gtttccatc ctcttcatc tggggccaga aggcttcggg
2701 gtgctgatgt tggatggcag taccgttagga cacactatca ttgacactgat gagcaaaaac
2761 tgctggggc tgctcgccca ctacctgaga gtactcatcc accagcatat cctgattcat
2821 ggcgatatacc gggaaaactac caagctcaat atcggggca ccgagattga agtggagaca
2881 ctcgtggagg acggggccga ggccggagca gtgaacaaag gcaactggcaa gtatgcctcc
2941 agagaatcc ttctgggtat gcccggacaaa atgaaggaga aaggcattga tgcgttgc
3001 agtaatgcca aagccgtcga gactgtatgt tag

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[55] A single mutant Chop2 of the invention may be encoded by the following Synthetic construct hVChR1-mKate-betaChR2(L132C) amino acid sequence (GenBank Accession No. AER29838, and SEQ ID NO: 12) with the following annotations, GFP sequence is in **bold**, L132C Chop2 sequence is underlined:

```

1 mdypvarsli vryptdlngt tvcmpqgcy cegwlrsgt siektiaitl qvvvfalsva
61 clgwyayqaw ratcweevy valiemmmksi ieafhefdsp atlwlssng vvvwmrygewl
121 ltcpvllihl snltglkddy skrtmgllvs dvgcivwgat samctgwtki lfflislsyg
181 mytyfhaavk yieafhtvpk gicrelrvrm awtfvawgm fpvlfllgte gfghispygs
241 aighsildli aknmwgvlgn ylrvihehi llygdirkkq kitiaqgome vetlvaeed
301 gtavatmvsk geelikenmh mklymegtvn nhhfkctseg egkpyegtgt mrikvveggp
361 lpfafdilat sfmygsktfi nhtqgipdff kqsfpiegftw ervttyedgg vltatqdtsl
421 qdgcliynvk irgvnfpstng pvmqkktlgw eastemlypa dgglegradm alklvggghl
481 icnlkttys kkpaknlkmp gvyyvdrrtle rikeadkety veqhevavar ycdlpsklgh
541 klnclqekks csqrmaefrq ycwnpdtggm lgrtparww islyyaafyv vmtglfalc
601 yvlmqtidpy tpdyqdqlks pgvtrlpdvy gerglqisyn isenssrqaq itgrpetetl
661 ppvdyggals avgrellfvt npvvvngsrl vpedqycag wiesrgtnga qtasnvlqwl
721 aagfsilllm fyayqtwkst cgweeiivca iemvkvilef ffefknpsml ylatghrvqw
781 lryaewlltc pvlilihsnl tglndysrr tmglvdsdig tivwgatsam atgyvkviff
841 clglcygant ffhaakayie gyhtvpkgrc rqvvtgmawf ffvswgmfpf lfilgpeffg
901 vlsvygstvg htiidlmksn cwglighylr vlihehilih gdirkttkln iggtieiev

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961 lvedeaeaga vnkgtgkyas resflvmrdk mkekgidvrc snakavetdv

[56] A L132C single mutant Chop2 of the invention may be encoded by the following amino acid sequence (positions 132 underlined and bolded, SEQ ID NO: 13):

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1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
121 YAEWLLTCPV ICIHLSNLTG LSNDYSRTM GLLVSDIGTI VWGATSAMAT GYVKVIFFCL
181 GLCYGANTFF HAAKAYIEGY HTVPKGCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
301 EDEAEAGAVN KGTGK

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[57] A T159C single mutant Chop2 of the invention may be encoded by the following amino acid sequence (positions 159 underlined and bolded, SEQ ID NO: 14):

```

1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
121 YAEWLLTCPV ILIHLSNLTG LSNDYSRTM GLLVSDIGCI VWGATSAMAT GYVKVIFFCL
181 GLCYGANTFF HAAKAYIEGY HTVPKGCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
301 EDEAEAGAVN KGTGK

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[58] A L132C/T159C double mutant Chop2 of the invention may be encoded by the following nucleotide sequence (SEQ ID NO: 15):

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1 atggactaacg ggggggctct gtctgctgtc gggagggAAC tgctgtttgt gactaacccct
61 gtcgtcgtga acggaggtgt gctggccct gaggaccagt gctactgtgc cggctggatc
121 gaatcacgcg gaaccaacgg ggcccagaca gctagcaatg tgctgcagtg gctggccgct
181 gggtttagta tcctgctgt gatgttctac gcctatcaga cttggaagtc aactgcggc
241 tgggagggaaa tctacgtgtg cgctatttgg atggtaaaag tgatctgga gttcttcttc
301 gagttcaaga acccaagcat gctgtacccg gctactggac accgagtgca gtggctgaga
361 tatgcagaat ggctgctgac atgccccgtc atctgcattc acctgtccaa cctgacaggc
421 ctgagcaatg actactccag gagaactatg ggactgctgg tgcccgacat cggctgcatt
481 gtctggggag caacttctgc tatggcaacc ggatacgtga aggtcatctt tttctgcctg
541 gggctgtgt atggcgcaaa taccttttc cacgcagcca aggcctacat tgaggggat
601 cataccgtgc caaaaggccc gtgcccgacag gtggcacag gaatggcttg gctttttc
661 gtcttttgg gaatgttcc catcctgttc attctggggc ctgaagggtt cggcgtgctg
721 tctgtctacg gaagttacgtt ggggcataact atcattgacc tgatgtccaa aaactgttgg
781 ggcctgtgg gacactatct gagagtgtcg atccacgcg atatcctgtat tcatggcgat
841 attcggaaaga ccacaaaact gaatatcggc ggaaccgaga ttgaagtgga aacactgggt
901 gaagacgagg ctgaggctgg ggctgtgaac aaggggactg gcaaa

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[59] A L132C/T159C double mutant Chop2 of the invention may be encoded by the following amino acid sequence (positions 132 and 159 underlined and bolded, SEQ ID NO: 16):

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1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR

```

121 YAEWLLTCPV **ICIHL**SNLTG LSNDYSRRTM GLLVSDIG**C**I VWGATSAMAT GYVKVIFFCL
 181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
 241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
 301 EDEAEAGAVN KGTGK

[60] A T159S single mutant Chop2 of the invention may be encoded by the following amino acid sequence (positions 159 underlined and bolded, SEQ ID NO: 17):

1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
 61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
 121 YAEWLLTCPV **ICIHL**SNLTG LSNDYSRRTM GLLVSDIG**S**I VWGATSAMAT GYVKVIFFCL
 181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
 241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
 301 EDEAEAGAVN KGTGK

[61] A L132C/T159S double mutant Chop2 of the invention may be encoded by the following nucleotide sequence (SEQ ID NO: 18):

1 atggactacg ggggggctct gtctgctgtc gggagggAAC tgctgtttgt gactaacccct
 61 gtcgtcgta acgggagtgt gctggccct gaggaccagt gctactgtgc cggctggatc
 121 gaatcacgcg gaaccaacgg gccccagaca gctagcaatg tgctgcagtg gctggccgct
 181 gggtttagta tcctgctgct gatgttctac gcctatcaga cttggaaagtc aacctgcggc
 241 tgggagggaaa tctacgtgtc cgctattgag atggtaaag tgatcctgga gttttcttc
 301 gagttcaaga acccaagcat gctgtacctg gctactggac accgagtgca gtggctgaga
 361 tatgcagaat ggctgctgac atgccccgtc atctgcattc acctgtccaa cctgacaggc
 421 ctgagcaatg actactccag gagaactatg ggactgctgg tgtccgacat cggcagcatt
 481 gtctggggag caacttctgc tatggcaacc ggatacgtga aggtcattttttctgcctg
 541 gggctgtgtct atggcgcaaa taccttttc cacgcagcca aggcctacat tgaggggtat
 601 cataccgtgc caaaaaggccg gtgccgacag gtggcacag gaatggcttg gctgtttttc
 661 gtctcttggg gaatgtttcc catcctgttc attctggggc ctgaagggtt cggcgtgctg
 721 tctgtctacg gaagtagactt ggggcataact atcattgacc tgatgtccaa aaactgttgg
 781 ggcctgctgg gacactatct gagagtgtct atccacgagc atatcctgat tcataggcgat
 841 attcggaaaga ccacaaaact gaatatcgcc ggaaccgaga ttgaagtggaa aacactggat
 901 gaagacgagg ctgaggctgg ggctgtgaac aaggggactg gcaaa

[62] A L132C/T159S double mutant Chop2 of the invention may be encoded by the following amino acid sequence (positions 132 and 159 underlined and bolded, SEQ ID NO: 19):

1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
 61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
 121 YAEWLLTCPV **ICIHL**SNLTG LSNDYSRRTM GLLVSDIG**S**I VWGATSAMAT GYVKVIFFCL
 181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
 241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
 301 EDEAEAGAVN KGTGK

[63] A L132A single mutant Chop2 of the invention may be encoded by the following amino acid sequence (position 132 underlined and bolded, SEQ ID NO: 20):

1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
 61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
 121 YAEWLLTCPV **I**AILHSLNLTG LSNDYSRRM GLLVSDIGTI VWGATSAMAT GYVKVIFFCL
 181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
 241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
 301 EDEAEAGAVN KGTGK

[64] A L132A/T159C double mutant Chop2 of the invention may be encoded by the following nucleotide sequence (SEQ ID NO: 21):

1 ATGGACTACG GGGGGGCTCT GTCTGCTGTC GGGAGGGAAC TGCTGTTTGT GACTAACCCCT
 61 GTCGTCGTGA ACGGGAGTGT GCTGGTCCCT GAGGACCAAGT GCTACTGTGC CGGCTGGATC
 121 GAATCACCGCG GAACCAACCGG GGCCCCAGACA GCTAGCAATG TGCTGCAGTG GCTGCCGCT
 181 GGGTTTAGTA TCCTGCTGCT GATGTTCTAC GCCTATCAGA CTTGGAAGTC AACCTGCGGC
 241 TGGGAGGAAA TCTACGTGTG CGCTATTGAG ATGGTGAAAG TGATCCTGGA GTTCTTCTTC
 301 GAGTTCAAGA ACCCAAGCAT GCTGTACCTG GCTACTGGAC ACCGAGTGCA GTGGCTGAGA
 361 TATGCAGAAT GGCTGCTGAC ATGCCCGTC ATCGCCATTG ACCTGTCCAA CCTGACAGGC
 421 CTGAGCAATG ACTACTCCAG GAGAACTATG GGACTGCTGG TGTCCGACAT CGGCTGCATT
 481 GTCTGGGGAG CAACATTCTGC TATGGCAACC GGATACGTGA AGGTCTACATT TTTCTGCCTG
 541 GGGCTGTGCT ATGGCGCAAA TACCTTTTC CACGCCAGCCA AGGCCTACAT TGAGGGGTAT
 601 CATACCGTGC CAAAAGGCCG GTGCCGACAG GTGGTCACAG GAATGGCTTG GCTGTTTTC
 661 GTCTCTTGGG GAATGTTCC CATCCTGTT ATTCTGGGGC CTGAAGGGTT CGGCGTGCCTG
 721 TCTGTCTACG GAAGTACAGT GGGGCATACT ATCATTGACC TGATGTCCAA AAACGTGTTGG
 781 GGCCTGCTGG GACACTATCT GAGAGTGCTG ATCCACGAGC ATATCCTGAT TCATGGCGAT
 841 ATTCGGAAGA CCACAAAAGT GAATATCGGC GGAACCGAGA TTGAAGTGGA AACACTGGTG
 901 GAAGACGAGG CTGAGGCTGG GGCTGTGAAC AAGGGGACTG GCAAA

[65] A L132A/T159C double mutant Chop2 of the invention may be encoded by the following amino acid sequence (positions 132 and 159 underlined and bolded, SEQ ID NO: 22):

1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
 61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
 121 YAEWLLTCPV **I**AILHSLNLTG LSNDYSRRM GLLVSDIG**C**I VWGATSAMAT GYVKVIFFCL
 181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
 241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
 301 EDEAEAGAVN KGTGK

[66] A T159A single mutant Chop2 of the invention may be encoded by the following amino acid sequence (position 159 underlined and bolded, SEQ ID NO: 23):

1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
 61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
 121 YAEWLLTCPV **I**LILHSLNLTG LSNDYSRRM GLLVSDIG**A**I VWGATSAMAT GYVKVIFFCL
 181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
 241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
 301 EDEAEAGAVN KGTGK

[67] A L132C/T159A double mutant Chop2 of the invention may be encoded by the following nucleotide sequence (SEQ ID NO: 24):

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1 atggactacg ggggggctct gtctgctgtc gggagggAAC tgctgttgt gactaaccct
61 gtcgtcgtga acggaggtgt gctggccct gaggaccagt gctactgtgc cggctggatc
121 gaatcacgcg gaaccaacgg ggcccagaca gctagcaatg tgctgcagtg gctggccgct
181 gggttttagta tcctgctgtc gatgttctac gcctatcaga ctggaaagtc aacctgcggc
241 tgggagggaaa tctacgtgtc cgctatttag gatggtaaag tgatcctgga gttcttcttc
301 gagttcaaga acccaagcat gctgtacctg gctactggac accgagtgcgatggctgaga
361 tatgcagaat ggctgctgac atgccccgtc atctgcattc acctgtccaa cctgacaggc
421 ctgagcaatg actactccag gagaactatg ggactgctgg tgtccgacat cggcgccatt
481 gtctggggag caacttctgc tatggcaacc ggatacgtga aggtcatctt tttctgcctg
541 gggctgtgtct atggcgcaaa taccttttc cacgcagccaa aggccatcat tgaggggtat
601 cataccgtgc caaaaggccg gtgcccacag gtggcacatggctg gctgttttc
661 gtcttttggg gaatgttcc catcctgttc attctggggc ctgaagggtt cggcgctg
721 tctgtctacg gaagtagtggctg gggcataact atcattgacc tgatgtccaa aaactgttgg
781 ggcctgctgg gacactatct gagagtgtctg atccacgcgat atacctgtat tcattggcgat
841 attcggaaaga ccacaaaact gaatatcggc ggaacccgaga ttgaagtggaa aacactggtg
901 gaagacgagg ctgaggctgg ggctgtgaac aaggggactg gcaaa

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[68] A L132C/T159A double mutant Chop2 of the invention may be encoded by the following amino acid sequence (positions 132 and 159 underlined and bolded, SEQ ID NO: 25):

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1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
121 YAEWLLTCPV ICIHSNLTG LSNDYSRRTM GLLVSDIGAI VWGATSAMAT GYVKVIFFCL
181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
301 EDEAEAGAVN KGTGK

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[69] A wild type (WT) Chop2 of the invention may be encoded by the following amino acid sequence (SEQ ID NO: 26):

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1 MDYGGALSAV GRELLFVTNP VVVNGSVLVP EDQCYCAGWI ESRGTNGAQT ASNVLQWLAA
61 GFSILLLMFY AYQTWKSTCG WEEIYVCAIE MVKVILEFFF EFKNPSMLYL ATGHRVQWLR
121 YAEWLLTCPV ILIHSNLTG LSNDYSRRTM GLLVSDIGTI VWGATSAMAT GYVKVIFFCL
181 GLCYGANTFF HAAKAYIEGY HTVPKGRCRQ VVTGMAWLFF VSWGMFPILF ILGPEGFGVL
241 SVYGSTVGHT IIDLMSKNCW GLLGHYLRVL IHEHILIHGD IRKTTKLNIG GTEIEVETLV
301 EDEAEAGAVN KGTGK

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[70] Mutant ChR2 proteins of the invention also demonstrate slower channel kinetics. Higher light sensitivity was found to correlate with slower channel kinetics, indicating a trade-off between light sensitivity and channel kinetics. Chop2 proteins that form the ChR2 proteins of the present invention may also comprise additional mutations or modifications that may improve channel kinetics, or increase the deactivation rate, of the ChR2. Particularly preferred ChR2 mutants balance the threshold of light sensitivity with channel kinetics.

Compositions and kits

[71] Compositions and kits of the invention comprise at least one nucleic acid

molecule or polypeptide molecule that encodes a mutant Chop2 protein, and the resulting ChR2, of the invention. The at least one nucleic acid molecule or polypeptide molecule that encodes a mutant Chop2 protein of the invention may further include a pharmaceutically-acceptable carrier. Kits of the invention further include instructions for administering a composition of the invention to a subject.

Therapeutic uses

[72] Mutations were made on a codon optimized Chop2-GFP fusion protein to create single and double mutations at the L132 (Leucine 132) and T159 (threonine 159) sites. The functional properties of each mutant ChR2, or a combination thereof, were first examined in HEK cells. AAV2 virus vectors carrying mutant Chop2-GFP constructs driven by CAG promoter were made and injected intravitreally into the eyes of adult mice. Mutant Chop2-mediated light responses were examined by using multi-electrode array recordings from whole-mount retinas.

[73] Single mutant ChR2, *i.e.*, L132 and T159C, markedly lower the threshold light intensity that is required to evoke a ChR2-mediated photocurrent. Moreover, several double mutant ChR2 variants, including L132C/T159C, L132A/T159C, and L132C/T159S, were found to further increase the photocurrent above the results of any single mutant ChR2 at low light intensities. The double mutants exhibited a slower off-rate, which is likely to contribute to the increased photocurrent at the low light intensities. Spiking activity of retinal ganglion cells mediated by the L132C/T159C double mutant was observed at the light intensity of 10^{13} photon/cm²/s and at the wavelength of 473 nm. This light level is about 1.5 to 2 log units lower than the light level that is required to elicit the spiking activity with wild-type ChR2. The spike firing of retinal ganglion cells expressing L132C/T159C could follow a light flicker frequency of up to 15 Hz. Ongoing studies are evaluating the long-term expression and safety of mutant ChR2s of the invention in retinal neurons.

[74] Furthermore, expression of the mutant Chop2 proteins, and the resulting ChR2 proteins, of the present invention was not found to cause neurotoxicity of up to two months after viral injection in mice, demonstrating the safety of the present invention for therapeutic use.

[75] Vectors for use in the present invention can include various viral vectors, such as plasmids and recombinant viruses, *i.e.*, recombinant adeno-associated virus (rAAV), recombinant adenoviruses, recombinant retroviruses, recombinant lentiviruses, and other viruses known in the art.

[76] In some embodiments, the expression of the Chop2 proteins of the present invention is driven by a constitutive promoter, *i.e.*, CAG promoter, CMV promoter, LTR. In other embodiments, the promoter is an inducible or a cell-specific promoter. Cell type-specific promoters that enable Chop2 protein expression in specific subpopulations of cells, *i.e.*, retinal neuron cells or degenerating cells, may be preferred. These cells may include, but are not limited to, a retinal ganglion cell, a photoreceptor cell, a bipolar cell, a rod bipolar cell, an ON-type cone bipolar cell, a retinal ganglion cell, a photosensitive retinal ganglion cell, a horizontal cell, an amacrine cell, or an AII amacrine cell. Cell type-specific promoters are well known in the art. Particularly preferred cell type-specific promoters include, but are not limited to mGluR6, NK-3, and Pcp2(L7).

[77] In some embodiments, use of different opsin genes in addition to the mutant Chop2 proteins of the present invention and targeted gene expression may further increase light sensitivity or improve vision. Visual information is processed through the retina through two pathways: an ON pathway which signals the light ON, and an OFF pathway which signals the light OFF. The existence of the ON and OFF pathway is important for the enhancement of contrast sensitivity. The visual signal in the ON pathway is relay from ON-cone bipolar cells to ON ganglion cells. Both ON-cone bipolar cells and ON-ganglion cells are depolarized in response to light. On the other hand, the visual signal in the OFF pathway is carried from OFF-cone bipolar cells to OFF ganglion cells. Both OFF-cone bipolar cells and OFF-ganglion cells are hypopolarized in response to light. Rod bipolar cells, which are responsible for the ability to see in dim light (scotopic vision), are ON bipolar cells (depolarized in response to light). Rod bipolar cells relay the vision signal through AII amacrine cells (an ON type retinal cells) to ON an OFF cone bipolar cells.

[78] Accordingly, a dual rhodopsin system can be used to recapitulate the ON and OFF pathways integral to visual processing and acuity. Briefly, a Chop2 protein of

the present invention can be specifically targeted to ON type retinal neurons (*i.e.*, ON type ganglion cells and/or ON type bipolar cells), while a hypopolarizing light sensor (*i.e.*, halorhodopsin or other chloride pump known in the art) can be targeted to OFF type retinal neurons (*i.e.* OFF type ganglion cells and/or OFF type bipolar cells) to create ON and OFF pathways. The specific targeting to preferred cell subpopulations can be achieved through the use of different cell type-specific promoters. For example, Chop2 expression may be driven by the mGluR6 promoter for targeted expression in ON-type retinal neurons (*i.e.*, ON type ganglion cells and/or ON type bipolar cells) while a hypopolarizing channel, such as halorhodopsin, expression is driven by the NK-3 promoter for targeted expression in OFF-type retinal neurons (*i.e.*, OFF type ganglion cells and/or OFF type bipolar cells).

[79] An alternative approach to restore ON and OFF pathways in the retina is achieved by, expressing a depolarizing light sensor, such as ChR2, to rod bipolar cells or AII amacrine. In this approach, the depolarization of rod bipolar cells or AII amacrine cells can lead to the ON and OFF responses at the levels of cone bipolar cells and the downstream retinal ganglion cells. Thus, the ON and OFF pathways that are inherent in the retina are maintained.

[80] The present invention can be formulated to a pharmaceutical composition or medicament suitable for administration into a subject or patient. Suitable routes of administration include, for example, intravitreal, intraocular, or subretinal injection.

[81] Such formulations comprise a pharmaceutically and/or physiologically acceptable vehicle, diluent, carrier or excipient, such as buffered saline or other buffers, *e.g.*, HEPES, to maintain physiologic pH. For a discussion of such components and their formulation, see, generally, Gennaro, AE., *Remington: The Science and Practice of Pharmacy*, Lippincott Williams & Wilkins Publishers; 2003 or latest edition). See also, WO00/15822. If the preparation is to be stored for long periods, it may be frozen, for example, in the presence of glycerol.

[82] The pharmaceutical composition described above is administered to a subject having a visual or blinding disease by any appropriate route, preferably by intravitreal or subretinal injection, depending on the retinal layer being targeted.

[83] Disclosures from Bennett and colleagues (cited herein) concern targeting of

retinal pigment epithelium – the most distal layer from the vitreal space. According to the present invention, the Chop2 construct or polypeptide is targeted to retinal cells, *i.e.*, retinal ganglion cells or bipolar cells. Such cells are known to be reasonably well-accessible to intravitreal injection as disclosed herein. Intravitreal and/or subretinal injection can provide the necessary access to the bipolar cells, especially in circumstances in which the photoreceptor cell layer is absent due to degeneration – which is the case in certain forms of degeneration that the present invention is intended to overcome.

[84] To test for the vector's ability to express the Chop2 mutants of the present invention, specifically in mammalian retinal neurons, by AAV-mediated delivery, a combination of a preferred promoter sequence linked to a reporter gene such as LacZ or GFP linked to a SV40 poly A sequence can be inserted into a plasmid and packaged into rAAV virus particles, concentrated, tested for contaminating adenovirus and titered for rAAV using an infectious center assay. The right eyes of a number of test subjects, preferably inbred mice, can be injected sub-retinally with about 1 μ l of the rAAV preparation (*e.g.*, greater than about 10¹⁰ infectious units ml). Two weeks later, the right (test) and left (control) eyes of half the animals may be removed, fixed and stained with an appropriate substrate or antibody or other substance to reveal the presence of the reporter gene. A majority of the test retinas in injected eyes will exhibited a focal stained region, *e.g.*, blue for LacZ/Xgal, or green for GFP consistent with a subretinal bleb of the injected virus creating a localized retinal detachment. All control eyes may be negative for the reporter gene product. Reporter gene expression examined in mice sacrificed at later periods is detected for at least 10 weeks post-injection, which suggests persistent expression of the reporter transgene.

[85] In one embodiment, the Chop2 constructs are packaged in adenoviral vectors for transgene delivery. An effective amount of rAAV virions carrying a nucleic acid sequence encoding the Chop2 DNA under the control of the promoter of choice, preferably a constitutive CMV promoter or a cell-specific promoter such as mGluR6, is preferably in the range of between about 10¹⁰ to about 10¹³ rAAV infectious units in a volume of between about 150 and about 800 μ l per injection. The rAAV

infectious units can be measured according to McLaughlin, SK *et al.*, 1988, *J Virol* 62:1963. More preferably, the effective amount is between about 10¹⁰ and about 10¹² rAAV infectious units and the injection volume is preferably between about 250 and about 500 μ l. Other dosages and volumes, preferably within these ranges but possibly outside them, may be selected by the treating professional, taking into account the physical state of the subject (preferably a human), who is being treated, including, age, weight, general health, and the nature and severity of the particular ocular disorder.

[86] It may also be desirable to administer additional doses (“boosters”) of the present nucleic acid(s) or rAAV compositions. For example, depending upon the duration of the transgene expression within the ocular target cell, a second treatment may be administered after 6 months or yearly, and may be similarly repeated. Neutralizing antibodies to AAV are not expected to be generated in view of the routes and doses used, thereby permitting repeat treatment rounds.

[87] The need for such additional doses can be monitored by the treating professional using, for example, well-known electrophysiological and other retinal and visual function tests and visual behavior tests. The treating professional will be able to select the appropriate tests applying routine skill in the art. It may be desirable to inject larger volumes of the composition in either single or multiple doses to further improve the relevant outcome parameters.

Ocular Disorders

[88] The ocular disorders for which the present Chop2 proteins, and the resulting ChR2 proteins, are intended and may be used to improve one or more parameters of vision include, but are not limited to, developmental abnormalities that affect both anterior and posterior segments of the eye. Anterior segment disorders include glaucoma, cataracts, corneal dystrophy, keratoconus. Posterior segment disorders include blinding disorders caused by photoreceptor malfunction and/or death caused by retinal dystrophies and degenerations. Retinal disorders include congenital stationary night blindness, age-related macular degeneration, congenital cone dystrophies, and a large group of retinitis-pigmentosa (RP)-related disorders. These

disorders include genetically pre-disposed death of photoreceptor cells, rods and cones in the retina, occurring at various ages. Among those are severe retinopathies, such as subtypes of RP itself that progresses with age and causes blindness in childhood and early adulthood and RP-associated diseases, such as genetic subtypes of LCA, which frequently results in loss of vision during childhood, as early as the first year of life. The latter disorders are generally characterized by severe reduction, and often complete loss of photoreceptor cells, rods and cones. (Trabulsi, EI, ed., *Genetic Diseases of the Eye*, Oxford University Press, NY, 1998).

[89] In particular, the Chop2 and ChR2 proteins of the present invention useful for the treatment and/or restoration of at least partial vision to subjects that have lost vision due to ocular disorders, such as RPE-associated retinopathies, which are characterized by a long-term preservation of ocular tissue structure despite loss of function and by the association between function loss and the defect or absence of a normal gene in the ocular cells of the subject. A variety of such ocular disorders are known, such as childhood onset blinding diseases, retinitis pigmentosa, macular degeneration, and diabetic retinopathy, as well as ocular blinding diseases known in the art. It is anticipated that these other disorders, as well as blinding disorders of presently unknown causation which later are characterized by the same description as above, may also be successfully treated by the Chop2 and ChR2 proteins of the present invention. Thus, the particular ocular disorder treated by the present invention may include the above-mentioned disorders and a number of diseases which have yet to be so characterized.

Optogenetics

[90] The emerging field of optogenetics involves the combination of genetic and optical methods to control specific events in targeted cells of a living tissue. Optogenetics may be used within freely moving mammals and other animals. Moreover, the temporal precision (millisecond-timescale) of optogenetic methods are sufficient to function within intact biological systems.

[91] The instant invention provides Chop2-gene therapy to retinal tissues of the

eye, by introducing into retinal cells a nucleic acid or polypeptide encoding for at least one mutant form of Chop2. Mutant Chop2/ChR2 proteins of the invention are specifically adapted to be light-activated at lower thresholds of light intensities than their wild type counterparts. Accordingly, the mutant Chop2/ChR2 proteins of the invention can be used to activate cells of the retina and visual system using less damaging sources of illumination. The mutant Chop2/ChR2 proteins also conduct larger photocurrents upon activation, resulting in a more robust or efficacious response from the mutant Chop2/ChR2-expressing cells.

[92] For example, mutant Chop2 proteins of the invention are administered to a subject through local, intravitreous or subretinal, injection of a nucleic acid molecule encoding a mutant Chop2, a mutant Chop2 polypeptide molecule, or a cell expressing a mutant Chop2/ChR2. Retinal cells of the subject express the mutant Chop2 proteins within the plasma membrane. When the transfected or transformed retinal cells encounter light radiation, the transfected or transformed retinal cells transduce an improved or restored signal.

[93] These methods may be used in subjects of normal and/or impaired vision. Chop2/ChR2 mutants of the invention may preserve, improve, or restore vision. Moreover, Chop2/ChR2 mutants of the invention are used to preserve, improve, or restore the transduction of non-visual information from photosensitive retinal ganglion cells to the brain.

[94] The term “vision” as used herein is defined as the ability of an organism to usefully detect light as a stimulus for differentiation or action. Vision is intended to encompass the following:

1. Light detection or perception - the ability to discern whether or not light is present;
2. Light projection - the ability to discern the direction from which a light stimulus is coming;
3. Resolution - the ability to detect differing brightness levels (*i.e.*, contrast) in a grating or letter target; and
4. Recognition - the ability to recognize the shape of a visual target by reference to the differing contrast levels within the target.

Thus, “vision” includes the ability to simply detect the presence of light. The polypeptides and polynucleotides encoding mutant Chop2 of the present invention can be used to improve or restore vision, wherein the improvement or restoration in vision includes, for example, increases in light detection or perception, increase in light sensitivity or photosensitivity in response to a light stimulus, increase in the ability to discern the direction from which a light stimulus is coming, increase in the ability to detect differing brightness levels, increase in the ability to recognize the shape of a visual target, and increases in visual evoked potential or transmission from the retina to the cortex. As such, improvement or restoration of vision may or may not include full restoration of sight, i.e., wherein the vision of the patient treated with the present invention is restored to the degree to the vision of a non-affected individual. The visual recovery described in the animal studies described below may, in human terms, place the person on the low end of vision function by increasing one aspect of vision (i.e., light sensitivity, or visual evoked potential) without restoring full sight.

Nevertheless, placement at such a level would be a significant benefit because these individuals could be trained in mobility and potentially in low order resolution tasks which would provide them with a greatly improved level of visual independence compared to total blindness. Even basic light perception can be used by visually impaired individuals, whose vision is improved using the present compositions and methods, to accomplish specific daily tasks and improve general mobility, capability, and quality of life.

[95] The degree of restoration of vision can be determined through the measurement of vision before, and preferably after, administering a vector comprising, for example, DNA encoding Chop2. Vision can be measured using any of a number of methods well-known in the art or methods not yet established. Vision, as improved or restored by the present invention, can be measured by any of the following visual responses:

1. a light detection response by the subject after exposure to a light stimulus – in which evidence is sought for a reliable response of an indication or movement in the general direction of the light by the subject individual when the light it is turned on;

2. a light projection response by the subject after exposure to a light stimulus in which evidence is sought for a reliable response of indication or movement in the specific direction of the light by the individual when the light is turned on;
3. light resolution by the subject of a light vs. dark patterned visual stimulus, which measures the subject's capability of resolving light vs dark patterned visual stimuli as evidenced by:
 - a. the presence of demonstrable reliable optokinetically produced nystagmoid eye movements and/or related head or body movements that demonstrate tracking of the target (see above) and/or
 - b. the presence of a reliable ability to discriminate a pattern visual stimulus and to indicate such discrimination by verbal or non-verbal means, including, for example pointing, or pressing a bar or a button; or
4. electrical recording of a visual cortex response to a light flash stimulus or a pattern visual stimulus, which is an endpoint of electrical transmission from a restored retina to the visual cortex, also referred to as the visual evoked potential (VEP). Measurement may be by electrical recording on the scalp surface at the region of the visual cortex, on the cortical surface, and/or recording within cells of the visual cortex.

[96] Thus, improvement or restoration of vision, according to the present invention, can include, but is not limited to: increases in amplitude or kinetics of photocurrents or electrical response in response to light stimulus in the retinal cells, increases in light sensitivity (*i.e.*, lowering the threshold light intensity required for initiating a photocurrent or electrical response in response to light stimulus, thereby requiring less or lower light to evoke a photocurrent) of the retinal cells, increases in number or amplitude of light-evoked spiking or spike firings, increases in light responses to the visual cortex, which includes increasing in visual evoked potential transmitted from the retina or retinal cells to the visual cortex or the brain.

[97] Both *in vitro* and *in vivo* studies to assess the various parameters of the present invention may be used, including recognized animal models of blinding human ocular disorders. Large animal models of human retinopathy, *e.g.*, childhood

blindness, are useful. The examples provided herein allow one of skill in the art to readily anticipate that this method may be similarly used in treating a range of retinal diseases.

[98] While earlier studies by others have demonstrated that retinal degeneration can be retarded by gene therapy techniques, the present invention demonstrates a definite physiological recovery of function, which is expected to generate or improve various parameters of vision, including behavioral parameters.

[99] Behavioral measures can be obtained using known animal models and tests, for example performance in a water maze, wherein a subject in whom vision has been preserved or restored to varying extents will swim toward light (Hayes, JM *et al.*, 1993, *Behav Genet* 23:395-403).

[100] In models in which blindness is induced during adult life or congenital blindness develops slowly enough that the individual experiences vision before losing it, training of the subject in various tests may be done. In this way, when these tests are re-administered after visual loss to test the efficacy of the present compositions and methods for their vision-restorative effects, animals do not have to learn the tasks *de novo* while in a blind state. Other behavioral tests do not require learning and rely on the instinctiveness of certain behaviors. An example is the optokinetic nystagmus test (Balkema GW *et al.*, 1984, *Invest Ophthalmol Vis Sci.* 25:795-800; Mitchiner JC *et al.*, 1976, *Vision Res.* 16:1169-71).

[101] The present invention may also be used in combination with other forms of vision therapy known in the art to improve or restore vision. For example, the use of visual prostheses, which include retinal implants, cortical implants, lateral geniculate nucleus implants, or optic nerve implants. Thus, in addition to genetic modification of surviving retinal neurons using the present methods, the subject being treated may be provided with a visual prosthesis before, at the same time as, or after the molecular method is employed. The effectiveness of visual prosthetics can be improved with training of the individual, thus enhancing the potential impact of the Chop2 transformation of patient cells as contemplated herein. Training methods, such as habituation training characterized by training the subject to recognize recognize (i) varying levels of light and/or pattern stimulation, and/or (ii) environmental

stimulation from a common light source or object as would be understood by one skilled in the art; and orientation and mobility training characterized by training the subject to detect visually local objects and move among said objects more effectively than without the training. In fact, any visual stimulation techniques that are typically used in the field of low vision rehabilitation are applicable here.

EXAMPLES

Example 1: Generation of labeled mutant Chop2 constructs.

[102] Mutations were made on a codon optimized Chop2-GFP fusion protein to create single and double mutations at the L132 (Leucine 132) and T159 (Threonine 159) sites. Several mutants were generated, for example, single mutants such as L132A, L132C, T159A, T159C, and T 159S, and double mutants such as L132C/T159C, L132C/T159S, L132A/T159C, and L132C/T159A. Chop2-GFP transgenes were cloned into a rAAV vector under the control of a CAG promoter using methods known in the art.

Example 2: In vitro analysis of mutant Chop2 constructs.

[103] The functional properties of each mutant Chop2, or a combination thereof, were first examined in HEK cells. Chop2 constructs were delivered to HEK cells by adenoviral infection, for example. Upon expression of the WT or mutant Chop2, functional WT and mutant ChR2 channels were formed. Measurements of the light sensitivity and other properties of the ChR2 channels were assessed as described herein. The light stimuli (photons/cm².s at 460 nm) were generated by a xenon arc lamp and attenuated by neutral density filters: ND4.0 (2.8x10¹⁴), ND3.0 (1.4x10¹⁵), ND2.5 (4.8x10¹⁵); ND2.0 (1.6x10¹⁶), ND1.0 (1.3x10¹⁷), ND0 (1.2x10¹⁸). Light evoked currents were measured from wild-type ChR2, T159C, L132C, L132C/T159C, and L132C/T159S. Patch clamp recordings were performed using methods known in the art.

[104] Representative recordings from this experiment comparing light sensitivity between the Chop2 constructs demonstrated that mutations at L132 alone or in combination with mutation at T159 show increased photocurrent in comparison to WT (Figure 1A and 1B). Figure 1B shows the same current traces at a different scale

to illustrate the difference in amplitude of the photocurrents between WT ChR2 and ChR2 mutants more clearly. Figure 1B specifically compares the current traces resulting from light stimulation using the neutral density filter (ND 2.5), equivalent to 4.8×10^{15} photos/cm²/s; the traces are designated by the arrows. The amplitude of the photocurrent of the L132C mutant is larger than that of WT; the amplitude of the photocurrent of double mutant L132C/T159C is larger than that of L132C; and the amplitude of the photocurrent of the L132C/T159S mutant larger than L132/T159C. The current traces of the ChR2 mutants, particularly double mutants L132C/T159C and L132C/T159S, also show slower deactivation kinetics when compared to WT and L132C.

[105] Figure 2 shows the representative recordings of light-evoked currents from WT ChR2, L132C, L132C/T159C, and L132C/T159S after stimulation by a 10 ms light pulse (1.2×10^{18} photons/cm²/s at 460 nm wavelength) to compare the deactivation time course, or decay time course after the light is off. Mutant ChR2 show longer deactivation time courses, with the double mutant L132C/T159S having the longest. Higher light sensitivity, as demonstrated by L132C/T159C and L132C/T159S, may be correlated with slower channel kinetics.

Example 3: In vivo ocular administration and analysis of mutant Chop2 constructs.

[106] AAV2 virus vectors carrying mutant Chop2-GFP constructs driven by CAG promoter were made and injected intravitreally into the eyes of C57BL/6J adult mice. Adult mice were anesthetized by IP injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Under a dissecting microscope, an incision was made by scissors through the eyelid to expose the sclera. A small perforation was made in the sclera region posterior to the lens with a needle and viral vector suspension of 0.8–1.5 μ l at the concentration of approximately 10^{11} genomic particles/ml was injected into intravitreal space through the hole with a Hamilton syringe with a 32-gauge blunt-ended needle. For each animal, usually only one eye was injected with viral vectors carrying a Chop2 construct, and the other eye was uninjected or injected with control viral vectors carrying GFP alone. Upon expression of the WT or mutant Chop2 of the present invention, functional WT or mutant ChR2 channels were formed utilizing

endogenous retinal, and the properties of these ChR2 proteins were assessed as described herein.

[107] ChR2-mediated light responses were examined by using multi-electrode array recordings from whole-mount retinas. Light stimuli (photons/cm²/s) was generated by a 473 nm blue laser and attenuated by neutral density filters: ND0 (6.3x10¹⁶), ND1.0 (7.4x10¹⁵), ND1.5 (2.7x10¹⁵), ND2.0 (7.3x10¹⁴), ND2.5 (3.2x10¹⁴), ND3.0 (8.5x10¹³), ND3.5 (3.8x10¹³), and ND4.0 (9.5x10¹²).

[108] The multielectrode array recordings were based on the procedures reported by Tian and Copenhagen (2003). Briefly, the retina was dissected and placed photoreceptor side down on a nitrocellulose filter paper strip (Millipore Corp., Bedford, MA). The mounted retina was placed in the MEA-60 multielectrode array recording chamber of 30 μ m diameter electrodes spaced 200 μ m apart (Multi Channel System MCS GmbH, Reutlingen, Germany), with the ganglion cell layer facing the recording electrodes. The retina was continuously perfused in oxygenated extracellular solution at 34°C during all experiments. The extracellular solution contained (in mM): NaCl, 124; KCl, 2.5; CaCl₂, 2; MgCl₂, 2; NaH₂PO₄, 1.25; NaHCO₃, 26; and glucose, 22 (pH 7.35 with 95% O₂ and 5% CO₂). Recordings were usually started 60 min after the retina was positioned in the recording chamber. The interval between onsets of each light stimulus was 10–15 s. The signals were filtered between 200 Hz (low cut off) and 20 kHz (high cut off). The responses from individual neurons were analyzed using Offline Sorter software (Plexon, Inc., Dallas, TX).

[109] Single mutant Chop2/ChR2 mutants, *i.e.*, L132 and T159C, markedly lower the threshold light intensity that is required to evoke a ChR2-mediated photocurrent. Moreover, several double mutants, including L132C/T159C, L132A/T159C, and L132C/T159S, were found to further increase the photocurrent at low light intensities. Different neutral density filters were used to attenuate the light stimuli to differentiate the light-evoked responses of the Chop2 constructs in low light. Spiking activity of retinal ganglion cells mediated by the mutants of the present invention was observed at the light intensities about 1.5 to 2 log units lower than the light level that is required to elicit the spiking activity with wild-type ChR2 (Figure 3). Specifically, WT ChR2

exhibited did not exhibit any spiking activity in response to light stimuli with neutral density filter 2.5 (3.2×10^{14} photons/cm²/s) while ChR2 mutants (L132C, L132C/T159C, and L132C/T159S) demonstrate spiking activity. In fact, the ChR2 mutants still exhibited spiking activity in response to light with neutral density filters 3.0 and 3.5. Therefore, ChR2 mutants of the present invention possess higher light sensitivity and, thus, a markedly lower threshold light intensity that is required to elicit a ChR2-mediated photocurrent. Moreover, ChR2 double mutants possess a higher light sensitivity than single mutants, i.e. L132C. In addition, the spike firing of retinal ganglion cells expressing L132C/T159C and L132C/T159S could follow a light flicker frequency of up to 15 Hz and 5 Hz, respectively (Figure 4).

[110] The L132C/T159A mutant shows high light sensitivity, probably the most light sensitive among these mutants, but it also shows extremely slow off-rate (the channel continues open for many sends after light off). Interestingly, it can be turned off more quickly using a light with long-wavelengths, such as yellow light. The L132C/T159A mutant (encoded by SEQ ID NOs: 24 and 25) demonstrates significant potential.

[111] Given the trade-off between light sensitivity and channel kinetics, Chop2/ChR2 mutants that demonstrate a balance between light sensitivity and channel kinetics, such as L132C/T159C or L132C/T159S, may be suitable for the application of vision restoration.

Example 4: Analysis of mutant Chop2 constructs in mouse models of disease.

[112] Mouse models of degenerative ocular diseases are known in the art. For example, homozygous *rd1* (*rd1/rd1*) mice are a commonly used photoreceptor degeneration model. *Rd1* mice carry a null mutation in a cyclic GMP phosphodiesterase, PDE6, similar to some forms of retinitis pigmentosa in humans. Other well-established mouse models of ocular disease that may be of particular interest to demonstrate ChR2 mutant safety and efficacy include *rds* (also known as *Prph^{Rd2}*), *rd3*, *rd4*, *rd5*, *rd6*, *rd7*, *rd8*, *rd9*, *Pde6b^{rd10}*, or *cpfl1* mice.

[113] The Chop2-GFP constructs of the present invention can be injected intravitreally into the eyes of newborn (P1) or adult mice at 2-12 months of age. GFP

signal can be observed in the Chop2-GFP-injected retinas, to determine the levels of ChR2 expression or expression in particular populations of cells, such as the retinal ganglion cells. Mutant Chop2-GFP expression can be monitored for a predetermined amount of time, i.e. 3-6 months, or 1 year after viral injection. Patch-clamp and multichannel array recordings can be performed using the methods known in the art and described herein to measure the light-evoked responses of mutant Chop2-GFP-expressing cells *in vivo*.

[114] Additional techniques and tests are well-established in the art to test for the restoration of light sensitivity or vision. Visual evoked potentials from the Chop2-GFP expressing cells or visual cortex can be examined, as described in PCT publication WO 2007/131180. Other tests include behavioral assessments of the visual acuity in the mice, i.e., virtual optomotor test and visual water maze.

Example 5: Analysis of long-term expression and safety of administration of mutant Chop2 constructs to retinal neurons.

[115] Neurotoxicity was assessed in C57BL/6J adult mice injected with Chop2 constructs of the present invention. The expression safety of Chop2 mutants in the retina was assessed by immunostaining and cell counting after exposure to strong blue light for two weeks. None of the mice were found to exhibit symptoms of neurotoxicity for up to two months after injection.

[116] Additional ongoing studies are evaluating the long-term expression and safety of Chop2/ChR2 mutants of the invention in retinal neurons.

OTHER EMBODIMENTS

[117] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

[118] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents

and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

[119] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. An isolated polypeptide molecule comprising SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L).
2. The polypeptide molecule of claim 1, wherein the amino acid at position 132 is cysteine (C) or alanine (A).
3. The polypeptide molecule of claim 2, wherein the amino acid at position 132 is cysteine (C), and wherein the polypeptide molecule comprises SEQ ID NO: 13.
4. The polypeptide molecule of claim 2, wherein the amino acid at position 132 is alanine (A), and wherein the polypeptide molecule comprises SEQ ID NO: 20.
5. An isolated polypeptide molecule comprising SEQ ID NO: 26 in which the amino acid at position 159 of SEQ ID NO: 26 is not a threonine (T).
6. The polypeptide molecule of claim 5, wherein the amino acid at position 159 is cysteine (C), serine (S), or alanine (A).
7. The polypeptide molecule of claim 6, wherein the amino acid at position 159 is cysteine (C), and wherein the polypeptide molecule comprises SEQ ID NO: 14.
8. The polypeptide molecule of claim 6, wherein the amino acid at position 159 is serine (S), and wherein the polypeptide molecule comprises SEQ ID NO: 17.
9. The polypeptide molecule of claim 6, wherein the amino acid at position 159 is alanine (A), and wherein the polypeptide molecule comprises SEQ ID NO: 23.

10. An isolated polypeptide molecule comprising SEQ ID NO: 26 in which the amino acid at position 132 of SEQ ID NO: 26 is not leucine (L) and the amino acid at position 159 is not threonine (T).
11. The polypeptide molecule of claim 10, wherein the amino acid at position 132 is cysteine (C), wherein the amino acid at position 159 is cysteine (C).
12. The polypeptide molecule of claim 10 or 11, wherein the polypeptide molecule comprises SEQ ID NO: 16.
13. An isolated nucleic acid molecule that encodes for the isolated polypeptide of claim 12.
14. The nucleic acid molecule of claim 13, wherein the nucleic acid molecule comprises SEQ ID NO: 15.
15. The polypeptide molecule of claim 10, wherein the amino acid at position 132 is cysteine (C), wherein the amino acid at position 159 is serine(S).
16. The polypeptide molecule of claim 10 or 15, wherein the polypeptide molecule comprises SEQ ID NO: 19.
17. An isolated nucleic acid molecule that encodes for the isolated polypeptide of claim 16.
18. The nucleic acid molecule of claim 17, wherein the nucleic acid molecule comprises SEQ ID NO: 18.
19. The polypeptide molecule of claim 10, wherein the amino acid at position 132 is alanine (A), wherein the amino acid at position 159 is cysteine (C).

20. The polypeptide molecule of claim 10 or 19, wherein the polypeptide molecule comprises SEQ ID NO: 22.
21. An isolated nucleic acid molecule that encodes for the isolated polypeptide of claim 22.
22. The nucleic acid molecule of claim 21, wherein the nucleic acid molecule comprises SEQ ID NO: 21.
23. The polypeptide molecule of claim 10, wherein the amino acid at position 132 is cysteine (C), wherein the amino acid at position 159 is alanine (A).
24. The polypeptide molecule of claim 10 or 23, wherein the polypeptide molecule comprises SEQ ID NO: 25.
25. An isolated nucleic acid molecule that encodes for the isolated polypeptide of claim 24.
26. The nucleic acid molecule of claim 25, wherein the nucleic acid molecule comprises SEQ ID NO: 24.
27. An isolated nucleic acid molecule that encodes for the isolated polypeptide of any one of claims 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 15, 16, 19, 20, 23, and 24.
28. An isolated nucleic acid molecule of claim 27, wherein the isolated polypeptide is about 315, 310, 300, 275, 250, 225, 200, 175, or 160 amino acids long.
29. The isolated nucleic acid molecule of claims 27 or 28, further comprising a pharmaceutically acceptable carrier.
30. The isolated polypeptide molecule of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9,

10, 11, 12, 13, 15, 16, 19, 20, 23, and 24, wherein the polypeptide molecule encodes for a mutant ChR2 protein elicits a current in response to a threshold intensity of light that is lower than the threshold of a wild type ChR2 protein.

31. The isolated polypeptide molecule of claim 30, wherein the polypeptide molecule is about 315, 310, 300, 275, 250, 225, 200, 175, or 160 amino acids long.

32. The isolated polypeptide molecule of claim 30 or 31, further comprising a pharmaceutically acceptable carrier.

33. A composition comprising an isolated nucleic acid molecule of any one of claims 27, 28, and 29.

34. A composition comprising an isolated polypeptide molecule of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 19, 20, 23, 24, 29, 30 and 31.

35. A cell comprising the isolated polypeptide molecule of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 19, 20, 23, 24, 29, 30 and 31.

36. A cell comprising an isolated nucleic acid molecule that encodes for the isolated polypeptide molecule of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 19, 20, 23, 24, 29, 30 and 31.

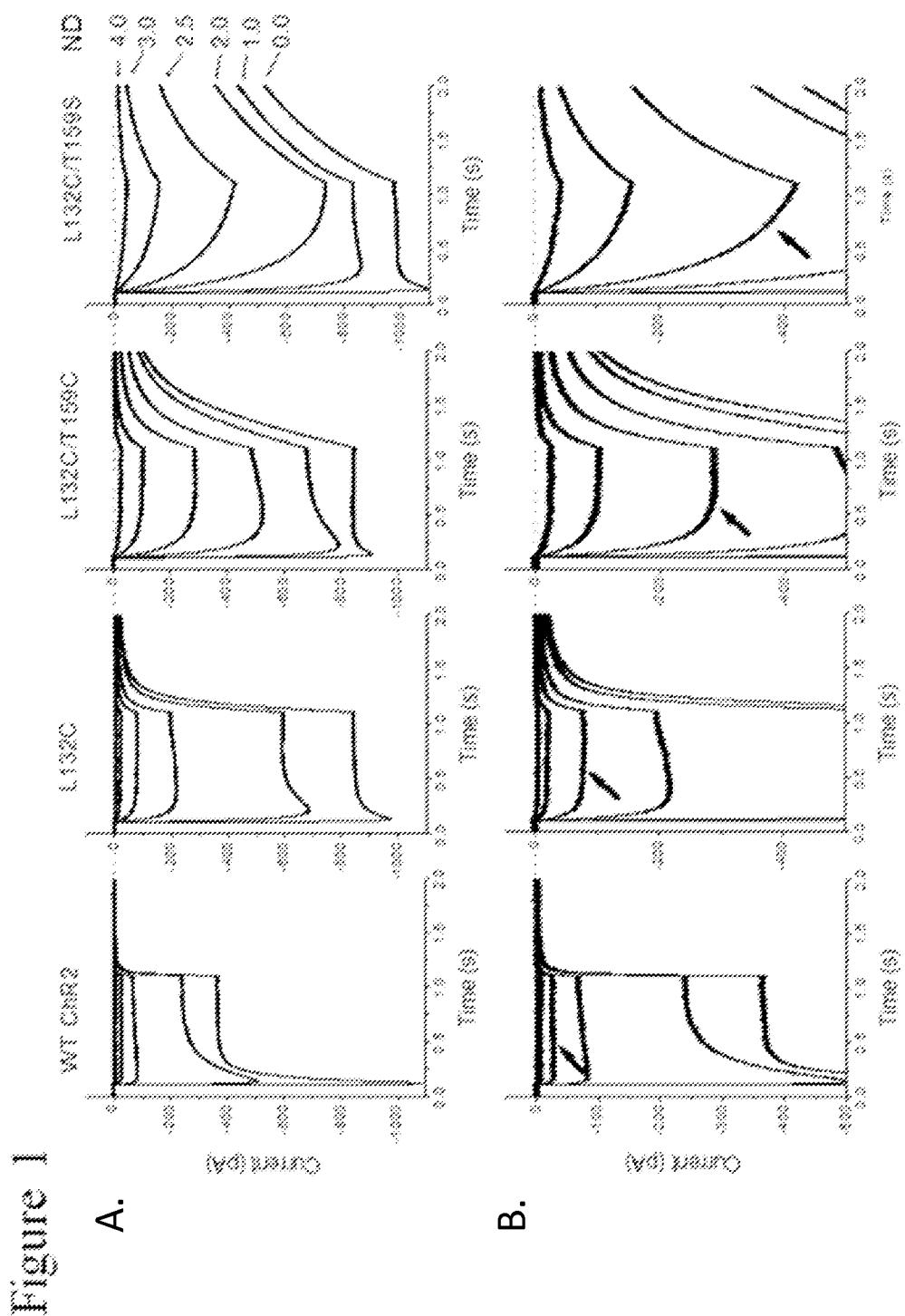
37. A cell comprising the isolated nucleic acid molecule of claim 27 or 28.

38. A composition comprising a cell of any one of claims 35, 36, and 37.

39. The cell of claim 35, 36, and 37, wherein the cell is contacted with the isolated polypeptide or an isolated nucleic acid encoding the polypeptide in vitro, ex vivo, in vivo, or in situ.

40. The cell of claim 35, 36, and 37, wherein the cell is a photoreceptor, a bipolar cell, a rod bipolar cell, an ON-type cone bipolar cell, a retinal ganglion cell, a photosensitive retinal ganglion cell, a horizontal cell, an amacrine cell, or an AII amacrine cell.
41. The cell of claim 40, wherein the cell is a retinal ganglion cell or a photosensitive retinal ganglion cell.
42. A method of improving or restoring vision, comprising administering to a subject the composition of claim 33.
43. A method of improving or restoring vision, comprising administering to a subject the composition of claim 34.
44. A method of improving or restoring vision, comprising administering to a subject the composition of claim 38.
45. The method of claim 42, 43 and 44, wherein the subject has normal vision.
46. The method of claim 42, 43 and 44, wherein the subject has impaired vision.
47. The method of claim 42, 43 and 44, wherein the subject is suffering from an ocular disease.
48. The method of claim 47, wherein the ocular disease is macular degeneration or retinitis pigmentosa.
49. The method of claim 42, 43 and 44, wherein the composition is administered by intravitreal or subretinal injection.
50. The method of claim 42, 43 and 44, wherein said improving or restoring

vision comprises any of the following: increasing light sensitivity; lowering the threshold light intensity required to elicit a photocurrent; and increasing visual evoked potential in the visual cortex.



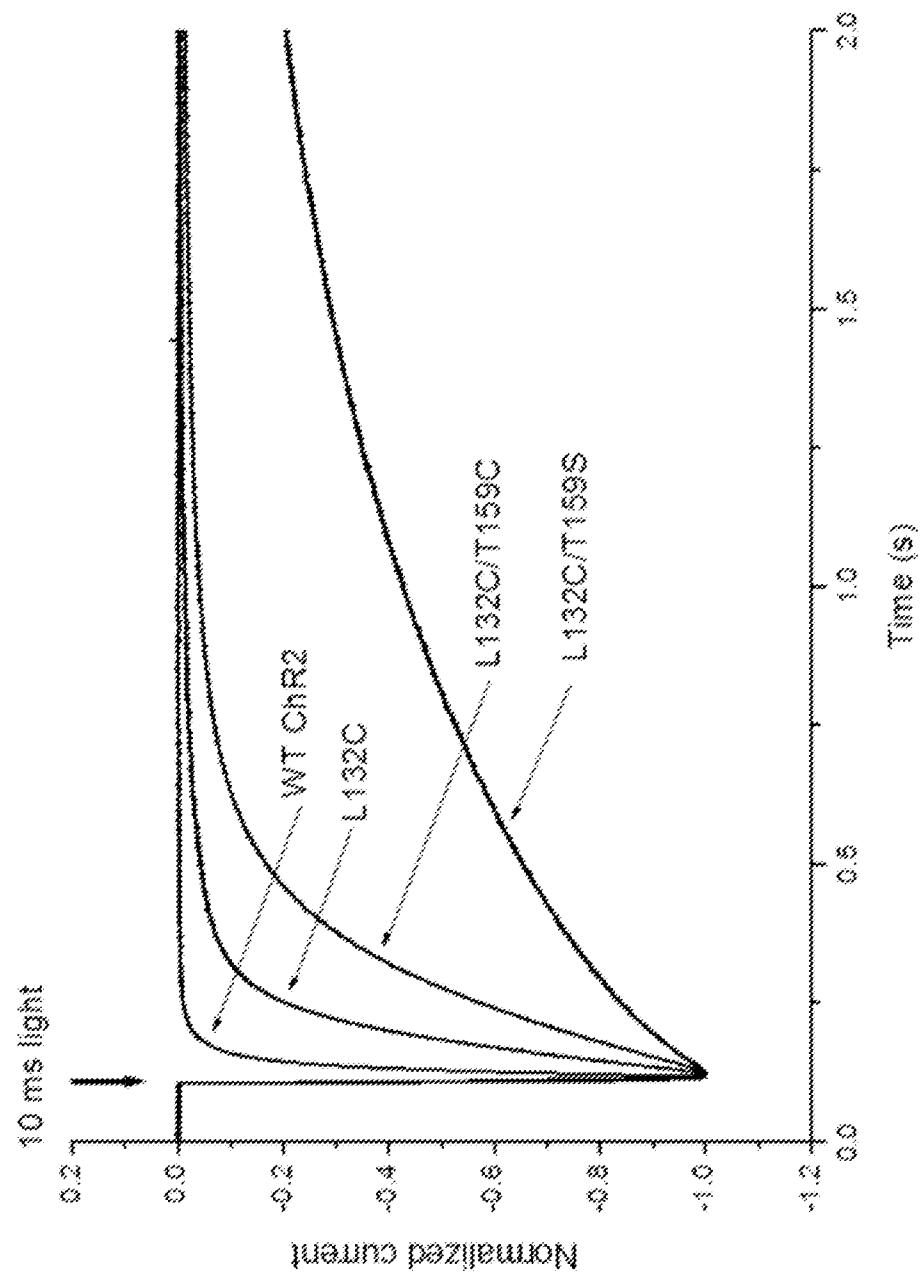


Figure 2

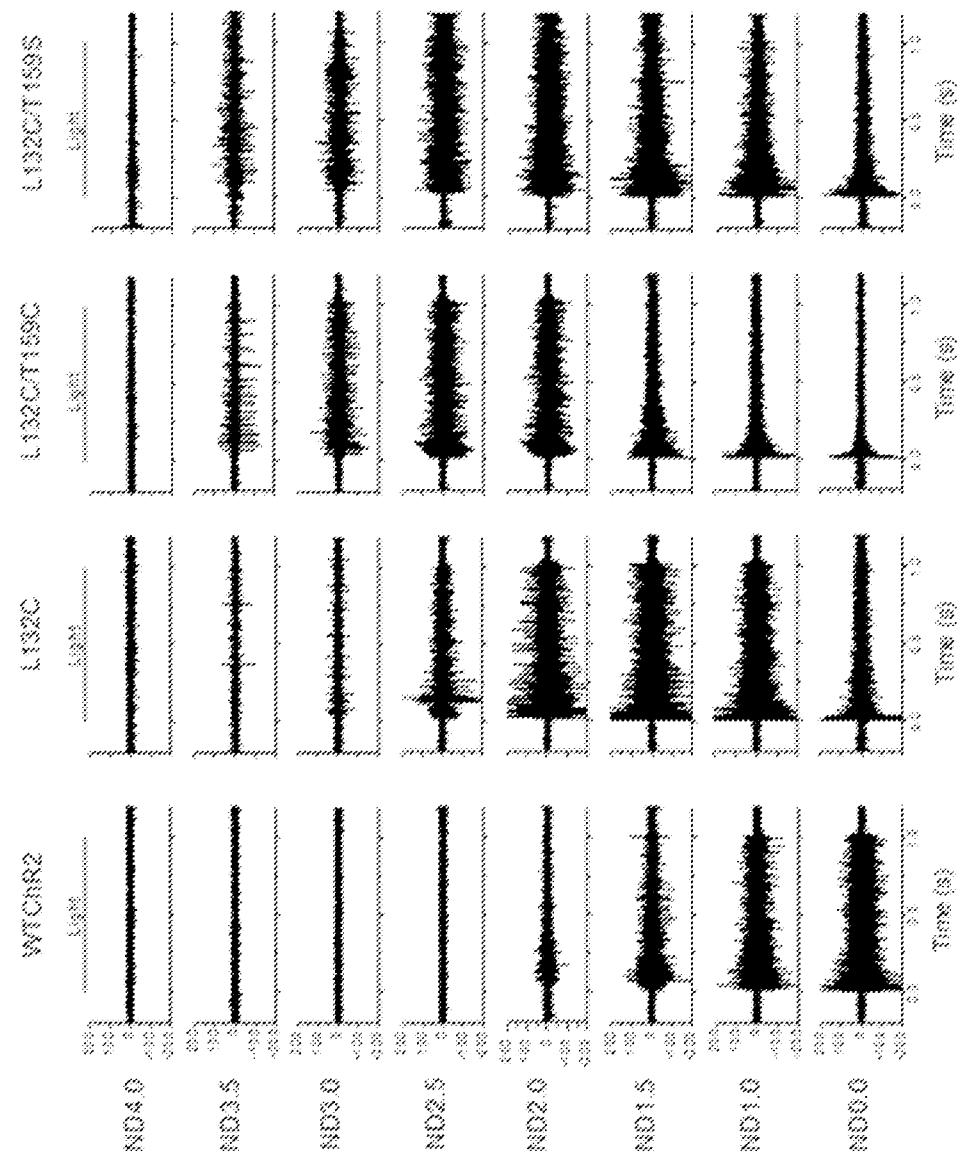
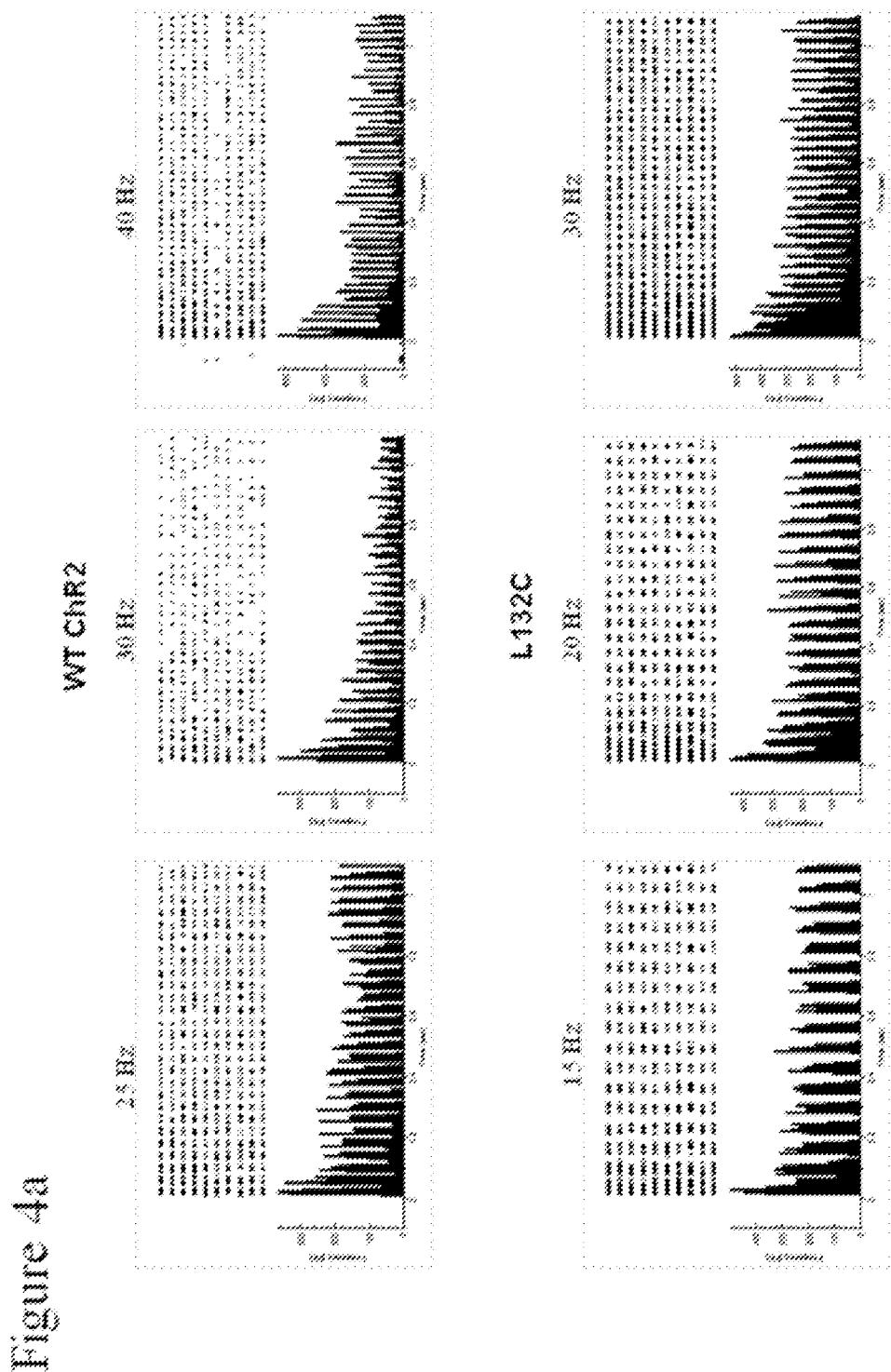
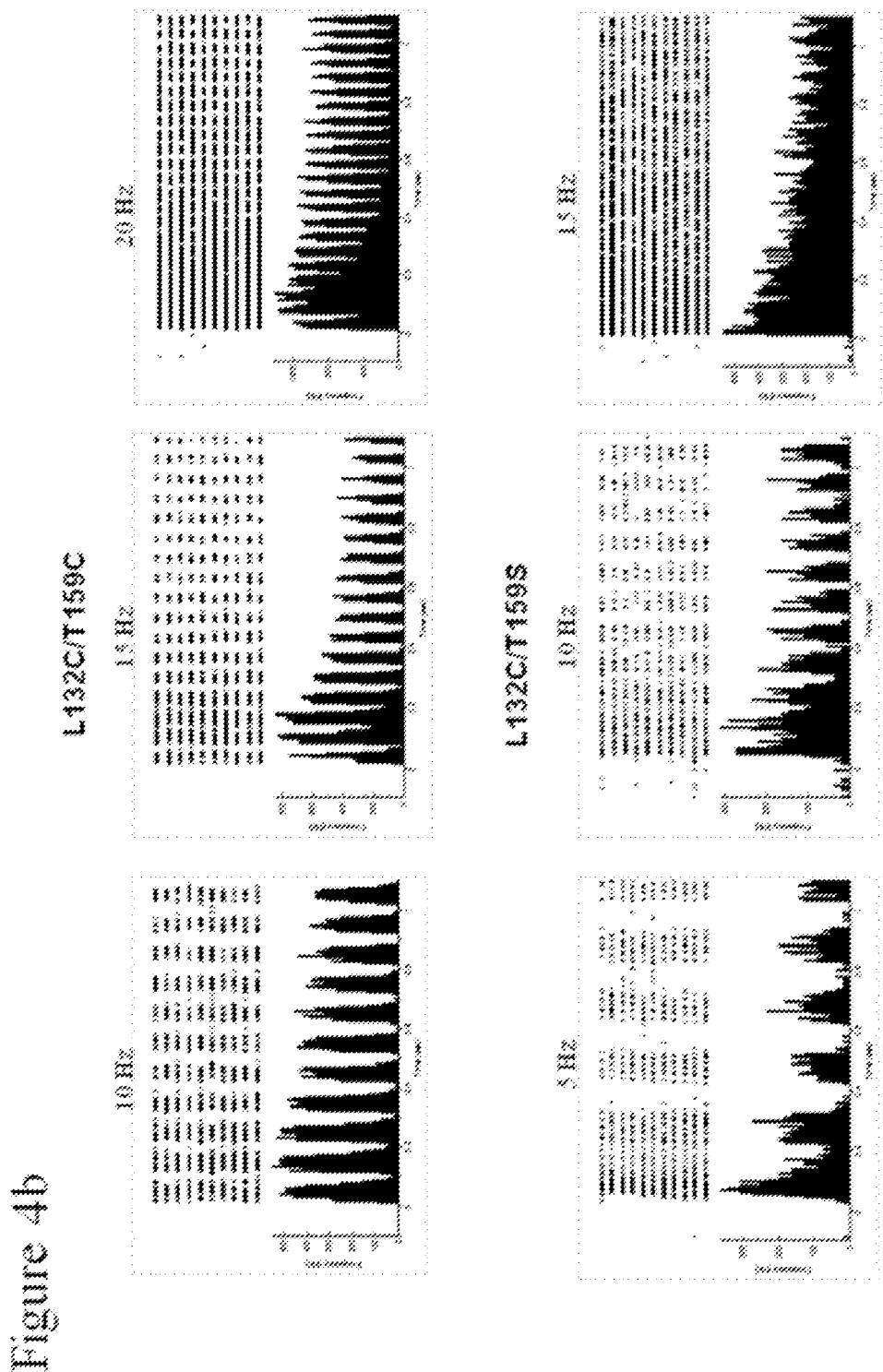


Figure 3





INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/029171

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/705
ADD.

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

B. FIELDS SEARCHED

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

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

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

EPO-Internal, Sequence Search, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SONJA KLEINLOGEL ET AL: "Ultra light-sensitive and fast neuronal activation with the Ca²⁺-permeable channelrhodopsin CatCh", NATURE NEUROSCIENCE, vol. 14, no. 4, 13 March 2011 (2011-03-13), pages 513-518, XP055009508, ISSN: 1097-6256, DOI: 10.1038/nn.2776 abstract pages 513, 517 figure 3</p> <p>-----</p> <p style="text-align: center;">-/-</p>	1-3, 27-50

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 the 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
14 August 2013	27/08/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Behrens, Joyce

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/029171

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.:
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:

see additional sheet

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 an 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/US2013/029171

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTIN L REIN ET AL: "The optogenetic (r)evolution", MOLECULAR GENETICS AND GENOMICS, SPRINGER, BERLIN, DE, vol. 287, no. 2, 20 December 2011 (2011-12-20), pages 95-109, XP035008231, ISSN: 1617-4623, DOI: 10.1007/S00438-011-0663-7 abstract table 1 pages 96, 106 -----	1-7, 10-14, 19-22, 42-50
X	WO 2011/140279 A1 (UNIV WAYNE STATE [US]; PAN ZHUO-HUA [US]) 10 November 2011 (2011-11-10) pages 17,54-56 sequence 20 claims 1-38 examples 1-2 -----	1-3, 27-50
X	A. BERNDT ET AL: "High-efficiency channelrhodopsins for fast neuronal stimulation at low light levels", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 108, no. 18, 3 May 2011 (2011-05-03), pages 7595-7600, XP055066271, ISSN: 0027-8424, DOI: 10.1073/pnas.1017210108 abstract -----	5-7, 27-41
X, P	WO 2012/032103 A1 (MAX PLANCK GESELLSCHAFT [DE]; BAMBERG ERNST [DE]; BAMANN CHRISTIAN [DE]) 15 March 2012 (2012-03-15) claims 1-17 pages 22-34 pages 14-17 & DATABASE Geneseq [Online] 26 April 2012 (2012-04-26), "Chlamydomonas reinhardtii mutant channelrhodopsin-2 sequence (L132C) #2.", retrieved from EBI accession no. GSP:AZU14264 Database accession no. AZU14264 sequence -----	1-3, 27-44, 46,50
		-/-

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/029171

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	ULLRICH SYBILLE ET AL: "Degradation of channelopsin-2 in the absence of retinal and degradation resistance in certain mutants", BIOLOGICAL CHEMISTRY, WALTER DE GRUYTER GMBH & CO, BERLIN, DE, vol. 394, no. 2, 1 February 2013 (2013-02-01), pages 271-280, XP009170311, ISSN: 1431-6730 abstract -----	5-7, 27-41
X,P	M. PRIGGE ET AL: "Color-tuned Channelrhodopsins for Multiwavelength Optogenetics", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 287, no. 38, 27 July 2012 (2012-07-27), pages 31804-31812, XP055075027, ISSN: 0021-9258, DOI: 10.1074/jbc.M112.391185 figures 2-4 page 31809 - page 31812 -----	5-7, 27-41
A	E. IVANOVA ET AL: "Evaluation of AAV-Mediated Expression of Chop2-GFP in the Marmoset Retina", INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE, vol. 51, no. 10, 1 October 2010 (2010-10-01), pages 5288-5296, XP055066268, ISSN: 0146-0404, DOI: 10.1167/iovs.10-5389 abstract -----	1-50
A	WO 2007/131180 A2 (UNIV WAYNE STATE [US]; PENNSYLVANIA COLLEGE OF OPTOME [US]; PAN ZHUO-H) 15 November 2007 (2007-11-15) claims 1-41 sequence 3 & DATABASE Geneseq [Online] 10 January 2008 (2008-01-10), "Chlamydomonas reinhardtii channelrhodopsin-2 polypeptide.", retrieved from EBI accession no. GSP:ANZ06204 Database accession no. ANZ06204 sequence ----- -/-	1-50

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/029171

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FENG ZHANG ET AL: "The Microbial Opsin Family of Optogenetic Tools", CELL, vol. 147, no. 7, 23 November 2011 (2011-11-23), pages 1446-1457, XP028348924, ISSN: 0092-8674, DOI: 10.1016/J.CELL.2011.12.004 [retrieved on 2011-12-08] -----	1-50

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4(completely); 10-50(partially)

An isolated polypeptide molecule comprising SEQ ID NO: 26, wherein the amino acid at position 132 is cysteine (C) or alanine (A).

2. claims: 5-9(completely); 10-50(partially)

An isolated polypeptide molecule comprising SEQ ID NO: 26, wherein the amino acid at position 159 is cysteine (C), serine (S) or alanine (A).

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/029171

Patent document cited in search report	Publication date	Patent family member(s)			Publication date
WO 2011140279	A1	10-11-2011			NONE
WO 2012032103	A1	15-03-2012	AU 2011298745 A1		21-03-2013
			CA 2810757 A1		15-03-2012
			EP 2614079 A1		17-07-2013
			SG 188409 A1		30-04-2013
			WO 2012032103 A1		15-03-2012
WO 2007131180	A2	15-11-2007	AU 2007247929 A1		15-11-2007
			CA 2685900 A1		15-11-2007
			CN 101484005 A		15-07-2009
			EP 2019588 A2		04-02-2009
			JP 2009536219 A		08-10-2009
			US 2010015095 A1		21-01-2010
			WO 2007131180 A2		15-11-2007