

PHOTORECEPTOR RESCUE CELL (PRC) COMPOSITIONS AND METHODS FOR TREATMENT OF OCULAR DISORDERS

RELATED APPLICATIONS

[001] This application claims the benefit of U.S. Provisional Application No.: 63/373,298, filed August 23, 2022 and U.S. Provisional Application No. 63/432,948, filed December 15, 2022, the entire contents of each of which are hereby incorporated by reference herein.

BACKGROUND

[002] There are a number of retinal diseases or disorders that can result in loss of vision or even blindness. Among the retinal diseases or disorders are rod or cone dystrophies, retinal degeneration, retinitis pigmentosa, diabetic retinopathy, macular degeneration (such as age-related macular degeneration (wet or dry), geographic atrophy secondary to AMD, Leber congenital amaurosis and Stargardt disease. Several retinal diseases or disorders are a result of cell loss in the nuclear layers, primarily in the outer nuclear layer, which includes photoreceptor cells. Replacement of degenerating photoreceptors with new cells offers a potential method of slowing or stopping cell degeneration and vision loss.

[003] A potential replacement source of photoreceptor cells includes stem cells. Early studies incorporated the use of mouse cells, mouse stem cells or heterogeneous populations of retinal progenitor cells as a possible source of cells for replacement of lost photoreceptors. These early studies described transplantation of photoreceptor precursor cells from postnatal day 1 mouse retina (Maclaren et al. Nature 444(9):203-207, 2006), *in vitro* generation of retinal precursor cells from mouse embryonic stem cells (Ikeda et al. Proc. Natl. Acad. Sci. 102(32):11331-11336, 2005), generation of retinal progenitor cells from postnatal day 1 mouse retinas (Klassen et al. Invest. Ophthalm. Vis. Sci. 45(11):4167-4175, 2004), implantation of bone marrow mesenchymal stem cells in an RCS rat model of retinal degeneration (Inoue et al. Exp. Eye Res. 8(2):234-241, 2007), production of retinal progenitor cells, including ganglion cells, amacrine cells, and photoreceptors wherein 0.01% of the total cells expressed S-opsin or rhodopsin, bipolar cells and horizontal cells, from the H1 human embryonic stem cell line (Lamba et al. Proc. Natl. Acad. Sci. 10(34):12769-12774, 2006) and induction of induced pluripotent stem cells (iPS) from human fibroblasts to produce retinal progenitor cells (Lamba et al. PLoS ONE 5(1):e8763. doi:10.1371/journal.pone.0008763). None of these approaches produced a homogeneous population of photoreceptor progenitor cells or photoreceptor cells for implantation. None of these approaches produced a population of photoreceptor progenitor cells or photoreceptor cells that showed *in vivo* rod or cone function (*e.g.*, detectable by conferring improvements in visual acuity). Differentiating stem cells into a population of photoreceptor rescue cells may provide an alternative to presently available therapeutic approaches.

SUMMARY

[004] The present invention provides a photoreceptor rescue cell (PRCs) composition comprising a plurality of heterogeneous photoreceptor rescue cells having unique marker portfolios and methods for their use in the treatment of ocular disorders.

[005] Accordingly, in one aspect, the present invention provides a photoreceptor rescue cell composition comprising a plurality of heterogeneous photoreceptor rescue cells, wherein the plurality of heterogeneous photoreceptor rescue cells cumulatively expresses at least two of the markers selected from the group consisting of FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA.

[006] In one embodiment, photoreceptor rescue cell composition further comprising a medium suitable for maintaining the viability of the cells.

[007] In one embodiment, the cells are generated by in vitro differentiation of pluripotent cells.

[008] In one embodiment, the pluripotent cells are embryonic cells (ESCs) or induced pluripotent stem cells (iPSCs).

[009] In one embodiment, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of cells in the composition are photoreceptor rescue cells.

[010] In one embodiment, the plurality of heterogeneous cells cumulatively expresses FOXG1 and MAP2.

[011] In one embodiment, (i) about 50% to about 100%, about 50% to about 90%, about 55% to about 85%, or about 55% to about 73% of cells in the composition express FOXG1; and/or (ii) at least about 50%, 55%, 57%, 60%, 65%, 70%, 71%, 75%, 78%, 79%, 80%, 81%, 85%, 87%, 90%, 92%, 95%, 97%, or 100% of cells in the composition express FOXG1, and/or (iii) about 55 transcripts per million (TPM) to about 200 TPM, about 60 TPM to about 170 TPM, about 140 TPM to about 165 TPM, or about 149 TPM to about 170 TPM of FOXG1 transcripts are expressed by the cells of the composition; and/or (iv) at least 55 TPM, 60 TPM, 70 TPM, 80 TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 180 TPM, 190 TPM, or 200 TPM of FOXG1 transcripts are expressed by the cells of the composition.

[012] In one embodiment, (i) about 75% to about 100%, about 75% to about 98%, about 75% to about 95%, about 77% to about 93%, of cells in the composition express MAP2; and/or (ii) at least about 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of cells in the composition express MAP2; and/or (iii) about 250 transcripts per million (TPM) to about 700 TPM, about 290 TPM to about 650 TPM, about 450 TPM to about 625 TPM, or about 490 TPM to about 615 TPM of MAP2 transcripts are expressed by the cells of the composition; and/or (iv) at least 250 TPM, 300 TPM, 350 TPM, 400 TPM, 450 TPM, 475 TPM, 490 TPM, 510 TPM, 525 TPM, 550 TPM, 575 TPM, 600 TPM, 610 TPM, 625 TPM, 650 TPM, 675 TPM, or 700 TPM of MAP2 transcripts are expressed by the cells of the composition.

[013] In one embodiment, the plurality of heterogeneous cells cumulatively expresses at least one additional marker selected from the group consisting of STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA. In one embodiment, the plurality of heterogeneous cells cumulatively expresses at least 3, 4, 5, 6 or 7 of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA.

[014] In one embodiment, (i) about 60% to about 95%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, about 65% to about 95%, about 65% to about 90%, about 65% to about 85%, about 65% to about 80%, about 70% to about 95%, about 70% to about 90%, about 70% to about 85%, about 70% to about 80%, about 75% to about 95%, about 75% to about 90%, about 75% to about 85%, or about 75% to about 80% of cells in the composition express STMN2; and/or (ii) at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, or 100% of cells in the composition express STMN2; and/or (iii) about 150 transcripts per million (TPM) to about 600 TPM, about 190 TPM to about 560 TPM, about 400 TPM to about 600 TPM, or about 450 TPM to about 560 TPM of STMN2 transcripts are expressed by the cells of the composition; and/or (iv) at least 150 TPM, 185 TPM, 200 TPM, 250 TPM, 300 TPM, 350 TPM, 400 TPM, 425 TPM, 450 TPM, 475 TPM, 500 TPM, 525 TPM, 550 TPM, 575 TPM, or 600 TPM of STMN2 transcripts are expressed by the cells of the composition.

[015] In one embodiment, (i) about 65% to about 95%, about 65% to about 85%, about 70% to about 95%, about 70% to about 90%, about 70% to about 89%, about 75% to about 95%, about 75% to about 90%, or about 75% to about 89% of cells in the composition express DCX; and/or (ii) at least about 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, or 95% of cells in the composition express DCX; and/or (iii) about 200 transcripts per million (TPM) to about 900 TPM, about 250 TPM to about 900 TPM, about 600 TPM to about 900 TPM, or about 750 TPM to about 850 TPM of DCX transcripts are expressed by the cells of the composition; and/or (iv) at least 200 TPM, 250 TPM, 350 TPM, 400 TPM, 450 TPM, 500 TPM, 550 TPM, 600 TPM, 650 TPM, 700 TPM, 750 TPM, 800 TPM, 850 TPM, or 900 TPM of DCX transcripts are expressed by the cells of the composition.

[016] In one embodiment, (i) about 65% to about 98%, about 65% to about 95%, about 70% to about 98%, about 70% to about 95%, about 70% to about 90%, about 75% to about 98%, about 75% to about 90%, or about 80% to about 95% of cells in the composition express LINC00461; and/or (ii) at least about 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, or 98% of cells in the composition express LINC00461; and/or (iii) about 50 transcripts per million (TPM) to about 100 TPM, about 50 TPM to about 95 TPM, about 85 TPM to about 95 TPM, or about 87 TPM to about 93 TPM of LINC00461 transcripts are expressed by the cells of the composition; and/or (iv) at least 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 85 TPM, 87 TPM, 89 TPM, 90 TPM, 92 TPM, 95 TPM, or 100 TPM of LINC00461 transcripts are expressed by the cells of the composition.

[017] In one embodiment, (i) about 1% to about 25%, about 1% to about 20%, 1% to about 18%, 1% to about 16%, about 1% to about 14%, about 1% to about 12%, 1% to about 10%, about 1% to

about 8%, about 1% to about 7%, about 1% to about 5% or about 2% to about 4% of cells in the composition express NEUROD2; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, or 25% of cells in the composition express NEUROD2; and/or (iii) about 0 transcripts per million (TPM) to about 10 TPM, about 0.01 TPM to about 9 TPM, about 0.2 TPM to about 2 TPM, or about 0.4 TPM to about 1.2 TPM of NEUROD2 transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 0.2 TPM, 0.4 TPM, 0.6 TPM, 0.8 TPM, 1.0 TPM, 1.2 TPM, 1.5 TPM, 2 TPM, 4 TPM, 6 TPM, 8 TPM, or 10 TPM of NEUROD2 transcripts are expressed by the cells of the composition.

[018] In one embodiment, (i) about 35% to about 70%, about 35% to about 68%, about 35% to about 67%, about 35% to about 66%, about 35% to about 65%, about 40% to about 70%, about 40% to about 68%, about 40% to about 67%, about 40% to about 66%, about 40% to about 65%, about 42% to about 70%, about 42% to about 68%, about 42% to about 67%, about 42% to about 66%, or about 42% to about 65% of cells in the composition express GAD1; and/or (ii) at least about 35%, 40%, 45%, 50%, 55%, 60%, 65%, 66%, 67%, 68%, 69%, or 70% of cells in the composition express GAD1; and/or (iii) about 10 transcripts per million (TPM) to about 50 TPM, about 12 TPM to about 45 TPM, about 12 TPM to about 40 TPM, or about 25 TPM to about 42 TPM of GAD1 transcripts are expressed by the cells of the composition; and/or (iv) at least 10 TPM, 12 TPM, 16 TPM, 18 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 35 TPM, 37 TPM, 40 TPM, 42 TPM, 45 TPM, 47 TPM, or 50 TPM of GAD1 transcripts are expressed by the cells of the composition.

[019] In one embodiment, (i) about 60% to about 95%, about 60% to about 90%, about 60% to about 89%, about 60% to about 88%, about 60% to about 87%, about 60% to about 86%, about 65% to about 95%, about 65% to about 90%, about 65% to about 89%, about 65% to about 88%, about 65% to about 87%, about 65% to about 86%, about 69% to about 90%, about 69% to about 89%, about 69% to about 88%, about 69% to about 87%, or about 69% to about 86% of cells in the composition express NFIA; and/or (ii) at least about 50%, 55%, 60%, 65%, 67%, 69%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 92%, or 95% of cells in the composition express NFIA; and/or (iii) about 30 transcripts per million (TPM) to about 120 TPM, about 33 TPM to about 117 TPM, about 60 TPM to about 85 TPM, or about 65 TPM to about 80 TPM of NFIA transcripts are expressed by the cells of the composition; and/or (iv) at least 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, or 120 TPM of NFIA transcripts are expressed by the cells of the composition.

[020] In one embodiment, the plurality of heterogeneous cells cumulatively expresses each of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA. In one embodiment, the heterogeneous cells each individually express at least one of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, or NFIA.

[021] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises one or more cell types selected from the group consisting of an inhibitory neuron, an excitatory neuron, a

progenitor, an astrocyte, and an alternative neuron. In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises each of an inhibitory neuron, an excitatory neuron, a progenitor, an astrocyte, and an alternative neuron.

[022] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises an inhibitory neuron expressing one or more markers selected from the group consisting of DLX5, TUBB3, SCGN, ERBB4, and CALB2. In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of inhibitory neurons that cumulatively expresses each of markers DLX5, TUBB3, SCGN, ERBB4, and CALB2.

[023] In one embodiment, (i) about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 52% to about 69%, about 52% to about 75%, about 54% to about 69%, about 54% to about 68%, or about 54% to about 66% of cells in the composition express DLX5; and/or (ii) at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 72%, 74%, 76%, 78%, or 80% of cells in the composition express DLX5; and/or (iii) about 30 transcripts per million (TPM) to about 150 TPM, about 50 TPM to about 140 TPM, about 80 TPM to about 138 TPM, or about 130 TPM to about 140 TPM of DLX5 transcripts are expressed by the cells of the composition; and/or (iv) at least 30 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 80 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, 115 TPM, 120 TPM, 130 TPM, 135 TPM, 140 TPM, or 150 TPM of DLX5 transcripts are expressed by the cells of the composition.

[024] In one embodiment, (i) about 60% to about 95%, about 70% to about 95%, about 72% to about 95%, about 75% to about 95%, about 76% to about 94%, about 77% to about 93%, about 78% to about 93%, about 70% to about 90%, about 72% to about 89%, about 73% to about 88%, about 74% to about 87%, about 75% to about 87%, about 76% to about 86%, about 77% to about 86%, or about 79% to about 86% of cells in the composition express TUBB3; and/or (ii) at least about 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 91%, 92%, 93%, or 95% of cells in the composition express TUBB3; and/or (iii) about 150 transcripts per million (TPM) to about 500 TPM, about 160 TPM to about 450 TPM, about 300 TPM to about 450 TPM, about 350 TPM to about 430 TPM, or about 375 TPM to about 430 TPM of TUBB3 transcripts are expressed by the cells of the composition; and/or (iv) at least 150 TPM, 175 TPM, 200 TPM, 225 TPM, 250 TPM, 275 TPM, 300 TPM, 325 TPM, 350 TPM, 375 TPM, 400 TPM, 425 TPM, 430 TPM, 450 TPM, 475 TPM, or 500 TPM of TUBB3 transcripts are expressed by the cells of the composition.

[025] In one embodiment, (i) about 45% to about 70%, about 45% to about 65%, about 50% to about 70%, about 50% to about 65%, about 50% to about 64%, about 50% to about 63%, about 50% to about 62%, about 50% to about 61%, about 46% to about 52%, about 47% to about 51%, about 48% to about 51%, or about 48% to about 50% of cells in the composition express SCGN; and/or (ii) at least about 45%, 47%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 65%, 67%, or 70% of cells in the composition express SCGN; and/or (iii) about 50 transcripts

per million (TPM) to about 200 TPM, about 70 TPM to about 180 TPM, about 75 TPM to about 175 TPM, or about 120 TPM to about 175 TPM of SCGN transcripts are expressed by the cells of the composition; and/or (iv) at least 50 TPM, 60 TPM, 70 TPM, 80 TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 171 TPM, 173 TPM, 175 TPM, 180 TPM, 185 TPM, 190 TPM, or 200 TPM of SCGN transcripts are expressed by the cells of the composition.

[026] In one embodiment, (i) about 60% to about 85%, about 60% to about 80%, about 60% to about 79%, about 60% to about 78%, about 60% to about 77%, about 60% to about 76%, about 63% to about 75%, about 63% to about 70%, about 63% to about 79%, about 63% to about 78%, about 63% to about 77%, about 63% to about 75%, about 63% to about 73%, about 63% to about 72%, about 63% to about 71%, about 65% to about 72%, or about 66% to about 71% of cells in the composition express ERBB4; and/or (ii) at least about 50%, 55%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 75%, 80%, or 85% of cells in the composition express ERBB4; and/or (iii) about 15 transcripts per million (TPM) to about 120 TPM, about 17 TPM to about 100 TPM, about 18 TPM to about 95 TPM, about 70 TPM to about 95 TPM, or about 73 TPM to about 94 TPM of ERBB4 transcripts are expressed by the cells of the composition; and/or (iv) at least 15 TPM, 30 TPM, 50 TPM, 70 TPM, 73 TPM, 75 TPM, 80 TPM, 82 TPM, 85 TPM, 88 TPM, 90 TPM, 91 TPM, 93 TPM, 95 TPM, 100 TPM, 110 TPM, or 120 TPM of ERBB4 transcripts are expressed by the cells of the composition.

[027] In one embodiment, (i) about 35% to about 75%, about 40% to about 70%, about 40% to about 65%, about 41% to about 75%, about 41% to about 70%, about 41% to about 65%, about 41% to about 64%, about 41% to about 63%, about 41% to about 62%, about 35% to about 55%, about 40% to about 52%, or about 41% to about 52% of cells in the composition express CALB2; and/or (ii) at least about 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 45%, 47%, 50%, 51%, 52%, 55%, 60%, 65%, 70%, or 75% of cells in the composition express CALB2; and/or (iii) about 30 transcripts per million (TPM) to about 220 TPM, about 50 TPM to about 200 TPM, about 70 TPM to about 200 TPM, or about 75 TPM to about 199 TPM of CALB2 transcripts are expressed by the cells of the composition; and/or (iv) at least 30 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, 125 TPM, 150 TPM, 175 TPM, 180 TPM, 185 TPM, 190 TPM, 195 TPM, 196 TPM, 220 TPM, 210 TPM, or 220 TPM of CALB2 transcripts are expressed by the cells of the composition.

[028] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises an excitatory neuron expressing one or more markers selected from the group consisting of NEUROD2, NEUROD6, SLA, NELL2, and SATB2. In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of excitatory neurons that cumulatively expresses each of markers NEUROD2, NEUROD6, SLA, NELL2, and SATB2.

[029] In one embodiment, (i) about 0.1% to about 30%, about 0.1% to about 25%, about 0.1% to about 24%, about 0.5% to about 30%, about 0.5% to about 25%, about 0.5% to about 24%, about 0.8% to about 30%, about 0.8% to about 25%, about 0.8% to about 24%, about 1% to about 5%, about 1% to about 4.5%, about 2% to about 5%, about 2% to about 4.5%, or about 2% to about 4% of cells in the composition express NEUROD6; and/or (ii) at least about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 1%, 2%, 4%, 5%, 7%, 10%, 12%, 15%, 17%, 20%, 22%, 23%, 24%, 26%, 28%, or 30% of cells in the composition express NEUROD6; and/or (iii) about 1 transcripts per million (TPM) to about 210 TPM, about 1 TPM to about 205 TPM, about 1 TPM to about 25 TPM, or about 10 TPM to about 20 TPM of NEUROD6 transcripts are expressed by the cells of the composition; and/or (iv) at least 1 TPM, 5 TPM, 10 TPM, 12 TPM, 15 TPM, 19 TPM, 50 TPM, 100 TPM, 150 TPM, 175 TPM, 200 TPM, 203 TPM, or 210 TPM of NEUROD6 transcripts are expressed by the cells of the composition.

[030] In one embodiment, (i) about 0.5% to about 20%, about 0.5% to about 15%, about 0.5% to about 10%, about 0.5% to about 5%, about 0.5% to about 4%, about 0.5% to about 3%, about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 5%, about 1% to about 4%, or about 1% to about 3% of cells in the composition express SLA; and/or (ii) at least about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 15%, 16%, 18%, or 20% of cells in the composition express SLA; and/or (iii) about 0.1 transcripts per million (TPM) to about 60 TPM, about 0.1 TPM to about 50 TPM, about 1 TPM to about 10 TPM, about 2 TPM to about 8 TPM, or about 3 TPM to about 6 TPM, of SLA transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 10 TPM, 20 TPM, 30 TPM, 40 TPM, 50 TPM, or 60 TPM of SLA transcripts are expressed by the cells of the composition.

[031] In one embodiment, (i) about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 20% to about 30%, or about 20% to about 28% of cells in the composition express NELL2; and/or (ii) at least about 10%, 12%, 15%, 17%, 20%, 21%, 24%, 25%, 27%, 30%, 32%, 35%, 40%, or 45% of cells in the composition express NELL2; and/or (iii) about 1 transcripts per million (TPM) to about 150 TPM, about 4 TPM to about 130 TPM, about 4 TPM to about 35 TPM, about 20 TPM to about 30 TPM, or about 25 TPM to about 28 TPM of NELL2 transcripts are expressed by the cells of the composition; and/or (iv) at least 1 TPM, 5 TPM, 15 TPM, 20 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, 120 TPM, or 150 TPM of NELL2 transcripts are expressed by the cells of the composition.

[032] In one embodiment, (i) about 1% to about 20%, about 1% to about 15%, about 1% to about 12%, about 1% to about 11%, about 2% to about 20%, about 2% to about 15%, about 2% to about 12%, about 2% to about 11%, about 3% to about 20%, about 3% to about 15%, about 3% to about

12%, about 3% to about 11%, about 2% to about 6%, about 2% to about 5%, or about 3% to about 4% of cells in the composition express SATB2; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 15%, 17%, or 20% of cells in the composition express SATB2; and/or (iii) about 0.1 transcripts per million (TPM) to about 30 TPM, about 0.5 TPM to about 20 TPM, about 1 TPM to about 5 TPM, or about 2 TPM to about 3 TPM of SATB2 transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 12 TPM, 15 TPM, 20 TPM, 25 TPM, or 30 TPM of SATB2 transcripts are expressed by the cells of the composition.

[033] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises a progenitor expressing one or more markers selected from the group consisting of VIM, MKI67, CLU, and GLI3. In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of progenitors that cumulatively expresses each of markers VIM, MKI67, CLU, and GLI3.

[034] In one embodiment, (i) about 30% to about 80%, about 30% to about 75%, about 30% to about 70%, about 40% to about 75%, about 40% to about 70%, about 40% to about 69%, about 40% to about 60%, or about 42% to about 47% of cells in the composition express VIM; and/or (ii) at least about 30%, 35%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 50%, 52%, 55%, 57%, 60%, 62%, 65%, 67%, 69%, 72%, 75%, or 80% of cells in the composition express VIM; and/or (iii) about 250 transcripts per million (TPM) to about 900 TPM, about 250 TPM to about 865 TPM, about 200 TPM to about 350 TPM, or about 250 TPM to about 340 TPM of VIM transcripts are expressed by the cells of the composition; and/or (iv) at least 250 TPM, 260 TPM, 270 TPM, 300 TPM, 320 TPM, 350 TPM, 370 TPM, 400 TPM, 500 TPM, 600 TPM, 700 TPM, 800 TPM, or 900 TPM of VIM transcripts are expressed by the cells of the composition.

[035] In one embodiment, (i) about 5% to about 20%, about 5% to about 15%, about 5% to about 12%, about 6% to about 15%, about 6% to about 12%, about 7% to about 15%, about 7% to about 12%, or about 6% to about 8% of cells in the composition express MKI67; and/or (ii) at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% of cells in the composition express MKI67; and/or (iii) about 5 transcripts per million (TPM) to about 40 TPM, about 10 TPM to about 35 TPM, about 15 TPM to about 25 TPM, or about 18 TPM to about 22 TPM of MKI67 transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 10 TPM, 12 TPM, 15 TPM, 17 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 32 TPM, 33 TPM, 35 TPM, 37 TPM, or 40 TPM of MKI67 transcripts are expressed by the cells of the composition.

[036] In one embodiment, (i) about 10% to about 60%, about 15% to about 55%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 40%, about 20% to about 35%, or about 25% to about 32% of cells in the composition express CLU; and/or (ii) at least about 10%, 15%, 17%, 20%, 22%, 25%, 27%, 30%, 32%, 35%, 37%, 40%, 42%, 45%, 47%, 50%, 55%, or 60% of cells in the composition express CLU; and/or (iii) about 30 transcripts per million

(TPM) to about 400 TPM, about 40 TPM to about 150 TPM, about 60 TPM to about 150 TPM, or about 60 TPM to about 105 TPM of CLU transcripts are expressed by the cells of the composition; and/or (iv) at least 30 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 80 TPM, 90 TPM, 100 TPM, 125 TPM, 150 TPM, 175 TPM, 200 TPM, 225 TPM, 250 TPM, 275 TPM, 300 TPM, 325 TPM, 350 TPM, 365 TPM, 375 TPM, or 400 TPM of CLU transcripts are expressed by the cells of the composition.

[037] In one embodiment, (i) about 10% to about 50%, about 10% to about 40%, about 10% to about 30%, about 12% to about 50%, about 12% to about 35%, about 12% to about 29%, about 15% to about 29%, about 15% to about 29%, or about 15% to about 17% of cells in the composition express GLI3; and/or (ii) at least about 10%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 35%, 40%, 45%, or 50% of cells in the composition express GLI3; and/or (iii) about 5 transcripts per million (TPM) to about 60 TPM, about 10 TPM to about 45 TPM, about 15 TPM to about 30 TPM, or about 20 TPM to about 25 TPM of GLI3 transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 10 TPM, 12 TPM, 15 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 25 TPM, 30 TPM, 35 TPM, 37 TPM, 40 TPM, 42 TPM, 45 TPM, 50 TPM, 55 TPM, or 60 TPM of GLI3 transcripts are expressed by the cells of the composition.

[038] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises an astrocyte expressing one or more markers selected from the group consisting of GFAP, LUCAT1, MIR99AHG, and FBXL7. In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of astrocytes that cumulatively expresses each of markers GFAP, LUCAT1, MIR99AHG, and FBXL7.

[039] In one embodiment, (i) about 1% to about 50%, about 1% to about 20%, about 1% to about 15%, about 1% to about 13%, about 1% to about 10%, about 1% to about 7%, about 1% to about 5%, about 1% to about 4%, or about 1% to about 3% of cells in the composition express GFAP; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of cells in the composition express GFAP; and/or (iii) about 0.1 transcripts per million (TPM) to about 150 TPM, about 0.1 TPM to about 125 TPM, about 1 TPM to about 20 TPM, or about 3 TPM to about 15 TPM of GFAP transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 0.2 TPM, 0.5 TPM, 1 TPM, 5 TPM, 7 TPM, 10 TPM, 12 TPM, 14 TPM, 16 TPM, 30 TPM, 40 TPM, 50 TPM, 80 TPM, 100 TPM, 110 TPM, 115 TPM, 120 TPM, 130 TPM, 140 TPM, or 150 TPM of GFAP transcripts are expressed by the cells of the composition.

[040] In one embodiment, (i) about 5% to about 20%, about 5% to about 17%, about 5% to about 15%, about 5% to about 13%, about 7% to about 20%, about 7% to about 17%, about 7% to about 15%, about 7% to about 13%, about 5% to about 12%, or about 7% to about 10% of cells in the

composition express LUCAT1; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 17%, or 20% of cells in the composition express LUCAT1.

[041] In one embodiment, (i) about 50% to about 100%, about 50% to about 90%, about 50% to about 88%, about 60% to about 100%, about 60% to about 90%, about 60% to about 88%, about 70% to about 90%, about 70% to about 88%, or about 75% to about 82% of cells in the composition express MIR99AHG; and/or (ii) at least about 50%, 60%, 65%, 70%, 72%, 75%, 77%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 92%, 94%, 96%, 98%, or 100% of cells in the composition express MIR99AHG; and/or (iii) about 5 transcripts per million (TPM) to about 40 TPM, about 5 TPM to about 30 TPM, about 6 TPM to about 25 TPM, or about 10 TPM to about 15 TPM of MIR99AHG transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 16 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 28 TPM, 30 TPM, 34 TPM, 38 TPM, or 40 TPM of MIR99AHG transcripts are expressed by the cells of the composition.

[042] In one embodiment, (i) about 20% to about 70%, about 20% to about 60%, about 25% to about 70%, about 25% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 40%, about 32% to about 39%, or about 34% to about 39% of cells in the composition express FBXL7; and/or (ii) at least about 20%, 22%, 25%, 27%, 30%, 32%, 34%, 35%, 37%, 38%, 39%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 60%, 65%, or 70% of cells in the composition express FBXL7; and/or (iii) about 5 transcripts per million (TPM) to about 40 TPM, about 5 TPM to about 30 TPM, about 7 TPM to about 25 TPM, about 10 TPM to about 15 TPM, or about 11 TPM to about 14 TPM of FBXL7 transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 16 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 28 TPM, 30 TPM, 34 TPM, 38 TPM, or 40 TPM of FBXL7 transcripts are expressed by the cells of the composition.

[043] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises an alternative neuron expressing one or more markers selected from the group consisting of MEIS2, PBX3, GRIA2, and CACNA1C. In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of alternative neurons that cumulatively expresses each of markers MEIS2, PBX3, GRIA2, and CACNA1C.

[044] In one embodiment, (i) about 30% to about 80%, about 30% to about 90%, about 40% to about 90%, about 45% to about 85%, about 45% to about 80%, about 49% to about 79%, or about 50% to about 78% of cells in the composition express MEIS2; and/or (ii) at least about 30%, 35%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 57%, 60%, 62%, 65%, 67%, 70%, 72%, 75%, 77%, 80%, 81%, or 82% of cells in the composition express MEIS2; and/or (iii) about 5 transcripts per million (TPM) to about 200 TPM, about 10 TPM to about 180 TPM, about 50 TPM to about 180 TPM, or about 60 TPM to about 173 TPM of MEIS2 transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 10 TPM, 15 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 80

TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 135 TPM, 136 TPM, 138 TPM, 140 TPM, 145 TPM, 150 TPM, 160 TPM, 170 TPM, 180 TPM, 190 TPM, or 200 TPM of MEIS2 transcripts are expressed by the cells of the composition.

[045] In one embodiment, (i) about 30% to about 90%, about 35% to about 85%, about 40% to about 85%, about 40% to about 80%, about 45% to about 75%, or about 49% to about 75% of cells in the composition express PBX3; and/or (ii) at least about 30%, 35%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 57%, 60%, 62%, 65%, 67%, 70%, 72%, 75%, 80%, 85%, or 90% of cells in the composition express PBX3; and/or (iii) about 5 transcripts per million (TPM) to about 100 TPM, about 5 TPM to about 90 TPM, about 25 TPM to about 90 TPM, or about 29 TPM to about 88 TPM of PBX3 transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 15 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, or 100 TPM of PBX3 transcripts are expressed by the cells of the composition.

[046] In one embodiment, (i) about 20% to about 60%, about 20% to about 50%, about 20% to about 50%, about 20% to about 48%, about 22% to about 55%, about 22% to about 50%, about 22% to about 47%, or about 23% to about 47% of cells in the composition express GRIA2; and/or (ii) at least about 20%, 22%, 25%, 27%, 30%, 32%, 34%, 35%, 37%, 38%, 39%, 40%, 42%, 45%, 46%, 47%, 50%, 52%, 54%, 56%, 58%, or 60% of cells in the composition express GRIA2; and/or (iii) about 2 transcripts per million (TPM) to about 40 TPM, about 2 TPM to about 30 TPM, about 4 TPM to about 35 TPM, or about 10 TPM to about 30 TPM of GRIA2 transcripts are expressed by the cells of the composition; and/or (iv) at least 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 15 TPM, 17 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 25 TPM, 26 TPM, 27 TPM, 28 TPM, 29 TPM, 30 TPM, 35 TPM, or 40 TPM of GRIA2 transcripts are expressed by the cells of the composition.

[047] In one embodiment, (i) about 20% to about 70%, about 30% to about 60%, about 35% to about 70%, about 35% to about 60%, about 33% to about 60%, about 35% to about 60%, or about 39% to about 60% of cells in the composition express CACNA1C; and/or (ii) at least about 20%, 25%, 30%, 32%, 34%, 35%, 37%, 38%, 39%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 65%, or 70% of cells in the composition express CACNA1C; and/or (iii) about 1 transcripts per million (TPM) to about 15 TPM, about 1 TPM to about 10 TPM, about 1 TPM to about 7 TPM, or about 3 TPM to about 7 TPM of CACNA1C transcripts are expressed by the cells of the composition; and/or (iv) at least 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 10 TPM, 12 TPM, or 15 TPM of CACNA1C transcripts are expressed by the cells of the composition.

[048] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises: (i) an inhibitory neuron expressing one or more markers selected from the group consisting of DLX5, TUBB3, SCGN, ERBB4, and CALB2; (ii) an excitatory neuron expressing one or more markers selected from the group consisting of NEUROD2, NEUROD6, SLA, NELL2, and SATB2; (iii) a

progenitor expressing one or more markers selected from the group consisting of VIM, MKI67, CLU, and GLI3; (iv) an astrocyte expressing one or more markers selected from the group consisting of GFAP, LUCAT1, MIR99AHG, and FBXL7; and (v) an alternative neuron expressing one or more markers selected from the group consisting of MEIS2, PBX3, GRIA2, and CACNA1C.

[049] In one embodiment, the plurality of heterogeneous photoreceptor rescue cells comprises: (i) a plurality of inhibitory neurons that cumulatively expresses each of markers DLX5, TUBB3, SCGN, ERBB4, and CALB2; (ii) a plurality of excitatory neurons that cumulatively expresses each of markers NEUROD2, NEUROD6, SLA, NELL2, and SATB2; (iii) a plurality of progenitors that cumulatively expresses each of markers VIM, MKI67, CLU, and GLI3; (iv) a plurality of astrocytes that cumulatively expresses each of markers GFAP, LUCAT1, MIR99AHG, and FBXL7; and (v) a plurality of alternative neurons that cumulatively expresses each of markers MEIS2, PBX3, GRIA2, and CACNA1C.

[050] In one embodiment, the composition comprises: (i) about 25% to about 55%, about 25% to about 50%, about 30% to about 55%, about 30% to about 50%, about 35% to about 55%, about 35% to about 50%, or about 38% to about 49% inhibitory neurons; and/or (ii) about 0% to about 15%, about 0% to about 12%, about 0% to about 10%, about 0% to about 8%, or about 0.5% to about 9% excitatory neurons; and/or (iii) about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 17% to about 45%, about 17% to about 40%, about 17% to about 35%, or about 20% to about 35% progenitors; and/or (iv) about 0% to about 6%, about 0% to about 5%, about 0% to about 4%, about 0% to about 3%, about 0% to about 2%, about 0.5% to about 6%, about 0.5% to about 5%, about 0.5% to about 4%, about 0.5% to about 4%, about 0.5% to about 3%, about 0.5% to about 2%, or about 0.5% to about 1.5% astrocytes; and/or (v) about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 12% to about 50%, about 12% to about 45%, about 12% to about 40%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, or about 17% to about 37% mixed neurons.

[051] In one embodiment, cells in the composition further express one or more eye field progenitor markers, rod/cone photoreceptor markers, and/or neuron markers. In one embodiment, the eye field progenitor markers are selected from the group consisting of PAX6, LHX2, SIX3, NES, and SOX2. In one embodiment, the plurality of heterogeneous cells cumulatively expresses at least 1, 2, 3 or 4 of the eye field progenitor markers PAX6, LHX2, SIX3, NES, or SOX2. In one embodiment, the plurality of heterogeneous cells cumulatively expresses at least each of the eye field progenitor markers PAX6, LHX2, SIX3, NES, and SOX2. In one embodiment, the plurality of heterogeneous cells cumulatively expresses SOX2. In one embodiment, the composition is substantially free of cells that express eye field progenitor markers RAX, SIX6, and/or TBX3.

[052] In one embodiment, (i) about 25% to about 60%, about 25% to about 55%, about 25% to about 52%, about 25% to about 45%, about 30% to about 60%, about 30% to about 55%, about 30%

to about 45%, , or about 30% to about 42% of cells in the composition express PAX6; and/or (ii) at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% of cells in the composition express PAX6; and/or (iii) about 20 transcripts per million (TPM) to about 125 TPM, about 30 TPM to about 110 TPM, about 35 TPM to about 100 TPM, about 70 TPM to about 80 TPM, or about 73 TPM to about 78 TPM of PAX6 transcripts are expressed by the cells of the composition; and/or (iv) at least 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 75 TPM, 78 TPM, 80 TPM, 82 TPM, 85 TPM, 87 TPM, 90 TPM, 92 TPM, 95 TPM, 97 TPM, 100 TPM, 105 TPM, 110 TPM, 115 TPM, 120 TPM, or 125 TPM of PAX6 transcripts are expressed by the cells of the composition.

[053] In one embodiment, (i) about 3% to about 35%, about 5% to about 35%, about 5% to about 35%, about 6% to about 35%, about 7% to about 35%, about 3% to about 30%, about 5% to about 30%, about 6% to about 30%, about 7% to about 30%, about 3% to about 25%, about 5% to about 25%, about 6% to about 25%, or about 7% to about 9% of cells in the composition express LHX2; and/or (ii) at least about 3%, 4%, 6%, 8%, 10%, 15%, 20%, 25%, 26%, 27%, 28%, 29%, 30%, 32%, or 35% of cells in the composition express LHX2 ; and/or (iii) about 5 transcripts per million (TPM) to about 40 TPM, about 5 TPM to about 36 TPM, about 5 TPM to about 10 TPM, or about 6 TPM to about 8 TPM of LHX2 transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 17 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 32 TPM, 36 TPM, 38 TPM, or 40 TPM of LHX2 transcripts are expressed by the cells of the composition.

[054] In one embodiment, (i) about 1% to about 25%, about 1% to about 20%, 1% to about 18%, 1% to about 16%, about 1% to about 14%, about 1% to about 12%, 1% to about 10%, about 2% to about 25%, about 2% to about 20%, 2% to about 18%, 2% to about 16%, about 2% to about 14%, about 2% to about 12%, or 2% to about 10%, of cells in the composition express SIX3; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, or 25% of cells in the composition express SIX3 ; and/or (iii) about 1 transcripts per million (TPM) to about 50 TPM, about 2 TPM to about 30 TPM, about 1 TPM to about 25 TPM, or about 5 TPM to about 20 TPM of SIX3 transcripts are expressed by the cells of the composition; and/or (iv) at least 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM 9 TPM, 10 TPM, 12 TPM, 15 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, or 50 TPM of SIX3 transcripts are expressed by the cells of the composition.

[055] In one embodiment, (i) about 15% to about 40%, about 15% to about 35%, about 15% to about 34%, about 15% to about 33%, about 15% to about 32% about 15% to about 31%, about 18% to about 40%, about 18% to about 35%, about 18% to about 34%, about 18% to about 33%, about 18% to about 32%, or about 18% to about 31% of cells in the composition express NES; and/or (ii) at least about 15%, 17%, 20%, 25%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, or 40% of cells in the composition express NES ; and/or (iii) about 5 transcripts per million (TPM) to about 35 TPM, about 5 TPM to about 28 TPM, about 7 TPM to about 15 TPM, about 10 TPM to about 15 TPM, or

about 11 TPM to about 13 TPM of NES transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 17 TPM, 19 TPM, 20 TPM, 22 TPM, 24 TPM, 25 TPM, 26 TPM, 27 TPM, 30 TPM, or 35 TPM of NES transcripts are expressed by the cells of the composition.

[056] In one embodiment, (i) about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 55% to about 90%, about 55% to about 85%, about 55% to about 80%, about 55% to about 75%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, or about 60% to about 75% of cells in the composition express SOX2; and/or (ii) at least about 50%, 55%, 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, or 90% of cells in the composition express SOX2; and/or (iii) about 50 transcripts per million (TPM) to about 250 TPM, about 90 TPM to about 200 TPM, about 125 TPM to about 190 TPM, or about 155 TPM to about 175 TPM of CACNA1C transcripts are expressed by the cells of the composition; and/or (iv) at least 50 TPM, 60 TPM, 70 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 180 TPM, 190 TPM, 200 TPM, 210 TPM, 220 TPM, 230 TPM, 240 TPM, or 250 TPM of CACNA1C transcripts are expressed by the cells of the composition.

[057] In one embodiment, the rod/cone photoreceptor markers are selected from the group consisting of ASCL1, RORB, NR2E3, and NRL. In one embodiment, the plurality of heterogeneous cells cumulatively expresses each of the rod/cone photoreceptor markers ASCL1, RORB, NR2E3, and NRL. In one embodiment, the composition is substantially free of cells that express rod/cone photoreceptor markers CRX, RHO, OPN1SW, PDE6B, RCVRN, ARR3, CNGB1, GNAT1, and GNAT2.

[058] In one embodiment, (i) about 10% to about 60%, about 20% to about 60%, about 20% to about 50%, about 20% to about 45%, about 22% to about 45%, about 22% to about 43%, about 25% to about 42%, or about 28% to about 30% of cells in the composition express ASCL1; and/or (ii) at least about 10%, 15%, 20%, 22%, 25%, 27%, 30%, 32%, 35%, 37%, 40%, 41%, 42%, 45%, 50%, 55%, or 60% of cells in the composition express ASCL1; and/or (iii) about 50 transcripts per million (TPM) to about 150 TPM, about 60 TPM to about 140 TPM, about 65 TPM to about 130 TPM, or about 95 TPM to about 130 TPM of ASCL1 transcripts are expressed by the cells of the composition; and/or (iv) at least 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, 120 TPM, 125 TPM, 130 TPM, 140 TPM, or 150 TPM of ASCL1 transcripts are expressed by the cells of the composition.

[059] In one embodiment, (i) about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 22%, about 10% to about 25%, or about 11% to about 22% of cells in the composition express RORB; and/or (ii) at least about 5%, 7%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 25%, 30%, 35%, 40%, 45%, or 50% of cells in the composition express RORB;

and/or (iii) about 0.1 transcripts per million (TPM) to about 20 TPM, about 0.1 TPM to about 10 TPM, about 2 TPM to about 8 TPM, or about 1 TPM to about 6 TPM of RORB transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 12 TPM, 14 TPM, 16 TPM, 18 TPM, or 20 TPM of RORB transcripts are expressed by the cells of the composition.

[060] In one embodiment, (i) about 1% to about 25%, about 1% to about 20%, 1% to about 18%, 1% to about 16%, about 1% to about 14%, about 1% to about 12%, 1% to about 10%, about 2% to about 25%, about 2% to about 20%, 2% to about 18%, 2% to about 16%, about 2% to about 14%, about 2% to about 12%, or about 2% to about 5% of cells in the composition express NR2E3; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, or 20% of cells in the composition express NR2E3; and/or (iii) about 0.1 transcripts per million (TPM) to about 10 TPM, about 0.1 TPM to about 9 TPM, about 0.1 TPM to about 3 TPM, or about 0.1 TPM to about 1 TPM of NR2E3 transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 0.4 TPM, 0.5 TPM, 0.6 TPM, 0.7 TPM, 0.8 TPM, 0.9 TPM, 1 TPM, 2 TPM, 5 TPM, 7 TPM, 9 TPM, or 10 TPM of NR2E3 transcripts are expressed by the cells of the composition.

[061] In one embodiment, (i) about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 2% to about 20%, about 2% to about 15%, about 2% to about 10%, about 2% to about 9%, or about 2% to about 8% of cells in the composition express NRL; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, or 20% of cells in the composition express NRL; and/or (iii) about 0.1 transcripts per million (TPM) to about 10 TPM, about 0.1 TPM to about 9 TPM, about 0.1 TPM to about 7 TPM, or about 0.1 TPM to about 2 TPM of NRL transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 0.4 TPM, 0.5 TPM, 0.6 TPM, 0.7 TPM, 0.8 TPM, 0.9 TPM, 1 TPM, 1.5 TPM, 2 TPM, 4 TPM, 6 TPM, 8 TPM, or 10 TPM of NRL transcripts are expressed by the cells of the composition.

[062] In one embodiment, the neuron markers are selected from the group consisting of TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5. In one embodiment, the plurality of heterogeneous cells cumulatively expresses each of the neuron markers TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

[063] In one embodiment, (i) about 50% to about 100%, about 60% to about 100%, about 60% to about 95%, about 70% to about 100%, about 70% to about 95%, about 80% to about 100%, about 80% to about 95%, or about 80% to about 90% of cells in the composition express NFIB; and/or (ii) at least about 50%, 55%, 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 98%, or 100% of cells in the composition express NFIB; and/or (iii) about 150 transcripts per million (TPM) to about 650 TPM, about 180 TPM to about 610 TPM, about 400 TPM to about 500 TPM, or about 400 TPM to about

480 TPM of NFIB transcripts are expressed by the cells of the composition; and/or (iv) at least 150 TPM, 175 TPM, 180 TPM, 185 TPM, 190 TPM, 200 TPM, 225 TPM, 250 TPM, 275 TPM, 300 TPM, 325 TPM, 350 TPM, 375 TPM, 400 TPM, 425 TPM, 450 TPM, 475 TPM, 500 TPM, 550 TPM, 600 TPM, 625 TPM, or 650 TPM of NFIB transcripts are expressed by the cells of the composition.

[064] In one embodiment, (i) about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 2% to about 20%, about 2% to about 15%, about 2% to about 10%, about 2% to about 9%, about 2% to about 8%, or about 2% to about 6% of cells in the composition express OTX2; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, or 20% of cells in the composition express OTX2; and/or (iii) about 5 transcripts per million (TPM) to about 50 TPM, about 8 TPM to about 40 TPM, about 8 TPM to about 25 TPM, or about 12 TPM to about 15 TPM of OTX2 transcripts are expressed by the cells of the composition; and/or (iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 15 TPM, 16 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 26 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, or 50 TPM of OTX2 transcripts are expressed by the cells of the composition.

[065] In one embodiment, (i) about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 78%, about 55% to about 90%, about 55% to about 85%, about 55% to about 80%, about 55% to about 78%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, or about 60% to about 78% of cells in the composition express ELAVL3; and/or (ii) at least about 50%, 55%, 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, or 90% of cells in the composition express ELAVL3; and/or (iii) about 10 transcripts per million (TPM) to about 120 TPM, about 15 TPM to about 100 TPM, about 20 TPM to about 90 TPM, about 60 TPM to about 90 TPM, or about 70 TPM to about 80 TPM of ELAVL3 transcripts are expressed by the cells of the composition; and/or (iv) at least 10 TPM, 15 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 71 TPM, 72 TPM, 73 TPM, 74 TPM, 75 TPM, 76 TPM, 77 TPM, 78 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, or 120 TPM of ELAVL3 transcripts are expressed by the cells of the composition.

[066] In one embodiment, (i) about 30% to about 90%, about 30% to about 85%, about 30% to about 80%, about 30% to about 78%, about 35% to about 90%, about 35% to about 85%, about 35% to about 80%, about 35% to about 78%, about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, or about 50% to about 65% of cells in the composition express ELAVL4; and/or (ii) at least about 30%, 40%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 75%, 80%, 85%, or 90% of cells in the composition express ELAVL4; and/or (iii) about 10 transcripts per million (TPM) to about 120 TPM, about 15 TPM to about 100 TPM, about 20 TPM to about 90 TPM, about 50 TPM to about 90 TPM, about 70 TPM to about 90 TPM, or about 79 TPM to about 85 TPM of ELAVL4 transcripts are

expressed by the cells of the composition; and/or (iv) at least 10 TPM, 15 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 71 TPM, 72 TPM, 73 TPM, 74 TPM, 75 TPM, 76 TPM, 77 TPM, 78 TPM, 80 TPM, 82 TPM, 84 TPM, 86 TPM, 90 TPM, 100 TPM, 110 TPM, or 120 TPM of ELAVL4 transcripts are expressed by the cells of the composition.

[067] In one embodiment, (i) about 40% to about 90%, about 40% to about 85%, about 40% to about 80%, about 40% to about 75%, about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 50% to about 73%, about 51% to about 73%, or about 52% to about 66% of cells in the composition express SLC1A2; and/or (ii) at least about 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 75%, 80%, 85%, or 90% of cells in the composition express SLC1A2; and/or (iii) about 10 transcripts per million (TPM) to about 120 TPM, about 10 TPM to about 90 TPM, about 20 TPM to about 90 TPM, about 40 TPM to about 70 TPM, about 50 TPM to about 70 TPM, or about 58 TPM to about 63 TPM of SLC1A2 transcripts are expressed by the cells of the composition; and/or (iv) at least 10 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, or 120 TPM of SLC1A2 transcripts are expressed by the cells of the composition.

[068] In one embodiment, (i) about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 11% to about 19%, or about 10% to about 12% of cells in the composition express SLC1A3; and/or (ii) at least about 5%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 25%, 30%, 35%, or 40% of cells in the composition express SLC1A3; and/or (iii) about 50 transcripts per million (TPM) to about 200 TPM, about 70 TPM to about 190 TPM, about 60 TPM to about 100 TPM, about 60 TPM to about 80 TPM, or about 72 TPM to about 79 TPM of SLC1A3 transcripts are expressed by the cells of the composition; and/or (iv) at least 50 TPM, 60 TPM, 65 TPM, 70 TPM, 71 TPM, 72 TPM, 73 TPM, 74 TPM, 75 TPM, 76 TPM, 77 TPM, 78 TPM, 79 TPM, 80 TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 175 TPM, 180 TPM, or 200 TPM of SLC1A3 transcripts are expressed by the cells of the composition.

[069] In one embodiment, (i) about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, or about 3% to about 4% of cells in the composition express HCN1; and/or (ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% of cells in the composition express HCN1; and/or (iii) about 0.1 transcripts per million (TPM) to about 10 TPM, about 0.1 TPM to about 5 TPM, about 0.1 TPM to about 1 TPM, or about 0.2 TPM to about 0.6 TPM of HCN1 transcripts are expressed by the cells of the composition; and/or (iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 0.4 TPM, 0.5 TPM, 0.6 TPM, 0.7

TPM, 0.8 TPM, 0.9 TPM, 1.0 TPM, 2 TPM, 4 TPM, 6 TPM, 8 TPM, or 10 TPM of HCN1 transcripts are expressed by the cells of the composition.

[070] In one embodiment, (i) about 5% to about 30%, about 5% to about 25%, about 1% to about 30%, about 1% to about 25%, about 5% to about 25%, about 10% to about 25%, or about 10% to about 15% of cells in the composition express HES5; and/or (ii) at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, or 30% of cells in the composition express HES5; and/or (iii) about 10 transcripts per million (TPM) to about 60 TPM, about 5 TPM to about 50 TPM, about 12 TPM to about 46 TPM, or about 15 TPM to about 39 TPM of HES5 transcripts are expressed by the cells of the composition; and/or (iv) at least 10 TPM, 15 TPM, 17 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 32 TPM, 35 TPM, 37 TPM, 40 TPM, 42 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM of HES5 transcripts are expressed by the cells of the composition.

[071] In one embodiment, the plurality of heterogeneous cells cumulatively expresses: (i) one or more of the eye field progenitor markers PAX6, LHX2, SIX3, NES, and SOX2; (ii) one or more of the rod/cone photoreceptor markers ASCL1, RORB, NR2E3, and NRL; and (iii) one or more of the neuron markers TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

[072] In one embodiment, the plurality of heterogeneous cells cumulatively expresses: (i) each of the eye field progenitor markers PAX6, LHX2, SIX3, NES, and SOX2; (ii) each of the rod/cone photoreceptor markers ASCL1, RORB, NR2E3, and NRL; and (iii) each of the neuron markers TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

[073] In one embodiment, the composition is substantially free of at least one cell type selected from the group consisting of pluripotent stem cells, retinal ganglion cells, photoreceptors and amacrine cells. In one embodiment, the composition is substantially free of pluripotent stem cells, retinal ganglion cells, photoreceptors and amacrine cells.

[074] In one embodiment, the composition is substantially free of retinal progenitors expressing VSX2 and/or POU5F1. In one embodiment, the composition has less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.5% of cells expressing SSEA4, optionally, determined by flow cytometry, or wherein the composition is free of cells expressing SSEA4, optionally, determined by flow cytometry.

[075] In one embodiment, the cells in the composition have phagocytic activity, optionally the ability to phagocytose isolated photoreceptor outer segments, dye conjugates or both.

[076] In one embodiment, the cells in the composition secrete one or more neuroprotective factors. In one embodiment, the neuroprotective factors are selected from the group consisting of CNTF, MIF, S100B, GFAP, TAU, NCAM1, and TNC.

[077] In one embodiment, at least 50%, at least 55%, at least 60%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 75%, or at least 80% of the cells in the

composition are viable. In one embodiment, about 50% to about 80%, about 55% to about 80%, about 60% to about 80%, about 65% to about 80%, about 70% to about 80%, about 75% to about 80%, about 50% to about 75%, about 55% to about 75%, about 60% to about 75%, about 65% to about 75%, about 70% to about 75%, about 50% to about 70%, about 55% to about 70%, about 60% to about 70%, about 65% to about 70%, about 50% to about 65%, about 55% to about 65%, about 60% to about 65%, about 50% to about 60%, about 55% to about 60%, or about 50% to about 55% of the cells in the composition are viable. In one embodiment, at least 50% of the cells in the composition are viable. In one embodiment, at least 55% of the cells in the composition are viable. In one embodiment, at least 60% of the cells in the composition are viable. In one embodiment, at least 65% of the cells in the composition are viable. In one embodiment, at least 68% of the cells in the composition are viable.

[078] In one embodiment, the composition is produced according to a method comprising: 1) culturing pluripotent stem cells in Rescue Induction Medium (RIM) and Noggin; 2) culturing the cells from step 1 in Neural Differentiation Medium (NDM) and Noggin; 3) expanding the cells from step 2 in NDM in the absence of Noggin, comprising a) culturing the cells in low-adherence or non-adherent conditions in NDM without Noggin, and b) culturing the cells in adherent conditions in NDM without Noggin.

[079] In one embodiment, the pluripotent stem cells are embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).

[080] In one embodiment, step 3 is performed at least 1 time, at least 2 times, at least 3 times, at least 4 times, at least 5 times, or at least 6 times. In one embodiment, the cells in the composition are harvested after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3. In one embodiment, the cells in the composition are harvested after the fifth repeat of step 3.

[081] In one embodiment, the harvested cells in the composition are cryopreserved. In one embodiment, the composition is cryopreserved between the first and second repeat of step 3, between the second and third repeat of step 3, between the third and fourth repeat of step 3, or between the fourth and fifth repeat of step 3. In one embodiment, the composition is cryopreserved after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3. In one embodiment, step 3 is repeated five times and wherein the composition is cryopreserved after the fifth repeat of step 3.

[082] In one embodiment, at least 50%, at least 55%, at least 60%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 75%, or at least 80% of the cells are viable after cryopreservation and thawing. In one embodiment, about 50% to about 80%, about 55% to about 80%, about 60% to about 80%, about 65% to about 80%, about 70% to about 80%, about 75% to about 80%, about 50% to about 75%, about 55% to about 75%, about 60% to about 75%, about 65% to about 75%, about 70% to about 75%, about 50% to about 70%, about 55% to about 70%, about 60% to about 70%, about 65% to about 70%, about 50% to about 65%, about 55% to about 65%,

about 60% to about 65%, about 50% to about 60%, about 55% to about 60%, or about 50% to about 55% viable after cryopreservation and thawing. In one embodiment, at least about 50% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, at least about 55% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, at least about 60% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, at least about 65% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, at least 68% of the cells in the composition are viable after cryopreservation and thawing.

[083] In one embodiment, the composition comprises cell spheres. In one embodiment, about 50% to about 100%, about 60% to about 100%, about 70% to about 100%, about 80% to about 100%, about 50% to about 90%, about 50% to about 80%, about 50% to about 70%, about 60% to about 90%, about 60% to about 80%, or about 70% to about 90% of the cells are cell spheres.

[084] In one embodiment, during steps 1, 2 and/or 3 the cells of the composition are cultured in a cell culture vessel selected from the group consisting of a culture dish, a culture flask, and a culture chamber. In one embodiment, the cell culture vessel has about 50 cm² to about 800 cm², about 60 cm² to about 800 cm², about 100 cm² to about 800 cm², about 150 cm² to about 800 cm², about 175 cm² to about 800 cm², about 200 cm² to about 800 cm², about 250 cm² to about 800 cm², about 300 cm² to about 800 cm², about 400 cm² to about 800 cm², about 500 cm² to about 800 cm², about 600 cm² to about 800 cm², about 700 cm² to about 800 cm², about 30 cm² to about 100 cm², about 50 cm² to about 100 cm², about 100 cm² to about 300 cm², about 150 cm² to about 250 cm², or about 150 cm² to about 200 cm² cell growth area. In one embodiment, the cell culture vessel has at least about 60 cm², about 175 cm², or about 636 cm² cell growth area.

[085] In one embodiment, the culture chamber is a stackable rectangular chamber. In one embodiment, step 3 has 1 to 40, 2 to 40, 5 to 40, 10 to 40, 20 to 40, 1 to 20, 2 to 20, 5 to 20, 10 to 20, 1 to 10, 2 to 10, 5 to 10, 1 to 5, or 1 to 2 culture chambers. In one embodiment, step 3 has at least 1, 2, 5, 10, or 40 culture chambers.

[086] In one embodiment, the cell culture vessel is coated for low-adherence or non-adherent cell culture for step 3a, and/or adherent cell culture for step 3b. In one embodiment, the cells are enzymatically disassociated from the plate into cell suspension. In one embodiment, the enzymatic disassociation utilizes an enzyme selected from the group consisting of thermolysin, liberase, accutase and a combination thereof. In one embodiment, the enzyme used to disassociate cells is accutase. In one embodiment, disassociation of the cells from the plate does not involve manual scraping.

[087] Accordingly, in another aspect, the present invention provides a pharmaceutical preparation comprising the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein and a pharmaceutically acceptable excipient. In one embodiment, the pharmaceutically acceptable excipient is suitable for ocular delivery.

[088] Accordingly, in another aspect, the present invention provides a formulation comprising: a) the composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein; and b) about 4-10% (v/v) cryoprotectant, about 2-3% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer.

[089] In one embodiment, the cryoprotectant is selected from DMSO, glycerol, and ethylene glycol. In one embodiment, the cryoprotectant is DMSO. In one embodiment, the formulation comprises about 4-6% (v/v) cryoprotectant. In one embodiment, the formulation comprises about 0.08-0.10% (w/v) glucose. In one embodiment, the formulation comprises about 5% (v/v) DMSO, about 2.5% (w/v) albumin, about 0.09% (w/v) glucose, and a buffer. In one embodiment, the formulation comprises about 0.6% (w/v) glucose. In one embodiment, the formulation comprises about 5% DMSO, about 2.5% albumin, about 0.6% glucose, and a buffer.

[090] In one embodiment, albumin is human albumin. In one embodiment, albumin is recombinant human albumin.

[091] In one embodiment, the buffer is buffered saline. In one embodiment, the buffer is phosphate-buffered saline (PBS). In one embodiment, the buffered saline comprises Ca^{2+} and Mg^{2+} . In one embodiment, the buffered saline does not comprise Ca^{2+} and Mg^{2+} . In one embodiment, the formulation is cryopreserved.

[092] Accordingly, in another aspect, the present invention provides a formulation comprising: a) the composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein; and b) a solution comprising (1) a buffer, maintaining the solution at a physiological pH; and (2) at least 2 mM or at least 0.05% (w/v) glucose; and (3) an osmotically active agent maintaining the solution at a physiological osmolarity.

[093] In one embodiment, the solution comprises at least 5 mM or at least 0.1% (w/v) glucose; or at least 7.5 mM or at least 0.14% (w/v) glucose; or at least 10 mM or at least 0.2% (w/v) glucose; or at least 15 mM or at least 0.25% (w/v) glucose; or at least 20 mM or at least 0.4% (w/v) glucose; or at least 25 mM or at least 0.5% (w/v) glucose.

[094] In one embodiment, the solution further comprises (4) a source of divalent cations, optionally wherein the source of divalent cations comprises a calcium and/or a magnesium source, and/or (5) an acetate buffer and/or a citrate buffer.

[095] Accordingly, in another aspect, the present invention provides a formulation comprising: a) the composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein; and b) a solution comprising (1) a buffer, maintaining the solution at a physiological pH, wherein the buffer is not a dicarbonate buffer; and (2) glucose; and (3) an osmotically active agent maintaining the solution at a physiological osmolarity;

and (4) a source of divalent cations, optionally wherein the source of divalent cations comprises a calcium source and/or a magnesium source, and/or wherein the buffer comprises an acetate buffer and/or a citrate buffer.

[096] In one embodiment, the calcium source comprises a pharmaceutically acceptable calcium salt, and/or the magnesium source comprises a pharmaceutically acceptable magnesium salt. In one embodiment, the pharmaceutically acceptable calcium and/or the pharmaceutically acceptable magnesium salt is selected from the group of calcium and/or magnesium salts formed with an acid selected from the group comprising acetic acid, ascorbic acid, citric acid, hydrochloric acid, maleic acid, oxalic acid, phosphoric acid, stearic acid, succinic acid, and sulfuric acid. In one embodiment, the calcium source comprises calcium chloride, optionally wherein the calcium source comprises calcium chloride dihydrate. In one embodiment, the magnesium source comprises magnesium chloride, optionally wherein the magnesium source comprises magnesium chloride hexahydrate. In one embodiment, the citrate buffer is provided as sodium citrate. In one embodiment, the glucose is D-glucose (Dextrose).

[097] In one embodiment, the osmotically active agent is a salt, optionally wherein the osmotically active agent is a sodium salt, further optionally wherein the osmotically active agent is sodium chloride. In one embodiment, the solution comprises calcium chloride, magnesium chloride, sodium citrate, sodium chloride, and glucose.

[098] In one embodiment, the pH of the solution is 6.8-7.8, or 7.4-7.5, or about 7.5.

[099] In one embodiment, the solution is isotonic or hypertonic.

[100] In one embodiment, the solution exhibits an osmolality of about 270-345 mOsm/l or about 315 mOsm/l.

[101] In one embodiment, the concentration of the calcium source is (a) 0.25-0.75 mM, or 0.4-0.65 mM, or 0.5-0.6 mM, or about 0.6 mM; or (b) 0.5-0.9 mM, or 0.6-0.8 mM, or about 0.7 mM.

[102] In one embodiment, the concentration of the magnesium source is 0.05-5 mM, or 0.1-0.3 mM, or about 0.3 mM. In one embodiment, the concentration of the glucose is 5-50 mM, or 10-25 mM, or 10-20 mM, or about 16 mM. In one embodiment, the concentration of the osmotically active agent is about 100-200 mM, or about 125-175 mM, or about 150 mM. In one embodiment, the concentration of citrate or acetate is 0.1-5 mM, or 0.5-2 mM, or about 1 mM.

[103] In one embodiment, the solution further comprises a potassium salt, optionally wherein the potassium salt is potassium chloride, further optionally wherein the concentration of KCl is 0.2-5 mM, or 1-2.5 mM, or about 2 mM.

[104] In one embodiment, the solution comprises (a) about 0.7 mM CaCl_2 (calcium chloride), about 0.3 mM MgCl_2 (magnesium chloride), about 1 mM sodium citrate, about 16 mM dextrose, and about 145 mM NaCl, or (b) about 0.5-0.9 mM CaCl_2 (calcium chloride), about 0.2-4 mM MgCl_2 (magnesium chloride), about 0.8-1.2 mM sodium citrate, about 13-19 mM dextrose, and about 116-174 mM NaCl, or (c) about 0.85% NaCl, about 0.01% CaCl_2 dihydrate (calcium chloride dihydrate),

about 0.006% MgCl_2 hexahydrate (magnesium chloride hexahydrate), about 0.035% sodium citrate dihydrate, and about 0.29% dextrose, or (d) about 0.68-1.02 % NaCl, about 0.008-0.012% CaCl_2 dihydrate (calcium chloride dihydrate), about 0.0048-0.0072% MgCl_2 hexahydrate (magnesium chloride hexahydrate), about 0.028-0.042% sodium citrate dihydrate, and about 0.23-0.35% dextrose.

[105] In one embodiment, the solution further comprises (a) about 2 mM KCl, and/or (b) a viscoelastic polymer, optionally wherein the polymer is hyaluronic acid or a salt or solvate thereof, further optionally wherein the polymer is sodium hyaluronate.

[106] In one embodiment, the polymer is present at a concentration effective to reduce the exposure of cells in the solution to shear stress, optionally wherein the concentration of the polymer is 0.005-5% w/v or about 0.05% w/v.

[107] In one embodiment, the solution comprises (a) about 0.7 mM CaCl_2 (calcium chloride), about 0.3 mM MgCl_2 (magnesium chloride), about 2 mM KCl, about 1 mM sodium citrate, about 16 mM dextrose, about 145 mM NaCl, and about 0.05% hyaluronic acid, or (b) about 0.5-0.8 mM CaCl_2 (calcium chloride), about 0.2-0.4 mM MgCl_2 (magnesium chloride), about 1.6-2.4 mM KCl, about 0.8-1.2 mM sodium citrate, about 13-19 mM dextrose, about 116-174 mM NaCl, and about 0.04-0.06% hyaluronic acid.

[108] In one embodiment, the solution does not comprise (a) a carbonate buffer, and/or (b) glutathione, or glutathione disulfide (GSSG), and/or (c) a zwitterionic organic buffer.

[109] In one embodiment, the solution can be (a) stored for at least 48 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 144 hours, at least one week, at least two weeks, at least three weeks, or at least one month at 25 °C without measurable precipitation of solutes and/or measurable loss of the capability of the solution to support survival and viability of cells stored in the solution, and/or (b) stored for at least 48 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 144 hours, at least one week, at least two weeks, at least three weeks, or at least one month at 2-8 °C without measurable precipitation of solutes and/or measurable loss of the capability of the solution to support survival and viability of cells stored in the solution.

[110] In one embodiment, the solution is (a) suitable for administration to a subject, suitable for administration to the eye of a subject, and/or suitable for transplanting cells into the eye of a subject, and/or (b) essentially pyrogen-free, and/or (c) sterile, and/or (d) for irrigation, cell reconstitution, cell storage, cell transport, and/or administration to a subject.

[111] Accordingly, in another aspect, the present invention provides a method of producing the composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, comprising 1) culturing pluripotent stem cells in Rescue Induction Medium (RIM) and Noggin; 2) culturing the cells from step 1 in Neural Differentiation Medium (NDM) and Noggin; 3) expanding the cells from step 2 in NDM without Noggin, comprising a) culturing the cells in low-adherence or non-adherent conditions in NDM without Noggin, and b) and culturing the cells in non-

adherent conditions in NDM without Noggin, thereby differentiating the pluripotent stem cells into photoreceptor rescue cells.

[112] Accordingly, in another aspect, the present invention provides a method of producing a photoreceptor rescue cell composition comprising a plurality of heterogeneous cells, the method comprising: 1) culturing pluripotent stem cells in Rescue Induction Medium (RIM) and Noggin; 2) culturing the cells in Neural Differentiation Medium (NDM) and Noggin; 3) expanding the cells in NDM without Noggin, comprising a) culturing the cells in low-adherence or non-adherent conditions in NDM without Noggin, and b) and culturing the cells in adherent conditions in NDM without Noggin, thereby differentiating the pluripotent stem cells into the plurality of cells of the photoreceptor rescue cell composition.

[113] In one embodiment, the pluripotent stem cells are embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).

[114] In one embodiment, step 3 is performed at least 1 times, at least 2 times, at least 3 times, at least 4 times, at least 5 times, or at least 6 times. In one embodiment, the cells in the composition are harvested after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3. In one embodiment, the composition are harvested after the fifth repeat of step 3.

[115] In one embodiment, the harvested cells in the composition are cryopreserved.

[116] In one embodiment, the composition is cryopreserved between the first and second repeat of step 3, between the second and third repeat of step 3, between the third and fourth repeat of step 3, or between the fourth and fifth repeat of step 3. In one embodiment, the composition is cryopreserved after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3. In one embodiment, step 3 is repeated five times and wherein the composition is cryopreserved after the fifth repeat of step 3.

[117] In one embodiment, at least 50%, at least 55%, at least 60%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 75%, or at least 80% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, about 50% to about 80%, about 55% to about 80%, about 60% to about 80%, about 65% to about 80%, about 70% to about 80%, about 75% to about 80%, about 50% to about 75%, about 55% to about 75%, about 60% to about 75%, about 65% to about 75%, about 70% to about 75%, about 50% to about 70%, about 55% to about 70%, about 60% to about 70%, about 65% to about 70%, about 50% to about 65%, about 55% to about 65%, about 60% to about 65%, about 50% to about 60%, about 55% to about 60%, or about 50% to about 55% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, less than about 50% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, less than about 55% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, less than about 60% of the cells in the composition are viable after cryopreservation and thawing. In one embodiment, less than about 65% of the cells in the composition are viable after cryopreservation and thawing. In one

embodiment, less than about 68% of the cells in the composition are viable after cryopreservation and thawing.

[118] In one embodiment, the composition comprises cell spheres. In one embodiment, about 50% to about 100%, about 60% to about 100%, about 70% to about 100%, about 80% to about 100%, about 50% to about 90%, about 50% to about 80%, about 50% to about 70%, about 60% to about 90%, about 60% to about 80%, or about 70% to about 90% of the cells are cell spheres.

[119] In one embodiment, during steps 1, 2 and/or 3 the cells of the composition are cultured in a cell culture vessel selected from the group consisting of a culture dish, a culture flask, and a culture chamber.

[120] In one embodiment, the cell culture vessel has about 50 cm² to about 800 cm², about 60 cm² to about 800 cm², about 100 cm² to about 800 cm², about 150 cm² to about 800 cm², about 175 cm² to about 800 cm², about 200 cm² to about 800 cm², about 250 cm² to about 800 cm², about 300 cm² to about 800 cm², about 400 cm² to about 800 cm², about 500 cm² to about 800 cm², about 600 cm² to about 800 cm², about 700 cm² to about 800 cm², about 30 cm² to about 100 cm², about 50 cm² to about 100 cm², about 100 cm² to about 300 cm², about 150 cm² to about 250 cm², or about 150 cm² to about 200 cm² cell growth area. In one embodiment, the cell culture vessel has at least about 60 cm², about 175 cm², or about 636 cm² cell growth area.

[121] In one embodiment, the culture chamber is a stackable rectangular chamber.

[122] In one embodiment, step 3 has 1 to 40, 2 to 40, 5 to 40, 10 to 40, 20 to 40, 1 to 20, 2 to 20, 5 to 20, 10 to 20, 1 to 10, 2 to 10, 5 to 10, 1 to 5, or 1 to 2 culture chambers. In one embodiment, step 3 has at least 1, 2, 5, 10, or 40 culture chambers.

[123] In one embodiment, the cell culture vessel is coated for low-adherence or non-adherent cell culture for step 3a, and/or adherent cell culture for step 3b.

[124] In one embodiment, the cells are enzymatically disassociated from the plate into cell suspension. In one embodiment, the enzyme used to disassociate cells is thermolysin, liberase, and/or accutase. In one embodiment, the enzyme used to disassociate cells is accutase. In one embodiment, disassociation of the cells from the plate does not involve manual scraping.

[125] Accordingly, in another aspect, the present invention provides a photoreceptor rescue cell composition produced by the method of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[126] Accordingly, in another aspect, the present invention provides a method of treating an eye disease or disorder in a subject, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[127] Accordingly, in another aspect, the present invention provides a method of increasing secretion of neuroprotective factors in an eye of a subject having an eye disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[128] In one embodiment, the method increases secretion of neuroprotective factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to secretion of neuroprotective factors prior to administration.

[129] Accordingly, in another aspect, the present invention provides a method of improving visual acuity in a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[130] In one embodiment, the method increases visual acuity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to visual acuity prior to administration.

[131] In one embodiment, visual acuity is measured by optometer response (OMR) and/or electroretinogram (ERG).

[132] In one embodiment, the treated eye has increased spatial frequency threshold measured by OMR of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to spatial frequency threshold prior to administration.

[133] In one embodiment, the treated eye has increased scotopic b-wave amplitude measured by ERG of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to scotopic b-wave amplitude prior to administration.

[134] Accordingly, in another aspect, the present invention provides a method of preventing or slowing loss of photoreceptor cells in a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or

the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[135] In one embodiment, preventing loss of photoreceptor cells is measured by the expression of CNFT. In one embodiment, the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% as compared to expression of CNFT in the eye prior to administration.

[136] Accordingly, in another aspect, the present invention provides a method of increasing phagocytic activity in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[137] In one embodiment, the method increases phagocytic activity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to phagocytic activity prior to administration.

[138] Accordingly, in another aspect, the present invention provides a method of inhibiting microglial activation in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[139] In one embodiment, inhibiting microglial activation is measured by the expression of CNFT and/or MIF. In one embodiment, the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration. In one embodiment, the method increases expression of MIF by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of MIF prior to administration.

[140] Accordingly, in another aspect, the present invention provides a method of decreasing oxidative stress in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the

formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[141] In one embodiment, decreasing oxidative stress is measured by the expression of CNFT. In one embodiment, the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration.

[142] Accordingly, in another aspect, the present invention provides a method of increasing expression of anti-apoptotic factors in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[143] In one embodiment, the method increases expression of anti-apoptotic factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of anti-apoptotic factors prior to administration.

[144] In one embodiment, the anti-apoptotic factor is S100B.

[145] Accordingly, in another aspect, the present invention provides a method of preventing degeneration of outer nuclear layer (ONL) in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical preparation of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[146] In one embodiment, the disease or disorder is rod or cone dystrophy, retinal degeneration, retinitis pigmentosa, diabetic retinopathy, macular degeneration, geographic atrophy secondary to macular degeneration, intermediate dry age-related macular degeneration (AMD), Leber congenital amaurosis or Stargardt disease. In one embodiment, the eye disease is macular degeneration or retinitis pigmentosa. In one embodiment, the disease is a retinal degenerative disease.

[147] In one embodiment, the disease is associated with loss of photoreceptor cells.

[148] In one embodiment, the disease is associated with loss of photoreceptor cells in the outer nuclear layer of the retina. In one embodiment, the photoreceptor rescue cell composition, formulation or pharmaceutical preparation is administered to the subretinal space, the suprachoroidal space, by depot to the eye, or by systemic delivery to other part of the body of the subject.

- [149] In one embodiment, the cell preparation or formulation is administered by injection or implantation.
- [150] In one embodiment, the injection is administered intraocularly.
- [151] In one embodiment, the intraocular administration comprises injection of an aqueous solution, optionally an isotonic solution and/or a saline solution, into the subretinal space, thereby forming a pre-bleb, and removal of said aqueous solution, prior to administration of said photoreceptor rescue cell composition into the same subretinal space as said aqueous solution.
- [152] In one embodiment, the cell preparation or formulation is administered within at least 1 week, at least 1 month, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years of onset of symptoms.
- [153] In one embodiment, (i) the subject has intermediate or near end-stage disease; (ii) the subject has a Best Corrected Visual Acuity (BCVA) ranging from 20/50 to 20/200; (iii) the subject has a BCVA worse than 20/200 but maintains light perception; or (iv) is diagnosed as having retinitis pigmentosa by genotyping.
- [154] In one embodiment, the method further wherein one or more anti-inflammatory agents are administered to the subject.
- [155] In one embodiment, the one or more anti-inflammatory agents are administered concurrently. In one embodiment, the one or more anti-inflammatory agents are administered separately.
- [156] In one embodiment, the one or more anti-inflammatory agents are administered prior to the cell preparation or formulation, or the cell preparation or formulation is administered prior to the one or more anti-inflammatory agents. In one embodiment, the one or more anti-inflammatory agents are administered before and after administration of the cell preparation or formulation. In one embodiment, the administration of the cell preparation or formulation is without one or more anti-inflammatory agents. In one embodiment, the one or more anti-inflammatory agents comprises dexamethasone and/or cyclosporine.
- [157] Accordingly, in another aspect, the present invention provides a population of extracellular vesicles (EVs) secreted from the photoreceptor rescue cell composition of various embodiments of the above aspects or any other aspect of the invention delineated herein.
- [158] In one embodiment, the EVs secreted from the photoreceptor rescue cell express at least one of the proteins selected from FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, NFIA, DLX5, TUBB3, SCGN, ERBB4, CALB2, NEUROD2, NEUROD6, SLA, NELL2, SATB2, VIM, MKI67, CLU, GLI3, GFAP, LUCAT1, MIR99AHG, FBXL7, MEIS2, PBX3, GRIA2, CACNA1C, PAX6, LHX2, SIX3, NES, SOX2, ASCL1, RORB, NR2E3, NRL, TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, HES5, AGT, ACBLN2, CDH7, DNAH11, EGR1, FAM216B, FOS, KCNC2, LGI2, LOC221946, LRRC4C, MAP3k19, OLFM3, PRND, PTGER3, RELN, TCERGIL, TSHR, UNC13C, TRb2, PDE6B, CNGb1, Tuj1, CHX10, Nestin, TRbeta2, MASH1, RORbeta, MAP2, ELAVL3, NFIA, DCX, LHX2, SLC1A2, ELAVL4, PAX6,

EMX2, ASCL1, DLL1, NFIB, ENOX1, TUBB3, MAP2, DCLK1/2, DCX, KALRN, LINC00461, C1orf61, NCAM1, SETBP1, PAK3, AKAP6, RTN1, CRMP1, FOXG1, TRIM2, BACH2, Recoverin, Opsin, Rhodopsin, rod and cone cGMP Phosphodiesterase.

[159] Accordingly, in another aspect, the present invention provides a pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein and a pharmaceutically acceptable carrier.

[160] Accordingly, in another aspect, the present invention provides a formulation comprising: a) the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the pharmaceutical composition of various embodiments of the above aspects or any other aspect of the invention delineated herein; and b) about 4-10% (v/v) cryoprotectant, about 2-3% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer.

[161] In one embodiment, the cryoprotectant is selected from DMSO, glycerol, and ethylene glycol. In one embodiment, the cryoprotectant is DMSO. In one embodiment, the formulation comprises about 4-6% (v/v) cryoprotectant. In one embodiment, the formulation comprises about 0.08-0.10% (w/v) glucose. In one embodiment, the formulation comprises about 5% (v/v) DMSO, about 2.5% (w/v) albumin, about 0.09% (w/v) glucose, and a buffer. In one embodiment, the formulation comprises about 0.6% (w/v) glucose. In one embodiment, the formulation comprises about 5% DMSO, about 2.5% albumin, about 0.6% glucose, and a buffer.

[162] In one embodiment, albumin is human albumin. In one embodiment, albumin is recombinant human albumin.

[163] In one embodiment, the buffer is buffered saline. In one embodiment, the buffer is phosphate-buffered saline (PBS). In one embodiment, the buffered saline comprises Ca^{2+} and Mg^{2+} . In one embodiment, the buffered saline does not comprise Ca^{2+} and Mg^{2+} .

[164] In one embodiment, the formulation is cryopreserved.

[165] Accordingly, in another aspect, the present invention provides a method of treating an eye disease in a subject, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[166] Accordingly, in another aspect, the present invention provides a method of increasing secretion of neuroprotective factors in an eye of a subject, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the

formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[167] In one embodiment, the method increases secretion of neuroprotective factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to secretion of neuroprotective factors prior to administration.

[168] Accordingly, in another aspect, the present invention provides a method of improving visual acuity in a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[169] In one embodiment, the method increases visual acuity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to visual acuity prior to administration. In one embodiment, visual acuity is measured by optometer response (OMR) and/or electroretinogram (ERG). In one embodiment, the treated eye has increased spatial frequency threshold measured by OMR of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to spatial frequency threshold prior to administration. In one embodiment, the treated eye has increased scotopic b-wave amplitude measured by ERG of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to scotopic b-wave amplitude prior to administration.

[170] Accordingly, in another aspect, the present invention provides a method of preventing or slowing loss of photoreceptor cells in a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[171] In one embodiment, preventing or slowing loss of photoreceptor cells is measured by the expression of CNFT. In one embodiment, the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% as compared to expression of CNFT in the eye prior to administration.

[172] Accordingly, in another aspect, the present invention provides a method of increasing phagocytic activity in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[173] In one embodiment, the method increases phagocytic activity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to phagocytic activity prior to administration.

[174] Accordingly, in another aspect, the present invention provides a method of inhibiting microglial activation in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[175] In one embodiment, inhibiting microglial activation is measured by the expression of CNFT and/or MIF. In one embodiment, the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration. In one embodiment, the method increases expression of MIF by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of MIF prior to administration.

[176] Accordingly, in another aspect, the present invention provides a method of decreasing oxidative stress in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[177] In one embodiment, decreasing oxidative stress is measured by the expression of CNFT. In one embodiment, the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at

least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration.

[178] Accordingly, in another aspect, the present invention provides a method of increasing expression of anti-apoptotic factors in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[179] In one embodiment, the method increases expression of anti-apoptotic factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of anti-apoptotic factors prior to administration. In one embodiment, the anti-apoptotic factor is S100B.

[180] Accordingly, in another aspect, the present invention provides a method of preventing degeneration of outer nuclear layer (ONL) in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, the pharmaceutical composition comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein, or the formulation comprising the population of EVs of various embodiments of the above aspects or any other aspect of the invention delineated herein.

[181] In one embodiment, the disease or disorder is rod or cone dystrophy, retinal degeneration, retinitis pigmentosa, diabetic retinopathy, macular degeneration, geographic atrophy secondary to macular degeneration, intermediate dry age-related macular degeneration (AMD), Leber congenital amaurosis or Stargardt disease. In one embodiment, the eye disease is macular degeneration or retinitis pigmentosa. In one embodiment, the disease is a retinal degenerative disease. In one embodiment, the disease is associated with loss of photoreceptor cells. In one embodiment, the disease is associated with loss of photoreceptor cells in the outer nuclear layer of the retina.

[182] In one embodiment, the population of EVs, the pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered to the subretinal space or to the suprachoroidal space of the subject.

[183] In one embodiment, the population of EVs, the pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered by injection or implantation.

[184] In one embodiment, the injection is administered intraocularly.

[185] In one embodiment, the intraocular administration comprises injection of an aqueous solution, optionally an isotonic solution and/or a saline solution, into the subretinal space, thereby forming a

pre-bleb, and removal of said aqueous solution, prior to administration of said photoreceptor rescue cell composition into the same subretinal space as said aqueous solution.

[186] In one embodiment, the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered within at least 1 week, at least 1 month, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years of onset of symptoms.

[187] In one embodiment, the method further wherein one or more anti-inflammatory agents are administered to the subject.

[188] In one embodiment, the one or more anti-inflammatory agents and the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs are administered concurrently.

[189] In one embodiment, the one or more anti-inflammatory agents and the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs are administered separately.

[190] In one embodiment, 1) the one or more anti-inflammatory agents are administered prior to the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs, or 2) the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered prior to the one or more anti-inflammatory agents.

[191] In one embodiment, the one or more anti-inflammatory agents are administered before and after administration of the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs.

[192] In one embodiment, the administration of the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is without one or more anti-inflammatory agents. In one embodiment, the one or more anti-inflammatory agents comprises dexamethasone and/or cyclosporine.

BRIEF DESCRIPTION OF THE DRAWINGS

[193] **FIG. 1A** depicts cell cluster analysis of ESC/PRE/PRCs. ESCs: Undifferentiated, research grade J1-ESCs and Kd-ESCs. RPE: J1-RPE at P3+4 months timepoint. PRC: J1-PRC (P4), Kd-PRC (P4), GB2R-PRC-2019 (P4), GB2R-PRC-PR1 (P4), GB2R-PRC-CN2 (P4), GB2R-PRC-CN3 (P4).

[194] **FIG. 1B** depicts qPCR validation of neuronal genes, including NANOG, NEUROD2, GAD1, DCX, MAP2, and NFIA.

[195] **FIG. 1C** depicts single cell analysis of PRCs-P4 (GB2R-PRC-2019 (P4), GB2R-PRC-PR1 (P4), GB2R-PRC-CN2 (P4), and GB2R-PRC-CN3 (P4)). Cells were categorized as inhibitory neurons, excitatory neurons, mixed neurons, progenitors, and astrocytes.

[196] **FIG. 1D** depicts qPCR identity assays comparing upregulation of markers, FOXG1, MAP2, STMN2, DCX, and LINC00461, in GB2R-PRC, GB2R-ESC, RPE, and HMC cells. GB2R-FCP: GB2R-Final Cell Product includes, GB2R-PRC-CN3, GB2R-PRC-CN2, Pioneer, and GB2R-PRC-2019.

[197] **FIG. 1E** shows heat map of expression of eye field progenitor markers, rod/cone photoreceptor markers, and neuronal markers in inhibitory neurons, excitatory neurons, alternative neurons, progenitors and astrocytes as shown in **FIG. 1C**.

[198] **FIGs. 1F-1T** show expression of cell markers from day 2 (D2), day 12 (D12), day 19 (D19), day 37 (D37/P0), day 55 (D55/P1), day 72 (D72/P2), day 90 (D90/P3) and day 107 (D107/P4) from PRC compositions listed in **Table 10**. PRC markers FOXG1, MAP2, STMN2, and DCX (**FIG. 1F**); PRC markers LINC00461, NEUROD2, GAD1, and NFIA (**FIG. 1G**); Inhibitory neuron markers DLX5, TUBB3, and SCGN (**FIG. 1H**); Inhibitory neuron markers ERBB4 and CALB2 (**FIG. 1I**); Excitatory neuron markers NEUROD2, NEUROD6, and SLA (**FIG. 1J**); Excitatory neuron markers NELL2, and SATB2 (**FIG. 1K**); Progenitor markers VIM, MKI67, CLU, and GLI3 (**FIG. 1L**); Astrocyte markers GFAP, MIR99AHG, and FBXL7 (**FIG. 1M**); Alternative neuron markers MEIS2, PBX3, GRIA2, and CACNA1C (**FIG. 1N**); Eye field progenitor markers PAX6, LHX2, and SIX3 (**FIG. 1O**); Eye field progenitor markers NES and SOX2 (**FIG. 1P**); Rod/cone photoreceptor markers ASCL1, RORB, NR2E3, and NRL (**FIG. 1Q**); Neuron markers TUBB3, NFIA, and NFIB (**FIG. 1R**); Neuron markers OTX2, ELAVL3, and ELAVL4 (**FIG. 1S**); and Neuron markers SLC1A2, SLC1A3, HCN1, and HES5 (**FIG. 1T**).

[199] **FIG. 2A** depicts brightfield image of PRC-P4 cells.

[200] **FIG. 2B** depicts scorecard analysis of PRCs differentiation looking at self-renewal, ectoderm, mesoderm, and endoderm markers.

[201] **FIG. 2C** depicts immunocytochemistry (ICC) staining in PRCs-P4 showing expression of PAX6/OTX2 in PRC-NPC cells (neural progenitor cells), and STMN2, CALB2, SCGN, and DCX in PRC-P4 cells.

[202] **FIG. 2D** depicts expression of markers NEUROD2, FOXG1, and HMGA1 in PRC-P4 cells indicating successful differentiation.

[203] **FIG. 2E** depicts flow cytometry of GB2R-CN3-P4 cells for purity based on FOXG1 and MAP2 expression. Percentage of cells expression purity markers: Mean % (GB2R-PRC-CN3 FCP, N = 3). FOXG1: 95.6 (94.2-96.7); MAP2: 88.4 (84.7-91.4); and SSEA4: 0.44 (0.15-0.88).

[204] **FIG. 2F** depicts differentially expressed genes between PRC-P3 and PRC-P4 determined via RNA-seq analysis. These genes have a log10 ratio of mean expression higher than 3 or lower than -3.

[205] **FIG. 3A** depicts stimulation of PRC using TBHP (Oxidative stress) and Luminex assay for detecting secreted factors from stimulated PRC. PRC: GB2R-PRC-CN3, GB2R-PRC-CN2, Pioneer, and GB2R-PRC-2019.

[206] **FIG. 3B** depicts level of secreted neuroprotective factors detected in the media when PRC cells are without oxidative stress. Stimulation of PRC using TBHP (Oxidative stress) and Luminex assay for detecting secreted factors from stimulated PRC. CNTF, MIF, and S100B were quantified in the media when unstimulated (without oxidative stress). PRC: GB2R-PRC-CN3, GB2R-PRC-CN2, Pioneer, and GB2R-PRC-2019.

[207] **FIG. 3C** shows confocal images demonstrating that subretinal engraftment of PRCs suppress microglial infiltration into the ONL (P60) in RCS rats. Stained retinal sections were imaged using confocal z-stack analysis on the Leica SP8.

[208] **FIG. 3D** shows stimulation of Microglia line SIM-A9 using LPS and inhibition using L-Carnitine.

[209] **FIG. 3E** depicts phagocytosis assay with GB2R-PRC-PR1 incubated with pHrodo *E. coli* bioparticles.

[210] **FIG. 3F** depicts internalization of rod outer segment (ROS) debris in RCS (Royal College of Surgeons) rat retina by PRCs. RHO+ debris observed in GFP+ PRC graft (left panel), and EM in right panel show ROS shed in PRC's cytoplasm. GFAP+ PRCs co-stain with HuNu (data not shown).

[211] **FIG. 4A** depicts schematic of RCS rat dosing and time course of *in vivo* experimentation.

[212] **FIG. 4B** depicts Optomotor response (OMR) and Electroretinogram (ERG) analysis of RCS rats injected at P25 with 100,000 cells/eye of GB2R-PRC-2019 PRCs. Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.) with test articles compared with uninjected and GS2 vehicle injected eyes. Cell Viability: 78.4% (manual).

[213] **FIG. 4C** shows representative images showing PRC engraftment significantly attenuates outer nuclear layer (ONL) degeneration out to 3 months post-transplantation. Confocal images show PRC engraftment is highly correlated with ONL preservation. Stained retinal sections were imaged using the Leica MDi8 epifluorescent microscope.

[214] **FIG. 4D** depicts quantification of outer nuclear layer (ONL) thickness showing PRC engraftment is correlated to significant preservation at P35, P60 and P120. Cell Viability: P35: 57.1%, 54%; P60: 57.1% (Cellometer); P120: >70% (Manual). n=148 ROI/ 7 eyes.

[215] **FIG. 4E** depicts area plot of GB2R-PRC-2019 (P4) engrafted PRCs vs. photoreceptor ONL in the P120 RCS rat model of retinal degeneration. Statistical analysis was performed using the Pearson correlation coefficient. Cell Viability: >70% (Manual). n = 4 eyes (12 sections/eye).

[216] **FIG. 5A** depicts immunohistochemical analysis of glial fibrillary acid protein (rabbit anti-GFAP; ABCAM) in the P120 RCS rat. GFAP is expressed in both Müller glia (MG) and optic nerve fiber astrocytes of the host rat retina (white arrows) as well as engrafted PRCs in the subretinal space. MG hypertrophy with increased expression of GFAP in MG and astrocytes, as well as expanded distribution of GFAP expression throughout the radial MG cell body, is characteristic of disease associated reactive gliosis and can be seen distal to the graft site (yellow arrows, white arrows). Upregulation and increased distribution of GFAP, MG hypertrophy, and outer nuclear layer

(ONL) degeneration is reduced or absent at the subretinal PRC graft site. Cell Viability: >70% (Manual).

[217] **FIG. 5B** depicts TUNEL quantification (TUNEL label Mix, Sigma catalog# 11767291910, TUNEL enzyme Sigma catalog# 11767305001) performed at P35 during which peak apoptotic activity is observed in the RCS retina. PRCs engrafted in the subretinal space were identified by Ku80+ staining (Abcam, ab80592) of adjacent sections (see below). In PRC engrafted areas, very few, if any TUNEL+ nuclei are observed, indicating little to no apoptotic activity at graft sites. However, non-engrafted areas contain significant TUNEL+ nuclei in the ONL, indicating widespread photoreceptor death at P35. Quantification of TUNEL+ nuclei in the ONL shows significant attenuation of apoptotic activity at graft sites vs non-graft areas. Retinal sections through the PRC graft were chosen for TUNEL quantification and stained with Ku80 to localize engrafted PRCs and DAPI to identify the ONL. A total of 3 to 4 regions of interest (ROIs) were imaged at central and peripheral regions per retina. TUNEL quantification was performed in a blinded fashion, using only the DAPI channel. ROIs were subsequently identified as “Graft” or “No Graft” by the presence of subretinal Ku80+ PRCs. Cell Viability: 3.5%/52.3% (Cellometer).

[218] **FIG. 5C** depicts extracellular vesicles (EVs) isolated from PRCs preserve OMR response out to P90 in RCS rats. EVs were isolated from PRC conditioned media and subretinally injected into RCS rats at a dose of 9.42×10^8 EVs/eye. Other RCS rats received subretinal injections of PRCs (100,000 cells/eye), or vehicle. Injections were performed at age P25. OMR analysis was performed at P60, 90, 120 and compared to non-injected (NI) animals at the same age.

[219] **FIG. 5D** depicts a single subretinal injection of PRC-EVs preserves ONL thickness out to P90. Quantification of retinal ONL thickness from PRC-EV injected RCS rats vs that of uninjected eyes. Retinal cryosections were stained with DAPI to visualize the ONL. Stained retinal sections were imaged using the Leica DMi8 epifluorescent scope. ONL thickness was determined by line graph measurements through the ONL at central and peripheral areas as described in **FIG. 4C**. P90: ONL preservation correlates with OMR preservation. ONL thickness in Long-evans Rat (sighted): $\sim 55 \mu\text{m}$ (Weber et al., 1996). P- prefix denotes Postnatal Age.

[220] **FIG. 5E** depicts single PRC-EV injection. ONL thickness preserved out to P90.

[221] **FIG. 5F** depicts ONL after no PRC-EV injection. Small patches of preserved ONL, on average 1-3 rows of nuclei.

[222] **FIG. 5G** depicts ONL after PRC-EV injection. Longer stretches of preserved ONL when compared to uninjected, on average 3-6 rows of nuclei.

[223] **FIG. 5H** depicts ONL after PRC-EV injection at P120. Single PRC-EV injection does not preserve ONL thickness at P120.

[224] **FIG. 5I** depicts optomotor responses after PRC-EV injection. Visual function was assessed by recording optomotor responses in PRC-EV injected rats at P60, P90 and P120.

- [225] **FIG. 5J** ONL analysis of PRC-EV-treated rats shows single injection of PRC-EVs confers morphological preservation of the ONL up to P90 but not at P120.
- [226] **FIG. 5K** depicts ONL after subretinal cell transplantation of PRCs. Subretinal cell transplantation of PRCs preserves ONL thickness up to P120.
- [227] **FIG. 6A** shows Toluidine blue staining of PRC engrafted areas and non-engrafted areas. ONL preservation is clearly observed whereas non-engrafted areas show severely degenerated ONL. ONL visualized by thick, dark band (long arrows) Note: the absence of ONL preservation on the non-injected side (short arrows).
- [228] **FIG. 6B** shows Transmission Electron Microscope (TEM) analysis of engrafted and non-engrafted site of the ONL. There was noticeable preservation of the ONL in engrafted areas compared to ONL sites with no PRC engraftment.
- [229] **FIG. 6C** shows TEM ultrastructural analysis. TEM shows overall morphological preservation of photoreceptors as well as finer structures such as the connecting cilium of the rod inner segment in the presence of subretinal PRCs. Finally, PRC engraftment minimizes the debris zone of the subretinal space. In the absence of subretinal PRCs, rod outer segment debris populates most of the subretinal space due to non-clearance after outer segment shedding. GB2R-PRC-2019 were injected at P25 postnatal. Black arrowheads pointing to preserved connecting cilium of the inner and outer segment. Cell Viability: 57.1% (Cellometer).
- [230] **FIG. 7A** depicts a schematic outline for administration of PRCs using an rd10 mouse model. On postnatal 14 days (P14), 100k cells were transplanted into subretinal space of the right eye, GS2 vehicle was injected into left eyes as a control. OMR were performed on P21, P28, P35, P42 and P49. Cell Viability: 68.5% (Manual).
- [231] **FIG. 7B** depicts a graph showing that at P21 and P28, both eyes show presence of tracking. At P35, P42 and P49 control eye became blind and cell-injected eye still show the capacity to track.
- [232] **FIG. 7C** depicts a graph showing morphometric analysis of photoreceptor outer nuclear layer (ONL) preservation in the P28 rd10 mouse. ONL preservation quantified as ONL area (mm^2) can be identified at the locus of PRC subretinal engraftment compared with adjacent nongrafted central and peripheral retina. Cell Viability: 73.3% (Manual).
- [233] **FIG. 7D** depicts a graph showing analysis of cone length in the P28 rd10 mouse. The length of cone arrestin labelled cone photoreceptors is significantly increased at the locus of PRC subretinal engraftment compared with adjacent regions of central and peripheral retina in which there is no subretinal engraftment. Cell Viability: 73.3% (Manual).
- [234] **FIG. 7E** depicts confocal imaging showing CtBP2/RIBEYE labelled rod synapses at the site of engraftment and in central and peripheral retinal loci without engraftment.
- [235] **FIG. 7F** depicts a graph quantifying rod synapses in the P28 rd10 mouse. The number of ribeye positive/cone arrestin negative rod synapses is significantly increased at the locus of PRC

engraftment compared with nongrafted central and peripheral retina indicating PRC associated preservation of rod photoreceptors in rd10 mouse retina. Cell Viability: 73.3% (Manual).

[236] **FIG. 8A** shows OMR at P35 with sub-70% viabilities from multiple PRC lots. The viabilities of 66.05%, 68.5% and 68.8% PRC showed similar preservations effects. PRC-injected eyes showed better OMR performance than control eyes.

[237] **FIG. 8B** shows anatomical preservation of PRC cells. Anatomical preservation was observed across multiple PRC lots with sub-70% viabilities. Histological analysis confirmed OMR data. At P35, PRCs that had been injected with 66.05% viability preserved the cone anatomy as assessed by ONL thickness, axon length and morphology. At P50, PRCs that had been injected with 68.5% viability preserved ONL thickness and density of ribeye+ synapses. At P70, PRCs that had been injected with 68.8% viability also preserved ONL thickness and density of ribeye+ synapse.

[238] **FIG. 9A** shows ONL preservation in RD10 mice. On postnatal 14 days (P14), 100k cells were transplanted into subretinal space of right eye, GS2 vehicle was injected into left eye as a control. At P50, eyes were collected for IHC staining. DAPI staining showed that only one row of photoreceptors was left in no graft retina, while multiple rows of photoreceptor were observed in graft retina. Quantification of data from 5 animals, 3 sections from each animal shows ONL thickness and area in graft retina are significantly higher than those in no graft retina (6um vs 18um; 5000um² vs 10000 um²). Cell Viability: 68.5% (Manual).

[239] **FIG. 9B** depicts ICC image staining for (Left panel) Cone arrestin (Millipore AB15282 1:500) which was used to detect the morphology of cones). In no graft retina, arrestin was only expressed in cell bodies of cones, outer segment and axons were degenerated. In graft retina, cones were preserved by maintaining normal morphology, arrestin were expressed in outer segment, cell body, axon and pedicles. Quantification of data shows that cones had significant longer axons in graft retina than those in no graft (8um vs 4um). (Right panel) Ribeye (also called ct-BP2, BD transduction Laboratories 612044, 1:500) is a marker of presynaptic structure located at the axon terminal of photoreceptors. It shows the synaptic connection between photoreceptor and horizontal cells. Quantification of data shows Ribeye puncta expression was higher in graft retina than that in no graft retina (40 vs 20/100um retina). Cell Viability: 68.5% (Manual).

[240] **FIG. 9C** depicts a schematic outline for administration of PRC cells to hemizygous rat. Summary of P23H rat dosing and time course of in vivo experimentation.

[241] **FIG. 9D** depicts a graph for OMR preservation of P23H hemizygous rats injected at P25 with 100,000 cells/eye of GB2R-PRC-P4-FDA PRCs that reveals statistically significant preservation of the OMR response compared with uninjected controls at P60, P90, and P120 and compared to vehicle injected controls at P90. Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.). Cell Viability: 71.7% (Manual).

[242] **FIG. 10A** depicts a schematic of HuNu+ cell quantification process. RCS rats, aged P23 to P25 were given subretinal injections of 100,000 GB2R PRCs or GS2 vehicle. Animals were sacrificed

at P120 for retinal tissue cryosectioning and histological analysis. Every 10th retinal section through the graft was chosen for quantification wherein HuNu+ PRCs were quantified. Cell numbers were interpolated between each section, and the sum total engraftment was calculated. Total PRC engraftment was calculated from n = 4.

[243] FIG. 10B depicts quantification of the number of engrafted PRCs in 4 transplanted animals at P120, or 3 months post-transplantation. Quantification of total HuNu+ PRCs in the subretinal grafts shows an average of 80,000 transplanted cells per eye which translates to an overall 80% rate of engraftment compared to an initial injection of 100,000 cells per injection.

[244] FIG. 10C depicts differential PRC engraftment in retinal degeneration models with varying disease onset and severity post-treatment. Specific lots and doses of PRCs used in this analysis are shown in the box at the very top of the figure. *Relative Subretinal Engraftment bar graph*: RCS rat and P23H hemizygous rat models of retinal degeneration demonstrate comparable engraftment of GB2R-PRCs with maximal engraftment found to cover 54 and 41% of the subretinal space respectively. rd10 mouse and P23H homozygous rat models of retinal degeneration show significantly less engraftment with 19% (rd10 mouse) and 2% (P23H homozygous rat) of the subretinal space showing PRC engraftment at the length maxima. *Relative Retinal Length Graft Coverage graph*: Relative ratio calculations present coverage relative to the different sizes of the mouse and rat eyes while plots of maximal lengths display absolute lengths of engraftment. The rd10 mouse and P23H rat show decreased engraftment in both relative and absolute terms compared to both RCS and P23H hemizygous rats.

[245] FIG. 11A depicts a graph showing proliferation in subretinal PRCs is downregulated by P120. HuNu (Millipore, MAB1281) and Ki67 (Abcam ab15580) double positive cells in the subretinal graft were quantified in retinal cross sections in P35, P60 (not shown) and P120 animals. A total of three animals were used for each timepoint. Confocal images show Ki67 and HuNu double-positive cells in the subretinal space at P35. However, by P120, proliferative (Ki67+) PRCs are not observed. Quantification reveals significant downregulation of Ki67 and HuNu double-positive PRCs by P120, or 3 months post-transplantation (bar graph in lower left panel). Cell Viability: P35: 43.5% and 52.3%; P60: 43.5% and 52.3% (Cellometer); and P120: > 70% (Manual).

[246] FIG. 11B shows confocal images of engrafted PRCs. The absence of Oct4 (Abcam ab27985) expression in engrafted, HuNu+ PRCs shows a lack of pluripotency at all-time points investigated after transplantation (P35, P60 and P120). A total of three animals were used for each timepoint. Cultured GMP1 iPSCs were used as a positive control for Oct4 expression, with WGA-647 (Thermo Scientific W32466) and DAPI staining for subcellular compartment localization (small images in lower middle panel).

[247] FIG. 12A depicts a schematic of a protocol to make PRC cells. There are approximately 200M cells at P4-PRC (DS), and currently scalable up to 500-600M cells at P4-PRC (DS). Prioritized process changes in protocol: i) replacement of culture dish (to T-flask), and ii) replacement of 2D to

3D lift-up method from mechanical to enzymatic dissociation (expected to be implemented in GLP Tox study and first-in-human studies). RIM: Rescue Induction Medium; NDM: Neural Differentiation Medium ICC: Immunocytochemistry; IFA: Immunofluorescence assay.

[248] **FIG. 12B** depicts a schematic of a protocol to make PRC cells including a cryopreservation step in between P3 and P4. There are approximately 200M cells at P4-PRC (DS), and currently scalable up to 500-600M cells and P4-PRC (DS) based. Implemented process changes during process development: i) replacement of culture dish (to T-flask), ii) replacement of 2D to 3D lift-up method from mechanical to enzymatic dissociation (expected to be implemented in GLP Tox study and first-in-human studies), and iii) implementation of a P3-sphere Cell Stock intermediate. RIM: Rescue Induction Medium; NDM: Neural Differentiation Medium ICC: Immunocytochemistry; IFA: Immunofluorescence assay.

[249] **FIG. 12C** depicts a schematic of a manufacturing process of PRCs.

[250] **FIG. 13** depicts a schematic of manufacturing, reconstitution and injection of PRC cells. CZ: Crystal Zenith; CRF: Controlled Rate Freezer.

[251] **FIG. 14A** depicts post-thaw cell viability of photoreceptor rescue cells (PRC) cells prepared in formulations containing 2.5% rHA, DPBS with Ca and Mg, 0.6% glucose, and different concentrations of DMSO (5% DMSO or 10% DMSO), and of PRC cells formulated in a commercially available CryoStor® CS10 cell freezing formulation which contains 10% DMSO, for comparison.

[252] **FIG. 14B** depicts percent viability of P4 PRC cells after cryopreservation in different conditions. Shown as mean $\pm$ SD.

[253] **FIG. 14C** depicts percent viability of P4 PRC cells after cryopreservation in different conditions selected from conditions in **FIG. 14B**. Shown as mean $\pm$ SD.

[254] **FIG. 15A** shows comparison of OMR response in animals subretinally injected with 100,000 or 200,000 GB2R PRCs. Twelve spatial frequencies (SP) were performed and the OMR response (tracking strength) was recorded in animals subretinally injected with either 100K (left panel) or 200K (middle panel) PRCs. A response of 1.2 on the Y axis is the threshold for OMR presence (shown by green dotted line). Any value below 1.2 means eyes are not able to track. At P35, P42 and P50, control eyes were blind, and 200k PRC cell-injected eyes consistently showed larger and broader amplitude curve than 100k cohort. SP threshold right shifted indicates better visual acuity in 200k eyes. Viability: 80.6%/76.64% (manual, 2 vials).

[255] **FIG. 15B** depicts a schematic timeline for injections and analysis in rd10 mice.

[256] **FIG. 15C** ICC analysis was used to show that ONL thickness in 200k-eyes was greater than that in 100k was confirmed by ICC analysis. Quantification of ONL thickness at graft sites vs non-graft sites in animals receiving 100K and 200K cells. The data is consistent with the hypothesis that higher dosing leads to greater efficacy. ONL Thickness at P70: OMR curves consistently with larger amplitude or broader in 200k vs. 100k group ONL preservation at P70 greater in 200k vs. 100k group.

Data consistent with hypothesis that greater retinal coverage (by higher dosing) leads to greater efficacy.

[257] **FIG. 16A** shows OMR recorded in 300k Cryopreserved PRC formulation (Cryo-PRC)-injected rd10 mice (same as for previous rd10 mice, subretinal injection at P14). Cryo-PRC were functionally efficacious at P35 and P42. But at P49, OMR is absent in both vehicle and Cryo-PRC eyes. Cryo: Cryopreservation formulation with PRCs or vehicle. Cell Viability: 69.56% (Manual). N=8.

[258] **FIG. 16B** shows OMR analysis of RCS rats injected at P25 with 100,000 cells/eye of GB2R-PRC (CN2 lot); GB2R-PRCs (P3 INT DS); or GB2R-PRCs (Cryo:Cryopreserved formulation). Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.) with test articles compared with uninjected and GS2 vehicle injected eyes.

[259] **FIG. 16C** shows ERG analysis of RCS rats injected at P25 with 100,000 cells/eye of GB2R-PRC (CN2 lot); GB2R-PRCs (P3 INT DS); or GB2R-PRCs (Cryo:Cryopreserved formulation). Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.) with test articles compared with uninjected and GS2 vehicle injected eyes.

[260] **FIGs. 17A and 17B** show OCT of eyes treated with GS2 buffer and supplementary IMT. **FIG. 17A** includes conditions GS2+/cyrobuffer (1:4) without IMT (left column), and GS2+/cyrobuffer (1:4) with Dex (right column). **FIG. 17B** shows GS2+/cyrobuffer (1:4) with Dex/CsA (left column), and BSS without IMT (right column).

[261] **FIGs. 18A-18C** show ERG responses for Group 1 (GS2+/cyrobuffer (1:4) without IMT); Group 2 (GS2+/cyrobuffer (1:4) with Dex); Group 3 (GS2+/cyrobuffer (1:4) with Dex/CsA); and Group 4 (BSS without IMT). **FIG. 18A** shows a-wave responses. **FIG. 18B** shows scotopic b-wave responses. **FIG. 18C** shows photopic b-wave responses.

[262] **FIG. 19** shows retinal morphology for Group 1 (GS2+/cyrobuffer (1:4) without IMT); Group 2 (GS2+/cyrobuffer (1:4) with Dex); Group 3 (GS2+/cyrobuffer (1:4) with Dex/CsA); and Group 4 (BSS without IMT).

[263] **FIG. 20** shows OCT images for Group 1 (GS2+/cyrobuffer (1:4) without IMT); Group 2 (GS2+/cyrobuffer (1:4) with Dex); Group 3 (GS2+/cyrobuffer (1:4) with Dex/CsA); and Group 4 (BSS without IMT).

[264] **FIG. 21** shows ONL thickness for Group 1 (GS2+/cyrobuffer (1:4) without IMT); Group 2 (GS2+/cyrobuffer (1:4) with Dex); Group 3 (GS2+/cyrobuffer (1:4) with Dex/CsA); and Group 4 (BSS without IMT).

[265] **FIG. 22** shows intraretinal migration after injection with PRCs frozen at intermediate step of P3 and further differentiated to P4 after thawing.

[266] **FIG. 23** shows OMR readings comparing P4(d) and P4(i) at time points P35, P42, and P49 after injection.

- [267] **FIG. 24** shows immunohistochemistry on inner plexiform layer (IPL) showing intraretinal migration between P4(d) and P4(i) injections.
- [268] **FIG. 25** shows immunohistochemistry on IPL showing enhanced migration into IPL after injection with P4(i) PRCs.
- [269] **FIG. 26** shows OMR readings of Cohort 1 dosed with high dose P4(d) or P4(i).
- [270] **FIG. 27** shows OMR readings of Cohort 1 dosed with high dose P4(d) or P4(i).
- [271] **FIG. 28** shows OMR readings of eyes injected with PRCs prepared using CellSTACK® or PDL-precoated flasks compared to CMC control (prepared using dishes as shown in **FIGs. 12A-12B**).
- [272] **FIG. 29** shows OCT images of retinas at D7 and D38 (days after injection) comparing preparations of PRCs using CellSTACK® and PDL-precoated flasks.
- [273] **FIGs. 30A-30B** shows immunohistochemistry staining for CAR/HuNu (**FIG. 30A and FIG. 30B**) comparing PRCs prepared using CellSTACK® or PDL-precoated flasks compared to CMC control. Both **FIG. 30A and FIG. 30B** show larger grafts and better cone preservation with PRCs prepared using CellSTACK®.
- [274] **FIG. 31** shows immunohistochemistry staining for GFAP and HuNu comparing PRCs prepared using CellSTACK® or PDL-precoated flasks compared to CMC control.
- [275] **FIG. 32** shows immunohistochemistry staining for IBA1 and HuNu comparing PRCs prepared using CellSTACK® or PDL-precoated flasks compared to CMC control.
- [276] **FIG. 33** shows immunohistochemistry staining for Ki67 and HuNu comparing PRCs prepared using CellSTACK® or PDL-precoated flasks compared to CMC control.
- [277] **FIG. 34** shows immunohistochemistry staining for OCT4 and HuNu comparing PRCs prepared using CellSTACK® or PDL-precoated flasks compared to CMC control.
- [278] **FIG. 35** shows a schematic of the experimental design for delayed injection of PRCs into RCS rats. Injections were at P25, P45, or P60 after disease onset. OMR and ERG readings were taken from P60 through P150.
- [279] **FIGs. 36A and 36B** show OMR (**FIG. 36A**) and ERG (**FIG. 36B**) readings at P60, P90, P120, and P150 after each injection timepoint.
- [280] **FIG. 37** shows immunohistochemistry staining STEM21 of delayed injection of PRCs at P45 in RCS rat.
- [281] **FIGs. 38A and 38B** show quantification of subretinal PRC engraftment for injection time points of P25, P45, and P60. **FIG. 38A** shows maximal graft length and **FIG. 38B** shows maximal graft/retina length ratio.
- [282] **FIGs. 39A and 39B** show quantification of ONL preservation for injection time points of P25, P45, and P60. **FIG. 39A** shows maximal preserved ONL length and **FIG. 39B** shows maximal ONL/retina length ratio. P25 Injected Gb2R-GMP-MCB PRC-P4 RCS (P150); P45 Injected Gb2R-
- [283] GMP-MCB PRC-P4 RCS (P150); P60 Injected Gb2R-GMP-MCB PRC-P4 RCS (P150).

[284] **FIGs. 40A-40D.** The optomotor response was preserved in PRC-injected eyes. The OMR response assay was performed at postnatal ages P28 (**FIG. 40A**), P35 (**FIG. 40B**), P42 (**FIG. 40C**), and P49 (**FIG. 40D**). Statistical significance against control eyes (vehicle or uninjected) was observed in Group 1 OD, while only a trend was observed in Group 2 OD against control eyes. No statistical difference was observed between cell-injected groups. * $p < 0.05$, ** $p < 0.01$.

[285] **FIGs. 41A-41F** show OCT images of retinas for D0 Group 1 (OD) $\geq 70\%$ (**FIG. 41A**); D0 Group 2 (OD) $\leq 60\%$ (**FIG. 41B**); D9 Group 1 (OD) $\geq 70\%$ (**FIG. 41C**); D9 Group 2 (OD) $\leq 60\%$ (**FIG. 41D**); D35 Group 1 (OD) $\geq 70\%$ (**FIG. 41E**); and D35 Group 2 (OD) $\leq 60\%$ (**FIG. 41F**).

[286] **FIGs. 42A and 42B.** **FIG. 42A** shows Positive HNA Staining was observed in cell-injected eyes from Groups 1 and 2. Red=HNA, Blue=DAPI. Scale bars are 50 μm . **FIG. 42B** shows Group 1 (OD) $> 70\%$ and Group 2 (OD) $< 60\%$.

[287] **FIGs. 43A-43F.** . Examples of subretinal PRC grafts associated with elongate cone morphology are found in treated eyes from Groups 1 and 2. Example images of cone morphology from eyes injected with $\geq 70\%$ viable PRCs (**FIG. 43A**), $\leq 60\%$ viable PRCs (**FIG. 43B**), or Vehicle (**FIG. 43C**). Green=CAR, Red=HNA. Scale bars are 50 μm . **FIG. 43D** shows Quantification of cone length. One-way ANOVA ($p < 0.05$). Error bars are SEM. See **FIG. 43E** (Group 1) and **FIG. 43F** (Group 2) for CAR staining for all eyes.

[288] **FIG. 44** shows a subretinal PRC graft was associated with thickened ONL at graft sites. One-way ANOVA ($p = 0.21$). Error bars are SEM.

[289] **FIG. 45** Muller glial GFAP immunoreactivity was substantial in all study eyes. Example GFAP staining in Study Group eyes indicating intense vertical banding characteristic of GFAP morphology in diseased retinas. Green=GFAP, Red=HNA. Scale bars are 50 μm .

[290] **FIGs. 46A-46J** show OCT imaging on D28 revealed surgery-associated damage at injection sites. OCT images displaying surgical damage and accumulation of subretinal material in injected eyes from Groups 1 (**FIG. 46A**, **FIG. 46B**, and **FIG. 46C**), 2 (**FIG. 46D**, **FIG. 46E**, and **FIG. 46F**), and 3 (**FIG. 46G**, **FIG. 46H**, and **FIG. 46I**), compared to naïve Group 4 eyes (**FIG. 46J**). The presence of subretinal material in Group 1 eyes suggested that the subretinal mass observed on OCT in cell-injected eyes (Groups 2 and 3) may contain a considerable number of host cells. In two eyes from separate female mice from Group 1, individual moderately sized regions of retinal atrophy were observed (red dashed line) at the injection center.

[291] **FIGs. 47A-47C** show ERG recordings (D29-32) reveal no statistically significant differences between study groups. **FIG. 47A** shows a-wave amplitude measurements via 2-way ANOVA; **FIG. 47B** show scotopic b-wave amplitude measurements via 2-way ANOVA; and **FIG. 47C** shows photopic b-wave amplitude measurements via 1-way ANOVA.

[292] **FIGs. 48A-48F** shows HNA immunostaining and retinal laminar structure at injection center. Nuclear HNA staining was not detected in Group 1 (**FIG. 48A** and **FIG. 48B**), Group 2 (**FIG. 48C**

and FIG. 48D), and Group 3 (FIG. 48E and FIG. 48F) (HNA channel alone, HNA and DAPI overlay, brightfield). However, subretinal graft-like structures (*) containing DAPI⁺ cells and autofluorescent debris were found in Groups 2 and 3 (FIG. 48C, FIG. 48E, and FIG. 48F). Additional brightly autofluorescent debris (#) was observed across Groups 1-3, either subretinally or embedded in the outer retina (FIG. 48B and FIG. 48D). Consistent with surgical damage, at the injection center, retinal lamination was focally disrupted across groups (HNA and DAPI overlay in FIGs. 48A-48F). In many cases, the brightest autofluorescent structures were yellow/pigmented on brightfield imaging (example in FIG. 48B). Red=HNA, Blue=DAPI. Scale bars are 100 μ m.

[293] FIGs. 49A-49H shows Müller glial GFAP immunoreactivity was enriched at injection sites in injected eyes, consistent with injection-associated damage. (A-H) Muller glial reactivity was assessed using GFAP immunostaining Group 1 (FIG. 49A and FIG. 49B), Group 2 (FIG. 49C and FIG. 49D), Group 3 (FIG. 49E and FIG. 49F), and Group 4 (FIG. 49G and FIG. 49H) (GFAP channel alone; GFAP and DAPI overlay). FIGs. 49C, 49E, and 49F GFAP also labeled subretinal cell masses with thin curled morphology, in contrast to Muller glial hypertrophy FIG. 49A. Green=GFAP, Blue=DAPI. Scale bar is 100 μ m.

[294] FIGs. 50A-50D show Iba1 immunoreactivity was elevated at injection sites in eyes of Group 1 (FIG. 50A), Group 2 (FIG. 50B), Group 3 (FIG. 50C), compared to naïve eyes in Group 4 (FIG. 50D), consistent with inflammation subsequent to injection-associated damage. #autofluorescent debris. Green=Iba1, Blue=DAPI. Scale bar is 100 μ m.

DETAILED DESCRIPTION

[295] The present invention is directed to a photoreceptor rescue cell composition including a plurality of heterogeneous photoreceptor rescue cells that possesses unique marker profiles. The compositions of heterogeneous photoreceptor rescue cells (PRCs) of the invention cumulatively express the markers: FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA. The photoreceptor rescue cell composition of the invention includes inhibitory neurons, excitatory neurons, progenitors, astrocytes, and mixed neurons. The PRC may be defined phenotypically, for example by intracellular or extracellular marker expression. The photoreceptor rescue cell may further be characterized by expression or lack of expression of eye field progenitor markers, neural markers, and/or rod/cone photoreceptor markers.

[296] These photoreceptor rescue cell compositions can be generated by *in vitro* differentiation from earlier progenitors including pluripotent stem cells, for example, embryonic stem cells (ESCs), cells that have undergone transdifferentiation or partial reprogramming to a progenitor state, and induced pluripotent stem cells (iPSCs). The invention provides a composition of photoreceptor rescue cells that have not been attained or are not attainable from primary sources and, as such, possess unique unnatural marker profiles.

[297] The photoreceptor rescue cell compositions may be used in a variety of *in vivo* and *in vitro* methods. For example, the photoreceptor rescue cells may be used to treat conditions of the retina, including, but not limited to, macular degeneration (including age-related macular degeneration (AMD), for example, wet and dry AMD, retinitis pigmentosa and geographic atrophy secondary to AMD). The photoreceptor rescue cells may be used *in vitro* in screening assays to identify putative therapeutic or prophylactic treatment candidates.

[298] Functionally, the PRCs exhibit the ability to treat eye diseases and to improve visual acuity in a subject having a retinal disease or disorder, for example, by increasing secretion of neuroprotective factors, by preventing or slowing loss of photoreceptor cells, by increasing phagocytic activity (*e.g.*, ability to phagocytose isolated photoreceptor outer segments), by inhibiting microglial activation (*e.g.*, by increasing expression of CNFT and/or MIF), by decreasing oxidative stress (*e.g.*, by increasing expression of CNFT), by increasing expression of anti-apoptotic factors, and/or by preventing degeneration of the outer nuclear layer.

Definitions

[299] As defined here, singular forms are provided for illustrative purposes, but may also apply to plural versions of the phrase. The following definitions are meant to supplement conventional definitions of the terms as they would be understood by persons of ordinary skill.

[300] As used herein, the term “photoreceptor rescue cell composition” refers to a composition comprising a heterogeneous combination of photoreceptor rescue cells (PRCs). The photoreceptor rescue cell composition, as used herein, includes heterogeneous cells including, but not limited to, inhibitory neurons, excitatory neurons, mixed neurons, progenitors, and astrocytes. The cells in the photoreceptor rescue cell composition cumulatively express at least 2, 3, 4, 5, 6 or 7 of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and/or NFIA. In one embodiment, the cells in the composition cumulatively express at least FOXG1 and MAP2. In another embodiment, the cells in the composition cumulatively express at least each of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA.

[301] Excitatory neurons in the photoreceptor rescue cell compositions of the invention, as used herein, refer to cells that express one or more of markers NEUROD2, NEUROD6, SLA, NELL2, and/or SATB2.

[302] Inhibitory neurons in the photoreceptor rescue cell compositions of the invention, as used herein, refer to cells that express one or more of markers DLX5, TUBB3, SCGN, ERBB4, and CALB2.

[303] Alternative neurons, as used herein, refer to cells in a photoreceptor rescue cell composition that express one or more markers MEIS2, PBX3, GRIA2, and CACNA1C.

[304] Progenitor cells, as used herein when in reference to the particular cell types contained in the photoreceptor rescue cell compositions of the invention refer to cells that express one or more of markers VIM, MKI67, CLU, and GLI3.

[305] Astrocytes in the photoreceptor rescue cell compositions of the invention, as used herein, express one or more of markers GFAP, LUCAT1, MIR99AHG, and FBXL7.

[306] A photoreceptor rescue cell composition may further contain photoreceptor rescue cells that express eye field progenitor markers, rod/cone photoreceptor markers, and/or neuronal markers.

[307] Exemplary eye field progenitor markers expressed by cells in the photoreceptor rescue cell composition of the invention include PAX6, LHX2, SIX3, NES, or SOX2. Several eye field progenitor markers, such as PAX6, LHX2, SOX2, NES, are widely expressed in many neuronal progenitor cells and are also highly expressed in PRCs of the present compositions. As such, in one embodiment, the compositions of the invention include heterogeneous photoreceptor rescue cells that cumulatively express at least PAX6, LHX2, SOX2, NES, and optionally, SIX3. However, certain genes that are specific eye field progenitor markers in neuronal progenitor cells, such as RAX, SIX6, and TBX3, have little to no expression in PRCs of the present compositions. Accordingly, in one embodiment, the compositions of the invention are substantially free of photoreceptor rescue cells, or cells generally, that express RAX, SIX6 or TBX3. In one embodiment, the compositions of the invention are substantially free of photoreceptor rescue cells, or cells generally, that express either RAX and/or TBX3.

[308] Exemplary rod/cone photoreceptor markers expressed by cells in the photoreceptor rescue cell composition of the invention include Mash1/ASCL1 and RORB. Several rod/cone photoreceptor markers, such as Mash1/ASCL1 and RORB are widely expressed in neuroectoderm and neural progenitors derived from neuroectoderm and are also highly expressed in PRCs of the present compositions. As such, in one embodiment, the compositions of the invention include heterogeneous photoreceptor rescue cells that cumulatively express at least Mash1/ASCL1 and RORB. However, certain genes that are specific rod/cone photoreceptor markers in neuroectoderm and neural progenitors derived from neuroectoderm, such as NRL and NR2E3 have little to no expression in PRCs of the present compositions. Accordingly, in one embodiment, the compositions of the invention are substantially free of photoreceptor rescue cells, or cells generally, that express CRX, RHO, OPN1SW, PDE6B, RCVRN, ARR3, CNGB1, GNAT1, and GNAT2.

[309] Exemplary neuronal markers expressed by cells in the photoreceptor rescue cell composition of the invention include TUBB3, NFIA, DCX, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5. Several neuronal markers are robustly expressed in PRCs of the present compositions, such as TUBB3, NFIA, DCX, and NFIB. As such, in one embodiment, the compositions of the invention include heterogeneous photoreceptor rescue cells that cumulatively express TUBB3, NFIA, DCX, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5. However certain genes that are specific neuronal markers in neuronal progenitor cells, such as

OTX2 have low expression in PRCs of the present composition. Accordingly, in one embodiment, the compositions of the invention are substantially free of photoreceptor rescue cells, or cells generally, that express OTX2.

[310] In further embodiments, the compositions of the invention are substantially free of photoreceptor rescue cells, or cells generally, that express either pluripotent markers SSEA4 and/or OCT4.

[311] “Retinal cell(s)” refers to the neural cells of the eye, which are layered into three nuclear layers comprised of photoreceptors, horizontal cells, bipolar cells, amacrine cells, Müller glial cells and ganglion cells. Retinal cells includes neural retinal cells (also referred to herein as photoreceptor cells), retinal pigment epithelial (RPE) cells, iris epithelial cells, and their precursors. “Retinal pigment epithelial (RPE) cells”, as used herein refer to cells of the outermost external layer of the retina. RPE cells function to provide support for the retinal photoreceptors and are responsible for the metabolic digestion of the discarded outer segments of the neural retina. “Neural retina cells”, as used herein, refer to the layer of photoreceptor cells (*i.e.*, rod and cone cells) underlying the RPE cell layer in the retina. Neural retina (NR) cells are modified light sensitive neurons. As used herein, the term “cumulatively” and grammatical variations thereof refer to the expression of markers across the population of heterogeneous cells in a composition. Specifically, cumulative expression refers to a composition where at least one cell in the composition expresses one of the markers such that the totality of cells in the composition expresses all the genes listed. By way of example, recitation that “the plurality of heterogeneous cells cumulatively expresses FOXG1 and MAP2” can refer to a composition in which at least one cell expresses FOXG1 and at least one cell expresses MAP2 or a composition in which at least a single cell expresses FOXG1 and MAP2 such that the plurality of cells in the composition cumulatively expresses both FOXG1 and MAP2. Further by way of example, recitation of “the plurality of heterogeneous cells cumulatively expresses FOXG1, MAP2, STMN2 and DCX” can refer to, but is not limited to, compositions in which at least one cell expresses FOXG1, at least one cell expresses MAP2, at least one cell expresses STMN2 and at least one cell expresses DCX. Alternatively, a composition in which at least one cell expresses FOXG1 and MAP2 and at least one other cell expresses STMN2 and DCX is intended to be encompassed by such phrase. Further by way of example, a composition in which at least one cell expresses FOXG1 and STMN2, at least one cell expresses DCX and at least one cell expresses MAP2 is intended to be encompassed by such phrase.

[312] The term “human neural stem cell” or “hNSC” is used herein to refer to a cell that is self-renewing, and generated throughout an adult's life via neurogenesis. These multipotent adult stem cells generate the main phenotype of the nervous system, differentiating into neurons, astrocytes, and oligodendrocytes.

[313] The term “neural progenitor cell” or NPC” is used herein to refer to progenitor cells of the central nervous system (CNS) that give rise to many, if not all, of the glial and neuronal cell types that populate the CNS.

[314] The term “plurality” is used herein to refer to the state of being plural, i.e., at least two, *e.g.*, cell types, *e.g.*, a plurality of heterogeneous photoreceptor rescue cells.

[315] The terms “substantially free” or “essentially free” are used herein to refer to more than about 95%, 96%, 97%, 98%, 99% or 100% free. By way of example, the phrase “wherein the composition is substantially free of cells that express progenitor markers RAX, SIX6, and/or TBX3” refers to a composition in which at least 95%, 96%, 97%, 98%, 99% or 100% of the cells do not express any of the foregoing markers.

[316] The compositions of the invention may be characterized as at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or about 100% of cells in the composition are PRCs. In certain embodiments, the methods described herein can produce compositions of the invention that may be characterized as about 50% to about 100%, about 50% to about 95%, about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 50% to about 55%, 55% to about 100%, about 55% to about 95%, about 55% to about 90%, about 55% to about 85%, about 55% to about 80%, about 55% to about 75%, about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, 60% to about 100%, about 60% to about 95%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, about 60% to about 75%, about 60% to about 70%, about 60% to about 65%, 65% to about 100%, about 65% to about 95%, about 65% to about 90%, about 65% to about 85%, about 65% to about 80%, about 65% to about 75%, about 65% to about 70%, 70% to about 100%, about 70% to about 95%, about 70% to about 90%, about 70% to about 85%, about 70% to about 80%, about 70% to about 75%, 75% to about 100%, about 75% to about 95%, about 75% to about 90%, about 75% to about 85%, about 75% to about 80%, 80% to about 100%, about 80% to about 95%, about 80% to about 90%, about 80% to about 85%, 85% to about 100%, about 85% to about 95%, about 85% to about 90%, 90% to about 100%, about 90% to about 95%, or about 95% to about 100% of cells in the composition are PRCs.

[317] The compositions of the invention may be characterized as at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, or at least about 85% of the cells in the composition are viable. In certain embodiments, the methods described herein can produce compositions of the invention that may be characterized as about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 50% to about 55%, about 55% to about 85%, about 55% to about 80%, about 55% to about 75%, about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, about 60% to about 85%, about 60% to about 80%, about 60% to about 75%, about 60% to about 70%, about 60% to about 65%, about 65% to about 85%,

about 65% to about 80%, about 65% to about 75%, about 65% to about 70%, about 70% to about 85%, about 70% to about 80%, about 70% to about 75%, about 75% to about 85%, about 75% to about 80%, or about 80% to about 85% of the cells in the composition are viable.

[318] As used herein, the phrase “substantially pure photoreceptor rescue cell composition” refers to a composition of heterogeneous photoreceptor rescue cells (*e.g.*, a composition comprising cells) wherein the composition includes a substantial percentage of cells, for example, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, about least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% that share identical expression as it relates to a particular marker profile. The ability of all or a majority of the PRC cells to be in contact with their surrounding medium and thus the factors in such medium to an approximately equal degree results in those progenitor cells differentiating at similar times and to similar degrees. This similar differentiation timeline for a population of PRC cells indicates that such cells are synchronized. The PRCs may be cell cycle synchronized in some instances also. Such synchronicity results in subpopulations of cells that are homogeneous or near homogeneous as it relates to particular marker expression profiles.

[319] By way of example, purity may refer to the percentage of photoreceptor cells in the composition that exhibit a particular expression profile. For example, a substantially pure photoreceptor rescue cell composition with respect to expression of FOXG1 and MAP2 may refer to a composition of heterogeneous photoreceptor rescue cells where at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, about least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% of the photoreceptor rescue cells in the composition express FOXG1 and MAP2. In some embodiments, the composition of heterogeneous photoreceptor rescue cells are at least 50% pure, at least 55% pure, at least 60% pure, at least 65% pure, at least 70% pure, at least 75% pure, about least 80% pure, at least 85% pure, at least 90% pure, or at least 95% pure. In some embodiments, the cells are about 50% pure to about 95% pure, about 55% pure to about 95% pure, about 60% pure to about 95% pure, about 70% pure to about 95% pure, about 75% pure to about 95% pure, about 80% pure to about 95% pure, about 85% pure to about 95% pure, about 90% pure to about 95% pure, about 50% pure to about 90% pure, about 55% pure to about 90% pure, about 60% pure to about 90% pure, about 65% pure to about 90% pure, about 70% pure to about 90% pure, about 75% pure to about 90% pure, about 80% pure to about 90% pure, about 85% pure to about 90% pure, about 50% pure to about 85% pure, about 55% pure to about 85% pure, about 60% pure to about 85% pure, about 65% pure to about 85% pure, about 70% pure to about 85% pure, about 75% pure to about 85% pure, about 80% pure to about 85% pure, about 50% pure to about 80% pure, about 55% pure to about 80% pure, about 60% pure to about 80% pure, about 65% pure to about 80% pure, about 70% pure to about 80% pure, about 75% pure to about 80% pure, about 50% pure to about 75% pure, about 55% pure to about 75% pure, about 60% pure to about 75% pure, about 65% pure to about 75% pure, about 70% pure to about 75% pure, about 50% pure to about 70% pure, about

55% pure to about 70% pure, about 60% pure to about 70% pure, about 65% pure to about 70% pure, about 70% pure to about 75% pure, about 50% pure to about 65% pure, about 55% pure to about 65% pure, about 60% pure to about 65% pure, about 50% pure to about 60% pure, about 55% pure to about 60% pure, or about 50% pure to about 55% pure.

[320] As used herein, a majority of cells means at least 50%, and depending on the embodiment may include at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or about 100% of cells.

[321] The degree of purity that may be achieved using the methods of the invention are particularly important where such cell populations are to be used *in vivo* for therapeutic or prophylactic purposes. The ability to obtain populations of high cellular purity avoids performing another manipulation such as an enrichment or selection step, which may result in unnecessary cell loss. This is particularly important where the cell population may be small or the cell number may be limited.

[322] For example, the level of purity may be quantified by determining the proportion of cells in the preparation that express one or more markers, such as those markers of PRCs (including those markers identified in this application), relative to the total number of cells in the preparation, *e.g.*, by detecting cells that do or do not express said one or more markers. Optionally expression of markers indicative of non-PRC cells may also be detected, thereby facilitating detection and/or quantitation of said cells. Exemplary methods that may be utilized to detect and/or quantitate marker expression include, without limitation, flow cytometry, Fluorescence Activated Cell Sorting (FACS), immunohistochemistry, in situ hybridization, scRNAseq, immunofluorescence, single cell proteomics (*e.g.*, via LC-MS), possibly single cell metabolomics, lipidomics, scPCR, and other suitable methods known in the art. Optionally, purity of the composition may be determined by the percentage of viable cells present in the composition.

[323] “Embryoid bodies” refers to clumps or clusters of pluripotent cells (*e.g.*, iPSC or ESC) which may be formed by culturing pluripotent cells under non-attached conditions, *e.g.*, on a low-adherent substrate or in a “hanging drop.” In these cultures, pluripotent cells can form clumps or clusters of cells denominated as embryoid bodies. See Itskovitz-Eldor et al., *Mol Med.* 2000 February; 6(2):88-95, which is hereby incorporated by reference in its entirety. Typically, embryoid bodies initially form as solid clumps or clusters of pluripotent cells, and over time some of the embryoid bodies come to include fluid filled cavities, the former being referred to in the literature as “simple” EBs and the latter as “cystic” embryoid bodies.

[324] The disclosure provides compositions of heterogeneous PRCs based on the ability of the disclosed methods to directly differentiate progenitor cells such as but not limited to pluripotent stem cells (*e.g.*, ESCs and iPSCs). As used herein, directed differentiation intends that the progenitor cell population differentiates into or towards a desired lineage, due in part to the factors or other stimuli provided to such progenitor cells, thereby avoiding differentiation into other undesired, and thus potentially contaminating, lineages. In some embodiments, the methods provided herein drive

differentiation of for example pluripotent stem cells to PRCs without generating embryoid bodies (EB). EBs, as described below, are three dimensional cell clusters that can form during differentiation of pluripotent stem cells including but not limited to embryonic stem (ES) cells and iPSCs, and that typically contain cells, including progenitors, of mesodermal, ectodermal and endodermal lineages. The three dimensional nature of the EB may create a different environment, including different cell-cell interactions and different cell-cell signaling, than occurs in the non-EB based methods described herein. In addition, cells within EBs may not all receive a similar dose of an exogenously added agent, such as a differentiation factor present in the surrounding medium, and this can result in various differentiation events and decisions during development of the EB.

[325] In contrast, the PRC cell culture methods of the invention do not require and preferably avoid EB formation. Instead, these methods culture cells in conditions that provide the cells with equal contact with the surrounding medium, including factors in such medium. In certain embodiments, the PRCs may grow as a monolayer or near monolayer attached to a culture surface, *e.g.*, adherent conditions. In some embodiments, the culture methods disclosed herein provide that the PRCs are cultured in non-adherent or low adherent conditions, *e.g.*, suspension.

[326] The term “embryonic stem cell” (ES cell or ESC) is used herein as it is used in the art. This term includes cells derived from the inner cell mass of human blastocysts or morulae, including those that have been serially passaged as cell lines. The ES cells may be derived from fertilization of an egg cell with sperm, as well as using DNA, nuclear transfer, parthenogenesis, or by means to generate ES cells with homozygosity in the HLA region. ES cells are also cells derived from a zygote, blastomeres, or blastocyst-staged mammalian embryo produced by the fusion of a sperm and egg cell, nuclear transfer, parthenogenesis, androgenesis, or the reprogramming of chromatin and subsequent incorporation of the reprogrammed chromatin into a plasma membrane to produce a cell. Embryonic stem cells, regardless of their source or the particular method used to produce them, can be identified based on (i) the ability to differentiate into cells of all three germ layers, (ii) expression of at least OCT 4 and alkaline phosphatase, and (iii) ability to produce teratomas when transplanted into immunodeficient animals. Embryonic stem cells that may be used in embodiments of the present invention include, but are not limited to, human ES cells (“ESC” or “hES cells”) such as MA01, MA09, ACT-4, No. 3, H1, H7, H9, H14 and ACT30 embryonic stem cells. Additional exemplary cell lines include NED1, NED2, NED3, NED4, NED5, and NED7. See also NIH Human Embryonic Stem Cell Registry. An exemplary human embryonic stem cell line that may be used is MA09 cells. The isolation and preparation of MA09 cells was previously described in Klimanskaya, et al. (2006) “Human Embryonic Stem Cell lines Derived from Single Blastomeres.” *Nature* 444: 481-485. The human ES cells used in accordance with exemplary embodiments of the present invention may be derived and maintained in accordance with GMP standards.

[327] The term “ES cells” does not infer, and should not be inferred to mean, that the cells were generated through the destruction of an embryo. To the contrary, various methods are available and

can be used to generate ES cells without destruction of an embryo, such as a human embryo. As an example, ES cells may be generated from single blastomeres derived from an embryo, in a manner similar to the extraction of blastomeres for pre-implantation genetic diagnosis (PGD). Examples of such cell lines include NED1, NED2, NED3, NED4, NED5, and NED7. An exemplary human embryonic stem cell line that may be used is MA09 cells. The isolation and preparation of MA09 cells was previously described in Klimanskaya, et al. (2006) "Human Embryonic Stem Cell lines Derived from Single Blastomeres." *Nature* 444: 481-485. See also Chung et al. 2008, *Cell Stem Cell*, 2:113. All of these lines were generated without embryo destruction. As used herein, the term "pluripotent stem cells" includes but is not limited to tissue-derived stem cells, embryonic stem cells, embryo-derived stem cells, induced pluripotent stem cells, and stimulus-triggered acquisition of pluripotency (STAP) cells, regardless of the method by which the pluripotent stem cells are derived. The term also includes pluripotent stem cells having the functional and phenotypic characteristics of the aforementioned cells, regardless of the method used to generate such cells. Pluripotent stem cells are defined functionally as stem cells that are: (a) capable of inducing teratomas when transplanted in immunodeficient (SCID) mice; (b) capable of differentiating to cell types of all three germ layers (*e.g.*, can differentiate to ectodermal, mesodermal, and endodermal cell types); (c) express one or more markers of embryonic stem cells (*e.g.*, express OCT4, alkaline phosphatase, SSEA-3 surface antigen, SSEA-4 surface antigen, Nanog, TRA-1-60, TRA-1-81, SOX2, REX1, etc.) and d) are capable of self-renewal. The term "pluripotent" refers to the ability of a cell to form all lineages of the body or soma (*i.e.*, the embryo proper). In certain embodiments, pluripotent stem cells express one or more markers selected from the group consisting of: OCT4, alkaline phosphatase, SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81. Exemplary pluripotent stem cells include embryonic stem cells derived from the ICM of blastocyst stage embryos, as well as embryonic stem cells derived from one or more blastomeres of a cleavage stage or morula stage embryo (optionally without destroying the remainder of the embryo). Further exemplary pluripotent stem cells include induced pluripotent stem cells (iPSCs) generated by reprogramming a somatic cell by expressing a combination of factors (herein referred to as reprogramming factors). The iPSCs can be generated using fetal, postnatal, newborn, juvenile, or adult somatic cells. As used herein, the term "pluripotent stem cells", "PS cells", or "PSCs" includes embryonic stem cells, induced pluripotent stem cells, and embryo-derived pluripotent stem cells, regardless of the method by which the pluripotent stem cells are derived. For example, embryonic stem cells and induced pluripotent stem cells are a type of pluripotent stem cells that are able to form cells from each of the three germ layers: the ectoderm, the mesoderm, and the endoderm. Pluripotency is a continuum of developmental potencies ranging from the incompletely or partially pluripotent cell which is unable to give rise to a complete organism to the more primitive, more pluripotent cell, which is able to give rise to a complete organism (*e.g.*, an embryonic stem cell). Exemplary pluripotent stem cells can be generated using, for example, methods known in the art. Exemplary pluripotent stem cells include, but are not limited to, embryonic stem cells derived from

the inner cell mass of blastocyst stage embryos, embryonic stem cells derived from one or more blastomeres of a cleavage stage or morula stage embryo (optionally without destroying the remainder of the embryo), induced pluripotent stem cells produced by reprogramming of somatic cells into a pluripotent state, and pluripotent cells produced from embryonic germ (EG) cells (*e.g.*, by culturing in the presence of FGF-2, LIF and SCF). Such embryonic stem cells can be generated from embryonic material produced by fertilization or by asexual means, including somatic cell nuclear transfer (SCNT), parthenogenesis, and androgenesis.

[328] In certain embodiments, factors that can be used to reprogram somatic cells to pluripotent stem cells include, for example, a combination of OCT 4 (sometimes referred to as OCT 3/4), SOX2, c-Myc, and KLF4. In other embodiments, factors that can be used to reprogram somatic cells to pluripotent stem cells include, for example, a combination of OCT 4, SOX2, Nanog, and Lin28. In certain embodiments, at least two reprogramming factors are expressed in a somatic cell to successfully reprogram the somatic cell. In other embodiments, at least three reprogramming factors are expressed in a somatic cell to successfully reprogram the somatic cell. In other embodiments, at least four reprogramming factors are expressed in a somatic cell to successfully reprogram the somatic cell. In other embodiments, additional reprogramming factors are identified and used alone or in combination with one or more known reprogramming factors to reprogram a somatic cell to a pluripotent stem cell. Induced pluripotent stem cells are defined functionally and include cells that are reprogrammed using any of a variety of methods (integrative vectors, non-integrative vectors, chemical means, etc.). Pluripotent stem cells may be genetically modified or otherwise modified to increase longevity, potency, homing, to prevent or reduce alloimmune responses or to deliver a desired factor via cells that are differentiated from such pluripotent cells (for example, photoreceptor rescue cells, photoreceptor progenitor cells, rods, cones, etc. and other cell types described herein, *e.g.*, in the examples). In an embodiment, pluripotent stem cells may be genetically engineered or otherwise modified, for example, to increase longevity, potency, homing, to prevent or reduce immune responses, or to deliver a desired factor in cells that are obtained from such pluripotent cells (for example, photoreceptor rescue cells or cells present in a composition of photoreceptor rescue cells). For example, the pluripotent stem cell and therefore, the resulting differentiated cell, can be engineered or otherwise modified to lack or have reduced expression of beta 2 microglobulin, class I genes including HLA-A, HLA-B, HLA-C, HLA-E, HLA-F and HLA-G, TAP1, TAP2, Tapasin, CTIIA, RFX5, TRAC, or TRAB genes. As described in WO2012145384 and WO2013158292, which are herein incorporated by reference in their entireties, in some embodiments, the cell, such as a pluripotent stem cell and the resulting differentiated cell such as a photoreceptor rescue cell or cells present in a composition of photoreceptor rescue cells, comprises a genetically engineered disruption in a beta-2 microglobulin (B2M) gene. In some embodiments, the cell further comprises a polynucleotide capable of encoding a single chain fusion human leukocyte antigen (HLA) class I protein comprising at least a portion of the B2M protein covalently linked, either directly or via a

linker sequence, to at least a portion of an HLA-1 α chain. In some embodiments, the HLA-1 α chain is selected from HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, and HLA-G. In some embodiments, the cell comprises a genetically engineered disruption in a human leukocyte antigen (HLA) class II-related gene. In some embodiments, the HLA class II-related gene is selected from regulatory factor X-associated ankyrin-containing protein (RFXANK), regulatory factor 5 (RFX5), regulatory factor X associated protein (RFXAP), class II transactivator (CIITA), HLA-DPA (α chain), HLA-DPB (β chain), HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DMA, HLA-DMB, HLA-DOA, and HLA-DOB. In some embodiments, the cell comprises one or more polynucleotides encoding a single chain fusion HLA class II protein or an HLA class II protein. In some embodiments the cell further comprises one or more factors selected from the group consisting of: CD200, CD24, PD-L 1, HLAG or H2-M3, Cd47, FASLG or FasL, Ccl21 or Ccl21b, Mfge8, Serpin B9 or Spi6 or DUX4.

[329] “Induced pluripotent stem cells” (iPS cells or iPSC) can be produced by protein transduction of reprogramming factors in a somatic cell. In certain embodiments, at least two reprogramming proteins are transduced into a somatic cell to successfully reprogram the somatic cell. In other embodiments, at least three reprogramming proteins are transduced into a somatic cell to successfully reprogram the somatic cell. In other embodiments, at least four reprogramming proteins are transduced into a somatic cell to successfully reprogram the somatic cell.

[330] The pluripotent stem cells can be from any species. Embryonic stem cells have been successfully derived from, for example, mice, multiple species of non-human primates, and humans, and embryonic stem-like cells have been generated from numerous additional species. Thus, one of skill in the art can generate embryonic stem cells and embryo-derived stem cells from any species, including but not limited to, human, non-human primates, rodents (mice, rats), ungulates (cows, sheep, etc.), dogs (domestic and wild dogs), cats (domestic and wild cats such as lions, tigers, cheetahs), rabbits, hamsters, gerbils, squirrel, guinea pig, goats, elephants, panda (including giant panda), pigs, raccoon, horse, zebra, marine mammals (dolphin, whales, etc.) and the like. In certain embodiments, the species is an endangered species. In certain embodiments, the species is a currently extinct species.

[331] Similarly, iPS cells can be from any species. These iPS cells have been successfully generated using mouse and human cells. Furthermore, iPS cells have been successfully generated using embryonic, fetal, newborn, and adult tissue. Accordingly, one can readily generate iPS cells using a donor cell from any species. Thus, one can generate iPS cells from any species, including but not limited to, human, non-human primates, rodents (mice, rats), ungulates (cows, sheep, etc.), dogs (domestic and wild dogs), cats (domestic and wild cats such as lions, tigers, cheetahs), rabbits, hamsters, goats, elephants, panda (including giant panda), pigs, raccoon, horse, zebra, marine mammals (dolphin, whales, etc.) and the like. In certain embodiments, the species is an endangered species. In certain embodiments, the species is a currently extinct species.

[332] Induced pluripotent stem cells can be generated using, as a starting point, virtually any somatic cell of any developmental stage. For example, the cell can be from an embryo, placenta, fetus, neonate, juvenile, or adult donor. Exemplary somatic cells that can be used include fibroblasts, such as dermal fibroblasts obtained by a skin sample or biopsy, synoviocytes from synovial tissue, foreskin cells, cheek cells, or lung fibroblasts. In some embodiments, the somatic cells are placenta cells. Although skin and cheek provide a readily available and easily attainable source of appropriate cells, virtually any cell can be used. In certain embodiments, the somatic cell is not a fibroblast. In certain embodiments, the somatic cell is from placenta.

[333] The induced pluripotent stem cell may be produced by expressing or inducing the expression of one or more reprogramming factors in a somatic cell. The somatic cell may be a fibroblast, such as a dermal fibroblast, synovial fibroblast, or lung fibroblast, or a non-fibroblastic somatic cell. The somatic cell may be reprogrammed through causing expression of (such as through viral transduction, integrating or non-integrating vectors, etc.) and/or contact with (*e.g.*, using protein transduction domains, electroporation, microinjection, cationic amphiphiles, fusion with lipid bilayers containing, detergent permeabilization, etc.) at least 1, 2, 3, 4, 5 reprogramming factors. The reprogramming factors may be selected from OCT 3/4, SOX2, NANOG, LIN28, C-MYC, and KLF4. Expression of the reprogramming factors may be induced by contacting the somatic cells with at least one agent, such as a small organic molecule agent, that induces expression of reprogramming factors.

[334] Further exemplary pluripotent stem cells include induced pluripotent stem cells generated by reprogramming a somatic cell by expressing or inducing expression of a combination of factors ("reprogramming factors"). iPS cells may be obtained from a cell bank. The making of iPS cells may be an initial step in the production of differentiated cells. iPS cells may be specifically generated using material from a particular patient or matched donor with the goal of generating tissue-matched photoreceptor rescue cells. iPSCs can be produced from cells that are not substantially immunogenic in an intended recipient, *e.g.*, produced from autologous cells or from cells histocompatible to an intended recipient.

[335] The somatic cell may also be reprogrammed using a combinatorial approach wherein the reprogramming factor is expressed (*e.g.*, using a viral vector, plasmid, and the like) and the expression of the reprogramming factor is induced (*e.g.*, using a small organic molecule.) For example, reprogramming factors may be expressed in the somatic cell by infection using a viral vector, such as a retroviral vector or a lentiviral vector. Also, reprogramming factors may be expressed in the somatic cell using a non-integrative vector, such as an episomal plasmid. See, *e.g.*, Yu et al., Science. 2009 May 8; 324(5928):797-801, which is hereby incorporated by reference in its entirety. When reprogramming factors are expressed using non-integrative vectors, the factors may be expressed in the cells using electroporation, transfection, or transformation of the somatic cells with the vectors. For example, in mouse cells, expression of four factors (OCT3/4, SOX2, C-MYC, and KLF4) using integrative viral vectors is sufficient to reprogram a somatic cell. In human cells, expression of four

factors (OCT3/4, SOX2, NANOG, and LIN28) using integrative viral vectors is sufficient to reprogram a somatic cell.

[336] Once the reprogramming factors are expressed in the cells, the cells may be cultured. Over time, cells with ES characteristics appear in the culture dish. The cells may be chosen and subcultured based on, for example, ES morphology, or based on expression of a selectable or detectable marker. The cells may be cultured to produce a culture of cells that resemble ES cells.

[337] To confirm the pluripotency of the iPS cells, the cells may be tested in one or more assays of pluripotency. For example, the cells may be tested for expression of ES cell markers; the cells may be evaluated for ability to produce teratomas when transplanted into SCID mice; the cells may be evaluated for ability to differentiate to produce cell types of all three germ layers. Once a pluripotent iPSC is obtained it may be used to produce cell types disclosed herein, *e.g.*, photoreceptor rescue cells.

[338] Stimulus-triggered acquisition of pluripotency (STAP) cells are pluripotent stem cells produced by reprogramming somatic cells with sublethal stimuli such as low-pH exposure. The reprogramming does not require nuclear transfer into or genetic manipulation of the somatic cells. Reference can be made to Obokata et al., *Nature*, 505:676-680, 2014.

[339] As used herein, the term “stem cell” refers to a master cell that can reproduce indefinitely to form the specialized cells of tissues and organs. A stem cell is a developmentally pluripotent or multipotent cell. A stem cell can divide to produce two daughter stem cells, or one daughter stem cell and one progenitor (“transit”) cell, which then proliferates into the tissue's mature, fully formed cells.

[340] As used herein, the term “adult stem cell” refers to a stem cell which is isolated from a tissue or organ (*e.g.*, bone marrow stem cells, cord blood stem cells and adipose stem cells) of an animal (*e.g.*, human) at a stage of growth later than the embryonic stage. In one aspect, the stem cells of the invention may be isolated at the post-natal stage. The cells may be isolated preferably from a mammal, such as a human. Adult stem cells are unlike embryonic stem cells, which are defined by their origin, the inner cell mass of the blastocyst. Adult stem cells according to the invention may be isolated from any non-embryonic tissue, and will include neonates, juveniles, adolescents and adult patients. Generally the stem cell of the present invention will be isolated from a non-neonate mammal, and more preferably from a non-neonate human. These adult stem cells are characterized in that, in their undifferentiated state, they express telomerase, and they do not show gap junctional intercellular communication (GJIC) and do not have a transformed phenotype.

[341] “Signs” of disease, as used herein, refers broadly to any abnormality indicative of disease, discoverable on examination of the patient; an objective indication of disease, in contrast to a symptom, which is a subjective indication of disease.

[342] “Symptoms” of disease as used herein, refers broadly to any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the patient and indicative of disease.

[343] “Therapy,” “therapeutic,” “treating,” “treat” or “treatment”, as used herein, refers broadly to treating a disease, arresting or reducing the development of the disease or its clinical symptoms, and/or relieving the disease, causing regression of the disease or its clinical symptoms. Therapy encompasses prophylaxis, prevention, treatment, cure, remedy, reduction, alleviation, and/or providing relief from a disease, signs, and/or symptoms of a disease. Therapy encompasses an alleviation of signs and/or symptoms in patients with ongoing disease signs and/or symptoms. Therapy also encompasses “prophylaxis” and “prevention”. Prophylaxis includes preventing disease occurring subsequent to treatment of a disease in a patient or reducing the incidence or severity of the disease in a patient. The term “reduced”, for purpose of therapy, refers broadly to the clinically significant reduction in signs and/or symptoms. Therapy includes treating relapses or recurrent signs and/or symptoms. Therapy encompasses but is not limited to precluding the appearance of signs and/or symptoms anytime as well as reducing existing signs and/or symptoms and eliminating existing signs and/or symptoms. Therapy includes treating chronic disease (“maintenance”) and acute disease. For example, treatment includes treating or preventing relapses or the recurrence of signs and/or symptoms.

[344] Conditions to be treated according to the invention and thus using one or more of the preparations provided herein include but are not limited macular degeneration including age-related macular degeneration, and such macular degeneration may be early or late stage. Other conditions to be treated include but are not limited to retinitis pigmentosa, retinal dysplasia, retinal degeneration, diabetic retinopathy, age related macular degeneration (e.g. wet or dry), geographic atrophy secondary to AMD, congenital retinal dystrophy, rod dystrophy, cone dystrophy, cone-rod dystrophy, Leber congenital amaurosis, Stargardt disease, retinal detachment, glaucoma, optic neuropathy, and trauma that affects the eye.

Cell Markers:

[345] The photoreceptor rescue cell composition comprising a plurality of heterogeneous photoreceptor rescue cells exhibit unique marker profiles that distinguishes them from naturally occurring cells. In particular, the composition of the invention are characterized by expression of FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, NFIA, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA. In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by the expression of FOXG1 and/or MAP2. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of FOXG1 and MAP2.

[346] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention include inhibitory neurons and are, thus, characterized by the expression of DLX5, TUBB3, SCGN, ERBB4, CALB2, or a combination thereof. In some embodiments, the plurality of photoreceptor

rescue cells in the composition of the invention are characterized by the cumulative expression of DLX5, TUBB3, SCGN, ERBB4, and CALB2.

[347] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention include excitatory neurons and are, thus, characterized by the expression of NEUROD2, NEUROD6, SLA, NELL2, SATB2, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of NEUROD2, NEUROD6, SLA, NELL2, and SATB2.

[348] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention include progenitors and are, thus, characterized by the expression of VIM, MKI67, CLU, GLI3, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of VIM, MKI67, CLU, and GLI3.

[349] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention include astrocytes and are, thus, characterized by the expression of GFAP, LUCAT1, MIR99AHG, FBXL7, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of GFAP, LUCAT1, MIR99AHG, and FBXL7.

[350] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention include alternative neurons and are, thus, characterized by the expression of MEIS2, PBX3, GRIA2, CACNA1C, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of MEIS2, PBX3, GRIA2, and CACNA1C.

[351] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by the expression of eye field progenitor markers selected from the group consisting of PAX6, LHX2, SIX3, NES, SOX2, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of PAX6, LHX2, SIX3, NES, and SOX2.

[352] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are substantially free of cells that express eye field progenitor markers RAX, SIX6, and/or TBX3. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by little to no cumulative expression of RAX, SIX6, and TBX3.

[353] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by the expression of rod/ cone photoreceptor markers selected from the group consisting of ASCL1, RORB, NR2E3, NRL, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of ASCL1, RORB, NR2E3, and NRL.

[354] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by the expression of neuron markers selected from the group consisting of TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, HES5, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by the cumulative expression of TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

[355] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are substantially free of cells that express CRX, RHO, OPN1SW, PDE6B, RCVRN, ARR3, CNGB1, GNAT1, GNAT2, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by little to no cumulative expression of CRX, RHO, OPN1SW, PDE6B, RCVRN, ARR3, CNGB1, GNAT1, AND GNAT2.

[356] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are substantially free of cells that express VSX2, POU5F1, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by little to no cumulative expression of VSX2 and POU5F1.

[357] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are substantially free of cells that express OCT4, SSEA4, or a combination thereof. In some embodiments, the plurality of photoreceptor rescue cells in the composition of the invention are characterized by little to no cumulative expression of OCT4 and SSEA4.

[358] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by the expression of AGT, ACBLN2, CDH7, DNAH11, EGR1, FAM216B, FOS, KCNC2, LGI2, LOC221946, LRRC4C, MAP3k19, OLFM3, PRND, PTGER3, RELN, TCERGIL, TSHR, UNC13C, TRb2, PDE6B, CNGB1, Tuj1, CHX10, Nestin, TRbeta2, MASH1, RORbeta, MAP2, ELAVL3, NFIA, DCX, LHX2, SLC1A2, ELAVL4, PAX6, EMX2, ASCL1, DLL1, NFIB, ENOX1, TUBB3, MAP2, DCLK1/2, DCX, KALRN, LINC00461, C1orf61, NCAM1, SETBP1, PAK3, AKAP6, RTN1, CRMP1, FOXG1, TRIM2, BACH2, Recoverin, Opsin, Rhodopsin, rod and cone cGMP Phosphodiesterase, which may be assessed at the protein and/or mRNA level (see Fischer A J, Reh T A, Dev Neurosci. 2001; 23(4-5):268-76; Baumer et al., Development. 2003 July; 130(13):2903-15, Swaroop et al., Nat Rev Neurosci. 2010 August; 11(8):563-76, Agathocleous and Harris, Annu. Rev. Cell Dev. Biol. 2009. 25:45-69, each of which is hereby incorporated by reference in its entirety).

[359] In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by assessing expression of cell marker in comparison to expression in pluripotent stem cells, e.g., iPSCs or embryo-derived PSCs. In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by the decreased expression of FAM216B, FOS, KCNC2, LGI2, LOC221946, LRRC4c, MAP3k19, OLFM3, PRND, PTGER3, RELN, TCERGIL, TSHR, UNC13C, and SSEA4 in comparison to pluripotent stem cells, e.g., iPSCs or

embryo-derived PSCs. In some embodiments, the cells in the photoreceptor rescue cell composition of the invention are characterized by the increased expression of AGT, ACBLN2, DCH7, DNA11, and EGR1 in comparison to ESCs or iPSCs. The markers are generally human, e.g., except where the context indicates otherwise. The cell markers can be identified using conventional immunocytochemical methods, conventional PCR methods, Transcriptomic analyses including RNAseq, quantitative real-time PCR, flow cytometry, FACS, scRNAseq, bulk RNAseq, single cell or bulk qRT-PCR, immunocytochemistry, immunofluorescence, single cell or bulk proteomics (eg, via LC-MS), possibly metabolomics, lipidomics, and other suitable methods known in the art.

[360] As used herein, the term “scRNAseq” refers to single cell RNA sequencing. In some embodiments, scRNAseq provides data to cluster single cells of a population of cells based on expression of gene markers. In some embodiments, scRNAseq provides data to determine percentage of single cells in a population, e.g., population of PRC cells, that express gene marker(s). Mapped sequence data for scRNAseq is filtered for quality control metrics, run through dimensional reduction for visualization, and clustered using a shared nearest neighbor method with the Louvain algorithm. From there, differential expression is conducted on the clusters, and the most strongly differentially expressed genes are compared between the clusters and several published data sources. Numerous human developmental biology reviews are used, as well as datasets for human retina (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE142526), human hippocampus (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119212), human midbrain (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76381), and human cortex (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132672), to assign best-guess cell identities to the clusters based on the differentially expressed genes, and expression patterns during development and in the reviewed datasets.

[361] As used herein, the term bulk “RNAseq” refers to RNA sequencing analysis of a population of cells. Bulk RNAseq provided transcripts per million (TPM), where for every 1,000,000 RNA molecules in the RNA-seq sample, an amount came from the gene of interest.

Cell Culture Media:

[362] In embodiments of the invention, the cells are stored, proliferated or differentiated in various cell culture media. Rescue induction medium is utilized for differentiation of a stem cell into early-stage neuronal progenitor cells. The rescue induction medium (RIM) may comprise D-glucose, N2 supplement (e.g. 0.1-5%), B27 supplement (e.g., 0.005 to 0.2%), MEM non-essential amino acids solution and optionally including insulin and/or Noggin, and may be in a DMEM/F12 (Invitrogen) or similar base medium. For example, the rescue induction medium may include at least insulin. Additionally, the insulin concentration may be varied or increased which may promote cell survival and/or yield of differentiated cells. For example, the insulin concentration may be varied across a range and survival and/or differentiation monitored in order to identify an insulin concentration which

improves either or both of these attributes. The addition of Noggin is believed not to be necessary but was observed to increase the expression of neuronal progenitor-associated transcription factors.

[363] The components of DMEM/F12, Neurobasal medium, N2 serum supplement, and B27 serum supplement are provided in Tables 1-4. It is to be understood that the invention contemplates the use of these particular media and supplements or media or supplements comprising, consisting essentially of, or consisting of these components.

Table 1

DMEM/F12 Components	Molecular Weight	Concentration (mg/L)	mM
Amino Acids			
Glycine	75	18.75	0.25
L-Alanine	89	4.45	0.05
L-Arginine hydrochloride	211	147.5	0.699
L-Asparagine-H ₂ O	150	7.5	0.05
L-Aspartic acid	133	6.65	0.05
L-Cysteine hydrochloride-H ₂ O	176	17.56	0.0998
L-Cystine 2HCl	313	31.29	0.1
L-Glutamic Acid	147	7.35	0.05
L-Glutamine	146	365	2.5
L-Histidine hydrochloride-H ₂ O	210	31.48	0.15
L-Isoleucine	131	54.47	0.416
L-Leucine	131	59.05	0.451
L-Lysine hydrochloride	183	91.25	0.499
L-Methionine	149	17.24	0.116
L-Phenylalanine	165	35.48	0.215
L-Proline	115	17.25	0.15
L-Serine	105	26.25	0.25
L-Threonine	119	53.45	0.449
L-Tryptophan	204	9.02	0.0442
L-Tyrosine disodium salt dihydrate	261	55.79	0.214
L-Valine	117	52.85	0.452
Vitamins			
Biotin	244	0.0035	1.43E-05
Choline chloride	140	8.98	0.0641
D-Calcium pantothenate	477	2.24	0.0047
Folic Acid	441	2.65	0.00601
Niacinamide	122	2.02	0.0166
Pyridoxine hydrochloride	206	2	0.00971
Riboflavin	376	0.219	0.000582
Thiamine hydrochloride	337	2.17	0.00644
Vitamin B12	1355	0.68	0.000502
i-Inositol	180	12.6	0.07
Inorganic Salts			
Calcium Chloride (CaCl ₂) (anhyd.)	111	116.6	1.05
Cupric sulfate (CuSO ₄ ·5H ₂ O)	250	0.0013	5.2E-06
Ferric Nitrate (Fe(NO ₃) ₃ ·9H ₂ O)	404	0.05	0.000124

Ferric sulfate (FeSO ₄ -7H ₂ O)	278	0.417	0.0015
Magnesium Chloride (anhydrous)	95	28.64	0.301
Magnesium Sulfate (MgSO ₄) (anhyd.)	120	48.84	0.407
Potassium Chloride (KCl)	75	311.8	4.16
Sodium Bicarbonate (NaHCO ₃)	84	1200	14.29
Sodium Chloride (NaCl)	58	6995.5	120.61
Sodium Phosphate dibasic (Na ₂ HPO ₄) anhydrous	142	71.02	0.5
Sodium Phosphate monobasic (NaH ₂ PO ₄ -H ₂ O)	138	62.5	0.453
Zinc sulfate (ZnSO ₄ -7H ₂ O)	288	0.432	0.0015
Sodium Bicarbonate (NaHCO ₃)	84	1200	14.29
Sodium Chloride (NaCl)	58	6995.5	120.61
Sodium Phosphate dibasic (Na ₂ HPO ₄) anhydrous	142	71.02	0.5
Sodium Phosphate monobasic (NaH ₂ PO ₄ -H ₂ O)	138	62.5	0.453
Zinc sulfate (ZnSO ₄ -7H ₂ O)	288	0.432	0.0015
Other Components			
D-Glucose (Dextrose)	180	3151	17.51
HEPES	238	3574.5	15.02
Hypoxanthine Na	159	2.39	0.015
Linoleic Acid	280	0.042	0.00015
Li pole Acid	206	0.105	0.00051
Phenol Red	376.4	8.1	0.0215
Putrescine 2HCl	161	0.081	0.000503
Sodium Pyruvate	110	55	0.5
Thymidine	242	0.365	0.00151

Table 2

Neurobasal Components	Molecular Weight	Concentration (mg/L)	mM
Amino Acids			
Glycine	75	30	0.4
L-Alanine	89	2	0.0225
L-Arginine hydrochloride	211	84	0.398
L-Asparagine-H ₂ O	150	0.83	0.00553
L-Cysteine	121	31.5	0.26
L-Histidine hydrochloride-H ₂ O	210	42	0.2
L-Isoleucine	131	105	0.802
L-Leucine	131	105	0.802
L-Lysine hydrochloride	183	146	0.798
L-Methionine	149	30	0.201
L-Phenylalanine	165	66	0.4
L-Proline	115	7.76	0.0675
L-Serine	105	42	0.4
L-Threonine	119	95	0.798
L-Tryptophan	204	16	0.0784
L-Tyrosine	181	72	0.398
L-Valine	117	94	0.803

Vitamins			
Choline chloride	140	4	0.0286
D Calcium pantothenate	477	4	0.00839
Folic Acid	441	4	0.00907
Niacinamide	122	4	0.0328
Pyridoxine hydrochloride	204	4	0.0196
Riboflavin	376	0.4	0.00106
Thiamine hydrochloride	337	4	0.0119
Vitamin B12	1355	0.0068	0.000005
i-Inositol	180	7.2	0.04
Inorganic Salts			
Calcium Chloride (CaCl ₂) (anhyd.)	111	200	1.8
Ferric Nitrate (Fe(NO ₃) ₃ ·9H ₂ O)	404	0.1	0.000248
Magnesium Chloride (anhydrous)	95	77.3	0.814
Potassium Chloride (KCl)	75	400	5.33
Sodium Bicarbonate (NaHCO ₃)	84	2200	26.19
Sodium Chloride (NaCl)	58	3000	51.72
Sodium Phosphate monobasic (NaH ₂ PO ₄ ·H ₂ O)	138	125	0.906
Zinc sulfate (ZnSO ₄ ·7H ₂ O)	288	0.194	0.000674
Other Components			
D-Glucose (Dextrose)	180	4500	25
HEPES	238	2600	10.92
Phenol Red	376.4	8.1	0.0215
Sodium Pyruvate	110	25	0.227

Table 3

N2 Supplement Components	Molecular Weight	Concentration (mg/L)	mM
Proteins			
Human Transferrin (Holo)	10000	10000	1
Insulin Recombinant Full Chain	5807.7	500	0.0861
Other Components			
Progesterone	314.47	0.63	0.002
Putrescine	161	1611	10.01
Selenite	173	0.52	0.00301

Table 4

B27 Components			
Vitamins	Proteins	Other Components	
Biotin	BSA, fatty acid free Fraction V	Corticosterone	Linoleic Acid
DL Alpha Tocopherol Acetate	Human Recombinant Insulin	D-Galactose	Linolenic Acid
DL Alpha-Tocopherol Vitamin A (acetate)	Catalase	Ethanolamine HCl	Progesterone
	Human Transferrin	Glutathione (reduced)	Putrescine 2HCl Sodium

	Superoxide Dismutase	L-Carnitine HCl	
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[364] The methods described herein may use human factors such as human Noggin, human insulin, and the like.

[365] Noggin is a secreted bone morphogenetic protein (BMP) inhibitor that reportedly binds BMP2, BMP4, and BMP7 with high affinity to block TGF β family activity. SB431542 is a small molecule that reportedly inhibits TGF β /Activin/Nodal by blocking phosphorylation of ACTRIB, TGF β R1, and ACTRIC receptors. SB431542 is thought to destabilize the Activin- and Nanog-mediated pluripotency network as well as suppress BMP induced trophoblast, mesoderm, and endodermal cell fates by blocking endogenous Activin and BMP signals. It is expected that agents having one or more of the aforementioned activities could replace or augment the functions of one or both of Noggin and SB431542, *e.g.*, as they are used in the context of the disclosed methods. For example, applicants envision that the protein Noggin and/or the small molecule SB4312542 could be replaced or augmented by one or more inhibitors that affect any or all of the following three target areas: 1) preventing the binding of the ligand (*e.g.*, bone morphogenetic proteins (BMPs), such as BMP2, BMP4, BMP5, BMP6, BMP7, BMP13, and BMP14) to the receptor (*e.g.*, bone morphogenetic protein receptors; 2) blocking activation of receptor (*e.g.*, dorsomorphin), and 3) inhibition of SMAD intracellular proteins/transcription factors. Exemplary potentially suitable factors include the natural secreted BMP inhibitors Chordin (which blocks BMP4) and Follistatin (which blocks Activin), as well as analogs or mimetics thereof. Additional exemplary factors that may mimic the effect of Noggin include use of dominant negative receptors or blocking antibodies that would sequester BMP2, BMP4, and/or BMP7. Additionally, with respect to blocking receptor phosphorylation, dorsomorphin (or Compound C) has been reported to have similar effects on stem cells. Inhibition of SMAD proteins may also be effected using soluble inhibitors such as SIS3 (6,7-Dimethoxy-2-((2E)-3-(1-methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl-prop-2-enoyl))-1,2,3,4-tetrahydroisoquinoline, Specific Inhibitor of Smad3, SIS3), overexpression of one or more of the inhibitor SMADs (*e.g.*, SMAD6, SMAD7, SMAD10) or RNAi for one of the receptor SMADs (SMAD1, SMAD2, SMAD3, SMAD5, SMAD8/9). Another combination of factors expected to be suitable for generating neural progenitors comprises a cocktail of Leukemia Inhibitory Factor (LIF), GSK3 inhibitor (CHIR 99021), Compound E (γ secretase inhibitor XXI) and the TGF β inhibitor SB431542 which has been previously shown to be efficacious for generating neural crest stem cells (Li et al., Proc Natl Acad Sci USA. 2011 May 17; 108(20):8299-304). Additional exemplary factors may include derivatives of SB431542, *e.g.*, molecules that include one or more added or different substituents, analogous functional groups, etc. and that have a similar inhibitory effect on one or more SMAD proteins. Suitable factors or combinations of factors may be identified, for example, by contacting pluripotent cells with said factor(s) and monitoring for adoption of early-stage neuronal

progenitor phenotypes, such as characteristic gene expression (including expression of the markers described herein, expression of a reporter gene coupled to an early-stage neuronal progenitor promoter, or the like) or the ability to form a cell type disclosed herein such as early stage neuronal progenitor cells, late-stage neuronal progenitor cells, retinal neural progenitor cells, photoreceptor progenitors, rod progenitors, cones, rods and/or photoreceptor rescue cells.

[366] Preferably, the cells are treated with or cultured in a rescue induction medium prior to culture with a neural differentiation medium (NDM). NDM may be utilized to promote further maturation of early-stage neural progenitors cells. In one embodiment, a neural differentiation medium is utilized to promote the differentiation and development of early-stage neuronal progenitor cells. The neural differentiation medium may comprise D-glucose, penicillin, streptomycin, GlutaMAX™, N2 supplement, B27 supplement, MEM non-essential amino acids solution and optionally including Noggin. The neural differentiation medium may also be utilized for differentiation and maturation of early-stage neuronal progenitor cells with transcriptional signatures of excitatory or inhibitory neurons, and neurons or “photoreceptor rescue cells”. but without the inclusion of Noggin. In certain embodiments, Noggin is not needed once PSCs and PSC subpopulations are no longer present in the maturing cell product.

[367] The neural differentiation medium constituents are as follows: N2: 1% (1 ml of N2 per 100 ml), B27: 2% (2 ml of B27 per 100 ml), and Noggin: 50 ng/ml.

[368] Noggin is not needed after cells have all become early-stage neuronal progenitors.

Stem Cells, Embryonic Stem Cells (ESCs) or Adult Stem Cells or Induced Pluripotent Stem Cells (iPS):

[369] The ESCs, or Adult Stem Cells or iPS cells utilized herein may be propagated on a feeder-free system, such as in Matrigel™ (a soluble preparation from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells) or another matrix. Additionally, or alternatively, said pluripotent cells may be cultured on a matrix which may be selected from the group consisting of laminin (*e.g.*, laminin-111, laminin-211, laminin-121, laminin-221, laminin-332/laminin-3A32, laminin-3B32, laminin-311/laminin-3A11, laminin-321/laminin-3A21, laminin-411, laminin-421, laminin-511 (*e.g.*, iMatrix™-511), laminin-521, laminin-213, laminin-432, laminin-522, laminin-532, and/or laminin fragments), fibronectin, vitronectin, proteoglycan, entactin, collagen, collagen I, collagen IV, collagen VIII, heparan sulfate, Matrigel™ (a soluble preparation from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells), CellStart, a human basement membrane extract, and any combination thereof. Said matrix may comprise, consist of, or consist essentially of Matrigel™ (a soluble preparation from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells). In some embodiments, the stem cells do not form embryoid bodies in culture, which is an improvement over the prior art. In an embodiment, stem cells, for example ESCs, or iPSCs differentiate into photoreceptor rescue cells in the presence of Noggin.

Stem Cells

[370] In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin or intestinal tissues.

[371] Pluripotent stem cells such as human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSC) are capable of long-term proliferation *in vitro*, while retaining the potential to differentiate into all cell types of the body, including photoreceptor rescue cells. Thus these cells could potentially provide an unlimited supply of patient-specific functional photoreceptor rescue cells for both drug development and transplantation therapies. The differentiation of pluripotent stem cells to photoreceptor rescue cells *in vitro* may involve the addition of different growth factors at different stages of differentiation and may require about 10-30 days of differentiation (see e.g. Figure 12). Pluripotent stem cells, with their unlimited proliferation ability, provide an advantage over somatic cells as the starting cell population for production of photoreceptor rescue cells.

[372] Pluripotent stem cells, *e.g.*, embryonic stem (ES) cells or iPS cells, may be the starting material of the disclosed method. In any of the embodiments herein, the pluripotent stem cell may be human pluripotent stem cells (hPSCs). Pluripotent stem cells (PSCs) may be cultured in any way known in the art, such as in the presence or absence of feeder cells. Additionally, PSCs produced using any method can be used as the starting material to produce photoreceptor rescue cells. For example, the hES cells may be derived from blastocyst stage embryos that were the product of *in vitro* fertilization of egg and sperm. Alternatively, the hES cells may be derived from one or more blastomeres removed from an early cleavage stage embryo, optionally, without destroying the remainder of the embryo. In still other embodiments, the hES cells may be produced using nuclear transfer. In a further embodiment, iPSCs may be used. As a starting material, previously cryopreserved PSCs may be used. In another embodiment, PSCs that have never been cryopreserved may be used.

[373] In one aspect of the present invention, PSCs are plated onto an extracellular matrix under feeder or feeder-free conditions. In an embodiment, the PSCs can be cultured on an extracellular matrix, including, but not limited to, laminin, fibronectin, vitronectin, Matrigel, CellStart, collagen, or gelatin. In some embodiments, the extracellular matrix is laminin with or without e-cadherin. In some embodiments, laminin may be selected from the group comprising laminin 521, laminin 511, or iMatrix511. In some embodiments, the feeder cells are human feeder cells, such as human dermal fibroblasts (HDF). In other embodiments, the feeder cells are mouse embryo fibroblasts (MEF).

[374] In certain embodiments, the media used when culturing the PSCs may be selected from any media appropriate for culturing PSCs. In some embodiments, any media that is capable of supporting

PSC cultures may be used. For example, one of skill in the art may select amongst commercially available or proprietary media.

[375] The medium that supports pluripotency may be any such medium known in the art. In some embodiments, the medium that supports pluripotency is Nutristem™. In some embodiments, the medium that supports pluripotency is TeSR™. In some embodiments, the medium that supports pluripotency is StemFit™. In other embodiments, the medium that supports pluripotency is Knockout™ DMEM (Gibco), which may be supplemented with Knockout™ Serum Replacement (Gibco), LIF, bFGF, or any other factors. Each of these exemplary media is known in the art and commercially available. In further embodiments, the medium that supports pluripotency may be supplemented with ROCK inhibitor, bFGF or any other factors. In an embodiment, bFGF may be supplemented at a low concentration (*e.g.*, 4ng/mL). In another embodiment, bFGF may be supplemented at a higher concentration (*e.g.*, 100 ng/mL), which may prime the PSCs for differentiation.

[376] The concentration of PSCs to be used in the production method of the present invention is not particularly limited. For example, when a 10 cm dish is used, 1×10^4 - 1×10^8 cells per dish, preferably 5×10^4 - 5×10^6 cells per dish, more preferably 1×10^5 - 1×10^7 cells per dish are used. For example, when a CellSTACK® vessel is used, 1×10^4 - 1×10^8 cells per vessel, preferably 5×10^4 - 5×10^6 cells per vessel or 1.5×10^6 - 2.2×10^6 cells per vessel, more preferably 1×10^5 - 1×10^7 cells per vessel are used. For example, when a flask (*e.g.*, PDL-precoated T175 flask) is used, 1×10^4 - 1×10^8 cells per flask, preferably 5×10^4 - 5×10^6 cells per flask or 4.3×10^5 - 6.2×10^6 cells per flask, more preferably 1×10^5 - 1×10^7 cells per flask are used.

[377] In some embodiments, hESCs are expanded using target seed densities of 3×10^3 and 6×10^3 cells/cm². In some embodiments, hESCs are expanded preferably using 6-well plates (3×10^4 - 6×10^4 cells per vessel, *e.g.*, 2×10^4 - 7×10^4 cells per vessel) or in T75 flasks (2×10^5 - 5×10^5 cells per vessel, *e.g.*, 1×10^5 and 6×10^5 cells per vessel). In some embodiments, a CellSTACK® vessel or T175 flask is used to expand hESCs.

[378] In some embodiments, hESCs are seeded to start the differentiation process towards PRCs. In some embodiments, the target seeding density is 3,000 cells/cm². This target density is approximately 1.5×10^6 – 2.2×10^6 cells per CellSTACK® vessel, and 4.3×10^5 – 6.2×10^6 cells per T175 flask.

[379] In some embodiments, the PSCs are plated with a cell density of about 1,000-100,000 cells/cm². In some embodiments, the PSCs are plated with a cell density of about 5000 – 100,000 cells/cm², about 5000 – 50,000 cells/cm², or about 5000 – 15,000 cells/cm². In other embodiments, the PSCs are plated at a density of about 10,000 cells/cm².

[380] In some embodiments, the medium that supports pluripotency, *e.g.*, StemFit™ or other similar medium, is replaced with a differentiation medium to differentiate the cells into photoreceptor rescue cells. In some embodiments, replacement of the media from the medium that supports pluripotency to a differentiation medium may be performed at different time points during the cell

culture of PSCs and may also depend on the initial plating density of the PSCs. In some embodiments, replacement of the media can be performed after 2-14 days of culture of the PSCs in the pluripotency medium. In some embodiments, replacement of the media may be performed at day 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

[381] In some embodiments, the stem cells useful for the method described herein include but are not limited to embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, bone-marrow derived stem cells, hematopoietic stem cells, chondrocyte progenitor cells, epidermal stem cells, gastrointestinal stem cells, neural stem cells, hepatic stem cells, adipose-derived mesenchymal stem cells, pancreatic progenitor cells, hair follicular stem cells, endothelial progenitor cells and smooth muscle progenitor cells.

[382] In some embodiments, the stem cells used for the method described herein are isolated from umbilical cord, placenta, amniotic fluid, chorion villi, blastocysts, bone marrow, adipose tissue, brain, peripheral blood, the gastrointestinal tract, cord blood, blood vessels, skeletal muscle, skin, liver and menstrual blood.

[383] The detailed procedures for the isolation of human stem cells from various sources are described in Current Protocols in Stem Cell Biology (2007), which is incorporated by reference in its entirety herein. Methods of isolating and culturing stem cells from various sources are also described in U.S. Patent Nos. 5,486,359, 6,991,897, 7,015,037, 7,422,736, 7,410,798, 7,410,773, 7,399,632; each of which is incorporated by reference in its entirety herein.

Somatic Cells

[384] In certain aspects of the invention, there may also be provided methods of transdifferentiation, *i.e.*, the direct conversion of one somatic cell type into another, *e.g.*, deriving photoreceptor rescue cells from other somatic cells.

[385] However, human somatic cells may be limited in supply, especially those from living donors. In order to provide an unlimited supply of starting cells for photoreceptor rescue cell differentiation, somatic cells may be immortalized by introduction of immortalizing genes or proteins, such as hTERT and/or other oncogenes. The immortalization of cells may be reversible (*e.g.*, using removable expression cassettes) or inducible (*e.g.*, using inducible promoters).

[386] Somatic cells in certain aspects of the invention may be primary cells (non-immortalized cells), such as those freshly isolated from an animal, or may be derived from a cell line (immortalized cells). The cells may be maintained in cell culture following their isolation from a subject. In certain embodiments the cells are passaged once or more than once (*e.g.*, between 2-5, 5-10, 10-20, 20-50, 50-100 times, or more) prior to their use in a method of the invention. In some embodiments the cells will have been passaged no more than 1, 2, 5, 10, 20, or 50 times prior to their use in a method of the invention.

[387] The somatic cells used or described herein may be native somatic cells, or engineered somatic cells, *i.e.*, somatic cells which have been genetically altered. Somatic cells of the present invention are typically mammalian cells, such as, for example, human cells, primate cells or mouse cells. They may be obtained by well-known methods and can be obtained from any organ or tissue containing live somatic cells, *e.g.*, blood, bone marrow, skin, lung, pancreas, liver, stomach, intestine, heart, reproductive organs, bladder, kidney, urethra and other urinary organs, *etc.*

[388] Mammalian somatic cells useful in the present invention include, but are not limited to, Sertoli cells, endothelial cells, granulosa epithelial cells, neurons, pancreatic islet cells, epidermal cells, epithelial cells, hepatocytes, hair follicle cells, keratinocytes, hematopoietic cells, melanocytes, chondrocytes, lymphocytes (B and T lymphocytes), erythrocytes, macrophages, monocytes, mononuclear cells, cardiac muscle cells, and other muscle cells, *etc.*

[389] Methods described herein may be used to program one or more somatic cells, *e.g.*, colonies or populations of somatic cells into photoreceptor rescue cells. In some embodiments a population of cells of the present invention is substantially uniform in that at least 90% of the cells display a phenotype or characteristic of interest. In some embodiments at least 95%, 96%, 97%, 98%, 99%, 99.5%, 99.8%, 99.9, 99.95% or more of the cells display a phenotype or characteristic of interest. In certain embodiments of the invention the somatic cells have the capacity to divide, *i.e.*, the somatic cells are not post-mitotic.

[390] Somatic cells may be partially or completely differentiated. As described herein, both partially differentiated somatic cells and fully differentiated somatic cells can be differentiated to produce photoreceptor rescue cells.

Photoreceptor Rescue Cells (PRCs):

[391] The PRCs may be differentiated from the pluripotent stem cells (*e.g.*, ESCs or iPSCs in the absence of Noggin and in neural differentiation medium). The PRCs are FOXG1(+) and MAP2(+) as determined by flow cytometry. In one embodiment, 60%, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the PRCs are FOXG1(+) and MAP2(+). The PRCs can also be STMN2 (+), DCX(+), LINC00461 (+), NEUROD2(+), GAD1(+), and/or NFIA(+). PRCs can also be SSEA4(-) and/or OCT4 (-) as determined by flow cytometry. The cells may be grown as spheres or neurospheres (*e.g.*, on low attachment plates or optionally on hanging drop cultures, in a low-gravity environment, aggrewwells, or other suitable culture condition).

[392] Schematics are shown of alternative PRC manufacturing process in **FIGs. 12A** and **12B**. In some embodiments, hESCs or pluripotent cells are expanded prior to PRC differentiation. Expansion of hESCs or pluripotent cells can be performed using a culture chamber (*e.g.*, culture dish, a culture flask, *e.g.*, iMatrix-coated culture T75 flasks, or a culture vessel, *e.g.*, TC-coated CellSTACK®). In some embodiments, the expansion of hESCs or pluripotent cells includes hESCs or pluripotent cells being thawed at day -10, counted and seeded on culture vessels coated with iMatrix (Laminin-511,

Matrixome) and cultured with StemFit™ medium (Ajinomoto) supplemented with 100 ng/mL bFGF (Peprotech) and 10 μ M ROCK inhibitor (Y-27632; Fujifilm/Wako) under feeder-free conditions. After four days of daily medium changes (StemFit+bFGF without ROCK inhibitor), hESCs or pluripotent cells are harvested using Cell Dissociation Buffer (Gibco) and reseeded using the above culture conditions.

[393] After an additional 4 days in culture (day -2), hESCs are harvested as above, percent viability and a viable cell count are obtained, and hESCs are seeded at 3,000 cells/cm² for PRC differentiation on a culture chamber (e.g., culture dish, a culture flask, e.g., iMatrix-coated culture T75 flasks, or a culture vessel, e.g., TC-coated CellSTACK®) in StemFit+bFGF medium supplemented with ROCK inhibitor (Y-27632). After day -2 seeding, cultures are fed with (1) Day -1 – StemFit + bFGF (without ROCK inhibitor); (2) Day 0 to day 3 (daily) – Rescue Induction Medium (RIM: DMEM/F12 + B27 + N2 + Non-essential amino acids (all from Gibco) + glucose (Sigma) + Insulin (Akron Biotech) + Noggin (Gibco)); and (3) Day 4 to day 19 (every 2–3 days) – Neural Differentiation Medium Plus Noggin (NDM+: Neurobasal Medium + B27 + N2 + Non-essential amino acids + glucose + glutamax (Gibco) + Noggin).

[394] At day 19, cultures are “lifted” into suspension culture (2D to 3D) through incubation with a combination of Liberase and Thermolysin enzymes (Roche Custom Labs) and seeded on a culture chamber (e.g., culture dish, a culture flask, e.g., ultra-low attachment T75 flasks, or a culture vessel, e.g., ultra-low attachment CellSTACK®). 3D cultures are maintained for 3-4 days using NDM minus Noggin (NDM-) to allow for the formation of neural spheroids (“spheres”). Then, spheres in suspension are seeded (3D to 2D) onto culture vessels coated with poly-D-lysine (Advanced Biomatrix), viral inactivated human fibronectin (Akron Biotech) and laminin-521 (Biolamina) to start Passage 0 (P0d0), and cultured under 2D conditions for 14 days (until P0d14) using NDM- (feeding every 2-3 days).

[395] 2D to 3D to 2D transitions are repeated three times (through P1, P2 and P3, 3-4 days in 3D and 14 days in 2D culture) until P3d14 is reached after 90 days of differentiation. P3d14 cultures are harvested using Accutase (Innovative Cell Technologies) and cultured in ultra-low attachment T75 flasks for 24 hours. Then, P3-spheres are cryopreserved by resuspending in Cryostor CS10 (Stemcell Technologies) and freezing to -80°C, and then transferred to the vapor phase of liquid nitrogen for storage. The cryopreserved P3 spheres are called Cell Stock (CS). In some embodiments, the cells in the composition are expanded by growing in culture, adherent conditions and then low-adherent conditions. In some embodiments, the repeat of culturing in adherent and then low-adherence conditions are performed at least 1 time, at least 2 times, at least 3 times, at least 4 times, at least 5 times, or at least 6 times. In some embodiments, the cells in the composition are harvested after the third repeat of culturing in adherent and then low-adherence conditions, after the fourth repeat of culturing in adherent and then low-adherence conditions, or after the fifth repeat of culturing in adherent and then low-adherence conditions.

[396] In some embodiments, the cells in the composition are harvested after the fifth repeat of culturing in adherent and then low-adherence conditions. In some embodiments, the harvested cells in the composition are cryopreserved.

[397] In some embodiments, the suspension culture to adherent culture (2D to 3D) are grown in a culture dish, a culture flask, or a culture vessel. In some embodiments, the culture vessel is a CellSTACK® vessel from Corning. In some embodiments, the CellSTACK® is treated with low adherence or adherent coating. In one embodiment, the cells are cultured in low-adherence conditions and the CellSTACK® is coated in an Ultra-Low Attachment coating. In one embodiment, the cells are cultured in adherent conditions and the CellSTACK® is coated in TC-treated coating.

[398] Examples of methods to “lift” cells into suspension include, but are not limited to, methods shown in **Table 13**.

Table 13

Time point		Method 1	Method 2	Method 3
day	Passage day			
19		Cell scraper	Liberase + Thermolysin	Accutase + ROCK inhibitor
37	P0d14	Cell scraper	Liberase + Thermolysin	Accutase
54	P1d14	Cell scraper	Liberase + Thermolysin	Accutase
72	P2d14	Cell scraper	Liberase + Thermolysin	Accutase
90	P3d14	Accutase	Accutase	Accutase
107	P4d14	Accutase	Accutase	Accutase

[399] To produce the composition of the invention, vials of CS are thawed in a water bath at 37°C, resuspended in NDM- and transferred to ultra-low attachment T75 flasks and cultured in 3D suspension for 2-3 days, followed by re-plating onto T75 flasks coated with poly-D-lysine/fibronectin/laminin-521 and culture under 2D conditions for 14 days with NDM- to complete Passage 4. At P4d14, cells are harvested using Accutase, resuspended with NDM-, triturated and filtered through a 40µm cell strainer to obtain a single cell suspension that constitutes the PRC composition of the invention, which can be subsequently formulated with cryopreservative agents.

[400] PRC manufacturing protocol can be altered to allow for scaling up. For example, the flasks used can be changed from T75 flasks to T225 flasks or cell stacks (*e.g.*, Corning® CellSTACK®).

[401] Additionally, the PRC manufacturing protocol can include an intermediary cryopreservation step at any one or more of between P0 and P1, between P1 and P2, between P2 and P3, and/or between P3 and P4. In some embodiments, the PRC manufacturing protocol can exclude an intermediary cryopreservation step.

[402] The intermediary cryopreservation step can include 5% DMSO instead of 10% DMSO. In some embodiments, the intermediary cryopreservation step can include about 1% DMSO, about 2% DMSO, about 3% DMSO, about 4% DMSO, about 5% DMSO, about 6% DMSO, about 7% DMSO, about 8% DMSO, about 9% DMSO, about 10% DMSO, about 11% DMSO, about 12% DMSO, about 14% DMSO, or about 15% DMSO.

[403] The intermediary cryopreservation step can include the cryopreservative formulation, as described herein. The PRC manufacturing protocol can include thermolysin and liberase at D19 and accutase followed by an overnight culture with Rock inhibitor. The lifted cells or cell clusters can be seeded in Aggrewwells™ to allow for uniformly sized spheroids to develop. At day 19, cultures are “lifted” into suspension culture (2D to 3D) through incubation with Accutase (Innovative Cell Technologies) and seeded on ultra-low attachment T75 flasks using NDM minus Noggin (NDM-) medium supplemented with Y-27632 (ROCK inhibitor, Fujifilm Wako). 24 hours later, the growth medium is replaced with Y-27632-free NDM- and 3D cultures are maintained for additional 2-3 days using NDM- to allow for the formation of neural spheroids (“spheres”). Then, spheres in suspension are seeded (3D to 2D) onto culture vessels coated with poly-D-lysine (Advanced Biomatrix), viral inactivated human fibronectin (Akron Biotech) and laminin-521 (Biolamina) to start Passage 0 (P0d0), and cultured under 2D conditions for 14 days (until P0d14) using NDM- (feeding every 2-3 days). 2D to 3D to 2D transitions are repeated three times (through P1, P2 and P3, 3-4 days in 3D and 14 days in 2D culture) until P3d14 is reached after 90 days of differentiation. P3d14 cultures are harvested using Accutase (Innovative Cell Technologies) and cultured in ultra-low attachment T75 flasks for 24 hours. Then, P3-spheres are cryopreserved by resuspending in Cryostor CS10 (Stemcell Technologies) and freezing to -80°C, and then transferred to the vapor phase of liquid nitrogen for storage. The cryopreserved P3 spheres are called Cell Stock (CS).

[404] In some embodiments, the PRC manufacturing protocol uses Aggrewwells. According to this method, day 19, cultures are “lifted” into suspension culture (2D to 3D) through incubation with Accutase (Innovative Cell Technologies) and seeded on Aggrewell plates (Stemcell Technologies) using NDM minus Noggin (NDM-) medium supplemented with Y-27632 (ROCK inhibitor, Fujifilm Wako) to allow for the formation of neural spheroids (“spheres”). After 24 hours, cells are harvested from Aggrewell plates and transferred to ultra-low attachment T75 flasks where 3D cultures are maintained for an additional 2-3 days using Y-27632-free NDM-. Then, spheres in suspension are seeded (3D to 2D) onto culture vessels coated with poly-D-lysine (Advanced Biomatrix), viral inactivated human fibronectin (Akron Biotech) and laminin-521 (Biolamina) to start Passage 0 (P0d0), and cultured under 2D conditions for 14 days (until P0d14) using NDM- (feeding every 2-3

days). 2D to 3D to 2D transitions are repeated three times (through P1, P2 and P3, 3-4 days in 3D and 14 days in 2D culture) until P3d14 is reached after 90 days of differentiation. P3d14 cultures are harvested using Accutase (Innovative Cell Technologies) and cultured in ultra-low attachment T75 flasks for 24 hours. Then, P3-spheres are cryopreserved by resuspending in Cryostor CS10 (Stemcell Technologies) and freezing to -80°C, and then transferred to the vapor phase of liquid nitrogen for storage. The cryopreserved P3 spheres are called Cell Stock (CS). The PRC composition can be pretreated with sucrose, for example at D14 and P4, before being formulated with cryoprotective agents.

[405] Lastly, the cryopreservation step of the final PRC preparation can include poloxamer 188, a nonionic block linear copolymer and/or sucrose.

Extracellular Vesicles (EVs)

[406] The present invention also provides extracellular vesicles secreted from photoreceptor rescue cells and their use in methods of treating an eye disease in a subject.

Extracellular Vesicles Secreted from Photoreceptor Rescue Cells

[407] The present invention also provides extracellular vesicles isolated, derived, secreted, or released from a cell, *e.g.*, the photoreceptor rescue cells of the present invention.

[408] As used herein, the term “extracellular vesicle” or “EV” refers to lipid bound vesicles secreted by cells into the extracellular space. The three main subtypes of EVs are microvesicles (MVs), exosomes, and apoptotic bodies, which are differentiated based upon their biogenesis, release pathways, size, content, and function (Zaborowski M.P., *et al. Bioscience*. 2015;65:783–797). Generally extracellular vesicles range in diameter from 20 nm to 5000 nm, and can comprise various macromolecular payload either within the internal space (*i.e.*, lumen), displayed on the external surface of the extracellular vesicle, and/or spanning the membrane. Said payload can comprise nucleic acids, *e.g.*, microRNAs (miRNA), long non-coding RNAs (lncRNA), mRNAs, DNA fragments; proteins, carbohydrates, lipids, small molecules, and/or combinations thereof. By way of example and without limitation, extracellular vesicles include apoptotic bodies, fragments of cells, vesicles derived/secreted from cells by direct or indirect manipulation (*e.g.*, by serial extrusion or treatment with alkaline solutions), vesiculated organelles, and vesicles produced by living cells (*e.g.*, by direct plasma membrane budding or fusion of the late endosome with the plasma membrane). Extracellular vesicles can be derived/secreted from a living or dead organism, explanted tissues or organs, prokaryotic or eukaryotic cells, and/or cultured cells.

[409] As used herein, the term “exosome” refers to a cell-derived small vesicle comprising a membrane that encloses an internal space (*i.e.*, lumen), and which is formed from said cell by direct plasma membrane budding or by fusion of the late endosome with the plasma membrane (Yáñez-Mó M., *et al. J. Extracell. Vesicles*. 2015;4:27066). Specifically, exosomes are involved in protein sorting, recycling, storage, transport, and release. Exosome are generally between 20-300 nm in diameter.

Exosomes are secreted by all cell types and have been found in plasma, urine, semen, saliva, bronchial fluid, cerebral spinal fluid (CSF), breast milk, serum, amniotic fluid, synovial fluid, tears, lymph, bile, and gastric acid.

[410] Exosomes have been found to participate in cell–cell communication, cell maintenance, and tumor progression. In addition, exosomes have been found to stimulate immune responses by acting as antigen-presenting vesicles (Bobrie A., *et al.*, *Traffic*. 2011;12:1659–1668). In the nervous system, exosomes have been found to help promote myelin formation, neurite growth, and neuronal survival, thus playing a role in tissue repair and regeneration (Faure J., *et al.* *Mol. Cell. Neurosci.* 2006;31:642–648). At the same time, exosomes in the central nervous system (CNS) have been found to contain pathogenic proteins, such as beta amyloid peptide, superoxide dismutase, and alpha synuclein that may aid in disease progression (Fevrier B., *et al.*, *Proc. Natl. Acad. Sci. USA*. 2004;101:9683–9688). Exosomes have also been shown as carriers for disease markers. The use of exosomes as carriers of biomarkers is ideal because these vesicles are found in bodily fluids, such as blood and urine, which allows for minimally to non-invasive “liquid biopsy” type methods to diagnose and even monitor a patient’s response to treatment.

[411] In addition to their natural role in cell-cell interactions, exosomes can be loaded with different cargos, *e.g.*, drugs and exogenous nucleic acids or proteins, and deliver this cargo to different cells. The cargo can be conjugated to an extracellular vesicle, embedded within an extracellular vesicle, encapsulated within an extracellular vesicle, or otherwise carried by an extracellular vesicle, or any combination thereof. Thus, as used herein, a reference to a cargo being “present” in an extracellular vesicle or its lumen is understood to include any of the foregoing means of carrying the cargo.

[412] A cargo can be an endogenous cargo, an exogenous cargo, or a combination thereof. Examples of cargos that can be conjugated, embedded, encapsulated within or otherwise carried by an extracellular vesicle described herein include, without limitation, nucleic acid molecules (*e.g.*, DNA, cDNA, antisense oligonucleotides, mRNA, inhibitory RNAs (*e.g.*, antisense RNAs, miRNAs, small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and agomiRs), antagomiRs, primary miRNAs (pri-miRNAs), long non-coding RNAs (lncRNAs), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), and microbial RNAs), polypeptides (*e.g.*, enzymes, antibodies), lipids, hormones, vitamins, minerals, small molecules, and pharmaceuticals, or any combination thereof. Importantly, exosomes, are natural carriers for miRNAs and other non-coding RNAs, and the direct membrane fusion with the target cell allows contents to be delivered directly into the cytosol. This makes exosomes an excellent delivery system for small molecules (Lai R.C., *et al.* *Biotechnol. Adv.* 2013;31:543–551).

[413] Microvesicles are EVs that form by direct outward budding, or pinching, of the cell’s plasma membrane. The size of microvesicles typically range from 100 nm up to 1000 nm in diameter. The route of microvesicle formation is not well understood, however, it is thought to require

cytoskeleton components, such as actin and microtubules, along with molecular motors (kinesins and myosins), and fusion machinery (SNAREs and tethering factors) (Cai H., *et al. Dev. Cell.* 2007;12:671–682). The number of microvesicles produced depends on the donor cell's physiological state and microenvironment (Zaborowski M.P., *et al. Bioscience.* 2015;65:783–797). Likewise, it has been previously demonstrated that the number of microvesicles consumed depends on the physiological state and microenvironment of recipient cells. Like exosomes, microvesicles are involved in cell–cell communication between local and distant cells. The ability of these EVs to alter the recipient cell has been well demonstrated (Harding C.V., *et al. J. Cell Biol.* 2013;200:367–371; White I.J., *et al., EMBO J.* 2006;25:1–12). The uniqueness of EVs is that they have the ability to package active cargo (proteins, nucleic acids, and lipids) and deliver it to another cell, neighboring or distant, and alter the recipient cell's functions with its delivery.

[414] Apoptotic bodies are released by dying cells into the extracellular space. They are reported to range in size from 50 nm up to 5000 nm in diameter, with the size of most apoptotic bodies tending to be on the larger side (Borges F., *et al. Braz. J. Med. Biol. Res.* 2013;46:824–830). These bodies form by a separation of the cell's plasma membrane from the cytoskeleton as a result of increased hydrostatic pressure after the cell contracts (Wickman G., *et al. Cell Death Differ.* 2012;19:735–742). The composition of apoptotic bodies is in direct contrast with exosomes and microvesicles. Unlike exosomes and microvesicles, apoptotic bodies contain intact organelles, chromatin, and small amounts of glycosylated proteins (Borges F., *et al., Braz. J. Med. Biol. Res.* 2013;46:824–830; Théry C., *et al. J. Immunol.* 2001;166:7309–7318).

Methods for Isolating Extracellular Vesicles

[415] The EVs of the invention can be isolated, secreted, derived, or separated, from a medium or other source material, *e.g.*, the photoreceptor rescue cells of the present invention, using routine methods known in the art (see, for example the techniques described in Taylor et al., *Serum/Plasma Proteomics*, Chapter 15, “Extracellular vesicle Isolation for Proteomic Analyses and RNA Profiling,” Springer Science, 2011; and Tauro et al., *Methods* 56 (2012) 293-304, and references cited therein) and as described in the Examples section below. The most commonly used method involves multiple centrifugation and ultracentrifugation steps.

[416] Physical properties of EVs may be employed for EV isolation, purification or enrichment, including separation on the basis of electrical charge (*e.g.*, electrophoretic separation), size (*e.g.*, filtration, molecular sieving, etc), density (*e.g.*, regular or gradient centrifugation), Svedberg constant (*e.g.*, sedimentation with or without external force, etc). Alternatively, or additionally, isolation may be based on one or more biological properties, and include methods that may employ surface markers (*e.g.*, for precipitation, reversible binding to solid phase, FACS separation, specific ligand binding, non-specific ligand binding, immuno-magnetic capture of EVs using magnetic beads coated with antibodies directed against proteins exposed on EV membranes, etc.).

[417] Methods based on the use of volume-excluding polymers, such as PEG, have been recently described by a number of different groups (U.S. Pat. Appl. 20130273544, U.S. Pat. Appl. 20130337440). Two such products are ExoQuick (System Biosciences, Mountain View, USA) and Total Exosome Isolation Reagent (Life Technologies, Carlsbad, USA). These polymers work by tying up water molecules and forcing less-soluble components such as extracellular vesicles, as well as proteins out of solution, allowing them to be collected by a short, low-speed centrifugation.

[418] In some embodiments, isolation, purification, and enrichment can be done in a general and non-selective manner (typically including serial centrifugation). Alternatively, isolation, purification, and enrichment can be done in a more specific and selective manner (e.g., using producer cell-specific surface markers). For example, specific surface markers may be used in immunoprecipitation, FACS sorting, affinity purification, or bead-bound ligands for magnetic separation.

[419] In some embodiments, tangential flow filtration may be used to isolate or purify the EVs.

[420] In some embodiments, size exclusion chromatography can be utilized to isolate or purify the EVs. Size exclusion chromatography techniques are known in the art. In some embodiments, density gradient centrifugation can be utilized to isolate the EVs. In some embodiments, the isolation of EVs may involve ion chromatography, such as anion exchange, cation exchange, or mixed mode chromatography. In some embodiments, the isolation of EVs may involve desalting, dialysis, tangential flow filtration, ultrafiltration, or diafiltration, or any combination thereof. In some embodiments, the isolation of EVs may involve combinations of methods that include, but are not limited to, differential centrifugation, size-based membrane filtration, concentration and/or rate zonal centrifugation. In some embodiments, the isolation of EVs may involve one or more centrifugation steps. The centrifugation may be performed at about 50,000 to 150,000×g. The centrifugation may be performed at about 50,000×g, 75,000×g, 100,000×g, 125,000×g, or 150,000×g. In another embodiment, EVs are separated from nonmembranous particles, using their relatively low buoyant density (Raposo et al., 1996; Escola et al., 1998; van Niel et al., 2003; Wubbolts et al., 2003). Kits for such isolation are commercially available, for example, from Qiagen, InVitrogen and SBI. Methods for loading EVs with a therapeutic agent are known in the art and include lipofection, electroporation, as well as any standard transfection method.

Applications and Uses

Screening Assays

[421] The present invention provides methods for screening various agents that modulate the differentiation of a retinal progenitor cell. It could also be used to discover therapeutic agents that support and/or rescue mature photoreceptors that are generated in culture from retinal progenitor cells. For the purposes of this invention, an “agent” is intended to include, but not be limited to, a biological or chemical compound such as a simple or complex organic or inorganic molecule, a peptide, a protein (e.g. antibody), a polynucleotide (e.g. anti-sense) or a ribozyme. A vast array of compounds

can be synthesized, for example polymers, such as polypeptides and polynucleotides, and synthetic organic compounds based on various core structures, and these are also included in the term “agent.” In addition, various natural sources can provide compounds for screening, such as plant or animal extracts, and the like. It should be understood, although not always explicitly stated, that the agent is used alone or in combination with another agent, having the same or different biological activity as the agents identified by the inventive screen.

[422] To practice the screening method *in vitro*, an isolated population of cells can be obtained as described herein. When the agent is a composition other than a DNA or RNA, such as a small molecule as described above, the agent can be directly added to the cells or added to culture medium for addition. As is apparent to those skilled in the art, an “effective” amount must be added which can be empirically determined. When the agent is a polynucleotide, it can be directly added by use of a gene gun or electroporation. Alternatively, it can be inserted into the cell using a gene delivery vehicle or other method as described above. Positive and negative controls can be assayed to confirm the purported activity of the drug or other agent.

Biocompatible Support for the Photoreceptor Rescue Cells

[423] A biocompatible support for the cells can be a biodegradable polyester film support for photoreceptor rescue cells. The biodegradable polyester can be any biodegradable polyester suitable for use as a substrate or scaffold. The polyester should be capable of forming a thin film, preferably a micro-textured film, and should be biodegradable if used for tissue or cell transplantation. Suitable biodegradable polyesters for use in the invention include polylactic acid (PLA), polylactides, polyhydroxyalkanoates, both homopolymers and co-polymers, such as polyhydroxybutyrate (PHB), polyhydroxybutyrate co-hydroxyvalerate (PHBV), polyhydroxybutyrate co-hydroxyhexanote (PHBHx), polyhydroxybutyrate co-hydroxyoctononate (PHBO) and polyhydroxybutyrate co-hydroxyoctadecanoate (PHBOd), polycaprolactone (PCL), polyesteramide (PEA), aliphatic copolyesters, such as polybutylene succinate (PBS) and polybutylene succinate/adipate (PBSA), aromatic copolyesters. Both high and low molecular weight polyesters, substituted and unsubstituted polyester, block, branched or random, and polyester mixtures and blends can be used. Preferably the biodegradable polyester is polycaprolactone (PCL).

[424] In certain embodiments, the biocompatible support is a poly(p-xylylene) polymer, such as parylene N, parylene D, parylene-C, parylene AF-4, parylene SF, parylene HT, parylene VT-4 and Parylene CF, and most preferably parylene-C.

[425] The polymeric support can typically be formed into a thin film using known techniques. The film thickness is advantageously from about 1 micron to about 50 microns, and preferably about 5 microns in thickness. The surface of the film can be smooth, or the film surface can be partially or completely micro-textured. Suitable surface textures include micro-grooves or micro-posts, for instance. The film can be cut and shaped to form a suitable shape for implantation.

[426] The photoreceptor rescue cells can be plated directly onto the film to form a biocompatible scaffold. Alternatively, the polymer film can be coated with a suitable coating material such as poly-D-lysine, poly-L-lysine, fibronectin, laminin (*e.g.*, laminin-111, laminin-211, laminin-121, laminin-221, laminin-332/laminin-3A32, laminin-3B32, laminin-311/laminin-3A11, laminin-321/laminin-3A21, laminin-411, laminin-421, laminin-511 (*e.g.*, iMatrix™-511), laminin-521, laminin-213, laminin-432, laminin-522, laminin-532, and/or laminin fragments), collagen I, collagen IV, vitronectin and Matrigel™. The cells can be plated to any desired density, but a single layer of PRC cells (a PRC monolayer) is preferred.

[427] Alternatively, the photoreceptor rescue cells (PRCs) may be administered together with other cell types, including other retinal cell types such as but not limited to retinal ganglion cells, retinal ganglion progenitor cells, retinal pigment epithelium (RPE) cells or RPE progenitors. The photoreceptor rescue cells (PRCs) may be administered with one or any combination of these different cell types, *e.g.*, corneal endothelial cells. The cells may be administered on a matrix or scaffold or membrane, as described above, or they may be administered as cell aggregates, or they may be administered as a dissociated cell suspension. In some embodiments, the cells may be administered on top of a monolayer of RPE cells, which itself may or may not be situated on a matrix or substrate. The cells to be administered may all be derived from *in vitro* differentiation of hES cells or iPS cells or in some instances they may derive or be obtained from other sources. Certain cells may be derived from *in vitro* differentiation of hES cells or iPS cells and other cells may derive or be obtained from other sources. At a minimum, the photoreceptor rescue cells are derived from *in vitro* differentiation of pluripotent stem cells such as hES cells or iPS cells. Any of these various cell combinations may be administered conjointly with other therapeutic agents such as those described herein.

[428] The photoreceptor rescue cells can be administered via a device, for example a syringe, the Oxulumis Illuminated Suprachoroidal Microcatheter (Oxular), POD3 Gold (Oxular), Oxuspheres (Oxular), SCS microinjector (Clearside Biomedical), Orbit SDS (Gyroscope), via an implant, via a cell seeded substrate or an implantable membrane, either alone or in combination with another cell type, for example retinal pigment epithelial cells.

Therapeutic Uses

[429] This invention also provides methods for improving the activity/function of photoreceptor cells in a patient in need of this treatment comprising administering a pharmaceutical preparation including the plurality of heterogeneous photoreceptor rescue cells of the present invention, derived therefrom or a combination thereof, to a patient. As described herein, the pharmaceutical preparation can be a suspension of cells or cells which are formed into transplantable tissue *in vitro* or on a substrate. In many instances, the cells will be administered to the sub-retinal space of a diseased or degenerated retina or the suprachoroidal space. However, as the plurality of heterogeneous

photoreceptor rescue cells of the present invention also have a neuroprotective effect, the cells can be administered locally but outside of the retina (such as in the vitreous), suprachoroidally, or by depot or systemic delivery to other parts of the body. For example, photoreceptor rescue cells of the invention could be administered via a patch or other implantable device wherein a population of cells secrete neuroprotective factors. In one embodiment, such device could be reloaded with photoreceptor cells for repeated administration to improve longevity of the therapeutic effect.

[430] The pharmaceutical preparations of the present invention can be used in a wide range of diseases and disorders that result in visual system deterioration, including retinal degeneration-related disease. Such diseases and disorders may be caused by aging, such that there appears to be an absence of an injury or disease that is identifiable as a substantial source of the deterioration. Skilled artisans will understand the established methods for diagnosing such disease states, and/or inspecting for known signs of such injuries. In addition, the literature is replete with information on age-related decline or deterioration in aspects of the visual systems of animals. The term “retinal degeneration-related disease” is intended to refer to any disease resulting from innate or postnatal retinal degeneration or abnormalities. Examples of retinal degeneration-related diseases include retinal dysplasia, retinal degeneration, aged macular degeneration (wet or dry), geographic atrophy secondary to AMD, diabetic retinopathy, retinitis pigmentosa, congenital retinal dystrophy, Leber congenital amaurosis, Stargardt disease, retinal detachment, glaucoma, optic neuropathy, and trauma.

[431] Additionally or alternatively, the deterioration of the visual system components, such as the neurosensory retina can be caused by injury, for example trauma to the visual system itself (*e.g.*, an eye), to the head or brain, or the body more generally. Certain such injuries are known to be age-related injuries, *i.e.*, their likelihood, or frequency increases with age. Examples of such injuries include retinal tears, macular holes, epiretinal membrane, and retinal detachments, each of which might occur in an animal of any age, but which are more likely to occur, or occur with greater frequency in aging animals, including otherwise healthy aging animals.

[432] The deterioration of the visual system or components thereof also can be caused by disease. Included among the diseases are various age-related diseases that impact the visual system. Such diseases occur with greater likelihood and/or frequency in older animals than in the young. Examples of diseases which may affect the visual system, including for example the neurosensory retinal layers, and cause deterioration thereof are various forms of retinitis, optic neuritis, macular degeneration (wet or dry), geographic atrophy secondary to AMD, proliferative or nonproliferative diabetic retinopathy, diabetic macular edema, progressive retinal atrophy, progressive retinal degeneration, sudden acquired retinal degeneration, immune-mediated retinopathy, retinal dysplasia, chorioretinitis, retinal ischemia, retinal hemorrhage (preretinal, intraretinal and/or subretinal), hypertensive retinopathy, retinal inflammation, retinal edema, retinoblastoma, or retinitis pigmentosa.

[433] Some of the foregoing diseases tend to be specific to certain animals such as companion animals, *e.g.*, dogs and/or cats. Some of the diseases are listed generically, *i.e.*, there may be many

types of retinitis, or retinal hemorrhage; thus some of the disease are not caused by one specific etiologic agent, but are more descriptive of the type of disease or the result. Many of the diseases that can cause decline or deterioration of one or more components of the visual system can have both primary and secondary or more remote effects on an animal's visual system.

[434] Advantageously, the pharmaceutical preparations of the present invention may be used to compensate for a lack of or a diminution of photoreceptor cell function. As illustrated in the Examples, the cells of the invention, including the photoreceptor rescue cells, can be used as a cell replacement therapy in subjects that have lost photoreceptor function, in whole or in part. Such subjects if human may have eyesight characterized as 20/60 or worse, including 20/80 or worse, or 20/100 or worse, or 20/120 or worse, or 20/140 or worse, or 20/160 or worse, or 20/180 or worse, or 20/200 or worse. Thus, this disclosure contemplates treatment of subjects having some level of visual acuity as well as those having no discernable visual acuity.

[435] The photoreceptor rescue cells of this disclosure may be characterized by their ability to reconstitute some level of visual acuity in animal models such as mouse models. In some instances, suitable animal models may be those having a visual impairment that manifests as an optomotor response that is 10% or less, 20% or less, 30% or less, 40% or less, or 50% or less than wild type response. Optomotor responses may be measured using assays such as described in the Examples. After transplantation of the photoreceptor rescue cells of the invention, such optomotor responses will increase, preferably by a statistically significant amount, as shown in the Examples.

[436] This disclosure therefore contemplates administration of the composition comprising a plurality of heterogeneous photoreceptor rescue cells described herein for the purpose of preventing, in whole or in part, disease progression, or improving vision in the recipient, or some combination thereof. The extent to which either mechanism contributes to the improved outcome will depend on the extent of retinal degeneration in the recipient.

[437] Preferably, the photoreceptor rescue cell compositions of the invention are administered to a subject who possesses functional photoreceptors remaining at the time of administration to preserve/protect. As such, the target patient population are subjects with intermediate stage disease. In a particular embodiment, the subjects do not have substantial or near complete loss of photoreceptors.

[438] Examples of retinal dysfunction that can be treated by the retinal stem cell populations and the photoreceptor rescue cell compositions and methods of the invention include but are not limited to: partial or complete photoreceptor degeneration (as occurs in, *e.g.*, retinitis pigmentosa, cone dystrophies, cone-rod and/or rod-cone dystrophies, and macular degeneration); retinal detachment and retinal trauma; photic lesions caused by laser or sunlight; a macular hole; a macular edema; night blindness and color blindness; ischemic retinopathy as caused by diabetes or vascular occlusion; retinopathy due to prematurity/premature birth; infectious conditions, such as, *e.g.*, CMV retinitis and toxoplasmosis; inflammatory conditions, such as the uveitides; tumors, such as retinoblastoma and

ocular melanoma; and for the replacement of inner retinal neurons, which are affected in ocular neuropathies including glaucoma, traumatic optic neuropathy, and radiation optic neuropathy and retinopathy.

[439] In one aspect, the photoreceptor rescue cells can treat or alleviate the symptoms of retinitis pigmentosa in a patient in need of the treatment. In another aspect, the cells can treat or alleviate the symptoms of macular degeneration, such as age-related macular degeneration (wet or dry), Stargardt disease, myopic macular degeneration or the like, in a patient in need of this treatment. For all of these treatments, the cells can be autologous or allogeneic to the patient. In a further aspect, the cells of the invention can be administered in combination with other treatments.

[440] Retinitis pigmentosa (RP) refers to a heterogeneous group of hereditary eye disorders characterized by progressive vision loss due to a gradual degeneration of photoreceptors. An estimated 100,000 people in the United States have RP. Classification of this group of disorders under one rubric is based on the clinical features most commonly observed in these patients. The hallmarks of RP are night blindness and reduction of peripheral vision, narrowing of the retinal vessels, and the migration of pigment from disrupted retinal pigment epithelium into the retina, forming clumps of various sizes, often next to the retinal blood vessels.

[441] Typically, patients first notice difficulty seeing at night due to the loss of rod photoreceptors; the remaining cone photoreceptors then become the mainstay of visual function. Over years and decades, however, the cones also degenerate, leading to a progressive loss of vision. In most RP patients, visual field defects begin in the midperiphery, between 30° and 50° from fixation. The defective regions gradually enlarge, leaving islands of vision in the periphery and a constricted central field (called tunnel vision). When the visual field contracts to 200 or less and/or central vision is 20/200 or worse, the patient becomes legally blind.

[442] Inheritance patterns indicate that RP can be transmitted in X-linked (XLRP), autosomal dominant (ADRP), or recessive (ARRP) modes. Among the three genetic types of RP, ADRP is the mildest. These patients often retain good central vision to 60 years of age and beyond. In contrast, patients with the XLRP form of the disease are usually legally blind by 30 to 40 years of age. However, the severity and the age of onset of the symptoms varies greatly among patients with the same genetic type of RP. This variation is apparent even within the same family when presumably all the affected members have the same genetic mutation. Many RP-inducing mutations have now been described. Of the genes identified so far, many encode photoreceptor-specific proteins, several being associated with phototransduction in the rods, such as rhodopsin, subunits of the cGMP phosphodiesterase, and the cGMP-gated Ca²⁺ channel. Multiple mutations in each of the cloned genes have been found. For example, in the case of the rhodopsin gene, 90 different mutations have been identified among ADRP patients.

[443] Regardless of the specific mutation, the vision loss that is most critical to RP patients is due to the gradual degeneration of cones. In many cases, the protein that the RP-causing mutation affects is

not even expressed in the cones; the prime example is rhodopsin—the rod-specific visual pigment. Therefore, the loss of cones may be an indirect consequence of a rod-specific mutation. The ability to replace damaged photoreceptors provides an approach to the treatment of this disease.

[444] In a particular embodiment, the subject is diagnosed as having RP, for example, by genotyping. Specifically, subjects are diagnosed as having RP based on the identification of mutations affecting RPE genes or photoreceptors prior to treatment. In specific embodiments, patients are vision-impaired, but not to the point where they have total blindness or No Light Perception (NLP) vision. Preferably, the subjects suitable for treatment have a Best Corrected Visual Acuity (BCVA) ranging from 20/50 (vision impaired) to 20/200 (legally blind, but not NLP). In other embodiments, the subjects suitable for treatment have vision worse than 20/200, but maintain light perception.

[445] Age-related macular degeneration (AMD) causes a progressive loss of central vision, and is the most common cause of vision loss in people over age 55. The underlying pathology is degeneration of the photoreceptors. Various studies have implicated hereditary factors, cardiovascular disease, environmental factors such as smoking and light exposure, and nutritional causes as contributing to the risk of developing AMD. RPE degeneration is accompanied by variable loss of both the overlying photoreceptors and the underlying choroidal perfusion. Visual acuity loss or visual field loss occurs when the RPE atrophies and results in secondary loss of the overlying photoreceptor cells that it supplies. The ability to replace RPE and/or photoreceptor cells provides a means of treating established AMD.

[446] AMD is diagnosed by fundus examination, and early/intermediate/late AMD is determined based on anatomical hallmarks of the disease stage that are observed on the fundus image. Intermediate AMD is defined by the presence of at least one large drusen ($>125\text{ }\mu\text{m}$) and/or pigmentary abnormalities. AMD pigmentary abnormalities are defined as hyperpigmentation or hypopigmentation present within 2 disc diameters of the center of the macula in eyes with drusen $\geq 63\text{ }\mu\text{m}$ in diameter and without known retinal disease entities or other reasons for such abnormalities. (See García-Layana A, Cabrera-López F, García-Arumí J, Arias-Barquet L, Ruiz-Moreno JM. Early and intermediate age-related macular degeneration: update and clinical review. Clin Interv Aging. 2017 Oct 3;12:1579-1587). This definition encompasses a broad range of clinical presentations for intermediate AMD, including just one large drusen ($>125\text{ }\mu\text{m}$) OR many large drusen and large amounts of pigmentary abnormality, as long as criteria for Geography Atrophy or neovascular AMD are not met, which are the two versions of late AMD.

[447] Macular degeneration is broadly divided into two types. In the exudative-neovascular form, or “wet” AMD, which accounts for 10% of all cases, abnormal blood vessel growth occurs under the macula. There is formation of a subretinal network of choroidal neovascularization often associated with intraretinal hemorrhage, subretinal fluid, pigment epithelial detachment, and hyperpigmentation. Eventually, this complex contracts and leaves a distinct elevated scar at the posterior pole. These

blood vessels leak fluid and blood into the retina and thus cause damage to the photoreceptors. Wet AMD tends to progress rapidly and can cause severe damage; rapid loss of central vision may occur over just a few months.

[448] The remaining 90% of AMD cases are atrophic macular degeneration (dry form), where there is pigmentary disturbance in the macular region but no elevated macular scar and no hemorrhage or exudation in the region of the macula. In these patients there is a gradual disappearance of the retinal pigment epithelium (RPE), resulting in circumscribed areas of atrophy. Since photoreceptor loss follows the disappearance of RPE, the affected retinal areas have little or no visual function. Vision loss from dry AMD occurs more gradually over the course of many years. These patients usually retain some central vision, although the loss can be severe enough to compromise performance of tasks that require seeing details.

[449] When the appropriate age and clinical findings are accompanied by the loss of visual acuity, visual field, or other visual functions, the condition often is classified as AMD. At times, the step prior to the onset of visual loss has been classified as AMD if the patient has characteristic drusen and relevant family history.

[450] Occasionally, macular degeneration occurs at a much earlier age. Many of these cases are caused by genetic mutations. There are many forms of hereditary macular degeneration, each with its own clinical manifestations and genetic cause. The most common form of juvenile macular degeneration is known as Stargardt disease, which is inherited as an autosomal recessive. Patients are usually diagnosed under the age of 20. Although the progression of vision loss is variable, most of these patients are legally blind by age 50. Mutations that cause Stargardt disease have been identified in the ABCR gene, which codes for a protein that transports retinoids across the photoreceptor membrane.

[451] The photoreceptor rescue cell composition of the present invention find use in the treatment of degenerative diseases. The cells are administered in a manner that permits them to graft or migrate to the intended retinal site, such as in the outer nucleated layer, and reconstitute or regenerate the functionally deficient area.

[452] The Examples demonstrate the ability of photoreceptor rescue cells disclosed herein to regenerate visual acuity in mouse models of blindness due to photoreceptor degeneration. Visual acuity in such mouse models may be assessed using optomotor responses (or optokinetic nystagmus responses).

[453] In another aspect the disclosure provides a method of drug delivery, comprising administering the photoreceptor rescue cell composition described herein or produced by any method described herein to said patient, wherein said photoreceptor rescue cell composition deliver said drug. A wide range of drugs can be used. The engineered photoreceptor rescue cell composition may be prepared so that they include one or more compounds selected from the group consisting of drugs that act at synaptic and neuroeffector junctional sites; drugs that act on the central nervous system; drugs that

modulate inflammatory responses such as anti-inflammatory agents including non-steroidal anti-inflammatory agents; drugs that affect renal and/or cardiovascular function; drugs that affect gastrointestinal function; antibiotics; anti-viral agents, anti-neoplastic and anti-cancer agents; immunomodulatory agents; anesthetic, steroidal agent, antigen, vaccine, antibody, decongestant, antihypertensive, sedative, birth control agent, progestational agent, anti-cholinergic, analgesic, anti-depressant, anti-psychotic, β -adrenergic blocking agent, diuretic, cardiovascular active agent, vasoactive agent, nutritional agent, drugs acting on the blood and/or the blood-forming organs; hormones; hormone antagonists; agents affecting calcification and bone turnover, vitamins, gene therapy agents; or other agents such as targeting agents, etc.

[454] For example, the photoreceptor rescue cell composition may be prepared so that they include one or more compounds selected from the group consisting of drugs that act at synaptic and neuroeffector junctional sites (*e.g.*, acetylcholine, methacholine, pilocarpine, atropine, scopolamine, physostigmine, succinylcholine, epinephrine, norepinephrine, dopamine, dobutamine, isoproterenol, albuterol, propranolol, serotonin); drugs that act on the central nervous system (*e.g.*, clonazepam, diazepam, lorazepam, benzocaine, bupivacaine, lidocaine, tetracaine, ropivacaine, amitriptyline, fluoxetine, paroxetine, valproic acid, carbamazepine, bromocriptine, morphine, fentanyl, naltrexone, naloxone); drugs that modulate inflammatory responses (*e.g.*, aspirin, indomethacin, ibuprofen, naproxen, steroids, cromolyn sodium, theophylline); drugs that affect renal and/or cardiovascular function (*e.g.*, furosemide, thiazide, amiloride, spironolactone, captopril, enalapril, lisinopril, diltiazem, nifedipine, verapamil, digoxin, isordil, dobutamine, lidocaine, quinidine, adenosine, digitalis, mevastatin, lovastatin, simvastatin, mevalonate); drugs that affect gastrointestinal function (*e.g.*, omeprazole, sucralfate); antibiotics (*e.g.*, tetracycline, clindamycin, amphotericin B, quinine, methicillin, vancomycin, penicillin G, amoxicillin, gentamicin, erythromycin, ciprofloxacin, doxycycline, acyclovir, zidovudine (AZT), ddC, ddI, ribavirin, cefaclor, cephalexin, streptomycin, gentamicin, tobramycin, chloramphenicol, isoniazid, fluconazole, amantadine, interferon); anti-cancer agents (*e.g.*, cyclophosphamide, methotrexate, fluorouracil, cytarabine, mercaptopurine, vinblastine, vincristine, doxorubicin, bleomycin, mitomycin C, hydroxyurea, prednisone, tamoxifen, cisplatin, decarbazine); immunomodulatory agents (*e.g.*, interleukins, interferons, GM-CSF, TNF α , TNF β , cyclosporine, FK506, azathioprine, steroids); drugs acting on the blood and/or the blood-forming organs (*e.g.*, interleukins, G-CSF, GM-CSF, erythropoietin, vitamins, iron, copper, vitamin B12, folic acid, heparin, warfarin, coumarin); hormones (*e.g.*, growth hormone (GH), prolactin, luteinizing hormone, TSH, ACTH, insulin, FSH, CG, somatostatin, estrogens, androgens, progesterone, gonadotropin-releasing hormone (GnRH), thyroxine, triiodothyronine); hormone antagonists; agents affecting calcification and bone turnover (*e.g.*, calcium, phosphate, parathyroid hormone (PTH), vitamin D, bisphosphonates, calcitonin, fluoride), vitamins (*e.g.*, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, biotin, choline, inositol, camitine, vitamin C, vitamin A, vitamin E, vitamin K), gene therapy agents (*e.g.*, viral vectors, nucleic-acid-bearing liposomes, DNA-protein conjugates, anti-

sense agents); or other agents such as targeting agents etc. The photoreceptor rescue cell composition of the present invention can be engineered to include one or more therapeutic agents which are released or secreted by these cells either in a passive manner (diffuse out of the cells over time) or in an active manner (upon deliberate rupture or lysis of the cells). hESCs and/or hiPSCs may be genetically modified and used to produce photoreceptor rescue cells (PRCs) that express a desired agent for treatment of a disease. In one aspect, hESCs, hiPSCs and/or multi-lymphoid progenitors (MLPs) could be genetically modified to express an antitumor agent. Photoreceptor rescue cells (PRCs) produced from such genetically modified hESCs and hiPSCs, adult stem cells and multi-lymphoid progenitors (MLPs) may be used to deliver such antitumor agent to a tumor for the treatment of a neoplastic disease, including for example retinoblastoma.

[455] In some embodiments the photoreceptor rescue cells of the invention are produced by increasing expression of one or more transcription factor selected from the group consisting of: PAX6, FOXG1, HMGA1/2, OTX2, ASCL1, POU3F2, NR2F1/2, NR6A1, MEIS2, NEUROD6, HES5, ATF4/5 and RXRG. In one embodiment, photoreceptor rescue cells are generated by increasing expression of one or more transcription factor selected from the group consisting of: PAX6, FOXG1, HMGA1/2, OTX2, ASCL1, POU3F2, NR2F1/2, NR6A1, MEIS2, NEUROD6, HES5, ATF4/5 and RXRG in pluripotent stem cells, early-stage neuronal progenitor cells or late-stage neuronal progenitor cell.

[456] In certain embodiments, the photoreceptor rescue cells (PRCs) have been engineered to include one or more therapeutic agents, such as a small molecule drug, aptamer or other nucleic acid agent, or recombinant proteins.

[457] Genetically engineered photoreceptor rescue cells can also be used to target gene products to sites of degeneration. These gene products can include survival-promoting factors to rescue native degenerating neurons, factors that can act in an autocrine manner to promote survival and differentiation of grafted cells into site-specific neurons or to deliver neurotransmitter(s) to permit functional recovery. *Ex vivo* gene therapy, *e.g.*, recombinantly engineering the pluripotent stem cells, progenitor cells, early-stage neural progenitor cells, late stage neural progenitors or photoreceptor rescue cells, could be used effectively as a neuroprotective strategy to prevent retinal cell loss in RP (retinitis pigmentosa), AMD, and glaucoma and in diseases that cause retinal detachment, by the delivery of growth factors and neurotrophins such as FGF2, NGF, ciliary neurotrophic factor (CNTF), and brain derived neurotrophic factor (BDNF), which factors have been shown to significantly slow the process of cell death in models of retinal degeneration. Therapy using PRCs engineered to synthesize a growth factor or a combination of growth factors can not only ensure sustained delivery of neuroprotectants, but may also reconstruct damaged retina.

[458] The photoreceptor rescue cells of the present invention thereof may be administered conjointly with one or more other therapeutic agents. As used herein, the phrases “conjoint administration” and “administered conjointly” refer to any form of administration in combination of

two or more different therapeutic entities such that the second agent is administered while the previously administered therapeutic agent (such as the cells) is still effective in the body (*e.g.*, the two therapeutics are simultaneously effective in the patient, which may include synergistic effects of the two agents). For example, the different therapeutic agents can be administered either in the same formulation, where the cells are amenable to co-formulation, or in separate formulations, either concomitantly or sequentially. Thus, an individual who receives such treatment can benefit from a combined effect of transplanted cells and one or more different therapeutic agents.

[459] One or more angiogenesis inhibitors may be administered in combination (*i.e.*, conjointly) with the preparations of cells, preferably in a therapeutically effective amount for the prevention or treatment of ocular disease, such as an angiogenesis-associated ocular disease. Exemplary ocular diseases include macular degeneration (*e.g.*, wet AMD or dry AMD), diabetic retinopathy, and choroidal neovascularization. Exemplary angiogenesis inhibitors include VEGF antagonists, such as inhibitors of VEGF and/or a VEGF receptor (VEGFR, *e.g.*, VEGFR1 (FLT1, FLT), VEGFR2 (KDR, FLK1, VEGFR, CD309), VEGFR3 (FLT4, PCL)), such as peptides, peptidomimetics, small molecules, chemicals, or nucleic acids, *e.g.*, pegaptanib sodium, aflibercept, bevasiranib, rapamycin, AGN-745, vatalanib, pazopanib, NT-502, NT-503, or PLG101, CPD791 (a di-Fab' polyethylene glycol (PEG) conjugate that inhibits VEGFR-2), anti-VEGF antibodies or functional fragments thereof (such as bevacizumab (AVASTIN®) or ranibizumab (LUCENTIS®)), or anti-VEGF receptor antibodies (such as IMC-1121(B) (a monoclonal antibody to VEGFR-2), or IMC-18F1 (an antibody to the extracellular binding domain of VEGFR-1)). Additional exemplary inhibitors of VEGF activity include fragments or domains of VEGFR receptor, an example of which is VEGF-Trap (Aflibercept), a fusion protein of domain 2 of VEGFR-1 and domain 3 of VEGFR-2 with the Fc fragment of IgG1. Another exemplary VEGFR inhibitor is AZD-2171 (Cediranib), which inhibits VEGF receptors 1 and 2. Additional exemplary VEGF antagonists include tyrosine kinase inhibitors (TKIs), including TKIs that reportedly inhibit VEGFR-1 and/or VEGFR-2, such as sorafenib (Nexavar), SU5416 (Semaxinib), SU11248/Sunitinib (Sutent), and Vandetanib (ZD 6474). Additional exemplary VEGF antagonists include Ly317615 (Enzastaurin), which is thought to target a down-stream kinase involved in VEGFR signaling (protein kinase C). Additional exemplary angiogenesis inhibitors include inhibitors of $\alpha 5\beta 1$ integrin activity, including and anti- $\alpha 5\beta 1$ integrin antibodies or functional fragments thereof (such as volociximab), a peptide, peptidomimetic, small molecule, chemical or nucleic acid such as 3-(2-{1-alkyl-5-[(pyridine-2-ylamino)-methyl]-pyrrolidin-3-yloxy}-acetylamino)-2-(alkyl-amino)-propionic acid, (S)-2-[(2,4,6-trimethylphenyl)sulfonyl]amino-3-[7-benzyloxycarbonyl-8-(2-pyridinylaminomethyl)-1-oxa-2,7-diazaspiro-(4,4)-non-2-en-3-yl]carbonylamino propionic acid, EMD478761, or RC*D(ThioP)C* (Arg-Cys-Asp-Thioproline-Cys; asterisks denote cyclizing by a disulfide bond through the cysteine residues). Additional exemplary angiogenesis inhibitors include 2-methoxyestradiol, $\alpha V\beta 3$ inhibitors, Angiopoietin 2, angiostatic steroids and heparin, angiostatin, angiostatin-related molecules, anti- $\alpha 5\beta 1$ integrin

antibodies, anti-cathepsin S antibodies, antithrombin III fragment, bevacizumab, calreticulin, canstatin, carboxyamidotriazole, Cartilage-Derived Angiogenesis Inhibitory Factor, CDAI, CM101, CXCL10, endostatin, IFN- α , IFN- β , IFN- γ , IL-12, IL-18, IL-4, linomide, maspin, matrix metalloproteinase inhibitors, Meth-1, Meth-2, osteopontin, pegaptanib, platelet factor-4, prolactin, proliferin-related protein, prothrombin (kringle domain-2), ranibizumab, restin, soluble NRP-1, soluble VEGFR-1, SPARC, SU5416, suramin, tecogalan, tetrathiomolybdate, thalidomide, lenalidomide, thrombospondin, TIMP, TNP-470, TSP-1, TSP-2, vasostatin, VEGFR antagonists, VEGI, Volociximab (also known as M200), a fibronectin fragment such as anastellin (see Yi and Ruoslahti, Proc Natl Acad Sci USA. 2001 Jan. 16; 98(2):620-4) or any combination thereof. Said angiogenesis inhibitor is preferably in an amount sufficient to prevent or treat proliferative (neovascular) eye disease, such as choroidal neovascular membrane (CNV) associated with wet AMD and other diseases of the retina. Additional exemplary angiogenesis inhibitors include: Lenvatinib (E7080), Motesanib (AMG 706), Pazopanib (Votrient), and an IL-6 antagonist such as anti-IL-6 antibody. Additional exemplary angiogenesis inhibitors include fragments, mimetics, chimeras, fusions, analogs, and/or domains of any of the foregoing. Additional exemplary angiogenesis inhibitors include combinations of any of the foregoing. In an exemplary embodiment, the photoreceptor rescue cell composition comprises an anti-VEGF antibody, *e.g.*, bevacizumab, such as between about 0.1 mg to about 6.0 mg, *e.g.*, about 1.25 mg and about 2.5 mg bevacizumab, per injection into the eye. In further exemplary embodiments, the photoreceptor rescue cell composition comprises one or more inhibitors of VEGF activity and one or more inhibitors of $\alpha 5 \beta 1$ integrin activity.

[460] One or more anti-inflammatory agents may be administered in combination with the photoreceptor rescue cell composition. Exemplary anti-inflammatory agents include: glucocorticoids, non-steroidal anti-inflammatory drugs, aspirin, ibuprofen, naproxen, cyclooxygenase (COX) enzyme inhibitors, aldosterone, beclometasone, betamethasone, corticosteroids, cortisol, cortisone acetate, deoxycorticosterone acetate, dexamethasone, fludrocortisone acetate, fluocinolone acetonide (*e.g.*, ILUVIEN®), glucocorticoids, hydrocortisone, methylprednisolone, prednisolone, prednisone, steroids, and triamcinolone.

[461] One or more antioxidants, antioxidant cofactors, and/or other factors contributing to increased antioxidant activity may be administered in combination with the photoreceptor rescue cell composition, examples of which may include OT-551 (Othera), vitamin C, vitamin E, beta carotene, zinc (*e.g.*, zinc oxide), and/or copper (*e.g.*, copper oxide).

[462] One or more macular xanthophylls (such as lutein and/or zeaxanthin) may be administered in combination with the photoreceptor rescue cell composition.

[463] One or more long-chain omega-3 fatty acids, such as docosahexaenoic acid (DHA) and/or eicosapentaenoic acid (EPA)), may be administered in combination with the photoreceptor rescue cell composition.

[464] One or more amyloid inhibitors, such as fenretinide, Arc-1905, Copaxone (glatiramer acetate, Teva), RN6G (PF-4382923, Pfizer) (a humanized monoclonal antibody versus ABeta40 and ABeta42), GSK933776 (GlaxoSmithKline) (anti-amyloid antibody), may be administered in combination with the photoreceptor rescue cell composition.

[465] One or more ciliary neurotrophic factor (CNTF) agonists (*e.g.*, CNTF which may be delivered in an intraocular device such as NT-501 (Neurotech)) may be administered in combination with the photoreceptor rescue cell composition.

[466] One or more inhibitors of RPE65, such as ACU-4429 (Aculea, Inc.) may be administered in combination with the photoreceptor rescue cell composition.

[467] One or more factors that target A2E and/or lipofuscin accumulation, such as Fenretinide, and ACU-4429, may be administered in combination with the photoreceptor rescue cell composition.

[468] One or more downregulators or inhibitors of photoreceptor function and/or metabolism, such as fenretinide and ACU-4429, may be administered in combination with the photoreceptor rescue cell composition.

[469] One or more α 2-adrenergic receptor agonists, such as Brimonidine tartrate, may be administered in combination with the photoreceptor rescue cell composition.

[470] One or more selective serotonin 1A agonists, such as Tandoospirone (AL-8309B), may be administered in combination with the photoreceptor rescue cell composition.

[471] In combination with the photoreceptor rescue cell composition, one or more factors targeting C-5, membrane attack complex (C5b-9) and/or any other Drusen component may be administered, examples of which include inhibitors of complement factors D, C-3, C-3a, C5, and C5a, and/or agonists of factor H, such as ARC 1905 (Ophthotec) (an anti-C5 Aptamer that selectively inhibits C5), POT-4 (Potentia) (a compstatin derivative that inhibits C3), complement factor H, Eculizumab (Soliris, Alexion) (a humanized IgG antibody that inhibits C5), and/or FCFD4514S (Genentech, San Francisco) (a monoclonal antibody against complement factor D).

[472] One or more immunosuppressants, such as Sirolimus (rapamycin), may be administered in combination with the photoreceptor rescue cell composition.

[473] One or more agents that prevent or treat the accumulation of lipofuscin, such as piracetam, centrophenoxine, acetyl-L-carnitine, Ginkgo Biloba or an extract or preparation thereof, and/or DMAE (Dimethylethanolamine), may be administered in combination with the photoreceptor rescue cell composition.

[474] Where one or more agent (such as angiogenesis inhibitors, antioxidants, antioxidant cofactors, other factors contribute to increased antioxidant activity, macular xanthophylls, long-chain omega-3 fatty acids, amyloid inhibitors, CNTF agonists, inhibitors of RPE65, factors that target A2E and/or lipofuscin accumulation, downregulators or inhibitors of photoreceptor function and/or metabolism, α 2-adrenergic receptor agonists, selective serotonin 1A agonists, factors targeting C-5, membrane attack complex (C5b-9) and/or any other Drusen component, immunosuppressants, agents that

prevent or treat the accumulation of lipofuscin, etc.) is administered in combination with the photoreceptor rescue cell composition, said agent may be administered concurrently with, prior to, and/or subsequent to said photoreceptor rescue cell composition. For example, said agent may be administered to the eye of the patient during the procedure in which said photoreceptor rescue cell composition is introduced into the eye of said patient. Administration of said agent may begin prior to and/or continue after administration of said cells to the eye of the patient. For example, said agent may be provided in solution, suspension, as a sustained release form, and/or in a sustained delivery system (*e.g.*, the Allergan Novadur™ delivery system, the NT-501, or another intraocular device or sustained release system).

[475] In certain embodiments, the cells may be engineered to include a recombinant expression construct, which when expressed by the cells *in vivo*, produces a recombinant version of an agent set out herein. In the case of antibody, this includes both two-chain monoclonal antibodies, as well as epitope binding fragments thereof, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), disulfide-linked Fvs (sdFv), and fragments comprising either a VL or VH domain, as well as fibronectin scaffolded and other antibody CDR mimetics. The engineered cells may include expression constructs encoding recombinant peptides and proteins, as well as constructs which, when transcribed, form transcripts which give rise to RNA interference agents (such as siRNA, hairpin RNA or the like), aptamers, decoys (bind to transcription factors and inhibit expression of native gene), antisense or the like. The recombinant gene can be operably linked to a transcriptional regulatory element, such as promoter and/or enhancer, which is active in the transplanted cell (such as a constitutively active or photoreceptor-active element) or which can be regulated by small molecules.

[476] Exemplary recombinant agents to be expressed by the transplanted cells include anti-angiogenic agents, such as those which reduce occurrence of choroidal neovascularization (wet AMD). These include agents which inhibit VEGF mediated vascularization of the eye, such as anti-VEGF antibodies and VEGF receptor traps. Such proteins include antibodies and antibody analogs (such as single chain antibodies, monobodies, antigen binding sites and the like) such as ranibizumab, VEGF-traps such as Aflibercept which are soluble proteins including ligand binding domains from VEGF receptors, which bind to either VEGF or the VEGF receptor and block receptor activation.

[477] Activation of alternative complement pathway is implicated in disease progression for certain patients, particularly in the case of dry AMD. Another class of exemplary recombinant agents to be expressed by the transplanted cells include complement inhibitors, such as complement Factor D, Factor C5 and/or Factor C3 Inhibitors. These may be, merely to illustrate, RNA agents or recombinant antibodies.

[478] Drusen deposits in dry AMD resemble amyloid deposits. Accordingly, the transplanted cells may be engineered to express an anti β -amyloid agent. These include recombinant antibodies, β -secretase inhibitors, and the like.

[479] The transplanted cells may also be engineered to express one or more anti-inflammatory agents, such as antagonists/inhibitors of proinflammatory cytokines such as IL-1, IL-2, IL-3, and TNF- α or anti-inflammatory cytokines such as IL-37. The antagonists/inhibitors of proinflammatory cytokines include recombinant antibodies, receptor traps, aptamers, etc. In one embodiment, the transplanted cells can be engineered to express recombinant lipocortin, a potent anti-inflammatory protein.

[480] In the methods of the invention, cells to be transplanted are transferred to a recipient in any physiologically acceptable excipient comprising an isotonic excipient prepared under sufficiently sterile conditions for human administration. For general principles in medicinal formulation, the reader is referred to *Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy*, by G. Morstyn & W. Sheridan eds, Cambridge University Press, 1996. Choice of the cellular excipient and any accompanying elements of the composition will be adapted in accordance with the route and device used for administration. The cells may be introduced by injection, catheter, or the like. The cells may be frozen at liquid nitrogen temperatures and stored for long periods of time, being capable of use on thawing. If frozen, the cells will usually be stored in a 10% DMSO, 50% FCS, 40% RPMI 1640 medium.

[481] The pharmaceutical preparations of the invention are optionally packaged in a suitable container with written instructions for a desired purpose. Such formulations may comprise a cocktail of retinal differentiation and/or trophic factors, in a form suitable for combining with PRCs. Such a composition may further comprise suitable buffers and/or excipients appropriate for transfer into an animal. Such compositions may further comprise the cells to be engrafted.

Pharmaceutical Preparations

[482] The PRCs may be formulated with a pharmaceutically acceptable carrier. For example, PRCs may be administered alone or as a component of a pharmaceutical formulation. The subject compounds may be formulated for administration in any convenient way for use in medicine. Pharmaceutical preparations suitable for administration may comprise the PRCs, in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions (*e.g.*, balanced salt solution (BSS)), dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes or suspending or thickening agents. Exemplary pharmaceutical preparations comprises the PRCs in combination with ALCON® BSS PLUS® (a balanced salt solution containing, in each mL, sodium chloride 7.14 mg, potassium chloride 0.38 mg, calcium chloride dihydrate 0.154 mg, magnesium chloride hexahydrate 0.2 mg, dibasic sodium phosphate 0.42 mg, sodium bicarbonate 2.1 mg, dextrose 0.92 mg, glutathione disulfide (oxidized glutathione) 0.184 mg, hydrochloric acid and/or sodium hydroxide (to adjust pH to approximately 7.4) in water).

[483] When administered, the pharmaceutical preparations for use in this disclosure may be in a pyrogen-free, physiologically acceptable form.

[484] The preparation comprising PRCs used in the methods described herein may be transplanted in a suspension, gel, colloid, slurry, or mixture. Further, the preparation may desirably be encapsulated or injected in a viscous form into the vitreous humor for delivery to the site of retinal or choroidal damage. Also, at the time of injection, cryopreserved PRCs may be resuspended with commercially available balanced salt solution to achieve the desired osmolality and concentration for administration by subretinal injection. The preparation may be administered to an area of the pericentral macula that was not completely lost to disease, which may promote attachment and/or survival of the administered cells.

[485] The PRCs may be frozen (cryopreserved) as described herein. Upon thawing, the viability of such cells may be at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% at least 95% or about 100% (*e.g.*, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% at least 95% or about 100% of the cells harvested after thawing are viable or at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% at least 95% or about 100% of the cell number initially frozen are harvested in a viable state after thawing). In some instances, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85% of the cells of the composition are viable prior to and after cryopreservation and thawing. In some instances, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, or about 85% of the cells of the composition are viable after thawing. In some instances, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 50% to about 60%, about 50% to about 55%, about 55% to about 85%, about 55% to about 80%, about 55% to about 75%, about 55% to about 70%, about 55% to about 65%, about 55% to about 60%, about 60% to about 85%, about 60% to about 80%, about 60% to about 75%, about 60% to about 70%, about 60% to about 65%, about 65% to about 85%, about 65% to about 80%, about 65% to about 75%, about 65% to about 70%, about 70% to about 85%, about 70% to about 80%, about 70% to about 75%, about 75% to about 85%, about 75% to about 80%, or about 80% to about 85% of the cells of the composition are viable prior to and after cryopreservation and thawing. In some instances, at least about 50% of the cells in the composition are viable after cryopreservation and thawing. In some instances, at least about 55% of the cells in the composition are viable after cryopreservation and thawing. In some instances, at least about 60% of the cells in the composition are viable after cryopreservation and thawing. In some instances, at least about 65% of the cells in the composition are viable after cryopreservation and thawing. In some instances, at least about 70% of the cells in the composition are viable after cryopreservation and thawing. In some instances, at least about 75% of the cells in the composition are viable after cryopreservation and thawing. In some instances, the viability of the cells

prior to and after thawing is about 80%. In some instances, at least 90% or at least 95% or about 95% of cells that are frozen are recovered. The cells may be frozen as single cells or as aggregates. For example, the cells may be frozen as neurospheres.

[486] The PRCs of the disclosure may be delivered in a pharmaceutically acceptable ophthalmic formulation by intraocular injection. When administering the formulation by intravitreal injection, for example, the solution may be concentrated so that minimized volumes may be delivered.

Concentrations for injections may be at any amount that is effective and non-toxic, depending upon the factors described herein. The pharmaceutical preparations of PRCs for treatment of a patient may be formulated at doses of at least about 10^4 cells/mL. The PRC preparations for treatment of a patient are formulated at doses of at least about 10^3 , 10^4 , 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , or 10^{10} PRC cells/mL. For example, the photoreceptor rescue cells may be formulated in a pharmaceutically acceptable carrier or excipient.

[487] The pharmaceutical preparations of PRCs described herein may comprise at least about 1,000, at least about 2,000, at least about 3,000, at least about 4,000, at least about 5,000, at least about 6,000, at least about 7,000, at least about 8,000, at least about 9,000 PRCs. The pharmaceutical preparations of PRCs may comprise at least about 1×10^4 , at least about 2×10^4 , at least about 3×10^4 , at least about 4×10^4 , at least about 5×10^4 , at least about 6×10^4 , at least about 7×10^4 , at least about 8×10^4 , at least about 9×10^4 , at least about 1×10^5 , at least about 2×10^5 , at least about 3×10^5 , at least about 4×10^5 , at least about 5×10^5 , at least about 6×10^5 , at least about 7×10^5 , at least about 8×10^5 , at least about 9×10^5 , at least about 1×10^6 , at least about 2×10^6 , at least about 3×10^6 , at least about 4×10^6 , at least about 5×10^6 , at least about 6×10^6 , at least about 7×10^6 , at least about 8×10^6 , at least about 9×10^6 , at least about 1×10^7 , at least about 2×10^7 , at least about 3×10^7 , at least about 4×10^7 , at least about 5×10^7 , at least about 6×10^7 , at least about 7×10^7 , at least about 8×10^7 , at least about 9×10^7 , at least about 1×10^8 , at least about 2×10^8 , at least about 3×10^8 , at least about 4×10^8 , at least about 5×10^8 , at least about 6×10^8 , at least about 7×10^8 , at least about 8×10^8 , at least about 9×10^8 , at least about 1×10^9 , at least about 2×10^9 , at least about 3×10^9 , at least about 4×10^9 , at least about 5×10^9 , at least about 6×10^9 , at least about 7×10^9 , at least about 8×10^9 , at least about 9×10^9 , at least about 1×10^{10} , at least about 2×10^{10} , at least about 3×10^{10} , at least about 4×10^{10} , at least about 5×10^{10} , at least about 6×10^{10} , at least about 7×10^{10} , at least about 8×10^{10} , or at least about 9×10^{10} PRCs. The pharmaceutical preparations of PRCs may comprise at least about 1×10^2 to about 1×10^3 , about 1×10^2 to about 1×10^4 , about 1×10^4 to about 1×10^5 , or about 1×10^3 to about 1×10^6 PRCs. The pharmaceutical preparations of PRCs may comprise at least about 10,000, at least about 20,000, at least about 25,000, at least about 50,000, at least about 75,000, at least about 100,000, at least about 125,000, at least about 150,000, at least about 175,000, at least about 180,000, at least about 185,000, at least about 190,000, or at least about 200,000 PRCs. For example, the pharmaceutical preparation of PRCs may comprise at least about 20,000 to about 200,000 PRCs in a volume at least about 50 to about 200 μ L. Further, the pharmaceutical preparation of PRCs may comprise about 50,000 PRCs in a volume of about 150

μL, about 200,000 PRCs or more in a volume of about 150 μL, or at least about 180,000 PRCs in a volume at least about 150 μL.

[488] In the aforesaid pharmaceutical preparations and compositions, the number of PRCs or concentration of PRCs may be determined by counting viable cells and excluding non-viable cells. For example, non-viable PRCs may be detected by failure to exclude a vital dye (such as Trypan Blue), or using a functional assay (such as the ability to adhere to a culture substrate, phagocytosis, etc.). Additionally, the number of PRCs or concentration of PRCs may be determined by counting cells that express one or more PRC markers and/or excluding cells that express one or more markers indicative of a cell type other than PRCs.

[489] The PRCs may be formulated for delivery in a pharmaceutically acceptable ophthalmic vehicle, such that the preparation is maintained in contact with the ocular surface for a sufficient time period to allow the cells to penetrate the affected regions of the eye, as for example, the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid, retina, sclera, suprachoroidal space, conjunctiva, subconjunctival space, episcleral space, intracorneal space, epicorneal space, pars plana, surgically-induced avascular regions, or the macula.

[490] The PRCs may be contained in a sheet of cells. For example, a sheet of cells comprising PRCs may be prepared by culturing PRCs on a substrate from which an intact sheet of cells can be released, *e.g.*, a thermoresponsive polymer such as a thermoresponsive poly(N-isopropylacrylamide) (PNIPAAm)-grafted surface, upon which cells adhere and proliferate at the culture temperature, and then upon a temperature shift, the surface characteristics are altered causing release of the cultured sheet of cells (*e.g.*, by cooling to below the lower critical solution temperature (LCST) (see da Silva et al., Trends Biotechnol. 2007 December; 25(12):577-83; Hsiue et al., Transplantation. 2006 Feb. 15; 81(3):473-6; Ide, T. et al. (2006); Biomaterials 27, 607-614, Sumide, T. et al. (2005), FASEB J. 20, 392-394; Nishida, K. et al. (2004), Transplantation 77, 379-385; and Nishida, K. et al. (2004), N. Engl. J. Med. 351, 1187-1196 each of which is incorporated by reference herein in its entirety). The sheet of cells may be adherent to a substrate suitable for transplantation, such as a substrate that may dissolve *in vivo* when the sheet is transplanted into a host organism, *e.g.*, prepared by culturing the cells on a substrate suitable for transplantation, or releasing the cells from another substrate (such as a thermoresponsive polymer) onto a substrate suitable for transplantation. An exemplary substrate potentially suitable for transplantation may comprise gelatin (see Hsiue et al., *supra*). Alternative substrates that may be suitable for transplantation include fibrin-based matrixes and others. The sheet of cells may be used in the manufacture of a medicament for the prevention or treatment of a disease of retinal degeneration. The sheet of PRCs may be formulated for introduction into the eye of a subject in need thereof. For example, the sheet of cells may be introduced into an eye in need thereof by subfoveal membranectomy with transplantation of the sheet of PRCs, or may be used for the manufacture of a medicament for transplantation after subfoveal membranectomy.

[491] The volume of preparation administered according to the methods described herein may be dependent on factors such as the mode of administration, number of PRCs, age and weight of the patient, and type and severity of the disease being treated. If administered by injection, the volume of a pharmaceutical preparations of PRCs of the disclosure may be from at least about 1 mL, about 1.5 mL, about 2 mL, about 2.5 mL, about 3 mL, about 4 mL, or 5 mL. The volume may be at least about 1 mL to about 2 mL. For example, if administered by injection, the volume of a pharmaceutical preparation of PRCs of the disclosure may be at least about 1 μ L, about 2 μ L, about 3 μ L, about 4 μ L, about 5 μ L, about 6 μ L, about 7 μ L, about 8 μ L, about 9 μ L, about 10 μ L, about 11 μ L, about 12 μ L, about 13 μ L, about 14 μ L, about 15 μ L, about 16 μ L, about 17 μ L, about 18 μ L, about 19 μ L, about 20 μ L, about 21 μ L, about 22 μ L, about 23 μ L, about 24 μ L, about 25 μ L, about 26 μ L, about 27 μ L, about 28 μ L, about 29 μ L, about 30 μ L, about 31 μ L, about 32 μ L, about 33 μ L, about 34 μ L, about 35 μ L, about 36 μ L, about 37 μ L, about 38 μ L, about 39 μ L, about 40 μ L, about 41 μ L, about 42 μ L, about 43 μ L, about 44 μ L, about 45 μ L, about 4 μ L 6, about 47 μ L, about 48 μ L, about 49 μ L, about 50 μ L, about 51 μ L, about 52 μ L, about 53 μ L, about 54 μ L, about 55 μ L, about 56 μ L, about 57 μ L, about 58 μ L, about 59 μ L, about 60 μ L, about 61 μ L, about 62 μ L, about 63 μ L, about 64 μ L, about 65 μ L, about 66 μ L, about 67 μ L, about 68 μ L, about 69 μ L, about 70 μ L, about 71 μ L, about 72 μ L, about 73 μ L, about 74 μ L, about 75 μ L, about 76 μ L, about 77 μ L, about 78 μ L, about 79 μ L, about 80 μ L, about 81 μ L, about 82 μ L, about 83 μ L, about 84 μ L, about 85 μ L, about 86 μ L, about 87 μ L, about 88 μ L, about 89 μ L, about 90 μ L, about 91 μ L, about 92 μ L, about 93 μ L, about 94 μ L, about 95 μ L, about 96 μ L, about 97 μ L, about 98 μ L, about 99 μ L, about 100 μ L, about 101 μ L, about 102 μ L, about 103 μ L, about 104 μ L, about 105 μ L, about 106 μ L, about 107 μ L, about 108 μ L, about 109 μ L, about 100 μ L, about 111 μ L, about 112 μ L, about 113 μ L, about 114 μ L, about 115 μ L, about 116 μ L, about 117 μ L, about 118 μ L, about 119 μ L, about 120 μ L, about 121 μ L, about 122 μ L, about 123 μ L, about 124 μ L, about 125 μ L, about 126 μ L, about 127 μ L, about 128 μ L, about 129 μ L, about 130 μ L, about 131 μ L, about 132 μ L, about 133 μ L, about 134 μ L, about 135 μ L, about 136 μ L, about 137 μ L, about 138 μ L, about 139 μ L, about 140 μ L, about 141 μ L, about 142 μ L, about 143 μ L, about 144 μ L, about 145 μ L, about 146 μ L, about 147 μ L, about 148 μ L, about 149 μ L, about 150 μ L, about 151 μ L, about 152 μ L, about 153 μ L, about 154 μ L, about 155 μ L, about 156 μ L, about 157 μ L, about 158 μ L, about 159 μ L, about 160 μ L, about 161 μ L, about 162 μ L, about 163 μ L, about 164 μ L, about 165 μ L, about 166 μ L, about 167 μ L, about 168 μ L, about 169 μ L, about 170 μ L, about 171 μ L, about 172 μ L, about 173 μ L, about 174 μ L, about 175 μ L, about 176 μ L, about 177 μ L, about 178 μ L, about 179 μ L, about 180 μ L, about 181 μ L, about 182 μ L, about 183 μ L, about 184 μ L, about 185 μ L, about 186 μ L, about 187 μ L, about 188 μ L, about 189 μ L, about 190 μ L, about 191 μ L, about 192 μ L, about 193 μ L, about 194 μ L, about 195 μ L, about 196 μ L, about 197 μ L, about 198 μ L, about 199 μ L, or about 200 μ L (microliters). For example, the volume of a preparation of the disclosure may be from at least about 10 μ L to about 50 μ L, about 20 μ L to about 50 μ L, about 25 μ L to about 50 μ L, or about 1 μ L to about 200 μ L. The volume of a preparation of the disclosure may be at least about 10 μ L, about 20

μL, about 30 μL, about 40 μL, about 50 μL, about 100 μL, about 110 μL, about 120 μL, about 130 μL, about 140 μL, about 150 μL, about 160 μL, about 170 μL, about 180 μL, about 190 μL, or about 200 μL, or higher.

[illegible]

9×10^3 to about 2×10^4 , about 1×10^4 to about 2×10^4 , about 1×10^3 to about 1×10^4 , about 2×10^3 to about 1×10^4 , about 3×10^3 to about 1×10^4 , about 4×10^3 to about 1×10^4 , about 5×10^3 to about 1×10^4 , about 6×10^3 to about 1×10^4 , about 7×10^3 to about 1×10^4 , about 8×10^3 to about 1×10^4 , about 9×10^3 to about 1×10^4 , about 1×10^3 to about 9×10^3 , about 2×10^3 to about 9×10^3 , about 3×10^3 to about 9×10^3 , about 4×10^3 to about 9×10^3 , about 5×10^3 to about 9×10^3 , about 6×10^3 to about 9×10^3 , about 7×10^3 to about 9×10^3 , about 8×10^3 to about 9×10^3 , about 1×10^3 to about 8×10^3 , about 2×10^3 to about 8×10^3 , about 3×10^3 to about 8×10^3 , about 4×10^3 to about 8×10^3 , about 5×10^3 to about 8×10^3 , about 6×10^3 to about 8×10^3 , about 7×10^3 to about 8×10^3 , about 1×10^3 to about 7×10^3 , about 2×10^3 to about 7×10^3 , about 3×10^3 to about 7×10^3 , about 4×10^3 to about 7×10^3 , about 5×10^3 to about 7×10^3 , about 6×10^3 to about 7×10^3 , about 1×10^3 to about 6×10^3 , about 2×10^3 to about 6×10^3 , about 3×10^3 to about 6×10^3 , about 4×10^3 to about 6×10^3 , about 5×10^3 to about 6×10^3 , about 1×10^3 to about 5×10^3 , about 2×10^3 to about 5×10^3 , about 3×10^3 to about 5×10^3 , about 4×10^3 to about 5×10^3 , about 1×10^3 to about 4×10^3 , about 2×10^3 to about 4×10^3 , about 3×10^3 to about 4×10^3 , about 1×10^3 to about 3×10^3 , about 2×10^3 to about 3×10^3 , or about 1×10^3 to about 2×10^3 PRCs per μL . The preparation may comprise about 2000 PRCs per μL , for example, about 100,000 PRCs per 50 μL or about 180,000 PRCs per 90 μL .

[493] The method of treating retinal degeneration may further comprise administration of an immunosuppressant. Immunosuppressants that may be used include but are not limited to anti-lymphocyte globulin (ALG) polyclonal antibody, anti-thymocyte globulin (ATG) polyclonal antibody, azathioprine, BASILIXIMAB® (anti-IL-2R α receptor antibody), cyclosporin (cyclosporin A), DACLIZUMAB® (anti-IL-2R α receptor antibody), everolimus, mycophenolic acid, RITUXIMAB® (anti-CD20 antibody), sirolimus, and tacrolimus. The immunosuppressants may be dosed at least about 1, 2, 4, 5, 6, 7, 8, 9, or 10 mg/kg. When immunosuppressants are used, they may be administered systemically or locally, and they may be administered prior to, concomitantly with, or following administration of the PRCs. Immunosuppressive therapy may continue for weeks, months, years, or indefinitely following administration of PRCs. For example, the patient may be administered 5 mg/kg cyclosporin for 6 weeks following administration of the PRCs.

[494] The method of treatment of retinal degeneration may comprise the administration of a single dose of PRCs. Also, the methods of treatment described herein may comprise a course of therapy where PRCs are administered multiple times over some period. Exemplary courses of treatment may comprise weekly, biweekly, monthly, quarterly, biannually, or yearly treatments. Alternatively, treatment may proceed in phases whereby multiple doses are administered initially (*e.g.*, daily doses for the first week), and subsequently fewer and less frequent doses are needed.

[495] If administered by intraocular injection, the PRCs may be delivered one or more times periodically throughout the life of a patient. For example, the PRCs may be delivered once per year, once every 6-12 months, once every 3-6 months, once every 1-3 months, or once every 1-4 weeks.

Alternatively, more frequent administration may be desirable for certain conditions or disorders. If administered by an implant or device, the PRCs may be administered one time, or one or more times periodically throughout the lifetime of the patient, as necessary for the particular patient and disorder or condition being treated. Similarly contemplated is a therapeutic regimen that changes over time. For example, more frequent treatment may be needed at the outset (*e.g.*, daily or weekly treatment). Over time, as the patient's condition improves, less frequent treatment or even no further treatment may be needed.

[496] Intraocular administration may comprise injection of an aqueous solution, optionally an isotonic solution and/or a saline solution, into the subretinal space, thereby forming a pre-bleb, and removal of said aqueous solution, prior to administration of the photoreceptor rescue cell population into the same subretinal space as said aqueous solution. Prior to cell administration, a subretinal bleb may be formed, *e.g.*, of by injection of saline or another suitable fluid (a "pre-bleb"), which may then be removed prior to cell administration. However, the cells may also be administered without pre-bleb formation. The cells may be administered in a bleb in a temporal foveal position. For example, the bleb may optionally extend within the arcade blood vessels. The bleb may be positioned such that it does not detach the central macula fovea.

[497] The methods described herein may further comprise the step of monitoring the efficacy of treatment or prevention by measuring electroretinogram responses, optomotor acuity threshold, or luminance threshold in the subject. The method may also comprise monitoring the efficacy of treatment or prevention by monitoring immunogenicity of the cells or migration of the cells in the eye.

[498] The PRCs may be used in the manufacture of a medicament to treat retinal degeneration. The disclosure also encompasses the use of the preparation comprising PRCs in the treatment of blindness. For example, the preparations comprising human PRCs may be used to treat retinal degeneration associated with a number of vision-altering ailments that result in photoreceptor damage and blindness, such as, diabetic retinopathy, macular degeneration (including age related macular degeneration, *e.g.*, wet age related macular degeneration and dry age related macular degeneration), retinitis pigmentosa, and Stargardt Disease (fundus flavimaculatus), night blindness and color blindness. The preparation may comprise at least about 5,000-500,000 PRCs (*e.g.*, 100,000 PRCs) which may be administered to the retina to treat retinal degeneration associated with a number of vision-altering ailments that result in photoreceptor damage and blindness, such as, diabetic retinopathy, macular degeneration (including age related macular degeneration), retinitis pigmentosa, and Stargardt Disease (fundus flavimaculatus).

[499] The PRCs provided herein may be derived from a mammal. Note, however, that the human cells may be used in human patients, as well as in animal models or animal patients. For example, the human cells may be tested in mouse, rat, cat, dog, or non-human primate models of retinal degeneration. Additionally, the human cells may be used therapeutically to treat animals in need thereof, such as in veterinary medicine. Examples of veterinary subjects or patients include without

limitation dogs, cats, and other companion animals, and economically valuable animals such as livestock and horses.

[500] This disclosure further provides novel cryoprotective formulations that minimally require a cryoprotectant, albumin, and optionally a sugar. The cryoprotectant may be DMSO, glycerol, ethylene glycol, trehalose, or taurine. In some embodiments, the cryoprotectant is DMSO. The albumin may be human albumin. The sugar may be glucose. The formulation may further comprise a buffer or a buffered saline such as but not limited to phosphate-buffered saline (PBS). The formulation may have a pH that is about a physiological pH (*e.g.*, in the range of 6-8). The cryoprotectant may be present in a range of about 1% to about 10% volume/volume (v/v). The cryoprotectant may be present in a range of about 2% to about 10% volume/volume (v/v). The cryoprotectant may be present in a range of about 3% to about 10% volume/volume (v/v). The cryoprotectant may be present in a range of about 4% to about 10% volume/volume (v/v), for example 4%, 5%, 6%, 7%, 8%, 9% or 10%. The albumin may be present in a range of about 2% to about 3% (w/v). The albumin may be present in a range of about 2% to about 10% (w/v), of about 2% to about 8% (w/v), of about 2% to about 7.5% (w/v), of about 3.5% to about 7.5% (w/v), of about 4% to about 7.5% (w/v), of about 4% to about 8% (w/v), of about 4% to about 8.5% (w/v), of about 4% to about 9% (w/v) or of about 4% to about 10% (w/v). The sugar may be present in a range of about 0% about 1.5% (w/v).

[501] In some embodiments, the cryopreservative formulation comprises about 4% to about 10% DMSO (v/v), about 2% to about 3% albumin (w/v), about 0-1.5% glucose (w/v) and buffer or buffered saline. The formulation may consist essentially of about 4% to about 10% DMSO (v/v), about 2% to about 3% albumin (w/v), 0-1.5% glucose (w/v) and buffer or buffered saline. The formulation may consist of about 4% to about 10% DMSO (v/v), about 2% to about 3% albumin (w/v), 0-1.5% glucose (w/v) and buffer or buffered saline. The albumin may be human albumin, including recombinant human albumin. The buffered saline may be phosphate buffered saline (PBS).

[502] In some embodiments, the cryopreservative formulation comprises about 4% to about 6% DMSO (v/v), about 2% to about 3% albumin (w/v), about 0.08% to about 0.1% glucose (w/v) and buffer or buffered saline. In some embodiments, the cryopreservative formulation consists essentially of about 4% to about 6 % DMSO (v/v), about 2% to about 3% albumin (w/v), about 0.08% to about 0.1% glucose (w/v) and buffer or buffered saline. In some embodiments, the cryopreservative formulation consists of about 4% to about 6 % DMSO (v/v), about 2% to about 3% albumin (w/v), about 0.08% to about 0.1% glucose (w/v) and buffer or buffered saline. The albumin may be human albumin, including recombinant human albumin. The buffered saline may be phosphate buffered saline (PBS).

[503] In some embodiments, the cryopreservative formulation comprises about 4% to about 10% DMSO (v/v), about 2% to about 7.5% albumin (w/v), about 0-1.5% glucose (w/v) and buffer or buffered saline. The formulation may consist essentially of about 4% to about 10% DMSO (v/v),

about 2% to about 7.5% albumin (w/v), about 0-1.5% glucose (w/v) and buffer or buffered saline. The formulation may consist of about 4% to about 10% DMSO (v/v), about 2% to about 7.5% albumin (w/v), about 0-1.5% glucose (w/v) and buffer or buffered saline. The albumin may be human albumin, including recombinant human albumin. The buffered saline may be phosphate buffered saline (PBS).

[504] In some embodiments, the cryopreservative formulation comprises about 4% to about 6% DMSO (v/v), about 2% to about 7.5% albumin (w/v), about 0.08% to about 0.1% glucose (w/v) and buffer or buffered saline. In some embodiments, the cryopreservative formulation consists essentially of about 4% to about 6% DMSO (v/v), about 2% to about 7.5% albumin (w/v), about 0.08% to about 0.1% glucose (w/v) and buffer or buffered saline. In some embodiments, the cryopreservative formulation consists of about 4% to about 6% DMSO (v/v), about 2% to about 7.5% albumin (w/v), about 0.08% to about 0.1% glucose (w/v) and buffer or buffered saline. The albumin may be human albumin, including recombinant human albumin. The buffered saline may be phosphate buffered saline (PBS).

[505] In some embodiments, particular formulations are associated with a reduced incidence of multinucleated photoreceptor rescue cells having more than 4 nuclei per cell, following freezing and thawing. For example, formulations comprising high levels of DMSO (*e.g.*, 5%) and optionally also containing ethylene glycol (*e.g.*, 5%).

[506] In some embodiments, the cryoprotectant is a cell-penetrating cryoprotectant such as DMSO, glycerol or ethylene glycol, all of which were found to be associated with reduced incidence of multinucleated RPE cells.

[507] Still other formulations comprise about 5% DMSO (v/v), about 2.5% albumin (w/v), about 0.09% glucose (w/v) and buffer or buffered saline. Other formulations comprise about 2.5% albumin (w/v), about 0.09% glucose (w/v), about 5% (v/v) glycerin, about 200 mM taurine, and buffer or buffered saline. Other formulation comprise 2.5% albumin (w/v), about 0.09% glucose (w/v), about 5% (v/v) glycerin, about 100 mM trehalose, and buffer or buffered saline.

[508] Still other formulations comprise about 5% DMSO (v/v), about 2.5% albumin (w/v), about 0.6% glucose (w/v) and buffer or buffered saline. Other formulations comprise about 2.5% albumin (w/v), about 0.6% glucose (w/v), about 5% (v/v) glycerin, about 200 mM taurine, and buffer or buffered saline. Other formulation comprise 2.5% albumin (w/v), about 0.6% glucose (w/v), about 5% (v/v) glycerin, about 100 mM trehalose, and buffer or buffered saline.

[509] Still other formulations comprise about 5% DMSO (v/v), about 7.5% albumin (w/v), about 0.6% glucose (w/v) and buffer or buffered saline. Other formulations comprise about 7.5% albumin (w/v), about 0.6% glucose (w/v), about 5% (v/v) glycerin, about 200 mM taurine, and buffer or buffered saline. Other formulation comprise 7.5% albumin (w/v), about 0.6% glucose (w/v), about 5% (v/v) glycerin, about 100 mM trehalose, and buffer or buffered saline.

[510] In some embodiments, the cryopreservative formulation comprises one or more of betaine, albumin, ethylene glycol and pluronic.

[511] The cryopreserved cell preparation may comprise, in some embodiments, the cells of interest (*e.g.*, photoreceptor rescue cells), 165 μg DMSO, 27 μg glucose, 750 μg recombinant human albumin, 110 μg sodium chloride, 26.6 μg sodium phosphate, 2.10 μg calcium chloride, 4.19 μg potassium chloride, 4.19 μg potassium phosphate monobasic, 2.10 μg magnesium chloride-6H₂O, 168 μg sodium chloride, and 24.1 μg sodium phosphate dibasic.

[512] Upon thaw and dilution in a diluent as provided herein, the final cell preparation to be administered to a subject may comprise, in some embodiments, the cells of interest (*e.g.*, photoreceptor rescue cells), sodium hyaluronate, sodium chloride, potassium chloride, sodium phosphate dibasic dodecahydrate, dextrose (anhydrous), calcium chloride dihydrate, magnesium chloride hexahydrate, sodium acetate trihydrate, and sodium citrate dihydrate.

[513] The cells may be cryopreserved at higher concentration (or density) compared to standard prior art techniques. For example, the cells may be cryopreserved at a concentration (or density) of 10^4 cells per μL or higher, including about 2×10^4 cells per μL , about 3×10^4 cells per μL , about 4×10^4 cells per μL , about 5×10^4 cells per μL , about 6×10^4 cells per μL , about 7×10^4 cells per μL , about 8×10^4 cells per μL , about 9×10^4 cells per μL , or about 10^5 cells per μL , or higher. In some embodiments, the cell density is in the range of about 10^4 cells per μL to about 6×10^4 cells per μL , including 1.5×10^4 cell per μL to about 5×10^4 cells per μL , including about 2×10^4 cells per μL to about 5×10^4 cells per μL , and about 3×10^4 cells per μL to about 5×10^4 cells per μL . In some embodiments, the number of cells per vial is about 450,000 to 1,500,000 cells per vial. In some embodiments, the number of cells per vial is about 1,750,000 to about 3,500,000 cells per vial.

[514] Additionally, the volume of the cryopreserved cell preparation may be reduced compared to standard prior art techniques. For example, the cells may be cryopreserved in volumes ranging from about 10 μL to about 100 μL , in some embodiments, including volumes of about 10 μL to about 50 μL , including volumes of about 10 μL , about 20 μL , about 30 μL , about 40 μL , about 50 μL , about 60 μL , about 70 μL , about 80 μL , about 90 μL , or about 100 μL . Since the cells can be frozen in large numbers yet in low volumes, the frozen preparation can then be diluted with a suitable diluent to arrive at a desired volume and concentration, and then directly administered to a subject without the need for performing a washing step. Standard prior art techniques typically freeze lower concentrations of cells in larger volumes (*e.g.*, on the order of 1 mL), and then require that the thawed preparation be washed, sometimes multiple times, in order to remove the cryoprotectant before administration to a subject.

[515] In some embodiments, the cryoprotective formulation comprises, consists essentially of, or consists of about 4% to about 10% DMSO (v/v), about 2% to about 3% albumin (w/v), 0-1.5% glucose (w/v) and buffer, and is used to cryopreserve photoreceptor rescue cells. This formulation results in viability of the photoreceptor rescue cells, post thaw, of at least 45%, at least 50%, at least

55%, at least 60%, or at least 65% or at least 70%, or at least 75%, or at least 80% or at least 85%, or at least 90% or at least 91% or at least 92% or at least 93% or at least 94%, at least 95% at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100%. The DMSO may be used at about 5% or about 10%. The cells may be frozen at a concentration of about 50×10^3 cells per μL . The formulation results in improved viability of the cells, post-thaw, compared to a commercially available cryoprotective formulation, CS10.

[516] In some embodiments, the cryoprotective formulation comprises, consists essentially of, or consists of about 4% to about 10% DMSO (v/v), about 2% to about 3% albumin (w/v), 0-1.5% glucose (w/v) and buffer or buffered saline. The DMSO may be present at about 4% (v/v), about 5% (v/v), and about 6% (v/v). The albumin may be human albumin, including recombinant human albumin, and may be present at about 2% (w/v), or about 2.5% (w/v), or about 3% (w/v). The glucose may be absent, or it may be present at about 0.08% (w/v) or about 0.09% (w/v), or about 0.1% (w/v). The buffered saline may be phosphate buffered saline. In some embodiments, the cryoprotective formulation comprises, consists essentially of, or consists of about 4% to about 6% DMSO (v/v), about 2% to about 3% albumin (w/v), 0-1.5% glucose (w/v) and buffer or buffered saline. The albumin may be present at about 2% (w/v), about 2.5% (w/v), or about 3% (w/v). The glucose may be present at about 0.08% (w/v), or about 0.09% (w/v), or about 0.10% (w/v).

[517] In some embodiments, the cryoprotective formulation comprises, consists essentially of, or consists of about 4% to about 10% DMSO (v/v), about 2% to about 3% albumin (w/v), 0-1.5% glucose (w/v) and buffer or buffered saline. The DMSO may be present at about 4% (v/v), about 5% (v/v), and about 6% (v/v). The albumin may be human albumin, including recombinant human albumin, and may be present at about 2% (w/v), or about 2.5% (w/v), or about 3% (w/v), or about 4% (w/v), or about 5% (w/v), or about 6% (w/v), or about 7% (w/v), or about 7.5% (w/v), or about 8% (w/v), or about 9% (w/v) or about 10% w/v). The glucose may be absent, or it may be present at about 0.08% (w/v) or about 0.09% (w/v), or about 0.1% (w/v). The buffered saline may be phosphate buffered saline.

[518] In some embodiments, the cryoprotective formulation comprises, consists essentially of, or consists of about 4% to about 6% DMSO (v/v), about 2% to about 3% albumin (w/v), 0-1.5% glucose (w/v) and buffer or buffered saline. The albumin may be present at about 2% (w/v), about 2.5% (w/v), or about 3% (w/v). In some embodiments, the cryoprotective formulation comprises, consists essentially of, or consists of about 4% to about 6% DMSO (v/v), about 2% to about 7.5% albumin (w/v), 0-1.5% glucose (w/v) and buffer or buffered saline. The albumin may be present at about 2% (w/v), about 5% (w/v), or about 7.5% (w/v). The glucose may be present at about 0.08% (w/v), or about 0.09% (w/v), or about 0.10% (w/v).

Cryopreservative Formulations

[519] In some embodiments, the formulations or preparations provided herein exhibit a physiological pH and a physiological osmotic pressure, also referred to as a physiological osmolality.

A physiological pH refers to a pH that is not cytotoxic and resembles the pH of the cell or tissue in its natural environment. For most cells and tissues, a physiological pH is a pH of about 6.8 to about 7.8, for example, a pH of about 7 to about 7.7, a pH of about 7.2 to about 7.6, a pH of about 7.2 to about 7.4, or a pH of about 7.4 to about 7.5. Accordingly, in some embodiments, the formulations or preparations provided herein exhibit a pH of about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, or about 7.8. A physiological osmotic pressure refers to an osmotic pressure that is not cytotoxic and resembles the osmotic pressure of the cell or tissue in its natural environment. For most cells and tissues, a physiological osmotic pressure is about 270 to about 345 mOsm/l, for example, about 280 to about 330 mOsm/l, about 290 to about 325 mOsm/l, about 300 to about 315 mOsm/l. In some embodiments, a physiological osmotic pressure is about 300, about 305, about 310, about 315, about 320, or about 325 mOsm/l. In some embodiments, the formulations or preparations provided herein exhibit a physiological pH and an osmotic pressure that is greater than physiological osmotic pressure, for example, about 350 to about 450 mOsm/l, for example, about 360 to about 440 mOsm/l, about 370 to about 430 mOsm/l, about 380 to about 420 mOsm/l, or about 390 to about 410 mOsm/l. In some embodiments, an osmotic pressure that is greater than physiological osmotic pressure is about 350, about 355, about 360, about 365, about 370, about 375, about 380, about 385, about 390, about 395, about 400, about 405, about 410, about 415, about 420, about 425, about 430, about 435, about 440, about 445 or about 450 mOsm/l. In some embodiments, the formulations or preparations provided herein have an osmolality of about 1250 mOsm/kg, for example about 1250 mOsm/kg, about 1000 mOsm/kg, about 900 mOsm/kg, about 800 mOsm/kg, about 700 mOsm/kg, about 600 mOsm/kg, about 500 mOsm/kg, about 400 mOsm/kg or about 300 mOsm/kg. In some embodiments, the formulations or preparation provided herein have an osmolality about 300-450 mOsm/kg, about 300-400 mOsm/kg, about 300-450 mOsm/kg, about 350-450 mOsm/kg or about 350-400 mOsm/kg.

[520] In some embodiments, the cryopreservative formulations provided herein comprise (a) a cryoprotectant; (b) albumin; (c) a buffer, maintaining the solution at a physiological pH; and (d) glucose.

In some embodiments, the formulations comprise a cryoprotectant. Cryoprotectants are used to protect cells from damage during the freezing process. In some embodiments, the formulations comprise a cryoprotectant selected from dimethyl sulfoxide (DMSO), glycerol, and ethylene glycol. In some embodiments, the cryoprotectant is DMSO. In some embodiments, the formulations comprise about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, or about 10% (volume/volume, v/v) DMSO. In some embodiments, the solution comprises about 4%-10%, about 4%-9.5%, about 4%-9%, about 4%-8.5%, about 4%-8%, about 4%-7.5%, about 4%-7%, about 4%-6.5%, about 4%-6%, about 4%-5.5%, about 4%-5%, about 4%-4.5%, about 4.5%-10%, about 4.5%-9.5%, about 4.5%-9%, about 4.5%-8.5%, about 4.5%-8%, about 4.5%-7.5%, about 4.5%-7%, about 4.5%-6.5%, about 4.5%-6%, about 4.5%-

5.5%, about 4.5%-5%, about 5%-10%, about 5%-9.5%, about 5%-9%, about 5%-8.5%, about 5%-8%, about 5%-7.5%, about 5%-7%, about 5%-6.5%, about 5%-6%, about 5%-5.5%, about 5.5%-10%, about 5.5%-9.5%, about 5.5%-9%, about 5.5%-8.5%, about 5.5%-8%, about 5.5%-7.5%, about 5.5%-7%, about 5.5%-6.5%, about 5.5%-6%, about 6%-10%, about 6%-9.5%, about 6%-9%, about 6%-8.5%, about 6%-8%, about 6%-7.5%, about 6%-7%, about 6%-6.5%, about 6.5%-10%, about 6.5%-9.5%, about 6.5%-9%, about 6.5%-8.5%, about 6.5%-8%, about 6.5%-7.5%, about 6.5%-7%, about 7%-10%, about 7%-9.5%, about 7%-9%, about 7%-8.5%, about 7%-8%, about 7%-7.5%, about 7.5%-10%, about 7.5%-9.5%, about 7.5%-9%, about 7.5%-8.5%, about 7.5%-8%, about 8%-10%, about 8%-9.5%, about 8%-9%, about 8%-8.5%, about 8.5%-10%, about 8.5%-9.5%, about 8.5%-9%, about 9%-10%, about 9%-9.5%, or about 9.5%-10% (v/v) DMSO. In some embodiments, the formulations comprise about 4%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5%, about 5.1%, about 5.2%, about 5.3%, about 5.4%, about 5.5%, about 5.6%, about 5.7%, about 5.8%, about 5.9%, about 6%, about 6.1%, about 6.2%, about 6.3%, about 6.4%, about 6.5%, about 6.6%, about 6.7%, about 6.8%, about 6.9%, about 7%, about 7.1%, about 7.2%, about 7.3%, about 7.4%, about 7.5%, about 7.6%, about 7.7%, about 7.8%, about 7.9%, about 8%, about 8.1%, about 8.2%, about 8.3%, about 8.4%, about 8.5%, about 8.6%, about 8.7%, about 8.8%, about 8.9%, about 9%, about 9.1%, about 9.2%, about 9.3%, about 9.4%, about 9.5%, about 9.6%, about 9.7%, about 9.8%, about 9.9% or about 10% (volume/volume, v/v) DMSO

[521] In some embodiments, the formulations comprise albumin. In some embodiments, the albumin is recombinant albumin. In some embodiments, the albumin is recombinant human (rh) albumin. The term “recombinant albumin” is interchangeably used with the term “rA” or “rAlbumin”. The term “recombinant human albumin” is interchangeably used with the term “rHA”, “rHSA”, “rhAlbumin”. In some embodiments, the cryopreservative formulations comprises about 2.0%, about 2.1%, about 2.2%, about 2.3%, about 2.4%, about 2.5%, about 2.6%, about 2.7%, about 2.8%, about 2.9%, about 3.0%, about 3.1%, about 3.2%, about 3.3%, about 3.4%, about 3.5%, about 3.6%, about 3.7%, about 3.8%, about 3.9%, about 4.0%, about 4.1%, about 4.2%, about 4.3%, about 4.4%, about 4.5%, about 4.6%, about 4.7%, about 4.8%, about 4.9%, about 5.0% about 5.1%, about 5.2%, about 5.3%, about 5.4%, about 5.5%, about 5.6%, about 5.7%, about 5.8%, about 5.9%, about 6.0%, about 6.1%, about 6.2%, about 6.3%, about 6.4%, about 6.5%, about 6.6%, about 6.7%, about 6.8%, about 6.9%, about 7.0%, about 7.1%, about 7.2%, about 7.3%, about 7.4%, about 7.5%, about 7.6%, about 7.7%, about 7.8%, about 7.9%, about 8.0%, about 8.1%, about 8.2%, about 8.3%, about 8.4%, about 8.5%, about 8.6%, about 8.7%, about 8.8%, about 8.9%, about 9.0%, about 9.1%, about 9.2%, about 9.3%, about 9.4%, about 9.5%, about 9.6%, about 9.7%, about 9.8%, about 9.9%, or about 10.0% albumin. In some embodiments, the cryopreservative formulations comprise about 2.0%-3.0%, about 2.0%-2.9%, about 2.0%-2.8%, about 2.0%-2.7%, about 2.0%-2.6%, about 2.0%-2.5%, about 2.0%-2.4%, about 2.0%-2.3%, about 2.0%-2.2%, about 2.0%-2.1%, about 2.1%-3.0%, about 2.1%-2.9%, about 2.1%-2.8%, about 2.1%-2.7%, about 2.1%-2.6%, about 2.1%-2.5%, about 2.1%-2.4%, about

2.1%-2.3%, about 2.1%-2.2%, about 2.2%-3.0%, about 2.2%-2.9%, about 2.2%-2.8%, about 2.2%-2.7%, about 2.2%-2.6%, about 2.2%-2.5%, about 2.2%-2.4%, about 2.2%-2.3%, about 2.3%-3.0%, about 2.3%-2.9%, about 2.3%-2.8%, about 2.3%-2.7%, about 2.3%-2.6%, about 2.3%-2.5%, about 2.3%-2.4%, about 2.4%-3.0%, about 2.4%-2.9%, about 2.4%-2.8%, about 2.4%-2.7%, about 2.4%-2.6%, about 2.4%-2.5%, about 2.5%-3.0%, about 2.5%-2.9%, about 2.5%-2.8%, about 2.5%-2.7%, about 2.5%-2.6%, about 2.6%-3.0%, about 2.6%-2.9%, about 2.6%-2.8%, about 2.6%-2.7%, about 2.7%-3.0%, about 2.7%-2.9%, about 2.7%-2.8%, about 2.8%-3.0%, about 2.8%-2.9%, or about 2.9%-3.0% albumin.

[522] In some embodiments, the cryopreservative formulations comprise a buffer. A buffer refers to an agent that can maintain the pH of a solution, preparation or formulation relatively stable by neutralizing added acid or base. Typically, a buffer comprises a weak conjugate acid-base pair, *i.e.*, either a weak acid and its conjugate base, or a weak base and its conjugate acid. In some embodiments, the buffer comprised in the cryopreservative formulations provided herein is a water-based salt solution comprising disodium hydrogen phosphate (Na_2HPO_4) and sodium chloride (NaCl). In some embodiments, the buffer comprised in the formulations provided herein further comprises potassium chloride (KCl) and potassium dihydrogen phosphate (KH_2PO_4). In some embodiments, the formulations comprise a buffered saline such as phosphate-buffered saline (PBS). In some embodiments, the buffer is Dulbecco's phosphate-buffered saline (DPBS).

[523] In some embodiments, the buffer comprises divalent cations. Suitable divalent cations include, without limitation, *e.g.*, Ca^{2+} , Mg^{2+} , Zn^{2+} , Fe^{2+} , Mn^{2+} , Cr^{2+} , Cu^{2+} , Ba^{2+} , and Sr^{2+} . In some embodiments, the divalent cations comprise calcium. In some embodiments, the divalent cations comprise magnesium. In some embodiments, the divalent cations comprise two or more different divalent cations, *e.g.*, calcium and magnesium. In some embodiments, the buffer comprises calcium. In some embodiments, the buffer comprises a pharmaceutically acceptable calcium salt. In some embodiments, the buffer comprises magnesium. In some embodiments, the buffer comprises a pharmaceutically acceptable magnesium salt. In some embodiments of the solutions provided herein, the buffer comprises calcium chloride. In some embodiments, the buffer comprises calcium chloride dihydrate. In some embodiments, the buffer comprises magnesium chloride. In some embodiments, the buffer comprises magnesium chloride hexahydrate.

[524] In some embodiments, the cryopreservative formulations provided herein comprise glucose. In some embodiments, the cryopreservative formulation comprises about 0%, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%, 2%, 2.1%, 2.2%, 2.3%, 2.4%, 2.5%, 2.6%, 2.7%, 2.8%, 2.9%, 3%, 3.5%, 4%, 4.5% or 5% glucose. In some embodiments, the solution comprises about 0%-1.5%, 0%-1.4%, 0%-1.3%, 0%-1.2%, 0%-1.1%, 0%-1.0%, 0%-0.90%, 0%-0.80%, 0%-0.70%, 0%-0.60%, 0%-0.50%, 0%-0.40%, 0%-0.30%, 0%-0.20%, 0%-0.10%, 0%-0.09%, 0%-0.08%, 0%-0.07%, 0%-0.06%, 0%-0.05%, 0%-0.04%, 0%-0.03%, 0%-0.02%, 0%-0.01%,

0.01%-1.5%, 0.01%-1.4%, 0.01%-1.3%, 0.01%-1.2%, 0.01%-1.1%, 0.01%-1.0%, 0.01%-0.90%, 0.01%-0.80%, 0.01%-0.70%, 0.01%-0.60%, 0.01%-0.50%, 0.01%-0.40%, 0.01%-0.30%, 0.01%-0.20%, 0.01%-0.10%, 0.01%-0.09%, 0.01%-0.08%, 0.01%-0.07%, 0.01%-0.06%, 0.01%-0.05%, 0.01%-0.04%, 0.01%-0.03%, 0.01%-0.02%, 0.02%-1.5%, 0.02%-1.4%, 0.02%-1.3%, 0.02%-1.2%, 0.02%-1.1%, 0.02%-1.0%, 0.02%-0.90%, 0.02%-0.80%, 0.02%-0.70%, 0.02%-0.60%, 0.02%-0.50%, 0.02%-0.40%, 0.02%-0.30%, 0.02%-0.20%, 0.02%-0.10%, 0.02%-0.09%, 0.02%-0.08%, 0.02%-0.07%, 0.02%-0.06%, 0.02%-0.05%, 0.02%-0.04%, 0.02%-0.03%, 0.03%-1.5%, 0.03%-1.4%, 0.03%-1.3%, 0.03%-1.2%, 0.03%-1.1%, 0.03%-1.0%, 0.03%-0.90%, 0.03%-0.80%, 0.03%-0.70%, 0.03%-0.60%, 0.03%-0.50%, 0.03%-0.40%, 0.03%-0.30%, 0.03%-0.20%, 0.03%-0.10%, 0.03%-0.09%, 0.03%-0.08%, 0.03%-0.07%, 0.03%-0.06%, 0.03%-0.05%, 0.03%-0.04%, 0.04%-1.5%, 0.04%-1.4%, 0.04%-1.3%, 0.04%-1.2%, 0.04%-1.1%, 0.04%-1.0%, 0.04%-0.90%, 0.04%-0.80%, 0.04%-0.70%, 0.04%-0.60%, 0.04%-0.50%, 0.04%-0.40%, 0.04%-0.30%, 0.04%-0.20%, 0.04%-0.10%, 0.04%-0.09%, 0.04%-0.08%, 0.04%-0.07%, 0.04%-0.06%, 0.04%-0.05%, 0.05%-1.5%, 0.05%-1.4%, 0.05%-1.3%, 0.05%-1.2%, 0.05%-1.1%, 0.05%-1.0%, 0.05%-0.90%, 0.05%-0.80%, 0.05%-0.70%, 0.05%-0.60%, 0.05%-0.50%, 0.05%-0.40%, 0.05%-0.30%, 0.05%-0.20%, 0.05%-0.10%, 0.05%-0.09%, 0.05%-0.08%, 0.05%-0.07%, 0.05%-0.06%, 0.06%-1.5%, 0.06%-1.4%, 0.06%-1.3%, 0.06%-1.2%, 0.06%-1.1%, 0.06%-1.0%, 0.06%-0.90%, 0.06%-0.80%, 0.06%-0.70%, 0.06%-0.60%, 0.06%-0.50%, 0.06%-0.40%, 0.06%-0.30%, 0.06%-0.20%, 0.06%-0.10%, 0.06%-0.09%, 0.06%-0.08%, 0.06%-0.07%, 0.07%-1.5%, 0.07%-1.4%, 0.07%-1.3%, 0.07%-1.2%, 0.07%-1.1%, 0.07%-1.0%, 0.07%-0.90%, 0.07%-0.80%, 0.07%-0.70%, 0.07%-0.60%, 0.07%-0.50%, 0.07%-0.40%, 0.07%-0.30%, 0.07%-0.20%, 0.07%-0.10%, 0.07%-0.09%, 0.07%-0.08%, 0.08%-1.5%, 0.08%-1.4%, 0.08%-1.3%, 0.08%-1.2%, 0.08%-1.1%, 0.08%-1.0%, 0.08%-0.90%, 0.08%-0.80%, 0.08%-0.70%, 0.08%-0.60%, 0.08%-0.50%, 0.08%-0.40%, 0.08%-0.30%, 0.08%-0.20%, 0.08%-0.10%, 0.08%-0.09%, 0.09%-1.5%, 0.09%-1.4%, 0.09%-1.3%, 0.09%-1.2%, 0.09%-1.1%, 0.09%-1.0%, 0.09%-0.9%, 0.09%-0.80%, 0.09%-0.70%, 0.09%-0.60%, 0.09%-0.50%, 0.09%-0.40%, 0.09%-0.30%, 0.09%-0.20%, 0.09%-0.10%, 1.0%-1.5%, 1.0%-1.4%, 1.0%-1.3%, 1.0%-1.2%, 1.0%-1.1%, 1.1%-1.5%, 1.1%-1.4%, 1.1%-1.3%, 1.1%-1.2%, 1.2%-1.5%, 1.2%-1.4%, 1.2%-1.3%, 1.3%-1.5%, 1.3%-1.4%, or 1.4%-1.5% v/v glucose.

[525] In some embodiments, the formulation comprises about 4-10% (v/v) cell cryoprotectant, about 2-3% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer, optionally as a buffered saline. In some embodiments, the formulation comprises about 4-10% (v/v) cell cryoprotectant, about 2-3% (w/v) albumin, about 0.08-0.10% (w/v) glucose, and a buffer, optionally as a buffered saline. In some embodiments, the formulation comprises about 4-6% (v/v) cell cryoprotectant, about 2-3% (w/v) albumin, about 0.08-0.10% (w/v) glucose, and a buffer, optionally as a buffered saline.

[526] In some embodiments, the formulation comprises about 4-10% (v/v) DMSO, about 2-3% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer. In some embodiments, the formulation comprises about 4-10% (v/v) DMSO, about 2-3% (w/v) albumin, about 0.08-0.10% (w/v) glucose,

and a buffer. In some embodiments, the formulation comprises about 4-6% (v/v) DMSO, about 2-3% (w/v) albumin, about 0.08-0.10% (w/v) glucose, and a buffer. In some embodiments, the formulation comprises about 5% (v/v) DMSO, about 2.5% (w/v) albumin, about 0.09% (w/v) glucose, and a buffer. In some embodiments, the formulation comprises about 5% (v/v) DMSO, about 2.5% (w/v) albumin, about 0.6% (w/v) glucose, and a buffer.

[527] In some embodiments, the formulation comprises about 4-10% (v/v) DMSO, about 2-8% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer. In some embodiments, the formulation comprises about 4-6% (v/v) DMSO, about 2-8% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer. In some embodiments, the formulation comprises about 5% (v/v) DMSO, about 2.5% (w/v) albumin, about 0.6% (w/v) glucose, and a buffer. In some embodiments, the formulation comprises about 5% (v/v) DMSO, about 7.5% (w/v) albumin, about 0.6% (w/v) glucose, and a buffer.

[528] In some embodiments, the formulations provided herein further comprise at least one further excipient. In some embodiments, the formulations further comprise taurine. In some embodiments, the formulations further comprise trehalose. In some embodiments, the formulations comprise a polymeric excipient. In some embodiments, the polymeric excipient is dextran. In some embodiments, the formulations do not comprise a polymeric excipient. In some embodiments, the formulations do not comprise dextran.

Cell Preparations

[529] Some aspects of this disclosure provide cell preparations comprising a population of cells or a tissue in a cryopreservative formulation as provided herein. In some embodiments, the cell preparation is cryopreserved. The cell preparation provided herein may be cryopreserved by being cryogenically frozen at any appropriate temperature known in the art. For example, in some embodiments, the cell preparations provided herein are cryopreserved at temperatures between -100 °C and -200 °C. In some embodiments the cell preparations provided herein are cryopreserved at temperatures below -125 °C. In some embodiments the cell preparations provided herein are cryopreserved at temperatures below -135 °C. In some embodiments the cell preparations provided herein are cryopreserved at temperatures below -150 °C.

[530] The cryopreservative formulations and cell preparations provided herein can be used in connection with photoreceptor rescue cells of the invention.

[531] In some embodiments, the cell preparation is suitable for transplantation into a subject once thawed and diluted with a diluent. In some embodiments, the diluent is GS2 or GS2 plus (see WO2017031312 incorporated by reference in its entirety, and **Table 5** below).

[532] GS2 medium, as used herein, refers to a medium for cell reconstitution, storage, transport, and/or administration to a subject. The medium is prepared as follows: 48.75 ml of 0.9% NaCl in water; 13.10 ml of Alcon Balanced Salt Solution (BSS®), 300 mOsm, in water; and 3.75 ml of 5%

dextrose in 0.9% NaCl (in water) (560 mOsm) were combined to obtain 65.6 mL medium with a final concentration of 0.29% dextrose and an osmolality of 315 mOsm.

[533] The basic GS2 medium thus comprises about 145 mM NaCl (about 0.85% NaCl), about 2 mM KCl (about 0.015% KCl), about 0.7 mM CaCl_2 (calcium chloride) (about 0.01% CaCl_2 dihydrate (calcium chloride dihydrate)), about 0.3 mM MgCl_2 (magnesium chloride) (about 0.006% MgCl_2 hexahydrate (magnesium chloride hexahydrate)), about 1 mM sodium citrate (about 0.035% sodium citrate dihydrate), and about 16 mM Glucose (about 0.29% dextrose), in water.

[534] The GS2 plus medium thus comprises about 145 mM NaCl (about 0.85% NaCl), about 2 mM KCl (about 0.015% KCl), about 0.7 mM CaCl_2 (calcium chloride) (about 0.01% CaCl_2 dihydrate (calcium chloride dihydrate)), about 0.3 mM MgCl_2 (magnesium chloride) (about 0.006% MgCl_2 hexahydrate (magnesium chloride hexahydrate)), about 1 mM sodium citrate (about 0.035% sodium citrate dihydrate), about 16 mM Glucose (about 0.29% dextrose), and about 3 mM of sodium phosphate monobasic dehydrate, in water.

[535] In some embodiments, the diluent is a solution comprising about 0.1 to about 1.2 mM CaCl_2 , about 0.05 to about 5 mM MgCl_2 , about 1 to about 2.5 mM KCl, about 0.5 to about 2 mM sodium citrate, about 14 to about 17 mM dextrose, and about 125 to about 175 mM NaCl. In some embodiments, the diluent is a solution comprising about 0.9 mM CaCl_2 , about 0.3 mM MgCl_2 , about 2 mM KCl, about 1.2 mM sodium citrate, about 15 mM dextrose, and about 145 mM NaCl. The diluent may further comprise sodium acetate. The diluent may further comprise a polymer such as hyaluronic acid or a solvate thereof such as sodium hyaluronate, optionally at a concentration of about 0.01 to about 0.05% (w/v), including about 0.05% (w/v). It may further comprise buffering components such as sodium phosphate dibasic heptahydrate and/or sodium phosphate monobasic monohydrate and/or sodium phosphate monobasic dihydrate.

[536] In some embodiments, the diluent is a solution comprising about 0.008% to about 0.012% CaCl_2 dihydrate, about 0.0048% to about 0.0072% MgCl_2 hexahydrate, about 0.012% to about 0.018% KCl, about 0.028% to about 0.042% sodium citrate dihydrate, about 0.23% to about 0.35% dextrose, and about 0.68% to about 1.02% NaCl. In some embodiments, the diluent is a solution that comprises about 0.01% CaCl_2 dihydrate, about 0.006% MgCl_2 hexahydrate, about 0.015% KCl, about 0.035% sodium citrate dihydrate, at least 0.25% dextrose, and about 0.85% NaCl. The diluent may further comprise sodium acetate. The diluent may further comprise a polymer such as hyaluronic acid or a solvate thereof such as sodium hyaluronate, optionally at a concentration of about 0.01 to about 0.05% (w/v), including about 0.05% (w/v). It may further comprise buffering components such as sodium phosphate dibasic heptahydrate and/or sodium phosphate monobasic monohydrate and/or sodium phosphate monobasic dihydrate.

[537] In some embodiments, the diluent comprises about 0.27% glucose, about 0.84% sodium chloride, about 0.016% potassium chloride, about 0.01% calcium chloride, about 0.006% magnesium chloride, about 0.036% sodium citrate, and optionally sodium acetate, (e.g., at about 0.08%),

optionally sodium hyaluronate (e.g., at about 0.049%), and optionally sodium phosphate dibasic heptahydrate (e.g., at about 0.0007%) and sodium phosphate monobasic monohydrate (e.g., at about 0.0001%). In some embodiments, the diluent comprises about 15 mM glucose, about 144 mM sodium chloride, about 2.1 mM potassium chloride, about 0.9 mM calcium chloride, about 0.3 mM magnesium chloride, about 1.2 mM sodium citrate, optionally sodium acetate (e.g., at about 6 mM), optionally sodium hyaluronate, and optionally sodium phosphate dibasic heptahydrate (e.g., at about 0.027 mM) and sodium phosphate monobasic monohydrate (e.g., at about 0.007 mM). Such diluents include GS2.

[538] In some embodiments, the diluent comprises about 0.27% glucose, about 0.84% sodium chloride, about 0.016% potassium chloride, about 0.01% calcium chloride, about 0.006% magnesium chloride, about 0.036% sodium citrate, and optionally sodium acetate (e.g., at about 0.08%), optionally sodium hyaluronate (e.g., at about 0.049%), and optionally sodium phosphate monobasic dihydrate (e.g., at about 0.047%). In some embodiments, the diluent comprises about 15 mM glucose, about 144 mM sodium chloride, about 2.1 mM potassium chloride, about 0.9 mM calcium chloride, about 0.3 mM magnesium chloride, about 1.2 mM sodium citrate, optionally sodium acetate (e.g., at about 6 mM), optionally sodium hyaluronate, and optionally sodium phosphate monobasic dihydrate (e.g., at about 3 mM). Such diluents include GS2 Plus.

[539] Optionally, the GS2 medium may further comprise a viscoelastic polymer in an amount effective to reduce shear stress on cells, e.g., at a final concentration of about 0.005-5% w/v. In some embodiments, the viscoelastic polymer is hyaluronic acid or a salt or solvate thereof.

[540] In a particular embodiment, the GS2 and GS2 plus mediums are as set forth in Table 5 below:

Table 5

Component	Percentage			MW	mM		
	GS2 (from IND)	GS2 from MBR	GS2 Plus		GS2 (from IND)	GS2 from MBR	GS2 Plus
Sodium hyaluronate	0.051	0.0490	0.0490	NA	NA	NA	NA
Dextrose hydrous	0.3			198.2	15.1	-	-
D(+)-Glucose anhydrous		0.2729	0.2729	180.16	-	15.1	15.1
Sodium chloride (NaCl)	0.8412	0.8414	0.8414	58.44	144	144	144
Potassium chloride (KCl)	0.01595	0.0159	0.0159	74.551	2.14	2.13	2.13
Calcium chloride dihydrate (CaCl ₂)	0.01021	0.0102	0.0102	110.98	0.92	0.918	0.918
Magnesium chloride, hexahydrate (MgCl ₂)	0.006374	0.0064	0.0064	203.3	0.314	0.313	0.313

Sodium acetate trihydrate	0.08296	0.0827	0.0827	136.08	6.1	6.08	6.08
Trisodium citrate dihydrate	0.03618	0.0361	0.0361	294.1	1.23	1.23	1.23
Sodium phosphate dibasic heptahydrate	0.000714	0.0007		268.07	0.0266	0.0256	-
Sodium phosphate monobasic monohydrate	0.0001	0.0001		137.99	0.0073	0.0071	-
Sodium phosphate monobasic dihydrate			0.0469	156.01	-		3

[541] In some embodiments, the diluent is a solution comprising (a) a buffer that maintains the solution at a physiological pH; and (b) at least 2 mM glucose; and (c) an osmotically active agent maintaining the solution at a physiological osmolarity. In some embodiments, the diluent is GS2 or GS2 plus. In some embodiments, the diluent is a solution comprising (a) a buffer that maintains the solution at a physiological pH; (b) at least 2 mM glucose; and (c) an osmotically active agent maintaining the solution at an osmolarity that is higher than physiological osmolarity, for example in the range of 350-450 mOsm/kg or an osmolality in the range of 1250 mOsm/kk –300 mOsm/kg. In some embodiments, the diluent further comprises divalent cations. In some embodiments, divalent cations comprises calcium and/or magnesium. In some embodiments, the diluent comprises calcium. In some embodiments, the diluent further comprises magnesium. In some embodiments, the diluent comprises an acetate buffer and/or a citrate buffer. In some embodiments, the diluent is a solution which combines two or more or any number of criteria (*e.g.*, pH, osmolarity, solutes (buffer, glucose, osmotically active agent, magnesium, calcium, potassium, polymer), concentrations, etc.). In some embodiments, the diluent is a solution comprising a buffering agent, glucose, and an osmotically active agent with or without added polymer, a solution comprising potassium and a solution not comprising potassium, as well as a solution comprising any combination of solutes at any concentration provided for the respective solute. It will also be understood that the disclosure embraces a solution comprising the listed solutes as well as a solution essentially consisting of or consisting of the listed solutes and a solvent, *e.g.*, water. These alternatives are not spelled out here for purposes of brevity.

[542] For example, in some embodiments, the diluent is a solution comprising or consisting essentially of calcium chloride, magnesium chloride, sodium citrate, sodium chloride, and glucose, *e.g.*, D-glucose, in water. In some embodiments, the diluent is a solution comprising or consisting essentially of calcium chloride, magnesium chloride, sodium citrate, sodium chloride, glucose, *e.g.*, D-glucose, and potassium chloride, in water. In some embodiments, the diluent is GS2 or GS2 plus, as described in WO2017/031312A1, the contents of which is incorporated by reference herein.

[543] In some embodiments, the diluent is a solution comprising about 0.1 to about 1.2 mM CaCl_2 , about 0.05 to about 5 mM MgCl_2 , about 1 to about 2.5 mM KCl, about 0.5 to about 2 mM sodium citrate, about 15 to about 17 mM dextrose, and about 125 to about 175 mM NaCl. The diluent may further comprise a polymer, and said polymer may be present at a concentration of about 0.01 to about 0.05% (w/v). The polymer may be hyaluronic acid or a solvate thereof such as sodium hyaluronate. In some embodiments, the diluent is a solution comprising about 0.9 mM CaCl_2 , about 0.3 mM MgCl_2 , about 2 mM KCl, about 1.2 mM sodium citrate, about 15 mM dextrose, and about 145 mM NaCl. The diluent may further comprise sodium acetate.

[544] In some embodiments, the diluent is a solution comprising about 0.008% to about 0.012% CaCl_2 dihydrate, about 0.0048% to about 0.0072% MgCl_2 hexahydrate, about 0.012% to about 0.018% KCl, about 0.028% to about 0.042% sodium citrate dihydrate, about 0.23% to about 0.35% dextrose, and about 0.68% to about 1.02% NaCl. In some embodiments, the diluent is a solution that comprises about 0.01% CaCl_2 dihydrate, about 0.006% MgCl_2 hexahydrate, about 0.015% KCl, about 0.035% sodium citrate dihydrate, at least 0.25% dextrose, and about 0.85% NaCl. The diluent may further comprise sodium acetate.

[545] In some embodiments, the cell preparations provided herein comprising photoreceptor rescue cells are formulated for administration to a subject, for example, for administration via injection, once thawed and diluted.

[546] Exemplary cell or tissue preparations, post-thaw may be formulated to be suitable for use in treating a human patient, *e.g.*, pyrogen-free or essentially pyrogen-free, pathogen-free, sterile, and at physiological pH and osmolarity. In some embodiments, the preparations provided herein are formulated for injection into a specific site, *e.g.*, in the case of ophthalmologic preparations for treating retinal diseases or disorders, into the vitreous humor for delivery to the site of retinal or choroidal damage, via subretinal delivery or via suprachoroidal delivery.

[547] Cell preparations provided by the present disclosure may include additionally therapeutic agents, for example, an immunosuppressant, a pro-angiogenic agent, or nutrients or growth factors supporting survival and/or implantation of the cells in the preparation.

[548] The volume and the number of cells in the cell preparations to be administered will depend on the specific application. Typically, for cell transplantation applications, it is desirable to reduce the volume administered as much as possible. Accordingly, the cell preparations may be formulated so that minimized volumes may be delivered. Cell concentrations for injection may be at any concentration that is effective and non-toxic. In some embodiments, the volume of the cell preparation to be administered is between 1 to 1000 μL . In some embodiments, the volume of the cell preparation to be administered is between 1 to 50 μL , or between 10 to 50 μL or between 25 to 50 μL or between 50 to 100 μL or between 50 to 200 μL or between 50 to 300 μL or between 50 to 400 μL or between 50 to 500 μL , or between 100 to 500 μL , or between 100 to 400 μL or between 100 to 300

μL, or between 100 to 300 μl or about 200 μL or about 150 μl. In some embodiments the volume of the cell preparation to be administered is about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 900, 950 or 1000 μl.

[549] In some embodiments, the number of cells and/or the concentration of cells in a cell preparation provided herein may be determined by counting viable cells and excluding non-viable cells. For example, non-viable cells may be detected by failure to exclude a vital dye (such as Trypan Blue), or using a functional assay (such as the ability to adhere to a culture substrate, phagocytosis, etc.). Additionally, the number of cells or the concentration of cells of a desired cell type may be determined by counting cells that express one or more cell markers characteristic of that cell type and/or excluding cells that express one or more markers indicative of a cell type other than the desired cell type. In some embodiments, the number of cells and/or the concentration of cells in a cell preparation may be determined by manual counting, ViCell Blu- 2 Program (counts live and dead cells separately) and/or ViCell Blu mammalian (default settings).

[550] In some embodiments, a cell preparation to be administered comprises about at least 1×10^4 , 2×10^4 , 3×10^4 , 4×10^4 , 5×10^4 , 6×10^4 , 7×10^4 , 8×10^4 , 9×10^4 , 1×10^5 , 2×10^5 , 3×10^5 , 4×10^5 , 5×10^5 , 6×10^5 , 7×10^5 , 8×10^5 , 9×10^5 , 1×10^6 , 2×10^6 , 3×10^6 , 4×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 2×10^7 , 3×10^7 , 4×10^7 , 5×10^7 , 6×10^7 , 7×10^7 , 8×10^7 , 9×10^7 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , 5×10^8 , 6×10^8 , 7×10^8 , 8×10^8 , 9×10^8 , 1×10^9 , 2×10^9 , 3×10^9 , 4×10^9 , 5×10^9 , 6×10^9 , 7×10^9 , 8×10^9 , 9×10^9 , 1×10^{10} , 2×10^{10} , 3×10^{10} , 4×10^{10} , 5×10^{10} , 6×10^{10} , 7×10^{10} , 8×10^{10} , or 9×10^{10} cells, or more cells.

[551] In some embodiments, the number of cells per vial is about 450,000 to 1,500,000 cells per vial prior to cryopreservation. In some embodiments, the number of cells per vial is about 1,750,000 to about 3,500,000 cells per vial prior to cryopreservation. In some embodiments, the number of cells per vial is about 450,000 to 1,500,000 cells per vial following cryopreservation, thawing and dilution. In some embodiments, the number of cells per vial is about 1,750,000 to about 3,500,000 cells per vial prior following cryopreservation, thawing and diluting.

[552] The afore-mentioned numbers of cells may be present in the cryopreserved cell preparation in a single cryovial. The cells may be present in about 10 to about 500 μL of cryoprotective formulation, including in about 10 to about 200 μL of cryoprotective formulation, or in about 10 to about 100 μL of cryoprotective formulation, or about 10 to about 50 μL of cryoprotective formulation, or about 20 to about 50 μL of cryoprotective formulation. In some embodiments, the population of photoreceptor rescue cells is suitable for transplantation into the eye of a subject. The cells so formulated may be generated by directed differentiation of pluripotent or multipotent stem cells, including human induced pluripotent stem cells (hiPSC), human embryonic stem cells (hESC) and somatic cells (including transdifferentiated cells and stem cells).

[553] In some embodiments, the cell preparations comprise a population of photoreceptor rescue cells in a cryopreservative formulation provided herein. Suitable photoreceptor rescue cells may be differentiated from pluripotent stem cells, such as human embryonic stem cells or iPS cells, and are molecularly distinct from embryonic stem cells, iPS cells, adult-derived photoreceptor rescue cells, and fetal-derived photoreceptor rescue cells. In some embodiments, adult-derived photoreceptor rescue cells, and fetal-derived photoreceptor rescue cells are used. Where ES cell derived photoreceptor rescue cells are used, the cell preparation, in some embodiments, does not comprise a detectable amount of residual ES cells, such that the cell preparations provided herein do not pose an unacceptable risk to a recipient of such preparation. Where iPS cell derived photoreceptor rescue cells are used, the cell preparation, in some embodiments, does not comprise a detectable amount of residual iPS cells, such that the cell preparations provided herein do not pose an unacceptable risk to a recipient of such preparation.

[554] In some embodiments, the cell preparation comprising a population of cells suitable for transplantation into the eye of a subject is suitable for injection into the eye of the subject. In some embodiments, such a cell preparation may be used for treating retinal degeneration diseases or disorders, including, but not limited to, retinal detachment, retinal dysplasia, Angioid streaks, Myopic Macular Degeneration, or retinal atrophy or associated with a number of vision-altering ailments that result in photoreceptor damage and blindness, such as, for example, choroideremia, diabetic retinopathy, macular degeneration (*e.g.*, age related macular degeneration), retinitis pigmentosa, and Stargardt's Disease (fundus flavimaculatus).

[555] The volume of a pharmaceutical composition provided by some embodiments of this disclosure depends on factors such as the mode of administration, number of cells to be delivered, age and weight of the patient, and type and severity of the disease being treated. For example, if administered by injection, the volume of a pharmaceutical composition of cells of the disclosure may be about 1-1000 μL . In some embodiments, the volume may be about 1-200 μL . For example, the volume of a composition of the disclosure may be about 10–50, 20–50, 25–50, or 1–200 μL . The volume of a composition of the disclosure may be about 10, 20, 30, 40, 50, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 μL , or higher.

[556] In some embodiments, the concentration of cells in the cryopreservative formulation (or the cryopreserved cell preparation) is about 10,000, 15,000, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 cells/ μL . In some embodiments, the concentration of cells in the cryopreservative formulation (or the cryopreserved cell preparation) is about 10,000-100,000 cells/ μL , 10,000-90,000 cells/ μL , 10,000-80,000 cells/ μL , 10,000-70,000 cells/ μL , 10,000-60,000 cells/ μL , 10,000-50,000 cells/ μL , 10,000-40,000 cells/ μL , 10,000-30,000 cells/ μL , 10,000-20,000 cells/ μL , 20,000-100,000 cells/ μL , 20,000-90,000 cells/ μL , 20,000-80,000 cells/ μL , 20,000-70,000 cells/ μL , 20,000-60,000 cells/ μL , 20,000-50,000 cells/ μL , 20,000-40,000 cells/ μL , 20,000-30,000 cells/ μL , 30,000-100,000 cells/ μL , 30,000-

90,000 cells/ μ L, 30,000-80,000 cells/ μ L, 30,000-70,000 cells/ μ L, 30,000-60,000 cells/ μ L, 30,000-50,000 cells/ μ L, 30,000-40,000 cells/ μ L, 40,000-100,000 cells/ μ L, 40,000-90,000 cells/ μ L, 40,000-80,000 cells/ μ L, 40,000-70,000 cells/ μ L, 40,000-60,000 cells/ μ L, 40,000-50,000 cells/ μ L, 50,000-100,000 cells/ μ L, 50,000-90,000 cells/ μ L, 50,000-80,000 cells/ μ L, 50,000-70,000 cells/ μ L, 50,000-60,000 cells/ μ L, 60,000-100,000 cells/ μ L, 60,000-90,000 cells/ μ L, 60,000-80,000 cells/ μ L, 60,000-70,000 cells/ μ L, 70,000-100,000 cells/ μ L, 70,000-90,000 cells/ μ L, 70,000-80,000 cells/ μ L, 80,000-100,000 cells/ μ L, 80,000-90,000 cells/ μ L, or 90,000-100,000 cells/ μ L. In some embodiments, the population of cells is at a concentration of about 300 cells – 10,000 cells/ μ L in the cell preparation. In some embodiments, the population of cells is at a concentration of about 3,000 cells – 5,000 cells/ μ L or 3,000 cells – 4,000 cells/ μ L in the cell preparation.

[557] In some embodiments, the pharmaceutical composition or the cell preparation is not diluted (e.g., not diluted prior to administration to a subject).

[558] In some embodiments, the preparation supports survival of the cells in the population of cells during storage of the preparation. In some embodiments, the preparation supports survival of the cells during storage for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 16 weeks, at least 20 weeks, at least 24 weeks, at least 28 weeks, at least 32 weeks, at least 36 weeks, at least 40 weeks, at least 44 weeks, at least 48 weeks, at least 1 year, at least 2 years, at least 3 years, at least 4 years at least 5 years or more.

[559] In some embodiments, at least 30% of the cells in the cell population are viable after about 1-10 years, about 1-9 years, about 1-8 years, about 1-7 years about 1-6 years, about 1-5 years, about 1-4 years, about 1-3 years or about 1-2 years or less of storage of the preparation at -100 to -200 °C, preferably at less than -135 °C. In some embodiments, at least 40% of the cells in the cell population are viable after about 1-10 years, about 1-9 years, about 1-8 years, about 1-7 years about 1-6 years, about 1-5 years, about 1-4 years, about 1-3 years or about 1-2 years or less of storage of the preparation at -100 to -200 °C, preferably at less than -135 °C. In some embodiments, at least 50% of the cells in the cell population are viable after about 1-10 years, about 1-9 years, about 1-8 years, about 1-7 years about 1-6 years, about 1-5 years, about 1-4 years, about 1-3 years or about 1-2 years or less of storage of the preparation at -100 to -200 °C, preferably at less than -135 °C. In some embodiments, at least 55% of the cells in the cell population are viable after about 1-10 years, about 1-9 years, about 1-8 years, about 1-7 years about 1-6 years, about 1-5 years, about 1-4 years, about 1-3 years or about 1-2 years or less of storage of the preparation at -100 to -200 °C, preferably at less than -135 °C. In some embodiments, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% or 100% of the cells in the cell population are viable after about 1-10 years, about 1-9 years, about 1-8 years, about 1-7 years about 1-6 years, about 1-5 years, about 1-4 years, about 1-3 years or about 1-2 years or less of storage of the preparation at -100 to -200 °C, preferably at less than -135 °C. In some embodiments,

the preparation supports maintenance of the plating efficiency of the population of cells during storage of the preparation. In some embodiments, after about 1-10 years, about 1-9 years, about 1-8 years, about 1-7 years, about 1-6 years, about 1-5 years, about 1-4 years, about 1-3 years or about 1-2 years or less of storage of the preparation at -100 to -200 °C, preferably at less than -135 °C, the population of cells exhibits at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% or 100% of its original plating efficiency, wherein the original plating efficiency refers to the plating efficiency of the population of cells at the beginning of the storage period. In some embodiments, the preparation is within a storage container. In some embodiments, the preparation is within a syringe or a cryovial.

[560] The cell preparations provided herein, post-thaw, are suitable for administration to a subject following minimal processing (*e.g.* dilution). In some embodiments, the preparation consists essentially of cells, a cell population, or a tissue and a cryopreservative formulation as provided herein. In some embodiments, the preparation comprises one or more pharmaceutically active ingredients, for example, a preservative, an antioxidant, a radical scavenger, an immunosuppressant, a pro-angiogenic factor, an anti-angiogenic factor, a growth hormone, or a cell nutrient or substrate supporting cell growth, survival, and implantation.

[561] The following are examples to illustrate the invention and should not be viewed as limiting the scope of the invention.

EXAMPLES

EXAMPLE 1: *In vitro* characterization of PRCs

[562] Cell identity and gene expression profiles for PRC cells, ESC, and retinal pigment epithelium (RPE) cells were determined by cell cluster analysis and quantification of selected neuronal genes.

Cell Cluster analysis of ESC/RPE/PRCs

[563] Cell Cluster analysis of ESC/RPE/PRCs was performed using single-cell suspensions processed according to manufacturer's instructions on a 10X Chromium Machine using a 10X Next GEM Chip G Single Cell Kit (10X Genomics #PN-1000120 or #PN-1000127) and a 10X Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit (10X Genomics #PN-1000121 or #PN-1000128) (**FIG. 1A**). The tagged cells were processed for library preparation according to kit instructions for Illumina Single Index Plate T Set A (Illumina #PN-2000240). Libraries were then shipped to Genewiz (now Azenta) for paired-end Illumina-based sequencing. Raw sequence data was mapped to the GRCh38 human genome using Cell Ranger-5.0.1. Mapped sequence data for scRNAseq was processed with Seurat and filtered for quality control metrics, run through dimensional

reduction for visualization. Cells were plotted in the first two UMAP dimensions and colored by sample. ESCs: Undifferentiated, research grade J1-ESCs and Kd-ESCs. RPE: J1-RPE at P3+4mos timepoint. PRC: J1-PRC (P4), Kd-PRC (P4), GB2R-PRC-2019 (P4), GB2R-PRC-PR1 (P4), GB2R-PRC-CN2 (P4), GB2R-PRC-CN3 (P4).

[564] PRC lots (e.g., PRC composition) include GB2R-PRC-2019 (or GB2R-2019), GB2R-PRC-CN1 (or GB2R-CN1), GB2R-PRC-CN2 (or GB2R-CN2), GB2R-PRC-CN3 (or GB2R-CN3), GB2R-PRC-PR1 (or GB2R-PR1), and GMP-MBC-GB2R.

[565] Expression of neuronal genes in ESC, RPE, and PRCs were quantified by qRNA validation (FIG. 1B). For RNA extraction and cDNA synthesis, wells were washed with PBS and 350ul of RLT buffer with 1:100 B-ME was added to the wells. Cells were disrupted by pipetting 20-30 times, samples were then transferred to 2.0 mL Eppendorf tubes and stored at -80°C until further use. RNA extraction was performed with QIAcube (Qiagen) and RNeasy Plus Mini Kit (Qiagen, Cat# 74134) using the manufacturer's recommendations. Measurement of RNA concentration was performed using Nanodrop 2000 (ThermoFisher, Cat# ND2000CLAPTOP). The Invitrogen™ SuperScript™ IV First-Strand Synthesis System cDNA synthesis with ezDNase™ Enzyme (ThermoFisher, Cat#11766500) was used according to the manufacturer's protocol. Briefly, 1 µg of total RNA was incubated for 2 minutes at 37°C with ezDNase buffer and ezDNase in GeneAmp® PCR system 2700. SuperScript™ IV VILO™ Master Mix with reverse transcriptase (RT) and water were then added according to the manufacturer's protocol. Samples were gently mix and incubated at 25°C for 10 minutes to anneal the primers, followed by a 10-minute incubation at 50°C to reverse transcribe the RNA, and an enzyme inactivation step at 85°C for 5 minutes. All reactions were performed in the GeneAmp® PCR system 2700. The RT reactions were diluted 1:20 and stored at -20°C until further use. PCR reactions- Individual taqman expression assays and a PCR custom plate, were used with Taqman fast advanced master mix (ThermoFisher, Cat#A44360), nuclease free water and 5ng of cDNA per reaction. The PCR reactions were run in Applied Biosystems™ Quant studio 7 Flex PCR machine (ThermoFisher, Cat#4485701) using a denaturation step of 95°C for 20 seconds following with 40 cycles with annealing at 95°C for 1 second and extension at 60°C for 20 seconds. See Table 6.

[566] Table 6 Assay ID for individual Taqman probes and the PCR custom plate.

Table 6

Individual Taqman probes	PCR custom plate
Gene	Gene
PAX6	STMN2
RAX	DCX
NES	MAP2
SOX2	C1ORF61
OCT4	LIN00461
LHX2	DLX2
SIX3	DLX5
SIX6	DLX1
RCVRN	C11ORF96

RHO	MART
OPN1MW	GAD2
OPN1SW	SCGN
ASCL1	ERBB4
CRX	CALB2
RORB	ELAVL4
OTX2	PRKCA
NR2E3	GAD1
NRL	NEUROD2
	NEUROD6
	TUJ1/TUBB3
	SLA
	SOX11
	NFIB
	NFIA
	EMX2
	OCT4
	BRN3A
	MATH5
	NANOG
	CHX10
	GAPDH
	C1ORF43

Bioinformatics analysis: Error propagation in qPCR validation of selected neuronal genes

[567] Relative qPCR expression of cell identity markers in GB2R PRCs as compared to their expression in hESCs was calculated as follows. Assuming that expression of the gene of interest (conditions of experiment, GB2R PRCs) defined as $TE = E(TE) + dTE$ and expression of the gene of interest (control conditions, hESCs) defined as $TC = E(TC) + dTC$, fold change between expression characterizing experiment and control conditions is given by the expression $FC = 2^{-ddCt + dFC}$, where $ddCt = E(TE) - E(TC) + dTE - dTC$. Base 10 logarithm of the fold change was then calculated revealing $\log_{10} FC \approx \log_{10} E(FC) + \ln 2 / \ln 10 (d(dCt) - d(dCtE))$. The relative expression to hESCs was thus defined as $\log_{10} E(FC)$ and the error bars for it – according to the prescription $s2\log FC \approx (\ln 2 / \ln 10)^2 (s2dCtC + s2dCtE - 2cov(dCtC, dCtE))$ where $s2dCtC$ is the squared standard deviation (across technical/biological replicates) for dCt for the gene of interest under conditions of experiment (GB2R PRCs) and $s2dCtE$ (across technical/biological replicates) is the squared standard deviation for dCt for the gene of interest under control conditions (hESCs).

Single cell analysis of PRCs-P4

[568] Single-cell suspensions of each sample were processed according to manufacturer's instructions on a 10X Chromium Machine using a 10X Next GEM Chip G Single Cell Kit (10X Genomics #PN-1000120 or #PN-1000127) and a 10X Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit (10X Genomics #PN-1000121 or #PN-1000128) (**FIG. 1C**). The tagged cells were processed for library preparation according to kit instructions for Illumina Single Index Plate T Set A (Illumina #PN-2000240). Libraries were then shipped to Genewiz (now Azenta) for paired-end Illumina-based sequencing. Raw sequence data was mapped to the GRCh38 human genome using

Cell Ranger-5.0.1. Mapped sequence data for scRNAseq was filtered for quality control metrics, run through dimensional reduction for visualization, and clustered using a shared nearest neighbor method with the Louvain algorithm. From there, differential expression was conducted on the clusters, and the most strongly differentially expressed genes were compared between the clusters and several published data sources. Numerous human datasets were used (see **Table 2**) to assign "best-guess" cell identities to the clusters based on the differentially expressed genes, and expression patterns during development and in the reviewed datasets. Cells were plotted in the first two UMAP dimensions, and colored by assigned cell type. Markers driving the "best-guess" assigned cell type are shown in **Table 8**.

[569] Mapped sequence data for scRNA-seq was filtered for quality control metrics, run through a dimensional reduction for visualization, and clustered using a shared nearest neighbor method with the Louvain algorithm. A differential expression analysis was conducted on the clusters and the most strongly differentially expressed genes (DEGs) were compared between the clusters and several published data sources. See **Table 7**.

Table 7: Reference data sets used for scRNAseq analysis

Cell type	Dataset used
Human retina	www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE142526
Human hippocampus	www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119212
Human midbrain	www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE76381
Human cortex	www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132672

Table 8: Markers driving cell identities in scRNAseq analysis

Cell type	Markers used to identify	Cell type range in PRCs
Whole PRC population	FOXG1, MAP2	86.7%-92.1% double positive range (98.5% is single positive for Map2)
Inhibitory neurons	DLX5, TUBB3, SCGN, ERBB4, CALB2	38.9%-48.6%
Excitatory neurons	NEUROD2, NEUROD6, SLA, NELL2, SATB2	0.7%-8.2%
Mixed neurons	MEIS2, PBX3, GRIA2, CACNA1C	17.9%-36.1%
progenitors	VIM, MKI67, CLU, GLI3	20.8%-34.1%

astrocytes	GFAP, LUCAT1, MIR99AHG, FBXL7	0.9%-1.4%
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qPCR Identity Assays

[570] Cells were collected in RNAprotect (Qiagen) following the manufacturer's instructions to perform qRNA identity assays (**FIG. 1D**). RNA was extracted using RNeasy Plus Mini kit (Qiagen, cat. 74134), and then RNA concentration was determined using NanoDrop (Thermo Fisher). The protocol for SuperScript IV VILO (Thermo cat. 11766050) was performed to make cDNA at a concentration of 1 ng/ul in nuclease free water. RNA expression for the candidate genes was tested by qPCR using TaqMan probes (Thermo Fisher). 1 ng of total cDNA per qPCR reaction well was used. Final reaction mixture per well consisted of cDNA, Fast Advanced Master Mix (Thermo Fisher), nuclease free water, and the respective TaqMan probe. The total reaction volume per well was 12ul. Comparative Ct program on Quant Studio 7 flex instrument was used to run the qPCR. qPCR cycle parameters were as following: "Fast" with a hold stage at 95°C for (00.20), 40 cycles of PCR stage (step 1) at 95°C for (00.01) and a PCR stage (step 2) at 60°C for (00.20). GAPDH was used as reference gene. mRNA expression values for the target genes normalized to reference gene were obtained by calculating ΔCt (Ct value for target gene – Ct value of reference gene), followed by derivation of $2^{-\Delta Ct}$ value.

[571] **Tables 9-12** shows the cell types identified in PRC preparations, the marker used to identify said cell type, and the expression of each marker. **FIG. 1E** shows expression of eye field progenitor markers, rod/cone photoreceptor markers, and neuronal markers in inhibitory neurons, excitatory neurons, alternative neurons, progenitors and astrocytes as shown in **FIG. 1C**. **Table 9** shows cell markers categorized by cell type markers and their expression throughout the PRC composition manufacturing protocol as disclosed herein. **Table 10** shows single cell-sequencing results providing the percentage of single cells in a PRC composition as disclosed herein that express a cell marker. **Table 11** shows transcripts per million (TPM) for bulk RNA-seq data for PRC compositions as disclosed herein expressing a cell marker. Expression of cell markers from day 2 (D2), day 12 (D12), day 19 (D19), day 37 (D37/P0), day 55 (D55/P1), day 72 (D72/P2), day 90 (D90/P3) and day 107 (D107/P4) from PRC compositions listed in **Table 10** are shown in **FIGs. 1F-1T**. **Table 12** shows cells that have been grouped based on expression of astrocytes, excitatory neurons, inhibitory neurons, alternative neurons, or progenitor markers and the percentage of those cells that express a particular marker as determined by single cell-sequencing.

Table 9

Cell type markers	Marker used to identify as said cell type	Expression
Eye field progenitor markers	Pax6	10,000x increase over hESC by RNAseq at d19-21; 85%+ by flow at d19-21; High expression in sc-qPCR at p4 vs no expression in J1 ESC, 60x increase vs GMP1 iPSCs in bulk qPCR at p4
	Rx1	trace/absent at p4 via sc and bulk qPCR similar to J1 hESCs and GMP1 iPSCs; Not assessed at protein level at d19-21 (only 14.8 TPM via RNAseq)
	Lhx2	89% by flow at d19-21; highly expressed in sc and bulk PCR in p4 J1 PRC and GMP1 PRC
	Six3 at d19-21	149.2 transcripts per million (TPM) RNAseq
	Six6 at d19-21	Trace/absent at gene level (qPCR/RNAseq)
	Tbx3 at d19-21	Trace/absent at gene level (qPCR/RNAseq)
	Nestin at d19-21	3.39X more than hESC via PCR at d19-21
	Sox2 at d19-21	Protein level: 78% by flow at d19-21 and 33% by flow at p3; Gene level, 1.87X more than hESC via PCR at d19-21
Rod/cone photoreceptor markers	MASH1	High expression vs hESC in scRNAseq at p4 and 12x increase vs GMP1-iPSC at p4, 364 TPM via RNAseq at p3 (no comparison to ESC)
	CRX	Absent, similar to J1 hESC/GMP1 iPSC by sc and bulk qPCR at p4; absent at p3 via RNAseq
	RORb	sc-qPCR: some detection in few cells, but less expression than J1 ESC; bulk qPCR: similar vs. GMP1-iPSC; very low via ICC at p3
	TRb2	Low at 14.9 transcripts per million by RNAseq (no hESC comparison) at p3
	NR2E3	Absent in sc and bulk qPCR, similar to J1 ESC and GMP1-iPSC
	NRL	Absent in sc and bulk qPCR, similar to J1 ESC and GMP1-iPSC
	rhodopsin	ICC at p4: No expression above background when using the 4D2 antibody. ICC at p4: When using the 1D4 antibody, signal is detected, but also detected in neural stem cells and GRC, so signal is non-specific staining (not actually rhodopsin). No expression <i>in vivo</i>
	Opsin(s)	sc-qPCR: Opn1mw - Comparable to J1 ESC, moderate signal detected; bulk qPCR: opn1mw - 10x increase vs. GMP1-iPSC
	PDE6B	sc-qPCR: minor population, less expression than J1 ESC; bulk qPCR: 40% of GMP1-iPSC at p4

	recoverin	Absent via RNAseq at p3; absent at p4 via sc and bulk qPCR
	Cone arrestin (ARR3)	sc-qPCR: Absent, similar to J1 ESC bulk qPCR: Absent, vs. GMP1-iPSC
	Cngb1	sc-qPCR: Absent, similar to J1 ESC bulk qPCR: Absent, vs. GMP1-iPSC
	Gnat1	sc-qPCR: Absent, similar to J1 ESC bulk qPCR: Absent, vs. GMP1-iPSC
	Gnat2	sc-qPCR: Absent, similar to J1 ESC bulk qPCR: Absent, vs. GMP1-iPSC
Neuronal markers	Tuj1 (mature)	At p0, 61x increase over hESC. At p4, sc-qPCR shows high expression, slightly more than the already high expression of J1 ESC; bulk qPCR: ~5x increase vs. GMP1-iPSC
	NFIA	60% by ICC at p4; sc-qPCR: High expression vs J1 ESC bulk qPCR: ~215x increase, vs. GMP1-iPSC
	DCX	sc-qPCR: High expression vs J1 ESC at p3 bulk qPCR: ~535x increase, vs. GMP1-iPSC at p3
	NFIB	sc-qPCR: High expression vs J1 ESC at p3 bulk qPCR: ~190x increase, vs. GMP1-iPSC at p3
	OTX2	86% by flow at p4; sc-qPCR: Downregulated vs. J1 ESC; bulk qPCR: Absent, vs. GMP1-iPSC; lower than in hESCs at 30.9 TPM in RNAseq
Retinal neuronal progenitor markers	CHX10	Only 2.1 transcripts per million (TPM) at p0 which is 73x more than hESC at p0. trace at p3 via RNAseq even though it is still 73X more than hESCs; earlier data set showed trace/absent at p4 (sc and bulk qPCR). ICC data at d19-21 shows trace/absent at the protein level
Pluripotent stem cell markers	SSEA4	Negative
	Oct4	Negative
PRC identity markers	MAP2, FoxG1	88% by flow cytometry for MAP2; 95% by flow for FoxG1. MAP2 by bulk qPCR 100x increase over iPSC
Other neuronal markers	Elavl3	sc-qPCR: High expression vs J1 ESC; bulk qPCR: huge increase vs. GMP1-iPSC
	Elavl4	sc-qPCR: High expression vs J1 ESC; bulk qPCR: huge increase vs. GMP1-iPSC
	Slc1a2	sc-qPCR: High expression vs J1 ESC; bulk qPCR: huge increase vs. GMP1-iPSC
	Slc1a3	sc-qPCR: High expression, similar to high expression of J1 ESC bulk qPCR: ~7x increase vs. GMP1-iPSC
	Hcn1	sc-qPCR: Absent, similar to J1 ESC; bulk qPCR: Absent, vs. GMP1-iPSC

	Hes5	sc-qPCR: increased expression vs J1 ESC bulk qPCR: ~180x increase, vs. GMP1-iPSC
Functional markers of neuronal electrophysiologic activity	Voltage-gated sodium current (Nav)	Manual Patch Clamp: sub-population express moderate amount. (photoreceptors don't express Nav)
	M-type potassium current	Manual Patch Clamp: Cells in PRC population that have Nav also have larger M-current
	A-type potassium current	Manual Patch Clamp: Expression enriched in population expressing Nav
	Hyperpolarization-induced current (Ih)	Manual Patch Clamp: not observed (photoreceptors have Ih, mediated by HCN1 channels)

Table 10: Percentage of cells expressing cell markers

Type of marker	Gene	GB2R-2019	GB2R-CN2	GB2R-CN3	GB2R-PR1	Mean
PRC marker	FOXP1	78.8	57.4	72.6	81.3	72.525
	MAP2	94.8	77.8	89.9	92.3	88.7
	STMN2	70.1	72	81.6	82.7	76.6
	DCX	88.3	75.9	85.9	88	84.525
	LINC00461	93.1	75.6	87.1	91.1	86.725
	NEUROD2	2	3.1	3	18.9	6.75
	GAD1	61.4	44.1	64.4	44.4	53.575
Inhibitory neuron marker	NFIA	69.4	69.1	71.1	85.8	73.85
	DLX5	68.7	54.9	65.6	61.1	62.575
	TUBB3	92.4	78.3	86	90	86.675
	SCGN	61.1	49.5	50.3	54.7	53.9
	ERBB4	67.8	66.9	71	63.9	67.4
Excitatory neuron marker	CALB2	62	51.4	42.7	54.2	52.575
	NEUROD2	2	3.1	3	18.9	6.75
	NEUROD6	0.8	4.2	2.4	23.4	7.7
	SLA	1.2	2.9	1.9	16.2	5.55
	NELL2	35.2	20.6	27.2	30.1	28.275
Progenitor marker	SATB2	3.3	4	3	11.3	5.4
	VIM	68.4	46.3	43.5	59.9	54.525
	MKI67	11.7	7.3	7	8.5	8.625
	CLU	50.3	31.3	26.5	46.5	38.65
Astrocyte marker	GLI3	21.9	15.3	16.9	28.3	20.6
	GFAP	13.1	3.3	1.7	3.2	5.325
	LUCAT1	12.8	7.6	10	14.7	11.275
	MIR99AHG	87.8	75.8	81.2	87.6	83.1
Alternative neuron marker	FBXL7	54.6	38.6	34.9	45.5	43.4
	MEIS2	81.6	50.4	77.6	62.2	67.95
	PBX3	70.3	49.7	74.5	48.1	60.65
	GRIA2	31.3	23.1	46.3	39.7	35.1

	CACNA1C	53	39.4	59.5	54.2	51.525
Eye field progenitor markers	PAX6	50.6	30.5	41.6	46.6	42.325
	LHX2	9	7.2	7	24.6	11.95
	SIX3	6.6	2.2	10	2.8	5.4
	NES	32.9	19.8	30.2	30.4	28.325
	SOX2	78	60.8	72.8	70.4	70.5
Rod/cone photoreceptor markers	ASCL1	26.9	29.4	29.7	41.3	31.825
	RORB	12.4	11.8	20.4	22	16.65
	NR2E3	2.9	2.1	3.7	3	2.925
	NRL	6.6	2.9	8	5.8	5.825
Neuron markers	TUBB3	92.4	78.3	86	90	86.675
	NFIA	69.4	69.1	71.1	85.8	73.85
	NFIB	94.9	83.4	89.6	94.4	90.575
	OTX2	5.7	2.2	2.8	4.1	3.7
	ELAVL3	84.8	60.9	76.3	82.5	76.125
	ELAVL4	51	51.8	60.4	71.4	58.65
	SLC1A2	71	51.9	66	72.9	65.45
	SLC1A3	17.7	11.8	11.2	23.1	15.95
	HCN1	1.5	3.3	3.9	4	3.175
	HES5	19.4	13.7	10.5	25	17.15
Negative markers	CRX	0.3	0.1	0.3	0.3	0.25
	RHO	0	0	0	0	0
	OPN1SW	1.1	0.7	1.2	1.8	1.2
	PDE6B	1	1.7	1.7	3.3	1.925
	RCVRN	0.5	0.2	0.2	0.3	0.3
	ARR3	0.8	0.9	0.9	1	0.9
	CNGB1	0.3	0.1	0.2	0.5	0.275
	GNAT1	0	0	0.3	0.1	0.1
	GNAT2	0.2	0.1	0.1	0.2	0.15
	VSX2	0.2	0	0.2	0.3	0.175
	POU5F1	0.2	0.3	0.4	0.5	0.35
	OCT4	#N/A	#N/A	#N/A	#N/A	
	SSEA4	#N/A	#N/A	#N/A	#N/A	
	RAX	0.1	0.1	0	0.1	0.075
	SIX6	0	0	0	0	0
	TBX3	0.1	0	0.2	0.3	0.15

Table 11: RNA Bulk Seq (transcript per million (TPM))

Type of marker	Gene	GB2R-2019	GB2R-CN1	GB2R-CN2	GB2R-CN3	GB2R-PR1	Mean
PRC marker	FOXG1	131.915	59.99	149.915	161.895	169.505	134.644
	MAP2	509.965	290.29	494.075	611.475	561.91	493.543
	STMN2	223.885	193.79	464.255	555.88	544.98	396.558
	DCX	485.58	259.43	781.595	847.215	701.575	615.079

	LINC00461	89.065	53.43	92.27	88.665	85.38	81.762
	NEUROD2	0.555	0.03	1.105	0.465	8.19	2.069
	GAD1	16.895	12.45	27.21	40.525	16.495	22.715
	NFIA	116.92	33.56	79.41	64.925	86.905	76.344
Inhibitory neuron marker	DLX5	88.92	50.78	137.29	131.595	82.535	98.224
	TUBB3	307.12	162.86	428.605	378.475	375.25	330.462
	SCGN	122.955	74.94	172.31	120.075	103.74	118.804
	ERBB4	36.235	18.24	93.655	73.075	57.945	55.83
	CALB2	89.615	53.85	196.435	76.895	94.915	102.342
Excitatory neuron marker	NEUROD2	0.555	0.03	1.105	0.465	8.19	2.069
	NEUROD6	5.45	1.2	19.85	12.525	203.175	48.44
	SLA	4.39	0.35	5.425	3.78	50.21	12.831
	NELL2	27.21	4.86	27.59	25.695	128.885	42.848
	SATB2	2.49	0.73	2.445	2.85	20.405	5.784
Progenitor marker	VIM	861.375	262.82	320.97	270.5	294.65	402.063
	MKI67	33.66	11.89	19.225	21.155	21.825	21.551
	CLU	365.23	46.78	101.825	63.205	89.87	133.382
	GLI3	43.93	11.95	23.48	23.345	37.635	28.068
Astrocyte marker	GFAP	122.39	0.48	14.305	3.93	16.89	31.599
	LUCAT1	N/A	N/A	N/A	N/A	N/A	N/A
	MIR99AHG	23.81	6.68	12.76	10.535	16.065	13.97
	FBXL7	23.785	7.72	13.91	11.94	14.385	14.348
Alternative neuron marker	MEIS2	138.325	15.28	61.52	173.24	67.2	91.113
	PBX3	45.6	5.49	29.995	88.11	12.635	36.366
	GRIA2	19.73	4.48	11.35	29.025	27.295	18.376
	CACNA1C	4.605	1.14	3.665	6.68	3.57	3.932
Eye field progenitor markers	PAX6	99.025	36.67	77.07	73.885	87.035	74.737
	LHX2	15.465	8.54	10.81	9.78	35.71	16.061
	SIX3	10.17	2.48	5.865	19.94	3.85	8.461
	NES	26.805	7.39	12.94	11.805	10.9	13.968
	SOX2	196.375	96.53	173.06	156.77	139.38	152.423
Rod/cone photoreceptor markers	ASCL1	111.61	68.31	127.745	98.52	122.375	105.712
	RORB	2.675	3.03	5.005	6.125	5.28	4.423
	NR2E3	0.415	0.17	0.35	0.395	0.505	0.367
	NRL	0.715	0.28	0.585	1.36	0.545	0.697
Neuron markers	TUBB3	307.12	162.86	428.605	378.475	375.25	330.462
	NFIA	116.92	33.56	79.41	64.925	86.905	76.344
	NFIB	486.145	187.76	473.24	405.535	605.53	431.642
	OTX2	22.915	8.13	14	13.415	16.94	15.08
	ELAVL3	60.595	22.61	74.855	78.615	79.42	63.219
	ELAVL4	38.49	28.91	80.165	84.065	83.235	62.973
	SLC1A2	87.305	22.2	59.16	62.325	68.415	59.881
	SLC1A3	176.995	108.35	73.53	78.325	79.625	103.365
	HCN1	0.13	0.14	0.49	0.345	0.54	0.329
	HES5	25.83	15.38	37.59	18.065	45.155	28.404

Negative markers	CRX	0.25	0	0.075	0	0.025	0.07
	RHO	0	0.02	0.015	0.025	0	0.012
	OPN1SW	0.05	0	0.085	0.065	0.17	0.074
	PDE6B	0.045	0.07	0.165	0.155	0.155	0.118
	RCVRN	0.1	0	0.085	0.02	0	0.041
	ARR3	0.16	0	0	0	0	0.032
	CNGB1	0.025	0.03	0.04	0.015	0.04	0.03
	GNAT1	0.02	0	0	0.02	0.09	0.026
	GNAT2	0.38	0	0	0.055	0	0.087
	VSX2	0.105	0.02	0.065	0.015	0.185	0.078
	POU5F1	1.41	5.42	2.42	3.545	3.93	3.345
	OCT4	#N/A	#N/A	#N/A	#N/A	#N/A	
	SSEA4	#N/A	#N/A	#N/A	#N/A	#N/A	
	RAX	0.145	0.02	0.09	0.1	0.255	0.122
	SIX6	0.2	0.02	0.045	0.015	0.035	0.063
	TBX3	0.075	0.04	0.02	0.09	0.09	0.063

Table 12: Percent of cells with marker expression > 0

Markers	Astrocyte	Excitatory Neuron	Inhibitory Neuron	Alternative Neuron	Progenitor
ARR3	1.7	0.2	1.1	1.0	0.4
ASCL1	12.1	20.2	42.4	9.6	41.9
CNGB1	0.0	1.1	0.2	0.2	0.4
CRX	06	0.5	0.3	0.2	0.3
GNAT1	0.0	0.4	0.1	0.1	0.1
GNAT 2	0.6	0.0	0.1	0.2	0.1
LHX2	48.6	56.8	2.7	2.9	29.3
NES	32.4	8.1	32.6	16.6	41.0
NR2E3	6.4	2.3	3.9	2.7	1.9
NRL	7.5	4.6	4.9	10.0	4.2
OPN1SW	4.6	0.5	1.1	1.0	1.6
PAX6	56.6	13.0	46.5	41.8	45.2
PDE6B	4.0	3.0	2.0	1.5	1.8
RAX	0.0	0.0	0.0	0.0	0.1
RCVRN	0.0	0.0	0.5	0.0	0.4
RHO	0.0	0.0	0.0	0.0	0.0
RORB	60.1	24.1	10.7	15.0	26.8
SIX3	9.2	0.4	1.6	11.7	7.1

SIX6	0.0	0.0	0.0	0.0	0.1
SOX2	66.5	12.8	88.5	62.5	63.7
TBX3	0.6	0.2	0.2	0.2	0.1
DCX	37.6	81.7	98.7	92.9	58.3
ELAVL3	61.3	70.8	92.1	82.0	51.4
ELAVL4	17.3	60.3	84.5	46.6	32.8
FOXG1	80.9	69.1	90.4	68.1	54.4
HCN1	1.2	0.9	5.7	1.8	0.8
HESS	42.2	12.8	10.1	6.1	41.3
MAP2	93.6	86.3	97.8	95.5	71.1
NFIA	100.0	71.2	86.5	51.8	77.5
NFIB	98.8	90.7	98.5	91.2	79.5
OTX2	30.6	1.8	0.5	0.4	12.2
POU5F1	1.2	0.2	0.2	0.4	0.5
SLC1A2	75.1	65.9	76.6	68.2	49.2
SLC1A3	96.5	35.0	3.8	4.6	43.0
SSEA4	NA	NA	NA	NA	NA
TUBB3	66.5	84.0	96.7	88.7	73.0
VSX2	0.0	0.2	0.1	0.3	0.2

Brightfield images of PRCs

[572] Brightfield images were taken with a Keyence microscope (BZ-X710) at 20x, 10x, and 4x magnification at different days of PRC differentiation. Image shown is a representative image taken of GB2R derived PRCs at P4 (d107) (**FIG. 2A**).

Scorecard analysis of PRC differentiation

[573] For Scorecard analysis, cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Thermofisher, Cat#4368813) according to the manufacturer's protocol (**FIG. 2B**). TaqMan hPSC Scorecard Assay (Thermofisher, Cat#A15870) was used with Taqman standard master mix (Thermofisher, Cat# 4369016) following the manufacturer's protocol. The PCR reactions were run in Applied Biosystems™ Quant studio 7 Flex using the manufacturer's recommended cycle parameters. Quantitative analysis of trilineage potential of PRC was performed using hPSC Scorecard analysis software (Thermofisher).

ICC expression in PRCs-P4

[574] Immunocytochemistry (ICC) staining of PRCs at P4 was performed using Ibidi μ -Plate 24 Well Black (Ibidi, Cat# 82406) (**FIG. 2C**). Cells were washed 3 times with PBS $-/-$, fixed with 4%

PFA and washed 3X with PBS-/. After removing PBS, cells were treated with permeabilization solution with Triton-X 0.2% for 10 minutes. Fixed cells were washed with PBS (with Calcium and Magnesium followed by blocking at room temperature for 1 hour with 5% Goat serum (+/- 5% donkey serum) in PBS with calcium and magnesium. Primary antibodies (Pax6 (Millipore ab#2237), Otx2 (Thermo, #MA5-15854), STMNN2 (ProteinTech cat. 10586-1-AP), CALB2 (Sigma cat. MAB51568), SCGN (Sigma cat. HPA006641), and DCX (Santa Cruz cat. SC-271390) were diluted in blocking solution and incubated at 4°C overnight. Samples were washed using PBS with 0.05% Tween (PBST) for 3 times for 5-10 minutes. Secondary antibodies were diluted in blocking solution and incubated for 1 hour at RT. Samples were washed afterwards with PBST for 15 minutes followed by two 5-minute washes in PBS with calcium and magnesium. ProlongGold with DAPI (Thermofisher, Cat#P36931) was added to the samples (or DAPI was previously mixed directly with secondary antibody). A Leica DMi8 microscope with LAS X software was used for imaging.

PRCs markers

[575] PRCs markers of success were identified analyzing the transcriptomes of PRCs during different stages. Transcriptomes of GB2R PRCs during different stages of their protocol were combined into a single dataset, and transcription factors were focused on as the main drivers of GB2R PRC differentiation and maturation process. PCA-based dimensionality reduction techniques were applied to it, and leading principal components identified revealing the trajectory of differentiation and maturation of GB2R PRCs in the latent space of principal components. A linear model describing changes of gene expressions along the maturation trajectory was constructed and transcription factors with expressions systematically and monotonously changing along the differentiation trajectory were identified. Transcription factors most differentially expressed along the trajectory of differentiation/maturation of PRC GB2Rs were identified as the PRC markers of success. **FIG. 2D** shows an increase of expression of NEUROD2 at P1, while FOXG1 and HMGA1 stayed mostly the same as P0.

[576] Flow cytometry was performed to determine percentage of cells expressing FOXG1, MAP2 and not expressing SSEA4 (**FIG. 2E**). ESC and PRC-P4 cells were thawed in a 15 ml tube and the cell density was adjusted to $1 \times 10^6/\text{ml}$ and processed as described below. Percentage of cells expression purity markers: Mean % (GB2R-PRC-CN3 FCP, N = 3). FOXG1: 95.6 (94.2-96.7); MAP2: 88.4 (84.7-91.4); and SSEA4: 0.44 (0.15-0.88).

[577] Bulk RNA-seq analysis for PRC-P3 and PRC-P4 were performed (**FIG. 2F**). 19 genes were identified to be differentially expressed between PRC-P3 and PRC-P4. AGT, ACBLN2, CDH7, DNAH11, and EGR1 had increased expression in P4 compared to P3. FAM216B, FOS, KCNC2, LGI2, LOC221946, LRRC4C, MAP3k19, OLFM3, PRND, PTGER3, RELN, TCERGIL, TSHR, and UNC13C had decreased expression in P4 compared to P3.

[578] *Live/dead cell staining:* Cells are centrifuged at 300g at room temperature for 5 min, and following aspiration 1 ml PBS containing 1:10000 diluted Fixable Red Dead Cell Stain Kit is added and the cells are incubated at 4°C for 30 min. 5 ml PBS is added and the sample is centrifuged at 300g at room temperature for 5 min. The sample is aspirated and the cells are resuspended in HBSS (Thermo Fisher Scientific, cat# 14025092) + 2% FBS to 3×10^6 /ml.

[579] *SSEA4 staining:* 100 μ l (3×10^5 cells) of above cell suspension are transferred to a “U” bottom 96 well plate. 1 μ l anti-SSEA4-FITC antibody is added (Miltenyi Biotech, 130-122-918) per well, mixed well and the cells are incubated at 4°C for 30 min. The cells are washed twice: 1st wash with 200 μ l HBSS + 2% FBS per well, followed by centrifuging at 2000 rpm at 4°C for 1 min, 2nd wash with 200 μ l PBS per well followed by centrifuging at 2000 rpm at 4°C for 1 min.

[580] *Fixation and permeabilization:* 100 μ l Fix/Perm buffer (Transcription Factor Buffer Set, BD Bioscience, cat# 562574) is added per well, mixed well, and the cells are incubated at 4°C for 30 min. The cells are washed twice: each wash with 200 μ l Fix/Perm buffer per well followed by centrifuging at 2000 rpm for 1 min at 4°C.

[581] *FOXG1/MAP2 staining:* Cells are resuspended with 50 μ l Fix/Perm buffer per well, 50 μ l 2 μ g/ml anti-FOXG1 (Abcam, cat# ab196868) or 2 μ g/ml anti-MAP2 (Cell Signaling, cat# 4542S) antibody is added per well, the cells are mixed well and incubated at 4°C for 30min. The cells are washed twice: each wash with 200 μ l Fix/Perm buffer per well followed by centrifuging at 2000rpm at 4°C for 1 min. 100 μ l Fix/Perm buffer containing 1:250 diluted anti-rabbit-IgG-PE antibody (Thermo Fisher Scientific, cat# A10542) is added per well, the cells are mixed well and incubated at 4°C for 30min; the cells are washed twice, each wash with 200 μ l Fix/Perm buffer per well followed by centrifuging at 2000rpm at 4°C for 1min. Flow cytometric analysis by Sony Cell Analyzer SA3800 is performed. Cells are resuspended with 200 μ l HBSS+2% FBS per well, mixed well and samples are analyzed with a Sony Cell Analyzer SA3800. 10,000 gated events per well are acquired and the data was analyzed with FlowJo software (ver. 10.8.1). Positive signal against the IgG control in each cell type is confirmed. The positive percentage in PRC-P4 for the population with higher signal intensity compared with ESC is calculated.

Stimulation of Neuroprotective Factors

[582] PRC cells were stimulated by oxidative stress by using tert-butyl hydroperoxide (TBHP) and secreted factors were detected using a Luminex assay (**FIG. 3A**). NRF2 mediated oxidative stress response pathway was activated ex vivo and *in vivo* RCS rat retina treated with hNPCs as shown by Jones et al., 2019, Proteomics, 19, e1800213.

[583] PRC-P4 cells were thawed on compliant matrix (PDL, Fibronectin and Laminin-521) at a density of 220K-250K cells per well of 24W plate (surface area of well – 1.9cm²). On the following day (D1) TBHP dilutions (10 μ M to 50 μ M) in the PRC media were prepared. TBHP solution is very concentrated (approx. 5.1M or 5100mM, refer to lot number for exact concentration), and 1:10 serial dilutions were used to get down to a targeted concentration. Final TBHP dilutions were done in the

PRC media. For vehicle control –decane solution was diluted in PRC media following the TBHP dilution steps up to 50uM TBHP dilution. Cells were treated with TBHP for 2 days and at D3 post seeding, the conditioned media from the wells was collected. The conditioned media was spun at 4000g for 10 minutes to get rid of debris. Conditioned media was aliquoted and stored at -80°C for Luminex/ELISA. The manufacturer's instructions for Luminex assay (Life technologies Procartaplex 6 Plex Luminex assay, Assay ID: MX323GF, Species: Human, Targets (Neuro): CNTF, GFAP, MIF, NCAM-1, S100B, Tau (Total)) were followed. Millipore Milliplex Analyzer with Luminex MAP technology was used to obtain Mean fluorescent intensity (MFI) for each sample and standards. MFI for the test samples was used for comparing the secretion levels for each cytokine in the test samples. To calculate the concentrations, standard curve was plotted for known concentrations against the readout (MFI) for each concentration. Sigmoidal 4PL (4 parameter logistic) method on GraphPad Prism was used for curve fitting and data analysis. Concentrations of the test samples were extrapolated on the standard curve.

[584] Results were compared to secreted factors from PRC cells without oxidative stress (**FIG. 3B**). PRC-P4 cells were thawed and seeded on a compliant matrix (PDL, Fibronectin and Laminin-521) at a density of 120K cells per well of 24W plate (surface area of well – 1.9cm²). PRC were fed with fresh media a day after seeding. Conditioned media was collected after 2 days of feeding or 3 days after seeding. The conditioned media was spun at 4000g for 10 minutes to get rid of debris. Conditioned media was aliquoted and stored at -80°C for Luminex assay. One to two wells of cells were counted using accutase. The cell count at the collection step was recorded. The manufacturer's instructions for Luminex assay (Life technologies Procartaplex 6 Plex Luminex assay, Assay ID: MX323GF, Species: Human, Targets (Neuro): CNTF, GFAP, MIF, NCAM-1, S100B, Tau (Total)) were followed. Millipore Milliplex Analyzer with Luminex MAP technology was used to obtain Mean fluorescent intensity (MFI) for each sample and standards. MFI for the test samples was used for comparing the secretion levels for each cytokine in the test samples. To calculate the concentrations, standard curve was plotted for known concentrations against the readout (MFI) for each concentration. Sigmoidal 4PL (4 parameter logistic) method on GraphPad Prism was used for curve fitting and data analysis. The concentrations of the test samples was extrapolated on the standard curve.

[585] Subretinal engraftment of PRCs suppress microglial infiltration into the ONL (**FIG. 3C**). In the RCS retina, microglia migrate to the outer retina in response to oxidative stress. Confocal imaging of retinal cross sections, shows a decrease in the infiltration of Iba1+ (Wako 019-19741) microglia to the outer retina in the presence of engrafted PRCs compared to non-engrafted areas where microglia thoroughly populate the subretinal space. The outer retina is demarcated at the anterior end by the outer plexiform layer (OPL) and enclosed by the RPE layer at the posterior end as shown by the yellow dashed lines. In addition, co-staining of HuNu+ PRCs with phosphorylated (or activated) nuclear factor-erythroid factor 2-related factor (Nrf2) (anti-pNrf2 antibody, Invitrogen PA5-67520)

and one of its downstream regulators, superoxide dismutase 2 (SOD2) (anti-SOD2, Abcam Ab110300), reveals a possible role of the Nrf2-mediated pathway in regulating the response of engrafted PRCs to oxidative stress. Stained retinal sections were imaged using confocal z-stack analysis on the Leica SP8.

[586] Stimulation of Microglia line SIM-A9 using LPS and inhibition using L-Carnitine Seed 60K SIM-A9 cells per well of 48W culture plates (surface area of well - 0.95cm²) (**FIG. 3D**). On the following day (D1 post seeding) L-Carnitine solutions at concentrations of 30mM, 10mM, 2mM, and 0mM (Vehicle – water) in PRC media (NDM) were prepared. The L-Carnitine solutions were diluted to half the original concentrations by adding equal volume of SIM-A9 media to L-Carnitine solutions. Final concentrations of L-Carnitine solutions are 15mM, 5mM, 1mM and 0mM. SIM-A9 cells were treated with L-Carnitine solutions for 48 hours.

[587] After 48 hours (D3 post seeding) of L-Carnitine treatment, the LPS solution at 10 ug/mL is made from 5 mg/mL LPS stock in SIM-A9 media. Carnitine solutions at concentrations of 30mM, 10mM, 2mM, and 0mM (Vehicle – water) in PRC media (NDM) were prepared. The L-Carnitine solutions were diluted to half the original concentrations by adding equal volume of SIM-A9 media with 10ug/ml LPS to L-Carnitine solutions. Final concentrations of L-Carnitine solutions are 15mM, 5mM, 1mM and 0mM, and final concentration of LPS is 5ug/ml in each L-Carnitine solution. SIM-A9 cells were treated with L-Carnitine solutions with LPS for 24 hours. Conditioned media was collected from the SIM-A9 wells. The conditioned media at was spun at 4000g for 10 minutes to get rid of debris. Conditioned media was aliquoted and then stored at -80C for TNF-alpha ELISA. Manufacturer's instructions for ELISA assay (Mouse TNF alpha ELISA Kit; Abcam ab208348) were followed. Spectramax-M5 plate reader from Molecular Devices was used to obtain optical density (OD) for each sample and standards. Standard curve for known concentrations was plotted against the readout (OD) for each concentration. Sigmoidal 4PL (4 parameter logistic) method on GraphPad Prism was used for curve fitting and data analysis. Concentrations of the test samples were extrapolated on the standard curve. After collecting the conditioned media from the SIM-A9 wells, the cells in the wells were collected in RNA-protect solution for RNA extraction, cDNA synthesis, and RNA expression analysis for IL1b and NOS2 by qPCR following the methodology explained in the "RNA expression analysis by qPCR" section.

[588] Decreased microglial activation was observed after hNSC/NPC transplantation (rd1 mouse, RCS rat). Suppressed microglial activation was seen upon co-culturing with hNSC. Suppression of microglial infiltration at PRC graft sites was observed. (Li et al., 2016, Cytotherapy, 18, 771-784; Jones et al., 2016, MolVis, 22, 472-490).

Phagocytosis assay

[589] PRCs were assessed on ability to phagocytize pHrodo *E. coli* particles (**FIG. 3E**). PRC-P4 cells were thawed and seeded on PDL + Fibronectin + Laminin-521 (PDL: Advanced biomatrix, cat#

5049-50ml; Human Fibronectin: Akron Biotech, cat# AK9715-0005; Laminin 521: Biolamina, cat# LN521) at a density of 2.5×10^5 cells per well in 24 well plate. Cells were refed on the next day, 0.5ml per well. pHrodo Bioparticles were prepared 2 days after plating the cells by adding 2 mL Neural Differentiation Medium (NDM) to one vial of pHrodo Bioparticles to make a 1 mg Bioparticles/mL suspension. Bioparticle vials were vortexed at 3,200 rpm for 45-50 min in 4°C confirming no aggregates. 2 days after plating the cells, 200 μ l NDM + 300 μ l (300 μ g) pHrodo Bioparticles were added per well. pHrodo Bioparticles and cells were incubated at 37°C or 13°C (lower temperature control) for 20-28 hours. To harvest and for flow cytometry analysis, cells were washed at least 3 times with 200 μ l PBS per well. Cells were dissociated with StemPro Accutase (Thermo Fisher Scientific, cat# A1110501) by adding 300 μ l per well and incubating at 37°C for 7 min. Single cell suspension was made by pipetting the cell suspension. 300 μ l NDM was added per well and collected cell suspension was transferred to “U” bottom polystyrene tubes. Cell suspensions were centrifuged at 160g for 5 minutes at RT. Supernatants were aspirated and 300 μ l HBSS + 2% FBS+ 1 μ g/ml propidium iodide (Thermo Fisher Scientific, cat# P3566) was added. Tubes were vortex-mixed for approximately three 3 seconds and samples were analyzed with Sony Cell Analyzer SA3800. 10,000 gated events per tube were acquired, and the data was analyzed by FlowJo (ver.10.8.1). The % of PRC-P4 incorporating pHrodo bioparticles was calculated by comparing with two negative controls; no bioparticle control (37°C without pHrodo bioparticles) or low temperature control (13°C with pHrodo bioparticles).

PRCs internalize rod outer segment (ROS) debris in RCS rat retinas

[590] Royal College of Surgeons (RCS) rats received either a subretinal injection of 100,000 GB2R-PRCs or GS2 vehicle at age P23 (**FIG. 3F**). Rats at ~P23 were anesthetized with ketamine (75mg/kg) and dexmedetomidine (0.25mg/kg) intraperitoneally (IP). The eye was dilated with 1% Tropicamide (Bausch & Lomb, Rochester, NY) or 1% atropine (Bausch & Lomb) or 2.5% phenylephrine hydrochloride (Bausch & Lomb Inc.). A non-absorbable suture (4-0) (Ethicon, Inc., Somerville, NJ) was put in place to hold the eyeball forward. A 25 5/8 G metal needle was used to make a sclerotomy at the upper dorsal temporal region of the eye. 2 μ l of cell suspension were drawn into a sterile glass pipette (internal diameter 50–150 μ m) via a plastic tube filled with GS that was attached to a 25 μ l Hamilton syringe. Cells or GS (2 μ L volume) were injected into the subretinal space through the site of the sclerotomy. To reduce intraocular pressure and to limit the efflux of cells, the cornea was punctured using 30 G metal needle. Immediately after injection, the fundus was examined for retinal damage or vascular distress. The wound was sutured with a non-absorbable surgical suture (10-0) (Ethicon, Inc.). The suture around the eyeball was removed and then the eyelid was put into its normal position. Finally, erythromycin ophthalmic ointment (Bausch & Lomb) was used locally. The animals recovered from anesthesia on warming pads (37°C). Animals received daily dexamethasone (American Regent, Inc., Shirley, NY) injections (1.6 mg/kg, i.p. (intraperitoneal injection)) for 2

weeks following the subretinal procedure. Additionally, animals received cyclosporine-A (Bedford Laboratories, Bedford, OH) in their drinking water (210 mg/L) throughout the course of the entire experiment.

[591] Animals were sacrificed at p35 for immunocytochemistry (ICC) and confocal microscopy analysis (**FIG. 3F**, left panel). Subretinally engrafted PRCs were identified by HuNu (Millipore, MAB1281) and GFAP (Abcam, ab4674) double positive ICC staining. Rhodopsin+ staining (Millipore, MABN15) identified rod outer segment debris that had been internalized by PRCs in the subretinal space. ICC staining was performed on retinal cryosections essentially as described herein and imaging was performed on a Leica SP8 confocal microscope. Animals were sacrificed at P60 for Transmission Electron Microscope ("EM") analysis. EM confirms the presence of multilamellar structures, identified as rod outer segment fragments, localized to the cytoplasm of engrafted PRCs in the subretinal space (**FIG. 3F**, right panel). Tissue processing and imaging was performed according to standard procedures and images were captured on a Phillips CM10.

EXAMPLE 2: *In vivo* characterization of PRC

Methods

Subretinal Injections

[592] Rats at ~P25 were anesthetized with ketamine (75mg/kg) and dexmedetomidine (0.25mg/kg) intraperitoneally (IP). The eye was dilated with 1% Tropicamide (Bausch & Lomb, Rochester, NY) or 1% atropine (Bausch & Lomb) or 2.5% phenylephrine hydrochloride (Bausch & Lomb Inc). A non-absorbable suture (4-0) (Ethicon, Inc., Somerville, NJ) was put in place to hold the eyeball forward. A 25 5/8 G metal needle was used to make a sclerotomy at the upper dorsal temporal region of the eye. 2 µl of cell suspension were drawn into a sterile glass pipette (internal diameter 50–150 µm) via a plastic tube filled with GS that was attached to a 25µl Hamilton syringe. Cells or GS (2uL volume) were injected into the subretinal space through the site of the sclerotomy. To reduce intraocular pressure and to limit the efflux of cells, the cornea was punctured using 30 G metal needle. Immediately after injection, the fundus was examined for retinal damage or vascular distress. The wound was sutured with a non-absorbable surgical suture (10-0) (Ethicon, Inc.). The suture around the eyeball was removed and then the eyelid was put into its normal position. Finally, erythromycin ophthalmic ointment (Bausch & Lomb) was used locally. The animals recovered from anesthesia on warming pads (37°C). Animals received daily dexamethasone (American Regent, Inc., Shirley, NY) injections (1.6 mg/kg, i.p.) for 2 weeks following the subretinal procedure. Additionally, animals received cyclosporine-A (Bedford Laboratories, Bedford, OH) in their drinking water (210 mg/L) throughout the course of the entire experiment.

Optomotor Response (OMR)

[593] OMR testing was performed on animals at P60, P90, P120, P210, and P270 using the OptoMotry testing apparatus (Cerebral Mechanics Inc., Lethbridge, Canada) (Prusky, 2004). The

OptoMotry device uses four computer monitors arranged in a square to project a virtual three-dimensional (3-D) space of a rotating cylinder lined with a vertical sine wave grating. Unrestrained animals were placed on a platform in the center of the square, where they tracked the grating with reflexive head movements. The spatial frequency of the grating was clamped at the viewing position by re-centering the 'cylinder' on the animal's head. The acuity threshold was quantified by increasing the spatial frequency of the grating using a psychophysics staircase progression until the following response was lost.

Electroretinography (ERG)

[594] ERG was performed on animals at P60, P90, P120, and P210. Animals were prepared for ERG recording under dim red light following overnight dark adaptation. After anesthesia injection using a mixture of ketamine (150 mg/kg i.p.) and xylazine (10 mg/kg i.p.), the head was secured with a stereotaxic device and the body temperature was kept at 38°C throughout the experiment using a homeothermic blanket. Pupils were dilated with 2.5% topical phenylephrine plus 1% atropine and a drop of 0.9% saline was applied on the cornea to prevent the dehydration and allow electrical contact with the recording electrode. Single flash presentations with a standard duration of 10 seconds were given and the responsiveness was recorded using Espion E3 Electroretinography System (Diagnosys LLC).

Tissue Preparation

[595] Animals were euthanized and then the eyes were removed, immersed in 2% paraformaldehyde for one hour, infiltrated with sequential intermediate incubation steps with 10% and/or 20% and/or 30% sucrose, and 30% sucrose overnight. Next day, eyes were embedded in OCT and cut on a cryostat (10 µm) onto Superfrost+ slides and stored at -80°C.

Immunocytochemistry (ICC)

[596] Sections were removed from -80°C and dried for ~10 min at room temperature (RT) then washed in 1x PBS for 5 minutes at RT. Boundaries around sections are marked using liquid blocker super pap pen (Fisher Scientific, Cat.no NC9827128). Slides are then incubated in blocking buffer: 1X PBS with 5% Horse serum and 0.3% Triton-X for 1 hour at either 4°C or RT. Blocking buffer is removed and primary antibody solution is added in a humidified chamber overnight at 4°C. Slides are then washed with 1xPBS and incubated with secondary antibody solution for 1 hour at 4°C plus 4',6-diamidino-2-phenylindole (DAPI) (1:1000). After washing, coverslips are mounted using Prolog Gold anti-fade mounting media (Invitrogen, Cat.no P36930) and allowed to cure for ~24 hours at RT.

Engraftment vs. ONL preservation Morphometry

[597] Sectioned tissue from FY2019 lot (GB2R-PRC-2019-P4, 100,000 cells/eye) injected P120 RCS rats was utilized for morphometric analysis. Whole area tracing of both graft and photoreceptor outer nuclear layer (ONL) from the entire sectioned slide series was utilized for morphometry, with no regional selection or engraftment biased selection in the imaging. N=6 evenly distributed slides covering the entire sequence of sections for each eye were utilized for morphometry: AS193R- every

10th slide was selected; AS193L- every 10th slide was selected; AS194R- every 15th slide was selected (to reflect the greater # of slides sectioned in the series while maintaining the same N=6 slides for analysis); AS194L- every 10th slide was selected. Sampling depth through the eye approximately equaled 2400 μm (AS193R, AS193L, AS194L) and 3600 μm (AS194R). Immunohistochemistry was performed as described above using antibodies selective for STEM121 (human cytoplasmic marker; Takara Inc.) and the tissue was counterstained with DAPI. 2 eyes/slide were imaged using a Leica DMI8 Imaging suite with motorized stage and tiling functionality, generating 12 images/eye for each RCS eye. The images were exported as scaled tiff images with embedded scale bars and then whole area traces were generated using ImageJ analysis software of the DAPI labeled ONL and the STEM121 labelled subretinal PRC graft. Only subretinal graft and preserved ONL lamina was measured; scattered individual photoreceptors were not traced out. Ocular graft vs. ONL area was plotted using GraphPad Prism8 statistical software.

Immunohistochemistry

[598] Imaging for Immunohistochemistry was performed using a Leica SP8 Confocal microscope operating the Leica LasX software suite. Image manipulation (re-sizing, cropping, color correction) was performed using ImageJ image processing and analysis software from the National Institutes of Health (NIH). Images were assembled for presentation using Microsoft PowerPoint.

Subretinal Injections of rd10 mice

[599] The dosing apparatus, micro syringes (Hamilton 600 series) with 34 ga blunt needles (Hamilton #207434), were prefilled with cell suspension or vehicle prior to the surgery. Test animals (P14 Rd10 mouse) were administered ketamine/xylazine cocktail. When a test animal achieved deep anesthesia indicated by slowed breathing and lack of response to a toe pinch, it is placed on its side under a dissecting microscope so that the eye to receive the procedure is positioned up. The skin above and below the eye is pulled back so that the eye proptoses (pops out). Betadine eye drops (5%, sterile ophthalmic grade) are administered to the eye for topical disinfection of the globe, and artificial tears can be applied to rinse the excess betadine from the eye. Proparacaine eye drops are applied to numb the eye and surrounding tissue, as well as phenylephrine (2.5%) and tropicamide eye drops to dilate the pupils. In between eyedrop/artificial tear applications, excess solution is removed using a fabric-tipped swab. For a subretinal injection, a hole is cut in the conjunctiva to expose the sclera. Using a 30 ga beveled needle, a pilot hole is made on the limbal area. The micro syringe with 34 ga needle containing PRC suspension or vehicle buffer is inserted into the hole under visual guidance and injection material is ejected into the eye via manual depression of the plunger. Following the injection, the needle is slowly removed from the eye, and the animal is placed on the opposite side to perform the injection procedures on the second eye. Immediately after injection procedures, OCT imaging will be performed to evaluate injection success. After imaging, erythromycin (0.5%) ophthalmic ointment is applied to the surgical site for both anti-microbial and lubricating functions. For successfully injected mice, Antisedan (atipamezole, 2 mg/kg) is administered IP to aid in the

recovery process. Subcutaneous saline may be administered for hydration. Finally, the animal is moved to a heated cage to prevent hypothermia until sternal recumbency, and subsequent return to its home cage

ONL and Cone Length Preservation Morphometry and Rod Synapse Counting

[600] Sectioned tissue from FY2019 lot PRC (GB2R-PRC-2019 (P4), 100,000 cells/eye) injected P28 rd10 mice were utilized for morphometric analysis and rod synapse counting. Area tracing of the photoreceptor outer nuclear layer (ONL) from regions of the eye with subretinal PRC engraftment and distal to the site of engraftment towards both the central and peripheral retina were utilized for morphometry. N=4 evenly distributed slides spanning the sequence of sections which include engraftment for each of three eyes were utilized for morphometry. Sampling depth through each eye approximately equaled 480 μm . Immunohistochemistry was performed using antibodies selective for STEM121 (human cytoplasmic marker; Takara Inc.) and the tissue was counterstained with DAPI. 2 eyes/slide were imaged generating 8 images/eye for each rd10 eye. The images were exported as scaled tiff images with embedded scale bars and ONL area traces were generated using ImageJ analysis software of the DAPI labeled ONL. ONL area was plotted and compared using GraphPad Prism8 statistical software. For cone length analysis serial sections were stained as described for morphometry with antibodies selective for cone arrestin (Millipore) and STEM1281 (human nuclear antigen; Millipore) and imaged. The axial length of labelled cones from outer segment to axonal pedicle was measured at the site of subretinal PRC engraftment and distal to the site of engraftment towards both the central and peripheral retina and compared using GraphPad Prism8 statistical software. For Rod synapse analysis, sections were prepared as described for morphometry and stained with antibodies selective for cone arrestin (MilliporeSigma) and ribeye/CtBP2 (BD Biosciences). Ribeye positive/cone arrestin negative synapse (rod synapses) were counted at the site of subretinal PRC engraftment and distal to the site of engraftment towards both the central and peripheral retina and compared using GraphPad Prism8 statistical software.

Subretinal injection of PRC-EVs in RCS rats and Optomotor Response (OMR)

[601] RCS rats received either a subretinal injection of PRC-EVs or GS2 (vehicle) at P23 to P25 (23 and 25 postnatal days, respectively). PRC-EVs were administered at a dose of 9.42×10^8 EVs per eye. Spatial frequency was measured by recording optomotor responses at P60, P90 and P120 (60, 90 and 120 postnatal days). Animals were sacrificed at P90 and P120 for histological analysis.

Imaging and ONL Quantification of PRC-EV injected eyes.

[602] Retinal cryosections of P90 and P120 PRC-EV injected eyes were stained with the nuclear stain DAPI (4,6-diamidino-2-phenylindole) to visualize the outer nuclear layer (ONL) of the retina. Stained retinal sections were imaged using the Leica DMI8 epifluorescent scope. ONL thickness was determined by line graph measurements through the ONL at central and peripheral areas for a total of 3 to 5 regions of interest (ROIs) per retina.

Subretinal injection of PRC-EVs in RCS rats and optomotor response (OMR) test

[603] RCS rats received either a subretinal injection of PRC-EVs or GS2 (vehicle) at post-natal days 23 to 25 (P23 to P25). PRC-EVs were administered at a dose of 9.42×10^8 EVs per eye. To assess visual function after PRC-EV injection, spatial frequency was measured by recording optomotor responses at P60, P90 and P120. Animals were sacrificed at P90 and P120 for histological analysis.

Imaging and Outer Nuclear Layer (ONL) Quantification of PRC-EV injected eyes

[604] Retinal cryosections were stained with the nuclear marker, 4,6-diamidino-2-phenylindole (DAPI) to visualize the ONL, and stained sections were imaged using the Leica DMi8 epifluorescent scope. ONL thickness was determined by line graph measurements through the ONL at central and peripheral areas for a total of 3 to 5 regions of interest (ROIs) per retina. Total ONL thickness was taken as the average ONL length from each ROI.

Results: RCS (homozygous) rats

[605] RCS (homozygous) rats were injected with 16.5-200k PRCs subretinally in one eye (Summary depicted in **FIG. 4A**). The control eye (partner eye) was injected with vehicle or was left uninjected. Injection was at P25. OMR and ERG tests were performed at P60, P90, and P120. Histology of injected and control eyes were performed at P35, P60, and P120.

[606] OMR and ERG analysis of RCS rats injected at P25 with 100,000 cells/eye of GB2R-PRC-P4-FDA PRCs (**FIG. 4B**). Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.) with test articles compared with uninjected and GS2 vehicle injected eyes. Cell viability was 78.4% and determined manually.

[607] Representative images showing PRC engraftment significantly attenuates outer nuclear layer (ONL) degeneration out to 3 months post-transplantation (**FIG. 4C**). Confocal images show PRC engraftment is highly correlated with ONL preservation. Although an overall gradual decrease in ONL thickness with the progression of age is observed in the "no graft" areas, there is significant preservation of the ONL at graft areas compared to non-engrafted areas in each age group. Stained retinal sections were imaged using the Leica MDi8 epifluorescent microscope.

[608] Quantification of ONL thickness show PRC engraftment is correlated to significant preservation at P35, P60 and P120 (**FIG. 4D**). Retinal cryosections through the PRC graft were chosen for ONL quantification and stained with HuNu (Millipore, MAB1281) to localize engrafted PRCs and DAPI to identify the ONL. ONL thickness was determined by line graph measurements through the ONL at central and peripheral areas for a total of 3 to 4 regions of interest (ROIs) per retina. Quantification of the ONL was performed in a blinded fashion, using only the DAPI channel. Regions of Interest were subsequently identified as "Graft" or "No Graft" by the presence of subretinal HuNu+ PRCs.

[609] Area plot of GB2R-PRC-2019 (P4) engrafted PRCs vs. photoreceptor ONL in the P120 RCS rat model of retinal degeneration (**FIG. 4E**). Statistical analysis was performed using the Pearson correlation coefficient.

[610] Immunohistochemical analysis of glial fibrillary acid protein (rabbit anti-GFAP; ABCAM) in the P120 RCS rat was performed (**FIG. 5A**). GFAP is expressed in both Müller glia (MG) and optic nerve fiber astrocytes of the host rat retina (white arrows) as well as engrafted PRCs in the subretinal space. MG hypertrophy with increased expression of GFAP in MG and astrocytes, as well as expanded distribution of GFAP expression throughout the radial MG cell body, is characteristic of disease associated reactive gliosis and can be seen distal to the graft site (yellow arrows, white arrows). Upregulation and increased distribution of GFAP, MG hypertrophy, and outer nuclear layer (ONL) degeneration is reduced or absent at the subretinal PRC graft site.

[611] TUNEL quantification (TUNEL label Mix, Sigma catalog# 11767291910, TUNEL enzyme Sigma catalog# 11767305001) was performed at P35 during which peak apoptotic activity is observed in the RCS retina (**FIG. 5B**). PRCs engrafted in the subretinal space were identified by Ku80+ staining (Abcam, ab80592) of adjacent sections (see below). In PRC engrafted areas, very few, if any TUNEL+ nuclei are observed, indicating little to no apoptotic activity at graft sites. However, non-engrafted areas contain significant TUNEL+ nuclei in the ONL, indicating widespread photoreceptor death at P35. Quantification of TUNEL+ nuclei in the ONL shows significant attenuation of apoptotic activity at graft sites vs non-graft areas. Retinal sections through the PRC graft were chosen for TUNEL quantification and stained with Ku80 to localize engrafted PRCs and DAPI to identify the ONL. A total of 3 to 4 regions of interest (ROIs) were imaged at central and peripheral regions per retina. TUNEL quantification was performed in a blinded fashion, using only the DAPI channel. ROIs were subsequently identified as “Graft” or “No Graft” by the presence of subretinal Ku80+ PRCs.

[612] Extracellular vesicles (EVs) isolated from PRCs preserve OMR response out to P90 in RCS rats (**FIG. 5C**). EVs were isolated from PRC conditioned media and subretinally injected into RCS rats at a dose of 9.42×10^8 EVs/eye. Other RCS rats received subretinal injections of PRCs (100,000 cells/eye), or vehicle. Injections were performed at age P25. OMR analysis was performed at P60, 90, 120 and compared to non-injected (NI) animals at the same age. A single injection of PRC-EVs were able to preserve OMR out to P90 as compared to NI and vehicle-injected animals whereas a single subretinal injection of the PRC cells preserved OMR out to P120. This suggests PRC-derived EVs can at least partially recapitulate efficacy observed with subretinally injected PRC cells and supports secretion of paracrine-acting factors as part of the PRC mechanism of action.

[613] A single subretinal injection of PRC-EVs preserves ONL thickness out to P90 (**FIG. 5D**). Retinal ONL thickness from PRC-EV injected RCS rats vs that of uninjected eyes was quantified. Retinal cryosections were stained with DAPI to visualize the ONL. Stained retinal sections were imaged using the Leica DMi8 epifluorescent scope. ONL thickness was determined by line graph measurements through the ONL at central and peripheral areas as described herein.

[614] Visual function was assessed by recording optomotor responses in PRC-EV injected rats at P60, P90 and P120 (**FIG. 5I**). Visual acuity was significantly preserved at P60 and P90, but this preservation was not maintained by P120. To further investigate morphological preservation of photoreceptors after PRC-EV injection, ONL thickness was measured in P90 (**FIGs. 5E, 5F, and 5G**) and P120 (**FIG. 5H**) rats. ONL analysis of EV-treated rats shows single injection of PRC-EVs confers morphological preservation of the ONL up to P90 but not at P120 (**FIG. 5J**), corresponding with the lack of OMR preservation in the latter age group. While PRC-EV treatment maintains visual function and preserves outer retinal morphology up to P90, subretinal cell transplantation of PRCs preserves ONL thickness up to P120 (**FIG. 5K**) confirming prolonged morphological preservation of the ONL with subretinal cell transplantation compared to treatment with single EV injection.

[615] Spatial frequency was measured by recording optomotor responses in PRC-EV injected and PRC-transplanted rats at P60, P90 and P120. OMR responses between EV and cell treatment remained comparable and were significantly preserved up to P90. However, preservation of the OMR was lost in EV-injected rats by P120. ONL analysis of EV-treated rats shows single injection of EVs significantly preserves ONL thickness up to P90 but not at P120, corresponding with the lack of OMR preservation in the latter age group.

[616] In PRC engrafted areas, as visualized by Toluidine blue stain, ONL preservation is clearly observed whereas non-engrafted areas show severely degenerated ONL (**FIG. 6A**). Rats at age P25 were subretinally injected with 100,000 GB2R PRCs or vehicle and then sacrificed at age P60. Retinal tissue was formalin-fixed paraffin-embedded and cross-sectioned for staining with Toluidine Blue (Polysciences, 1234) to visualize the ONL. P60 eyes were processed for TEM analysis and imaged on a Phillips CM10. All tissue processing and imaging was performed by the UMass Electron Microscopy Core Facility.

[617] Transmission Electron Microscope (TEM) analysis confirms noticeable preservation of the ONL in engrafted areas compared to ONL sites with no PRC engraftment (**FIG. 6B**).

[618] TEM ultrastructural analysis shows overall morphological preservation of photoreceptors as well as finer structures such as the connecting cilium of the rod inner segment in the presence of subretinal PRCs (**FIG. 6C**). Finally, PRC engraftment minimizes the debris zone of the subretinal space. In the absence of subretinal PRCs, rod outer segment debris populates most of the subretinal space due to non-clearance after outer segment shedding.

Results: rd10 Mouse (homozygous)

[619] On postnatal 14 days (P14), 100k cells were transplanted into subretinal space of the right eyes of rd10 mice, GS2 vehicle was injected into the left eyes as a control. OMR were performed on P21, P28, P35, P42 and P49 (**FIG. 7A**).

[620] OMR analysis shows that at P21 and P28 both eyes show presence of tracking (**FIG. 7B**).

Cell injected eye showed capacity to track at P35, P42 and P49, in comparison to control eyes, which are blind.

[621] Morphometric analysis of photoreceptor outer nuclear layer (ONL) preservation in the P28 rd10 mouse (**FIG. 7C**). ONL preservation quantified as ONL area (mm²) can be identified at the locus of PRC subretinal engraftment compared with adjacent nongrafted central and peripheral retina.

[622] Analysis of cone length in the P28 rd10 mouse (**FIG. 7D**). The length of cone arrestin labelled cone photoreceptors is significantly increased at the locus of PRC subretinal engraftment compared with adjacent regions of central and peripheral retina in which there is no subretinal engraftment. Confocal imaging shows CtBP2/RIBEYE labelled rod synapses at the site of engraftment and in central and peripheral retinal loci without engraftment (**FIG. 7E**).

[623] Quantification of rod synapses in the P28 rd10 mouse (**FIG. 7F**). The number of ribeye positive/cone arrestin negative rod synapses is significantly increased at the locus of PRC engraftment compared with nongrafted central and peripheral retina indicating PRC associated preservation of rod photoreceptors in rd10 mouse retina.

[624] OMR at P35 with sub-70% viabilities from multiple PRC lots was compared (**FIG. 8A**). The viabilities of 66.05%, 68.5% and 68.8% PRC showed similar preservations effects. PRC-injected eyes showed better OMR performance than control eyes.

[625] Anatomical preservation observed across multiple PRC lots with sub-70% viabilities (**FIG. 8B**). Histological analysis confirmed OMR data. At P35, PRCs that had been injected with 66.05% viability preserved the cone anatomy as assessed by ONL thickness, axon length and morphology. At P50, PRCs that had been injected with 68.5% viability preserved ONL thickness and density of ribeye+ synapses. At P70, PRCs that had been injected with 68.8% viability also preserved ONL thickness and density of ribeye+ synapse.

[626] On postnatal 14 days (P14), 100k cells were transplanted into subretinal space of the right eye of RD10 mice, GS2 vehicle was injected into the left eye as a control (**FIG. 9A**). At P50, eyes were collected for IHC staining. DAPI staining showed that only one row of photoreceptors was left in no graft retina, while multiple rows of photoreceptor were observed in graft retina. Quantification of data from 5 animals, 3 sections from each animal shows ONL thickness and area in graft retina are significantly higher than those in no graft retina (6um vs 18um; 5000um² vs 10000 um²).

[627] ICC image staining for Cone arrestin (Millipore AB15282 1:500) was used to detect the morphology of cones (**FIG. 9B**, left panel). In no graft retina, arrestin was only expressed in cell bodies of cones, outer segment and axons were degenerated. In graft retina, cones were preserved by maintaining normal morphology, and arrestin was expressed in outer segment, cell body, axon and pedicles. Quantification of data shows that cones had significantly longer axons in graft retina than those in no graft (8um vs 4um). Ribeye (also called ct-BP2, BD transduction Laboratories 612044,

1:500), is a marker of presynaptic structure located at the axon terminal of photoreceptors, that shows the synaptic connection between photoreceptor and horizontal cells (**FIG. 9B**, right panel).

Quantification of data shows Ribeye puncta expression was higher in graft retina than that in no graft retina (40 vs 20/100um retina).

[628] **FIG. 9C** shows a schematic of dosing P23H rats. A P23H rat is an autosomal dominant retinitis pigmentosa (RP) model, where a mutated mouse rhodopsin (*Rho*) transgene is incorporated in the wild-type Sprague Dawley rat. P23 hemizygous rats were injected at P25 subretinally with 100K cells in one eye. Vehicle or non-injection was used for the partner eye as a control. OMR and ERG were performed at P60, P90, and P120 on both eyes, and histology analysis was performed at P120.

[629] OMR analysis of P23H hemizygous rats injected at P25 with 100,000 cells/eye of GB2R-PRC-P4-FDA PRCs reveals statistically significant preservation of the OMR response compared with uninjected controls at P60, P90, and P120 and compared to vehicle injected controls at P90 (**FIG. 9D**). Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.).

EXAMPLE 3: Pharmacokinetics

Methods

[630] Subretinal injections and immunohistochemistry (IHC) as described above.

Comparative Subretinal Engraftment Morphometry

[631] Evenly distributed slides spanning the sequence of sections displaying subretinal engraftment were stained for each of the RCS rat, P23H homozygous rat, P23H hemizygous rat, and rd10 mouse injected with 100,000 cells/eye of FY19 GB2R-PRC-2019 (P4) PRCs using antibodies selective for human cytoplasmic antigen (STEM121; Takara Inc.) and counterstained with DAPI. Eyes were imaged using a Leica DMI8 Imaging suite with motorized stage and tiling functionality. Images from the Slides showing the maximal extent of subretinal engraftment (maximal length of subretinal coverage relative to retinal length) were identified and used to calculate relative maximal length of subretinal graft coverage. N=4; N=3; N=3, and N=5 eyes (RCS, rd10, P23H homozygous, and P23H hemizygous eyes respectively) were used in calculations. The maximal length of the subretinal graft was measured as was the total length of overlaying retina. Data was plotted to show the relative retinal length vs. subretinal graft coverage. Additionally, the ratio of maximal subretinal engraftment vs. retinal length was calculated and plotted. One-way ANOVA with Tukey's multiple comparison test was utilized to compare differences in ratios of maximal engraftment between models.

Results

[632] Schematic depiction of HuNu+ cell quantification process (**FIG. 10A**). RCS rats, aged P23 to P25 were given subretinal injections of 100,000 GB2R PRCs or GS2 vehicle. Animals were sacrificed at P120 for retinal tissue cryosectioning and histological analysis. Every 10th retinal section through the graft was chosen for quantification wherein HuNu+ PRCs were quantified. Cell numbers were

interpolated between each section, and the sum total engraftment was calculated. Total PRC engraftment was calculated from $n = 4$.

[633] The number of engrafted PRCs was quantified in 4 transplanted animals at P120, or 3 months post-transplantation (**FIG. 10B**). Quantification of total HuNu+ PRCs in the subretinal grafts shows an average of 80,000 transplanted cells per eye which translates to an overall 80% rate of engraftment compared to an initial injection of 100,000 cells per injection.

[634] Differential PRC engraftment in retinal degeneration models with varying disease onset and severity post-treatment (**FIG. 10C**). Specific lots and doses of PRCs used in this analysis are shown in the box at the very top of the figure. Relative Subretinal Engraftment bar graph: RCS rat and P23H hemizygous rat models of retinal degeneration demonstrate comparable engraftment of GB2R-PRCs with maximal engraftment found to cover 54 and 41% of the subretinal space respectively. rd10 mouse and P23H homozygous rat models of retinal degeneration show significantly less engraftment with 19% (rd10 mouse) and 2% (P23H homozygous rat) of the subretinal space showing PRC engraftment at the length maxima. Relative Retinal Length Graft Coverage graph: Relative ratio calculations present coverage relative to the different sizes of the mouse and rat eyes while plots of maximal lengths display absolute lengths of engraftment. The rd10 mouse and P23H rat show decreased engraftment in both relative and absolute terms compared to both RCS and P23H hemizygous rats.

EXAMPLE 4: *In vivo* Safety Analysis

[635] PRCs have limited proliferation, do not display markers of pluripotency with no safety abnormalities observed in PRC-treated RCS. Proliferation in subretinal PRCs is downregulated by P120 (**FIG. 11A**). HuNu (Millipore, MAB1281) and Ki67 (Abcam ab15580) double positive cells in the subretinal graft were quantified in retinal cross sections in P35, P60 (not shown) and P120 animals. A total of three animals were used for each timepoint. Confocal images show Ki67 and HuNu double-positive cells in the subretinal space at P35. However, by P120, proliferative (Ki67+) PRCs are not observed. Quantification reveals significant downregulation of Ki67 and HuNu double-positive PRCs by P120, or 3 months post-transplantation (bar graph in lower left panel).

[636] The absence of Oct4 (Abcam ab27985) expression in engrafted, HuNu+ PRCs shows a lack of pluripotency at all time points investigated after transplantation (P35, P60 and P120) (**FIG. 11B**). A total of three animals were used for each timepoint. Cultured GMP1 iPSCs were used as a positive control for Oct4 expression, with WGA-647 (Thermo Scientific W32466) and DAPI staining for subcellular compartment localization (small images in lower middle panel).

[637] Summary of general welfare, ocular exam, and histopathology report from board-certified veterinary histopathologist indicates subretinally transplanted PRCs are generally well-tolerated with no adverse effects. P270 RCS rats (8 mo. Post-Transplant (Tx)) 1) maintained stable body weight in the last month of life ~similar to naïve RCS rats; 2) no welfare issues were observed; 3) no PRC

associated abnormalities observed via ophthalmoscopy; and 4) no macroscopic or microscopic PRC-Tx associated observations. P50 rd10 mice (36 dy Post-DP Tx) 1) maintained steady growth during study; 2) no welfare issues were observed; and 3) no abnormalities observed via ophthalmoscopy when PRCs in cryopreservative were transplanted.

Conclusion

[638] The effects of PRCs on 3 specific targets in the degenerating retina may be responsible for PRC efficacy in delaying vision loss: (1) Maintaining health of photoreceptors, which are important for initiation of visual phototransduction cascade; (2) Limiting microglial overactivity, which otherwise may exacerbate the degenerative process; and (3) Reducing accumulation of degenerative debris through phagocytosis, which otherwise contributes to further loss of photoreceptors.

[639] Methods of action related to target biology include (1) Paracrine-mediated neuroprotection of photoreceptors; data with PRC-secreted EVs suggests neuroprotection is a major contributing factor to PRC efficacy while other mechanisms may play a supportive role, such as; (2) PRC-associated reduction in glial cell reactivity and expression of anti-oxidant factors; and (3) PRC phagocytosis of degenerative debris.

[640] Target indication/patients for treatment with PRCs are patients diagnosed with Retinitis Pigmentosa with BCVA of 20/100 or worse regardless of genetic mutation. PRCs are expected to maintain or slow down loss of visual acuity as assessed by BCVA or other visual test (TBD). For example, slowing down retinal degeneration via fundus autofluorescence, spectral domain (SD)-OCT, and/or microperimetry.

[641] In RP, genetic mutations in rod photoreceptors lead to their degeneration in the peripheral retina followed by secondary degeneration of cones in the central retina (Humphries, 1992). Activation of microglia is thought to be a protective response to injury/degeneration, yet it often becomes exacerbated/dysregulated in chronic retinal degeneration and contributes to this cascading loss of photoreceptors as does the accumulation of degenerative debris. Progressive loss of photoreceptors impairs the visual phototransduction pathway, leading to loss of vision, therefore the ability of PRCs to maintain the health and function of photoreceptors is thought to be critically important. Evidence suggests various neuroprotective molecules can support the health and function of photoreceptors, helping to slow down retinal degeneration and vision loss (Kutluer 2020). Suppression of microgliosis and expression of anti-oxidant molecules may also help slow down the degenerative process by limiting stress and additional loss of photoreceptors (Murakami 2020; Peng, 2014; Rashid 2019; Newton 2020). Phagocytosing debris from dying photoreceptors may also help clear toxic signals, thus limiting secondary damage to nearby photoreceptors (Newton 2020). PRCs target all of these biological processes.

EXAMPLE 5: PRC Manufacturing

[642] Schematics are shown of alternative PRC manufacturing process in **FIGs. 12A** and **12B**. hESCs from the GMP-GB2R-MCB are thawed (day -10), counted and seeded on culture vessels coated with iMatrix (Laminin-511, Matrixome) and cultured with StemFit medium (Ajinomoto) supplemented with 100 ng/mL bFGF (Peprotech) and 10 μ M ROCK inhibitor (Y-27632; Fujifilm/Wako) under feeder-free conditions. After four days of daily medium changes (StemFit+bFGF without ROCK inhibitor), hESCs are harvested using Cell Dissociation Buffer (Gibco) and reseeded using the above culture conditions. After an additional 4 days in culture (day -2), hESCs are harvested as above, % viability and a viable cell count are obtained, and hESCs are seeded at 3,000 cells/cm² for PRC differentiation on iMatrix-coated culture T75 flasks in StemFit+bFGF medium supplemented with ROCK inhibitor (Y-27632). After day -2 seeding, cultures are fed with (1) Day -1 – StemFit + bFGF (without ROCK inhibitor); (2) Day 0 to day 3 (daily) – Rescue Induction Medium (RIM: DMEM/F12 + B27 + N2 + Non-essential amino acids (all from Gibco) + glucose (Sigma) + Insulin (Akron Biotech) + Noggin (Gibco)); and (3) Day 4 to day 19 (every 2–3 days) – Neural Differentiation Medium Plus Noggin (NDM+: Neurobasal Medium + B27 + N2 + Non-essential amino acids + glucose + glutamax (Gibco) + Noggin).

[643] At day 19, cultures are “lifted” into suspension culture (2D to 3D) through incubation with a combination of Liberase and Thermolysin enzymes (Roche Custom Labs) and seeded on ultra-low attachment T75 flasks. 3D cultures are maintained for 3-4 days using NDM minus Noggin (NDM-) to allow for the formation of neural spheroids (“spheres”). Then, spheres in suspension are seeded (3D to 2D) onto culture vessels coated with poly-D-lysine (Advanced Biomatrix), viral inactivated human fibronectin (Akron Biotech) and laminin-521 (Biolamina) to start Passage 0 (P0d0), and cultured under 2D conditions for 14 days (until P0d14) using NDM- (feeding every 2-3 days). 2D to 3D to 2D transitions are repeated three times (through P1, P2 and P3, 3-4 days in 3D and 14 days in 2D culture) until P3d14 is reached after 90 days of differentiation. P3d14 cultures are harvested using Accutase (Innovative Cell Technologies) and cultured in ultra-low attachment T75 flasks for 24 hours. Then, P3-spheres are cryopreserved by resuspending in Cryostor CS10 (Stemcell Technologies) and freezing to -80°C, and then transferred to the vapor phase of liquid nitrogen for storage. The cryopreserved P3 spheres are called Cell Stock (CS).

[644] Examples of methods to “lift” cells into suspension include, but are not limited to, methods shown in **Table 13**.

Table 13

Time point		Method 1	Method 2	Method 3
day	Passage day			
19		Cell scraper	Liberase + Thermolysin	Accutase + ROCK inhibitor
37	P0d14	Cell scraper	Liberase + Thermolysin	Accutase
54	P1d14	Cell scraper	Liberase + Thermolysin	Accutase
72	P2d14	Cell scraper	Liberase + Thermolysin	Accutase
90	P3d14	Accutase	Accutase	Accutase
107	P4d14	Accutase	Accutase	Accutase

[645] To produce the composition of the invention, vials of CS are thawed in a water bath at 37°C, resuspended in NDM- and transferred to ultra-low attachment T75 flasks and cultured in 3D suspension for 2-3 days, followed by re-plating onto T75 flasks coated with poly-D-lysine/fibronectin/laminin-521 and culture under 2D conditions for 14 days with NDM- to complete Passage 4. At P4d14, cells are harvested using Accutase, resuspended with NDM-, triturated and filtered through a 40µm cell strainer to obtain a single cell suspension that constitutes the PRC composition of the invention, which can be subsequently formulated with cryopreservative agents.

[646] PRC manufacturing protocol can be altered to allow for scaling up. For example, the flasks used can be changed from T75 flasks to T225 flasks or cell stacks (*e.g.*, Corning® CellSTACK®).

[647] Additionally, the PRC manufacturing protocol can include an intermediary cryopreservation step at any one or more of between P0 and P1, between P1 and P2, between P2 and P3, and/or between P3 and P4. In some embodiments, the PRC manufacturing protocol can exclude an intermediary cryopreservation step.

[648] The intermediary cryopreservation step can include 5% DMSO instead of 10% DMSO. In some embodiments, the intermediary cryopreservation step can include about 1% DMSO, about 2% DMSO, about 3% DMSO, about 4% DMSO, about 5% DMSO, about 6% DMSO, about 7% DMSO, about 8% DMSO, about 9% DMSO, about 10% DMSO, about 11% DMSO, about 12% DMSO, about 14% DMSO, or about 15% DMSO.

[649] The intermediary cryopreservation step can include the cryopreservative formulation, as described herein. The PRC manufacturing protocol can include thermolysin and liberase at D19 and accutase followed by an overnight culture with Rock inhibitor. The lifted cells or cell clusters can be seeded in Aggrewells™ to allow for uniformly sized spheroids to develop. At day 19, cultures are “lifted” into suspension culture (2D to 3D) through incubation with Accutase (Innovative Cell Technologies) and seeded on ultra-low attachment T75 flasks using NDM minus Noggin (NDM-) medium supplemented with Y-27632 (ROCK inhibitor, Fujifilm Wako). 24 hours later, the growth

medium is replaced with Y-27632-free NDM- and 3D cultures are maintained for additional 2-3 days using NDM- to allow for the formation of neural spheroids (“spheres”). Then, spheres in suspension are seeded (3D to 2D) onto culture vessels coated with poly-D-lysine (Advanced Biomatrix), viral inactivated human fibronectin (Akron Biotech) and laminin-521 (Biolamina) to start Passage 0 (P0d0), and cultured under 2D conditions for 14 days (until P0d14) using NDM- (feeding every 2-3 days). 2D to 3D to 2D transitions are repeated three times (through P1, P2 and P3, 3-4 days in 3D and 14 days in 2D culture) until P3d14 is reached after 90 days of differentiation. P3d14 cultures are harvested using Accutase (Innovative Cell Technologies) and cultured in ultra-low attachment T75 flasks for 24 hours. Then, P3-spheres are cryopreserved by resuspending in Cryostor CS10 (Stemcell Technologies) and freezing to -80°C, and then transferred to the vapor phase of liquid nitrogen for storage. The cryopreserved P3 spheres are called Cell Stock (CS).

[650] In some embodiments, the PRC manufacturing protocol uses Aggrewells. According to this method, day 19, cultures are “lifted” into suspension culture (2D to 3D) through incubation with Accutase (Innovative Cell Technologies) and seeded on Aggrewell plates (Stemcell Technologies) using NDM minus Noggin (NDM-) medium supplemented with Y-27632 (ROCK inhibitor, Fujifilm Wako) to allow for the formation of neural spheroids (“spheres”). After 24 hours, cells are harvested from Aggrewell plates and transferred to ultra-low attachment T75 flasks where 3D cultures are maintained for an additional 2-3 days using Y-27632-free NDM-. Then, spheres in suspension are seeded (3D to 2D) onto culture vessels coated with poly-D-lysine (Advanced Biomatrix), viral inactivated human fibronectin (Akron Biotech) and laminin-521 (Biolamina) to start Passage 0 (P0d0), and cultured under 2D conditions for 14 days (until P0d14) using NDM- (feeding every 2-3 days). 2D to 3D to 2D transitions are repeated three times (through P1, P2 and P3, 3-4 days in 3D and 14 days in 2D culture) until P3d14 is reached after 90 days of differentiation. P3d14 cultures are harvested using Accutase (Innovative Cell Technologies) and cultured in ultra-low attachment T75 flasks for 24 hours. Then, P3-spheres are cryopreserved by resuspending in Cryostor CS10 (Stemcell Technologies) and freezing to -80°C, and then transferred to the vapor phase of liquid nitrogen for storage. The cryopreserved P3 spheres are called Cell Stock (CS). The PRC composition can be pretreated with sucrose, for example at D14 and P4, before being formulated with cryoprotective agents.

[651] Lastly, the cryopreservation step of the final PRC preparation can include poloxamer 188, a nonionic block linear copolymer and/or sucrose.

EXAMPLE 6: Formulation and Formulated PRC dosing

[652] The culture of cells are cryopreserved and prepared as the composition at the manufacturing site. Cells are harvested at passage 4 day 14 (approximately Day 107 as shown in **FIG. 12C**) using Accutase enzymes and are isolated into a single cell suspension. Harvested cells are then subjected to a wash step to separate live cells from any dead cells and debris generated during the harvest step.

Next, cells are mixed with the cryopreservation buffer and distributed into the final product container. The final composition is then frozen using a controlled rate freezer.

[653] Frozen composition is then sent out to the clinical site, where the PRC composition would be rapidly thawed and diluted using the GS2 Diluent for Subretinal Injection (GS2+). The diluted PRC composition is then mixed before being loaded into a syringe and delivered to the patient via sub-retinal injection.

[654] **FIG. 13** is a schematic demonstrating the manufacturing, reconstitution and injection process flow for formulated photoreceptor rescue cells.

[655] Viability of PRCs tested in different formulations was measured post thaw of P4 (**FIGs. 14A and 14B**). In this experiment, PRCs were cultured through passage 4 day 14 (P4d14), then were harvested and isolated into single cells. The cell suspension was then washed using an OptiPrep™ (STEMCELL Technologies, Inc., Vancouver, Canada) density gradient to separate live and dead cells. The live cells were collected, washed in growth media, and resuspended in 0.5% rHA/DPBS. Cells were then distributed into different cryoprotectant-containing buffer at a volume ratio of 1:2, where each formulation contained a final concentration of 2.5% rHA and 0.6% glucose in DPBS with Ca/Mg, with different concentrations of penetrating and non-penetrating cryoprotective agents, as shown in **FIG. 14B**. For comparison, PRC cells were also formulated in a commercially available CryoStor® CS10 cell freezing medium (STEMCELL Technologies, Inc., Vancouver, Canada), which contains 10% DMSO. Post-thaw cell viability was evaluated following rapid thawing of the cells at 37°C and dilution into GS2+ diluent buffer. Post thaw viability was evaluated using the Trypan Blue cell counting method. The formulation containing 5% DMSO showed similar viability with the formulation of the invention containing 10% DMSO (see **FIG. 14B**), while both formulations showed higher viability than the commercially-available CryoStor® CS10. Top performing formulations were selected for further analysis.

[656] As a follow-up experiment, 6 different formulations were selected for further analysis. Cells were prepared using a similar procedure, and post-thaw analysis was again assessed using Trypan blue exclusion. During this experiment, 10% Ethylene Glycol + 0.2M sucrose had a higher post-thaw viability than the 5% DMSO formulation.

[657] Comparison of OMR response in animals subretinally injected with 100,000 or 200,000 GB2R PRCs (**FIG. 15A**). 12 spatial frequencies (SP) were performed and the OMR response was recorded (tracking strength) in animals subretinally injected with either 100K (left panel) or 200K (middle panel) PRCs. A response of 1.2 on the Y axis is the threshold for OMR presence (shown by green dotted line). Any value below 1.2 means eyes are not able to track. At P35, P42 and P50, control eyes were blind, 200k cell-injected eyes consistently showed larger and broader amplitude curve than 100k cohort. SP threshold right shifted indicates better visual acuity in 200k eyes.

[658] RD10 mice were subretinally injected with 100k or 200k PRCs and OMR was performed at P28, P35, P35, and P50 (**FIG. 15B**).

[659] ICC analysis confirmed ONL thickness in 200k-eyes was greater than that in 100k (FIG. 15C). Quantification of ONL thickness at graft sites vs non-graft sites in animals receiving 100K and 200K cells. The data is consistent with the hypothesis that higher dosing leads to greater efficacy.

[660] OMR was recorded in 300k rd10 mice injected with PRCs in cryopreservative (same as for previous rd10 mice, subretinal injection at P14) (FIG. 16A). PRCs in cryopreservative were functionally efficacious at P35 and P42. But at P49, OMR is absent in both vehicle and PRCs in cryopreservative eyes. OMR analysis of RCS rats injected at P25 with 100,000 cells/eye of GB2R-PRC (CN2 lot); GB2R-PRCs (P3 INT DS); or GB2R-PRCs (PRCs in cryopreservative) (FIG. 16B). Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.) with test articles compared with uninjected and GS2 vehicle injected eyes. ERG analysis of RCS rats injected at P25 with 100,000 cells/eye of GB2R-PRC (CN2 lot); GB2R-PRCs (P3 INT DS); or GB2R-PRCs (PRCs in cryopreservative) (FIG. 16C). Statistical analysis was performed by using 2-way ANOVA with Tukey's multiple comparison test (M.C.T.) with test articles compared with uninjected and GS2 vehicle injected eyes.

EXAMPLE 7: Supplemental Immunosuppressive Drug Therapy (IMT) and Cell Formulation

[661] Previously, exaggerated responses were seen after surgical procedure in NIH-III mice. Supplemental immunosuppressive drug therapy (IMT) was tested to see if exaggerated response could be mitigated. Table 14 below shows GS2 buffer only and different conditions including supplemental IMT. No PRCs were used in the following experiments.

Table 14

Group	Total No. of Mice	Test Article	Supplementary IMT	Age at Dosing	Necropsy (post-inj.)
1	10 (M + F)	5% DMSO cryobuffer/ GS2+ (1:4)	N/A	6-8 wk	1 mo
2	10 (M + F)	5% DMSO cryobuffer/ GS2+ (1:4)	7 Days Dex (IP, 2.5 mg/kg/day)	6-8 wk	1 mo
3	10 (M + F)	5% DMSO cryobuffer/ GS2+ (1:4)	7 Days Dex (IP, 2.5 mg/kg/day); CsA (in drinking water, 300 mg/L*)	6-8 wk	1 mo
4	8 (M + F)	BSS	N/A	6-8 wk	1 mo

[662] **FIGs. 17A and 17B** show ONL after injection of GS2 with or without IMT (e.g., dexamethasone (Dex) and/or cyclosporine (CsA)). GS2 buffer without supplemental IMT displayed least damaged morphology on optical coherence tomography (OCT) overall, including compared to balance salt solution (BSS).

[663] **FIGs. 18A-18C** show ERG responses for each of Groups 1-4 listed above. Supplemental IMT does not significantly affect a-wave, scotopic b-wave, and photopic b-wave levels between each group.

[664] **FIG. 19** retinal morphology of each of Groups 1-4. GFAP and DAPI staining show that each group has similar morphology and the addition of supplementary IMT does not alter retinal morphology.

[665] Further, tests were performed using C57BL/6 mice to investigate GS2 buffer only and final concentration of DMSO. **Table 15** shows the experimental groups.

Table 15

Group	Total No. of Mice	Test Article	Cryobuffer:GS2+ration (for dilution)	Final DMSO %	Age at Dosing	Necropsy (post-inj.)
1	8 (M + F)	GS2+ (lot 9201)	N/A	0	6-8 wk	1 mo
2	8 (M + F)	GS2+ / 5% DMSO cryobuffer	1:04	1%	6-8 wk	1 mo
3	8 (M + F)	GS2+ / 5% DMSO cryobuffer	1:14	0.33%	6-8 wk	1 mo
4	8 (M + F)	BSS	N/A	0	6-8 wk	1 mo

[666] **Table 16** shows the evaluation parameters and intervals of Groups 1-4 studied in **Table 15**.

Table 16: Evaluation Parameters and Intervals

Parameters	Approximate Intervals (Days are Post-Injection)
Mortality/Moribundity	At least once daily
Clinical observation	At least once daily for the first three days after surgery, then three times per week for the remainder of the study
Body weight	Weekly
Pre-dose Eye Inspection	OU: Pre-dose
Eye Examination	OU: Day 28±5, post dosing
Optical Coherence Tomography (OCT)	OD: At day 1±1, 14±3, 28±5 post dosing
Electroretinogram (ERG)	OU: At day 28±5 post dosing
Full necropsy	At termination, day 30±5 post dosing, including noting gross lesions

Tissue Collection	Eyes, Tissues will be fixed in 4% paraformaldehyde and stored at 4°C.
Immunohistochemistry	Eyes

[667] C57BL/6 mice were injected between 6-8 weeks, and the mice were examined after 4 weeks. The GS2 buffer or control buffer was injected monocular. IMT, dexamethasone at 2.5 mg/kg/day was administered for 7 days, and cyclosporine at 300 mg/L was administered from D0 throughout the duration of the experiment.

[668] **FIGs. 20 and 21** show similar retinal morphology between Groups 1-3 compared to control Group 4.

EXAMPLE 8: Evaluation of Cell-Stock Derived PRCs

[669] PCRs were prepared using the protocol described above and in **FIGs. 12A-12C**, and included a freezing step after P3, thaw, and the protocol was completed through P4. Specifically P4 PRCs with a direct/continuous process were compared to P4 PRCs that had been frozen down at the P3 passage step and thawed later to complete the differentiation.

[670] RCS rat eyes received P4 derived via P4(i) (P4(i) refers to PRCs at P4 that were frozen and thawed between P3 and P4; “indirect”) and displayed increased focal retinal disruption at injection site with enhanced intraretinal PRC migration (**FIG. 22**).

[671] **Table 17** shows the experimental design comparing P4(i)(“indirect”) and P4(d) (“direct”) PRC preparations.

Table 17

Group	Total # of Mice Rd10	Test Article	Dose	Dosing volume(ul)	IMT	Age at dosing	Necropsy
Cohort 1							
1	6	P4(d)	126k	1ul	dex +CsA	P14	P50
2	6	P4(i)	118k	1ul	dex +CsA	P14	P50
Cohort 2							
1	10	P4(d)	110k	1ul	dex +CsA	P14	P50
2	10	P4(i)	110k	1ul	dex +CsA	P14	P50

[672] Immunosuppressive regimen included i.p. injection of dexamethasone (5 mg/kg) daily for 7 days post-surgery. Oral cyclosporine A was administered in drinking water (300mg/L), and administered from P21 to the day of sacrifice.

[673] **FIG. 23** shows the ratio of correct versus incorrect eye movement (OMR analysis) comparing the PRC-injected right eye and the left eye control. The dose injected was 110k PRCs as shown for

Cohort 2 in Table 16. P4(i) PRCs showed reduced efficacy compared to P4(d) PRCs at P35, P42, and P49 age.

[674] **FIG. 24** shows intraretinal migration is similar between P4(i) and P4(d) using Cohort 1. However, **FIG. 25** shows enhanced migration into inner plexiform layer (IPL) after treatment with P4(i) PRCs in Cohort 2.

[675] **Table 18** shows Cohort 1 and Cohort 2 to evaluate P4(i) PRCs and investigate increased intraretinal migration/increased focal disruption in rd10 mouse model compared to rats.

Table 18

1st cohort	Test Articles	Direct P4 (P4d)/P4 via cell stock (P4i); CMC Lot 2
	Dose	62.5k/125k
	Status	Complete
	Results Summary (n=4-6)	Histology: Intraretinal migration/injection site disruption similar between P4d/P4i OMR: Low doses poor tracking; high dose: similar between groups, trend of P4d stronger than P4i Ocular Exam: Some increased incidence of vitreal cells/retinal detachment with P4i
2nd cohort	Test Articles	Direct P4 (P4d)/P4 via cell stock (P4i); CMC Lot 3
	Dose	125k
	Status	In-life Complete
	Results Summary (n=10/10)	Histology: <i>Analysis ongoing</i> OMR: P4d tracking is stronger than P4i. Suggests efficacy reduced by cell stock incorporation Ocular Exam: Incidence of vitreal cells/retinal detachment similar between P4d/P4i

[676] **FIGs. 26 and 27** show Cohort 1 and Cohort 2, respectively. P4(i) (P4 vis cell stock) showed reduced efficacy when injected for both Cohorts tested.

EXAMPLE 9: Evaluation of CellSTACK® Vessels for Cell Culture

[677] Rd10 mice were tested with PRCs prepared using PDL-coated T175 Flasks compared to PRCs prepared using CellSTACK® vessels (Corning®) (**FIG. 28**). Control PRC preparation was CMC lot #3. OMR was analyzed for each PRC preparation at P35, P42, and P49 postnatal age. **FIG. 28** represents the following: Uninjected eyes N=10; GS2 eyes N=11; PRC-CTL (CMC lot 3) N=5; CellSTACK N=10; and PDL-precoating N=6.

[678] **FIG. 29** shows eyes injected with PRCs prepared using CellSTACK® vessels compared to PDL-precoated flasks at D7 and D38 (7 days post injection and 38 days post injection, respectively). The graft size in 6 out of 10 eyes was increased when using CellSTACK® PRCs, and one eye had an abnormally large graft when comparing D7 and D38.

[679] Subretinal injections and immunohistochemistry (IHC) were performed as described above. **FIGs. 30-34** shows IHC marker to evaluate engraftment after injection at D38. Markers stained for include: Human Nuclear Antigen (HuNu) (for evaluating engraftment) (**FIGs. 30A and 30B**); Cone Arrestin (CAR) (for evaluating morphological preservation of cones) (**FIGs. 30A and 30B**); DAPI (for evaluating ONL Thickness and preservation of rods/cones); GFAP (for evaluating Müller gliosis, also secondary marker for PRC) (**FIG. 31**); IBA1 (for evaluating microglia/macrophage) (**FIG. 32**); Ki67 (for evaluating proliferation) (**FIG. 33**); and Oct4 (for evaluating pluripotency) (**FIG. 34**). Eyes were harvested at P52. All three groups, including PRCs prepared using PDL-precoated flasks, CellSTACK®, and the control, showed engraftment. CellSTACK® PRCs had induced noticeably larger grafts compared to either PDL-precoated flask PRCs or control PRCs (**FIGs. 30A and 30B**).

[680] Arrows in **FIG. 30B** are pointing to examples of good cone preservation. Examples of preserved cone processes were more frequent when treated with CellSTACK® PRCs. Examples where ONL is 3-5+ layers thick across wide region more frequent in CellSTACK® group.

[681] **FIG. 31** shows IHC of GFAP in each test group. PRCs continue to express GFAP and show Müller glia activation.

[682] **FIG. 32** shows IBA staining in each test group. The CellSTACK® showed slightly higher IBA infiltration compared to the other groups. Arrows show microglial infiltration.

[683] **FIG. 33** shows Ki67 levels in each of the three groups. Higher levels of Ki67 was found in the CellSTACK® group. However, Ki67+ cells are a minority, suggesting limited proliferation over time.

[684] **FIG. 34** shows PRCs from all three groups are negative for the pluripotent marker Oct4.

Example 10: Delayed Injection of PRCs

[685] A Delayed injection study was performed in RCS rats to demonstrate that the PRCs can have activity after the onset of degeneration. Conclusions from this study: 1) As typical with a neuroprotective approach, early intervention is best, however 2) late intervention can still evoke efficacy, even after the onset of disease.

[686] **FIG. 35** shows a schematic of the experimental design for delayed injection assays. Rats were injected at P25, P45, and P60 after disease onset. OMR and ERG readings were taken between P60 and P150, after which the eyes were harvested for IHC.

[687] **FIG. 36A** shows OMR readings taken at P60 (note for **FIG. 36A** injected eyes were analyzed at P62), P90, P120, and P190. OMR readings taken on eyes that had been injected the earliest, at P25, showed steady threshold response over the timecourse. Whereas, eyes which were injected at the later timepoint of P60 showed reduced threshold response over the timecourse, but higher threshold response compared to negative controls.

[688] **FIG. 36B** shows ERG readings taken at P60, P90, P120, and P150. Delayed injection does not result in detectable preservation of ERG. Injection at P25 resulted in detectable levels of ERG that

decreased over the timecourse. Preservation of photoreceptors by later injections could be below detection by ERG.

[689] **FIG. 37** shows IHC of engraftment and preservation. Quantification of engraftment and preservation is shown in **FIGs. 38A-39B**. Engraftment is calculated by the following formula:

$$\text{Engraftment} = \text{length of subretinal graft in section} / \text{length of retina}$$

[690] ONL Preservation is calculated by the following formula:

$$\text{ONL Preservation} = \text{length of ONL in section} / \text{length of retina}$$

Example 11: Low Viability Studies rd10 Mouse Model

Summary

[691] This study was performed to evaluate the efficacy of low viable ($\leq 60\%$) PRC drug product (Group 1) against high viable ($\geq 70\%$) PRC drug product (Group 2) in the rd10 mouse model.

[692] Additionally, some data was obtained to evaluate the safety of a low viable PRC drug product. Early on, a higher than anticipated attrition was observed within the first few days of the study, which was considered a result of the perioperative analgesic in the juvenile mice and affected both groups evenly (each group lost 3 mice). Through the in-life portion of the study, aside from an initial slow growth, particularly after weaning from P21 to P28, body weights increased steadily across both treatment groups, and no major welfare complications were reported. Upon examination, some treated eyes from both groups displayed instances of increased cells in the vitreous or retinal detachment, but no other complications across the 17 parameters evaluated. The frequency of complications was similar in both treated groups. On the optomotor response (OMR) assay, while vehicle-treated or untreated eyes displayed marked reduction and loss of tracking over time, tracking in cell-treated eyes from both groups through P49 resulted in either statistically significant retention of tracking or a nonsignificant but clear tracking function, reflected by an increase in tracking magnitude at 0.1-0.2 cycles/degree with drop-off to baseline on either side of the peak. Upon necropsy, no major abnormalities were observed, and aside from a slight increase in thymus weight in Group 1, the weights of various major organs were similar between groups and compared to naïve mice. Upon immunohistochemistry assessment, engraftment was confirmed by anti-Human Nuclear Antigen (HNA) staining in both groups. The rate of engraftment was similar between cell-injected groups, with 2 of 4 eyes for Group 1 and 3 of 4 eyes from Group 2 displaying HNA+ cell grafts. The injection of PRCs was associated with nonsignificant trends toward enrichment in elongate cone morphology when staining for cone arrestin, or thickened ONL when evaluating the DAPI stain. The intensity of GFAP staining in Muller glia was significant in all study eyes, consistent with the phenotype of this model and the retinal degeneration.

[693] The data obtained in this study supports the hypothesis that PRC drug product with higher than 70% viability and PRC drug product with less than 60% viability (i.e. 50-60%) are similarly efficacious. Additional safety data collected also support the safety of the product at either viability

level. Due to the higher than anticipated perioperative attrition, statistical significance was not observed in many evaluation parameters, but overall, the observed trends are consistent with the stated conclusions.

Reference PRCs and Test PRCs

[694] *Reference Article: Preparation of GS2+/cryobuffer injection mixture.* Cryobuffer was added to the GS2+ medium at a 1:4 ratio to generate the injection mixture. The GS2+/cryobuffer injection mixture was utilized as diluent for the Test Articles (ASP1819/PRC) as well as the vehicle control for injections in this study.

[695] *Test Articles: Preparation of ASP1819/PRC Cells.* Cryopreserved ASP1819/PRCs were stored in the vapor phase of a liquid nitrogen (LN2) storage tank at temperatures $\leq -135^{\circ}\text{C}$ until the day of transplantation. The ASP1819/PRC cells were thawed and diluted with GS2+ medium. Reconstituted cell suspensions were stored at $2-8^{\circ}\text{C}$ and assessed for viable cell number. After concentration to the target cell density (approximately 125,000 live cells/ μL) and viability (approximately 70% or $\leq 60\%$) in GS2+/cryobuffer injection mixture, the test articles were delivered for injection within ~4 hours of being concentrated.

Animal System

[696] Species: Mouse

[697] Strain: Pde6brd10 homozygous (C57BL6/J background)

[698] Sex: M and F

[699] Age Range: P14-15 days at dosing

[700] Age at tissue harvest: P51, P52

[701] Weight Range: Approximately 6-12 g at dosing

[702] Source: rd10 mouse colony at AIRM, originally The Jackson Laboratory (#004297)

[703] Number of Study Animals: 14 (Group 1: 2M, 5F, Group 2: 1M, 6F)

Cell preparation and handling

[704] Cells were thawed from frozen vials to generate the test articles for Groups 1 and 2. Cells were manually counted using a hemocytometer and trypan blue exclusion. To engineer specific cell viabilities for Groups 1 and 2, the initial cell suspension was subjected to Optiprep, an iodixanol-based density gradient, to isolate dead/live cells. The appropriate amount of dead and live cells were then combined to generate the test articles for injection. The details of cell preparation are specified in the batch records.

Study Design

[705] The cohort of 14 rd10 mice was randomized to two dose groups as described in **Table 18**. All mice received subretinal injection of 1 μL PRC cells (either $\geq 70\%$ or $\leq 60\%$ viability) in the right eye (OD) via transcleral route of administration under anesthesia. The left eye (OS) was either injected with the vehicle solution, or remained uninjected. From the injection date through the terminal sacrifice, animals were evaluated as indicated in **Table 19**. Analyses compared measurements

between the 4 groups of eyes ($\geq 70\%$ PRC, $\leq 60\%$ PRC, Vehicle, Uninjected). Mice were euthanized at day 37 ± 3 after surgery, or roughly at age P50.

Table 18: Experimental Study Design

Group	Number of Mice	Target Number of cells (1 μ L volume)	Right Eye (OD)	Left Eye (OS)
1	7 (M or F)	125,000	Injection with $\geq 70\%$ viable cells (70% buffer)	Vehicle buffer (3), or No injection (4)
2	7 (M or F)	125,000	Injection with $\leq 60\%$ viable cells (60% buffer)	Vehicle buffer (4), or No injection (3)

Table 19: Evaluation Parameters and Intervals

Parameters	Approximate Intervals (Days are Post-Injection)
Mortality/Moribundity	At least once daily
Clinical observation	At least once daily for the first three days after surgery, then three times per week for the remainder of the study
Body weight	Weekly
Pre-dose Eye Inspection	OU: Pre-dose (Day 0), for gross abnormalities
Eye Examination	OU: Day 35 ± 3 post dosing
Optomotor Response (OMR)	OU: At day 14 ± 3 , 21 ± 3 , 28 ± 3 , and 35 ± 3 post dosing
Optical Coherence Tomography (OCT)	OU: At day 1 ± 1 , 7 ± 3 , and 31 ± 3 post dosing
Full necropsy	At termination, day 37 ± 3 post dosing, including noting gross lesions
Tissue Collection	Eyes, optic nerves, brain, mandibular lymph nodes, heart, liver, kidneys, spleen, lungs, ovaries, testes, thymus
Organ Weights	Brain, heart, liver, kidneys, spleen, lungs, ovaries, testes,
Immunohistochemistry	Eyes

Immunosuppressive protocol

[706] An intraperitoneal injection of dexamethasone (2.5 mg/kg/day) was administered once daily for 7-8 days after surgery (from ~P14-21). Once weaned at ~P21, animals were maintained on oral cyclosporine A (CsA) administered in the drinking water (300 mg/L) for the remainder of the study until euthanasia.

Subretinal Injections

[707] The dosing apparatus, micro syringes (Hamilton 600 series) with 34 ga blunt needles (Hamilton #207434), was prefilled with cell suspension or vehicle prior to the surgery. Test animals

were administered ketamine/xylazine cocktail for anesthesia, and Ethiqs for pre-operative analgesia. When a test animal achieved deep anesthesia indicated by slowed breathing and lack of response to a toe pinch, it was placed on its side under a dissecting microscope so that the eye to receive the procedure was positioned up. The skin above and below the eye was pulled back so that the eye proptoses. Betadine eye drops (5%, sterile ophthalmic grade) were administered to the eye for topical disinfection of the globe, and artificial tears were applied to rinse the excess betadine from the eye. Proparacaine eye drops were applied to numb the eye and surrounding tissue, as well as phenylephrine (2.5%) and tropicamide eye drops to dilate the pupils. In between eyedrop/artificial tear applications, excess solution was removed using a fabric-tipped swab. For a subretinal injection, a hole was cut in the conjunctiva to expose the sclera. Using a 30 ga beveled needle, a pilot hole was made on the limbal area. The micro syringe with 34 ga needle containing cell suspension or vehicle buffer was inserted into the hole under visual guidance and injection material is ejected into the eye via manual depression of the plunger. Following the injection, the needle was slowly removed from the eye, and the animal was placed on the opposite side to perform the injection procedures on the second eye (if applicable, for Vehicle injections). Immediately after injection procedures, OCT imaging was performed to evaluate injection success. After imaging, erythromycin (0.5%) ophthalmic ointment was applied to the surgical site for both anti-microbial and lubricating functions. Antisedan (atipamezole, 2 mg/kg) is administered IP to aid in the recovery process. Subcutaneous saline was administered for hydration. Finally, the animal was moved to a heated cage to prevent hypothermia until sternal recumbency, and subsequent return to its home cage.

Optomotor Response (OMR)

[708] The optomotor response was measured using the Phenosys qOMR system. Head tracking responses to drifting gratings of varying spatial frequencies (0.05-0.6 cycles/degree, in 0.05 cycles/degree increments) was measured for all animals twice on separate days at 4 timepoints (Days 14±3, 21±3, 28±3, and 35±3 post surgery), and values were averaged to generate OMR response curves. Mice were placed unrestrained on an elevated platform in the center of an arena enclosed on 4 sides by monitors to simulate the presentation of a rotating cylinder with vertical sine wave gratings. The ratio of time spent with head movements in the preferred direction, over that in the non-preferred direction, was calculated, and the value of that ratio was considered the strength of tracking. Averaged values greater than 1.2 indicated the presence of tracking at that spatial frequency. Prior to the first timepoint collection (once 1-7 days prior), animals will be introduced to the apparatus for acclimation.

Optical Coherence Tomography (OCT)

[709] Spectral domain optical coherence tomography (SD-OCT) using a Leica Bioptigen Envisu system was performed on Days 1±3, 7±3, and 31±3 post surgery. Mice were anesthetized via IP injection or inhalation of anesthetic agents. Phenylephrine and tropicamide were applied to eyes to dilate pupils for better imaging of the retina. Lubricating eye drops (Gentel) were applied to maintain ocular clarity for imaging. B-scans were acquired using the imaging system at the injection site to

visualize the cell graft. At the end of imaging sessions, mice were moved to a heated cage to prevent hypothermia until sternal recumbency, and subsequent return to their home cages.

Immunohistochemistry

[710] For animals undergoing scheduled euthanasia at the terminal timepoint, double immunofluorescence staining was performed on slides prepared from all PRC-injected eyes using anti-Human Nuclear Antigen (HNA) staining to confirm PRC engraftment, and anti-cone arrestin (CAR), to evaluate cone preservation and measurement of cone axons. Additionally, analysis of outer nuclear layer (ONL) thickness was performed using DAPI to evaluate overall photoreceptor preservation. Quantification of cone axons or ONL thickness was performed blinded to sample identity. For vehicle-injected or uninjected eyes, CAR-staining and ONL thickness analyses were performed, but HNA-staining was omitted. Immunostaining for GFAP was performed to evaluate Muller gliosis and confirm PRC identity.

Cell Count and Viability

[711] Cells were counted and viability was measured as indicated in the batch record. Final counts and viabilities are indicated below in **Table 20**.

Table 20: Cell Counts and Viability

Group Number	Target Viable Cell Count (cells/ μ L)	Sample Cell Count (cells/ μ L)	Target Viability	Sample Viability
1	112,500-137,300	125,000*	$\geq 70\%$	68.4%#
2	112,500-137,300	125,000*	53-57%	53.5%

*Adjusted to value after final count by addition of GS2 buffer, confirmatory count was not performed to conserve cells. #Due to challenges of generating the $\geq 70\%$ viable preparation, this viability was deemed acceptable on the day of surgery.

Clinical Observation and Body Weight

[712] No abnormal clinical signs of distress were observed during the course of the study. After an initial slow period of growth during the first two weeks post-injection, body weight steadily increased in all surviving mice through the remainder of the study. No significant body weight differences were observed between Groups 1 and 2 at any timepoint.

Eye Examination

[713] A 17 point eye examination was performed (**Table 21**). No abnormalities were observed in the ocular surface or anterior segment. Two cell-injected eyes from Group 1 displayed cells in the vitreous, with one displaying a mild degree (11-20 cells), and another displaying a mild degree, but slightly more cells (21-30). In one cell-injected eye from Group 2, there was a mild degree of cells observed in the vitreous (11-20 cells).

[714] Retinal detachments were observed in both groups in cell-treated eyes. In Group 1, a moderate detachment was observed in one eye that also displayed a retinal tear at the graft site. In Group 2, two eyes displayed large detachments, and a third eye displayed a small detachment.

[715] While the presence of these complications was restricted to cell-treated eyes, complications were similarly frequent in either 60% viable or 70% viable groups. Additionally, retinal detachments are a known phenotype of rd10 mice (Pennesi et al., 2012, IOVS).

Table 21: Scores for Vitreous Cells and Retinal Detachment from Eye Examination. Eyes displaying abnormalities are indicated in red font. Data for full eye examination

Group (OD)	Animal	Sex	OS	Vitreous Cell (OS/OD)	Retinal Detachment (OS/OD)
1 (≥70%)	1	F	Veh	0/0	0/0
	6	F	Uninjected	0/2	0/0
	9	F	Uninjected	0/0	0/0
	10	F	Uninjected	0/1	0/2
2 (≤60%)	2	M	Uninjected	0/1	0/1
	3	F	Veh	0/0	0/0
	5	F	Veh	0/0	0/3
	8	F	Uninjected	0/0	0/3

Optomotor Response (OMR)

[716] The optomotor response assay was performed at postnatal ages P28, P35, P42 and P49 (**FIGs. 40A -40D**). For cell-injected eyes (70% or 60% viable eyes), there was a noticeable tracking response at all four timepoints evaluated, observed as an inverted U-shaped curve centered around 0.1-0.2 cycles per degree of spatial frequency. Meanwhile in vehicle, tracking was either weak (low-amplitude) or non-existent. The only significantly different values observed after statistical analysis were at P35 (*p=0.0376, 70% + buffer vs. buffer+Vehicle; *p=0.0345, 70% + buffer vs. uninjected) and at P49 (*p=0.0446, 70% +buffer vs. Buffer+ vehicle). No statistical significance was observed in the cell-treated eyes between the experimental groups at any timepoint.

Optical Coherence Tomography (OCT)

[717] OCT was performed on D0, 9, and 35 (**FIGs. 41A-41F**). D0 OCT confirmed subretinal bleb formation in right eyes from Group 1, except for animal 11-F, which was ultimately found dead on D1. For Group 2, D0 OCT confirmed subretinal bleb formation in all eyes in that group. On D9, OCT confirmed subretinal material persisting in all of 4 right eyes from surviving mice in Group 1, and all of 4 right eyes from surviving mice in Group 2. Mouse #8 from group 2 displayed a large retinal detachment at D9, that persisted through D35, at which point the graft appeared to round up. By D35, OCT confirmed varying sizes of subretinal material across both cell-treated groups.

Necropsy and Organ Weights

[718] Necropsy was performed at the terminal timepoint, on Day 38. No abnormalities were observed. Weights from brains, hearts, livers, kidneys, spleens, lungs, thymus, ovaries and testes were taken. Outside of a decrease in thymus weight between Groups 1 and 2 (0.081 ± 0.012 g (Grp. 1) vs. 0.0447 ± 0.0085 g (Grp. 2)), no other organs were statistically different between experimental groups. Values were similar compared to those from a naïve NIH-III mouse (data not shown).

*Immunohistochemistry**Human Nuclear Antigen (HNA)*

[719] HNA immunostaining was performed to confirm engraftment of administered PRCs. From cell treated eyes in Group 1, 2 of 4 eyes displayed engrafted cells. One of those eyes displayed a large number of cells (**FIG. 42A, Group 1**), while the other contained a small graft. In Group 2, amongst cell-treated eyes, 3 out of 4 eyes displayed engrafted cells. Two grafts were large in size, while that in the 3rd eye was small. In one eye with a large graft from Group 2, the graft appeared as a round ball of cells in the subretinal space within what appeared to be a large retinal detachment (**FIG. 42A, Group 2**).

Cone Arrestin (CAR)

[720] CAR immunostaining was performed to evaluate preservation of cones. In both experimental groups, examples of elongate cone morphology can be observed adjacent to PRC graft sites, indicating focal cone preservation proximal to PRC grafts can occur when PRCs are injected at high and low viabilities (**FIGs. 43A and 43B**, vs. **FIG. 43C**). In eyes injected with vehicle or left uninjected, the overall morphology of the cones was exclusively a flattened monolayer composed of short stubby cells (**FIG. 43D**). While the quantification of the cone axon length did not unveil significant differences between the groups, the one-way ANOVA was significant at $p < 0.05$, and there was a trend of increased cone axon length in cell-treated eyes compared to controls, suggesting a protective effect over cone morphology by PRC administration at either viability.

Quantification of Outer Nuclear Layer (ONL)

[721] Using DAPI to visualize the ONL, the thickness of the ONL was measured blinded at arbitrary sites proximal to the graft. No statistical significance was observed, but there was a trend in increased ONL thickness in both cell-treated groups compared to uninjected or vehicle-injected controls (**FIG. 44**).

Glial Acidic Fibrillary Protein (GFAP)

[723] GFAP staining was performed to evaluate Muller gliosis in retinas (**FIG. 45**). The intensity of GFAP staining in Muller glia was significant in all eyes, expressed as vertical bands spanning the thickness of the retina, consistent with the rd10 mouse model at the evaluated age (~P50). The persistence of GFAP immunoreactivity in Muller glia following PRC treatment suggests that host retinas continue to experience some degree of disease. Engrafted PRCs also expressed GFAP at a high level, consistent with previous observations after in vivo administration.

Conclusion

[724] The data obtained in this study supports the hypothesis that PRC drug product with higher than 70% viability and PRC drug product with less than 60% viability (i.e. 50-60%) are similarly efficacious. Additional safety data collected also support the safety of the product at either viability level.

Example 12: Low Viability Studies NIH-III Mouse Model

[725] This study was performed to evaluate the safety of low viable (<60%) PRC drug product against high viable (>70%) PRC drug product in the immunodeficient NIH-III mouse model. Aside from one death in Group 3 (<60%) associated with anesthesia during ERG recording, no other deaths occurred during the study. Through the in-life portion of the study, body weights increased steadily across groups, and no major welfare complications were reported. Upon ophthalmic examination, some treated eyes from all three injected groups (Groups 1-3) displayed instances of vitreal hemorrhaging, which was attributed to surgical complications. Additionally, within injected groups (Groups 2 and 3), some treated eyes exhibited cells in the vitreous, although the frequency was similar in both Group 2 (high-viable) and Group 3 (low-viable) groups. Aside from those observations, no other complications across the 17 parameters evaluated.

[726] OCT imaging at Day 28 identified pockets of retinal damage at injection centers in Groups 1-3, likely associated with the surgical procedure. Subretinal material of varying sizes were also identified at injection sites in Groups 1-3 on OCT. The observation of this subretinal material in the vehicle-injected group (Group 1) suggests the tendency of host cells, possibly immune cells, to infiltrate the injection site in this model after the procedure. Electroretinogram (ERG) recordings taken approximately at Day 30 did not reveal significant differences between study groups, suggesting no global effects over retinal function after administration of the cells or vehicle buffer. The ERG result is consistent with the ophthalmic observation and OCT imaging, which similarly did not identify global effects. No adverse findings were observed upon necropsy at 36 days post-administration, and organ weights were similar across all four groups. Hematology and clinical chemistry analyses did not reveal any major differences between groups.

[727] Upon histological examination of tissues harvested on Day 36, subretinally located GFAP immunostaining with distinctive PRC morphology (non-Muller glial morphology) was observed in 1 sample from Group 2 and 2 samples in Group 3. However, nuclear anti-HNA staining was not observed in any sample, and therefore, human cells could not be confirmed. At injection sites in Groups 1-3, focal Muller glial GFAP immunoreactivity and limited retinal laminar disruption displayed at a similar level across all three groups was consistent with surgery-induced injection site damage. Additionally, similar levels of elevated Iba1 immunoreactivity was detected at the injection sites within Groups 1-3. Lastly, CD45, Bestrophin-1, and Ter119 staining was also performed to

interrogate the composition of the subretinal grafts/cell masses, but none of those markers were appreciably expressed in the subretinal graft.

[728] Altogether, the data obtained in this study support the safety of a PRC drug product with <60% viability (i.e. 50-60%), when compared against PRCs with >70% viability.

Test Animals

[729] Species: Mouse

[730] Strain: NIH-III nude mouse

[731] Sex: M and F

[732] Age Range: 6-8 weeks at dosing

[733] Weight Range: Approximately 18-33 g at dosing

[734] Source: Charles River Laboratories (Strain Code: 201, homozygous)

[735] Number of Study Animals: 36

[736] *Study Design*

[737] Thirty-six NIH-III nude mice were randomized to four groups as described in **Table 22**. To facilitate cell preparation and injection procedures, mice were separated into two injection cohorts on consecutive days, with all cell-injected animals injected in the first cohort due to the complexity of the cell preparation with targeted viabilities. Mice in Groups 1-3 received subretinal injection of 1.5 μ L vehicle or PRC in the right eye (OD) via transcleral route of administration under anesthesia. The left eye (OS) remained uninjected. Mice in Group 4 remained uninjected. From the injection date through the terminal sacrifice, animals were evaluated as indicated in **Table 23**. Analyses compared measurements between the 4 groups. Mice were euthanized approximately 5 weeks after injection.

Table 22: Experimental Study Design

Group	Total No. of Mice	Test Article	Target Viability	Dose (# of cells)	Dosing Volume (μ L)	Injected Cell Density (cells/ μ L)	Age at Dosing	Necropsy (post-inj.)
1	10 (4M + 6F)	Vehicle	N/A	N/A	1.5	N/A	6-8 wk	5 wk
2	10 (4M + 6F)	PRC (>70% Viability)	70-80%	125k	1.5	83,333	6-8 wk	5 wk
3	10 (4M + 6F)	PRC (<60% Viability)	50-60%	125k	1.5	83,333	6-8 wk	5 wk
4	6 (1M + 5F)	N/A (Naïve)	N/A	N/A	N/A	N/A	N/A (age-matched)	5 wk

Table 23: Evaluation Parameters and Intervals

Parameters	Approximate Intervals (Days are Post-Injection)
Mortality/Moribundity	At least once daily

Clinical observation	At least once daily for the first three days after surgery, then three times per week for the remainder of the study
Body weight	Weekly
Pre-dose Eye Inspection	OU: Pre-dose (Day 0), for gross abnormalities
Eye Examination	OU: Day 30±3, post dosing
Optical Coherence Tomography (OCT)	OD: At day 1±1, 14±3, 30±3 post dosing
Electroretinogram (ERG)	OU: At day 30±3 post dosing
Clinical Pathology	At termination, day 35±3 post dosing
Full necropsy	At termination, day 35±3 post dosing, including noting gross lesions
Tissue Collection	Eyes, optic nerves. Brain, mandibular lymph nodes, heart, liver, kidneys, spleen, lungs, ovaries, testes, thymus, or any organ with gross lesions, will be preserved tissues for archival purposes. Tissues will be fixed in 4% paraformaldehyde and stored at 4°C. Use for archive purposes. Tissues will be provided to Sponsor.
Organ Weights	Brain, heart, liver, kidneys, spleen, lungs, ovaries, testes, thymus
Immunohistochemistry	Eyes

Subretinal Injections

[738] The dosing apparatus, micro syringes (Hamilton 600 series) with 34 ga blunt needles (Hamilton #207434), was prefilled with cell suspension or vehicle prior to the surgery. Test animals were administered ketamine/xylazine cocktail. When a test animal achieved deep anesthesia indicated by slowed breathing and lack of response to a toe pinch, it was placed on its side under a dissecting microscope so that the eye to receive the procedure was positioned up. The skin above and below the eye was pulled back so that the eye proptoses. Betadine eye drops (5%, sterile ophthalmic grade) were administered to the eye for topical disinfection of the globe, and artificial tears were applied to rinse the excess betadine from the eye. Proparacaine eye drops were applied to numb the eye and surrounding tissue, as well as phenylephrine (2.5%) and tropicamide eye drops to dilate the pupils. In between eyedrop/artificial tear applications, excess solution was removed using a fabric-tipped swab. For a subretinal injection, a hole was cut in the conjunctiva to expose the sclera. Using a 30 ga beveled needle, a pilot hole was made on the limbal area. The micro syringe with 34 ga needle containing cell suspension or vehicle buffer was inserted into the hole under visual guidance and injection material is ejected into the eye via manual depression of the plunger. Following the injection, the needle was slowly removed from the eye. Immediately after injection procedures, OCT imaging was performed to evaluate injection success. After imaging, erythromycin (0.5%) ophthalmic ointment was applied to the surgical site for both anti-microbial and lubricating functions. Antisedan (atipamezole, 2 mg/kg) is administered IP to aid in the recovery process. Subcutaneous saline was

administered for hydration. Finally, the animal was moved to a heated cage to prevent hypothermia until sternal recumbency, and subsequent return to its home cage.

Eye Examination

[739] The Scientific Lead performed eye examinations at 30 ± 3 days post-surgery using the Phoenix Micron IV Imaging Microscope. Eyes were dilated for examination using 1% tropicamide and/or 2.5% phenylephrine, instilled as 1 drop each/eye. Under anesthesia, the anterior (cornea and lens) and posterior (retina, vitreous) segments were examined, and checked for pupillary response, conjunctival discharge, conjunctival congestion, conjunctival swelling, cornea, surface area of cornea involvement, pannus, aqueous flare, aqueous cell, iris involvement, lens, vitreal flare, vitreal cells, vitreal hemorrhage, retinal detachment, retinal hemorrhage, and choroidal/retinal Inflammation. All abnormalities were recorded by the examiner.

Optical Coherence Tomography (OCT)

[740] Spectral domain optical coherence tomography (SD-OCT) using a Leica Bioptigen Envisu system was performed on Days 1 ± 3 , 14 ± 3 , and 30 ± 3 post surgery. Mice were anesthetized via IP injection or inhalation of anesthetic agents. Phenylephrine and tropicamide were applied to eyes to dilate pupils for better imaging of the retina. Lubricating eye drops (Genteal) were applied to maintain ocular clarity for imaging. B-scans were acquired using the imaging system at the injection site to visualize the cell graft. At the end of imaging sessions, mice were moved to a heated cage to prevent hypothermia until sternal recumbency, and subsequent return to their home cages.

Electroretinogram (ERG)

[741] ERG was performed on Day 30 ± 3 post surgery. Animals were kept in complete darkness overnight (at least 12 hours) for dark adaptation. Throughout recordings, animals were anesthetized by isoflurane at the recording platform, or were anesthetized by IP injection of ketamine/xylazine. Experimental procedures were performed under dim red illumination. Phenylephrine (2.5%) and tropicamide (1%) drops were applied to dilate the pupils, while hypromellose (0.3%) drops were applied for lubrication. After eyedrop application, the recording electrode contact lens (LKC) were placed on the animal's eyes. Full field illumination was delivered by LED or xenon bulb flashes via a Ganzfeld stimulator, and ERG responses were amplified, digitized, and recorded via the UTAS Bigshot ERG system (LKC). Responses were averaged depending on the stimulus intensity (3-20 stimulus presentations) to account for noise in the signal. Subcutaneous saline was administered for hydration (optional). Finally, the animal was moved to a heated cage to prevent hypothermia until sternal recumbency, and subsequent return to its home cage. Antisedan (atipamezole, 2 mg/kg) was administered IP to aid in the recovery process if ketamine/xylazine was used for anesthesia.

Immunohistochemistry

[742] For animals undergoing scheduled euthanasia at the terminal timepoint, immunofluorescence staining was performed on slides prepared from all PRC-injected eyes using anti-Human Nuclear Antigen (HNA) staining to confirm human origin of cell grafts. Vehicle-injected or uninjected eyes

may be used as controls. Additionally, all samples will be evaluated using anti-GFAP and anti-Iba1 staining, to evaluate Muller gliosis and microglial/macrophage infiltration, respectively. Some or all eyes will be evaluated for the presence of cells of hematopoietic origin using anti-CD45 staining, for presence of erythrocytes using anti-TER119 staining, and for the presence of retinal pigment epithelium using anti-Bestrophin staining.

Results

Cell Count and Viability

[743] Cells were counted and viability was measured as indicated in the batch records. Final counts and viabilities are indicated below in **Table 24**.

Table 24

Group Number	Target Viable Cell Count (cells/ μ L)	Sample Cell Count (cells/ μ L)	Dose in 1.5 μ L (Viable Count)	Target Viability	Sample Viability
2 (>70%)	75,000-91.666	75,250	112,875	$\geq 70\%$	75.9%
3 (<60%)	75,000-91.666	75,500	113,250	53-60%	58.0%
2 (>70%)	75,000-91.666	75,375	113,063	$\geq 70\%$	73.7%
3 (<60%)	75,000-91.666	76,125	114,188	53-60%	58.9%

Eye Examination

[744] A 17 point eye examination was performed on Day 28 (**Table 25**). No abnormalities were observed in the ocular surface or anterior segment. Two cell-injected eyes from Group 2 displayed a moderate degree of cells in the vitreous (~100 or >100). Meanwhile, in one cell-injected eye from Group 3, there was a moderate degree of cells observed in the vitreous (~100 cells).

[745] Vitreal hemorrhaging was observed in 4 injected eyes across Groups 1-3. Two examples were found in Group 1, with hemorrhages covering 10-20% of the fundus image. In Group 2, 1 example was found with ~10% coverage of the fundus image. In Group 3, 1 example was found with ~5% coverage of the fundus image by the vitreal hemorrhage.

[746] Aside from surgery-induced lesions, no additional retinal abnormalities were observed.

[747] While the presence of vitreal cells was restricted to cell-treated eyes, complications were similarly frequent in either 60% viable or 70% viable groups. With vitreal hemorrhages, due to the observation within the vehicle-injected group (Group 1), it appeared that this may be a risk caused by the subretinal injection procedure/surgery in the NIH-III strain, with no additional risk posed by administration of either the high or low viable PRC drug product.

[748] **Table 25** shows values are indicated as the number of mice with observed complications over the total number of animals in the group. Groups with animals displaying abnormalities are indicated in bold font.

Table 25: Scores for Vitreous Cells and Vitreal Hemorrhage from Eye Examination.

Group	Vitreous Cell	Vitreal Hemorrhage
1 (Veh)	0/10	2/10
2 (>70%)	2/10	1/10
3 (<60%)	1/10	1/10
4 (Naïve)	0/6	0/6

Optical Coherence Tomography (OCT)

[749] OCT was performed on D0, 14, and 30. D0 OCT confirmed subretinal bleb formation in right eyes from all mice in Groups 1-3. In a few eyes across all three groups, some hyperreflective material was detected in the vitreous, suggesting either partial misinjection into the vitreous or possibly the presence of bleeding associated with the surgery.

[750] In D14 OCT images from Groups 1-3, the resorption of the blebs is observed across all injected mice. A degree of focal surgical damage is observed in all vehicle- or cell-injected eyes, which is characterized by thinning of the retina across a limited retinal surface area, particularly of the outer nuclear layer (ONL). In Groups 2-3, some subretinal hyperfluorescent material is observed (either small/thin or large in size), consistent with the injection of PRCs. Unexpectedly, in Group 1, varying amounts of subretinal material are observed as well, suggesting that the injection of the vehicle buffer resulted in the subretinal accumulation of host cells, possibly of immune origin. Additional evidence for inflammation was the observation of scattered hyperfluorescence in the vitreous in roughly half of the eyes across Groups 1-3. In two eyes in Group 1, the lamination of the retina was absent at the injection site, and the increased thickness of the retina in one of those eyes suggested a degree of edema.

[751] By D30, observations made at D14 were generally also observed at the final OCT timepoint, particularly with regard to focal retinal thinning, subretinal material accumulation, and intravitreal hyperfluorescence. In the two eyes with retinal delamination in Group 1 from D14, at D30, those portions of the retina displayed severe focal atrophy with thick fibrous morphology suggestive of scarring.

[752] In total, OCT imaging revealed a degree of surgical damage is incurred by the retina in the NIH-III model, associated with what is likely the infiltration of immune cells despite the model's immune deficiency. The observation of these anomalies in Group 1 eyes, injected with the vehicle buffer, being as severe or in some cases more so than those made for Groups 2 and 3, support the

safety of the cells within the injected drug project, at either cell viability (>70% or <60%). Data shown in **FIGs. 46A-46J**.

Electroretinogram (ERG)

[753] Full field ERGs were performed on both eyes in all study animals at ~D30 under scotopic and photopic conditions. Comparisons of treated right eyes (Groups 1-3) were made against contralateral uninjected eyes, as well as against the right eyes from all other groups, including those of the naïve Group 4 (**FIGs. 47A-47C**).

[754] For scotopic recordings (a- or b-wave), there were no statistically significant differences, either between responses of left vs. right eyes, or right eyes against those of other groups.

[755] For photopic recordings, Groups 1 and 2 displayed statistically significant reductions in the treated eye against the uninjected contralateral eye (~20-25%). However, against all other right eyes across Groups 1-4, there were no statistically significant differences.

[756] Altogether, the ERG recordings did not reveal systematic effects of any injected test articles over the amplitude of ERG responses.

Necropsy and Organ Weights

[757] Necropsy was performed at the terminal timepoint, on Day 36. No abnormalities were observed. Weights from brains, hearts, livers, kidneys, spleens, lungs, thymus, ovaries and testes were taken. No organ weights were statistically different between experimental groups (data not shown).

Immunohistochemistry

Human Nuclear Antigen (HNA)

[758] HNA immunostaining was performed to confirm engraftment of administered PRCs in Groups 2 and 3. Additionally, samples were stained in Group 1 for comparison/control.

[759] Across Groups 1-3, no appreciable positive nuclear staining for HNA was observed, indicated by lack of DAPI co-localization (**FIGs. 48A-48F**). However, in 1 sample from Group 2 and 2 samples from Group 3, cell-sized autofluorescent structures were observed within subretinal masses consistent with the morphology of subretinal PRC grafts from past studies in retinal degeneration mice. Despite the lack of nuclear HNA staining, based on their morphology and pattern of GFAP expression, we suspected that these grafts in Groups 2 and 3 were of human origin.

[760] Also, across Groups 1-3, either within subretinal masses or embedded in the outer retina, additional autofluorescent cells and/or debris were observed. These brightly autofluorescent structures, often displaying yellow/pigmented coloration in brightfield imaging (**FIG. 48B**, brightfield) were suspected damaged or dying photoreceptors due to their localization approximating the outer nuclear/inner segment/outer segment lamina. Given that this morphology was observed across groups, this damage may have been caused by the surgical injection procedure.

Glial Acidic Fibrillary Protein (GFAP)

[761] GFAP staining was performed to evaluate Müller gliosis in retinas, as well as to provide a secondary marker for PRCs. Focally at injection sites in Groups 1-3, Müller glia were brightly labeled

by GFAP, vertically banded across the thickness of the retina, suggesting gliosis likely associated with damage due to the injection procedure (**FIGs. 49A-49H**). The degree of Müller glial GFAP immunoreactivity was comparable between the three injected groups, varying roughly from 100 μm laterally to several hundred microns in length along the retina. In the naïve animals of Group 4, GFAP was restricted to the retinal nerve fiber layer, consistent with healthy naïve retinas.

[762] Furthermore, the 3 subretinal DAPI+ graft-like structures in Groups 2 and 3 were also GFAP+ (**FIGs. 49C, 49E, and 49F**), with thin curled finger-like omnidirectional morphology of the stain consistent with that of PRCs after subretinal transplantation. GFAP expression in those grafts was not contiguous with retinal Müller glial GFAP immunoreactivity, suggesting a distinct source from Müller glia. Additionally, the ONL overlying those grafts was low GFAP-expressing, in contrast to examples of GFAP hypertrophy, where GFAP is enriched in the area of damaged ONL (**FIG. 49A**).

[763] In one sample in Group 3, GFAP labeled a structure with morphology consistent with the optic nerve head (ONH), which is typical staining for this structure in healthy retinas.

[764] Overall, the pattern of GFAP immunoreactivity within the retina at the injection sites in Groups 1-3 was consistent with surgical damage induced by injection procedures. Furthermore, in 1 sample in Group 2 and 2 samples in Group 3, the disorganized pattern of GFAP expression was consistent with expression of suspected PRCs after subretinal transplantation.

Iba1 (Microglia/Macrophage)

[765] Anti-Iba1 staining was performed to evaluate the extent of microglial activation between groups. In the injected groups (Groups 1-3), there is an enrichment for Iba1+ cells at the injection site, whereas the immunoreactivity in the naïve eyes (Group 4) is very low (**FIGs. 50A-50D**). In general, the morphology of the Iba1 staining in Groups 1-3 is consistent with that of activated microglia, having bushy or amoeboid morphology as opposed to that of resting microglia with thin ramified processes. Overall, Iba1 expression and morphology is similar between Groups 1-3, and is suspected to be induced by the surgical procedure.

CD45, Ter119, and Bestrophin-1

[766] Due to the observation on OCT of subretinal accumulation in Group 1, and presence of yellow or weakly pigmented material/cells observed within Group 1 sections, immunostaining for CD45, Ter119 and Bestrophin-1 was performed in a subset of samples across groups to determine whether subretinal masses contained hematopoietic cells (including T-cells), erythrocytes, or retinal pigment epithelium, respectively.

[767] CD45 was largely negative across groups. In subretinal grafts, in a few samples across groups 1-3, bright clusters of autofluorescence were observed that did not co-label with DAPI. Occasionally, a few cells were observed in the retina or subretinal graft that expressed the appropriately localized membrane-bound CD45 signal of hematopoietic cells.

[768] Aside from occasional clusters of subretinal autofluorescence observed in injected eyes, expression of Ter119 was localized to single cells within the retina across Groups 1-4, consistent with blood cells localized within retinal vasculature.

[769] Bestrophin-1 was negative across samples, either in the subretinal graft or host retinal pigment epithelium. (Data not shown).

[770] Altogether, within the subretinal grafts, staining for CD45, Ter119, and Bestrophin-1 was largely negative or brightly autofluorescent, suggesting that the subretinal material across Groups 1-3 was largely not of hematopoietic origin, or composed of erythrocytes or retinal pigment epithelium.

Conclusion

[771] The data obtained in this study support the safety of a PRC drug product with <60% viability (i.e. 50-60%), when compared against PRCs with >70% viability.

CLAIMS

1. A photoreceptor rescue cell composition comprising a plurality of heterogeneous photoreceptor rescue cells, wherein the plurality of heterogeneous photoreceptor rescue cells cumulatively expresses at least two of the markers selected from the group consisting of FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA.
2. The photoreceptor rescue cell composition of claim 1, further comprising a medium suitable for maintaining the viability of the cells.
3. The photoreceptor rescue cell composition of claim 1 or 2, wherein the cells are generated by *in vitro* differentiation of pluripotent cells.
4. The photoreceptor rescue cell composition of claim 3, wherein the pluripotent cells are embryonic cells (ESCs) or induced pluripotent stem cells (iPSCs).
5. The photoreceptor rescue cell composition of any one of the preceding claims, wherein at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of cells in the composition are photoreceptor rescue cells.
6. The photoreceptor rescue cell composition of any one of the preceding claims, wherein the plurality of heterogeneous cells cumulatively expresses FOXG1 and MAP2.
7. The photoreceptor rescue cell composition of any one of the preceding claims, wherein
 - (i) about 50% to about 100%, about 50% to about 90%, about 55% to about 85%, or about 55% to about 73% of cells in the composition express FOXG1; and/or
 - (ii) at least about 50%, 55%, 57%, 60%, 65%, 70%, 71%, 75%, 78%, 79%, 80%, 81%, 85%, 87%, 90%, 92%, 95%, 97%, or 100% of cells in the composition express FOXG1, and/or
 - (iii) about 55 transcripts per million (TPM) to about 200 TPM, about 60 TPM to about 170 TPM, about 140 TPM to about 165 TPM, or about 149 TPM to about 170 TPM of FOXG1 transcripts are expressed by the cells of the composition; and/or
 - (iv) at least 55 TPM, 60 TPM, 70 TPM, 80 TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 180 TPM, 190 TPM, or 200 TPM of FOXG1 transcripts are expressed by the cells of the composition.

8. The photoreceptor rescue cell composition of any one of the preceding claims, wherein
- (i) about 75% to about 100%, about 75% to about 98%, about 75% to about 95%, about 77% to about 93%, of cells in the composition express MAP2; and/or
 - (ii) at least about 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of cells in the composition express MAP2; and/or
 - (iii) about 250 transcripts per million (TPM) to about 700 TPM, about 290 TPM to about 650 TPM, about 450 TPM to about 625 TPM, or about 490 TPM to about 615 TPM of MAP2 transcripts are expressed by the cells of the composition; and/or
 - (iv) at least 250 TPM, 300 TPM, 350 TPM, 400 TPM, 450 TPM, 475 TPM, 490 TPM, 510 TPM, 525 TPM, 550 TPM, 575 TPM, 600 TPM, 610 TPM, 625 TPM, 650 TPM, 675 TPM, or 700 TPM of MAP2 transcripts are expressed by the cells of the composition.
9. The photoreceptor rescue cell composition of claim 6, wherein the plurality of heterogeneous cells cumulatively expresses at least one additional marker selected from the group consisting of STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA.
10. The photoreceptor rescue cell composition of any one of the preceding claims, wherein the plurality of heterogeneous cells cumulatively expresses at least 3, 4, 5, 6 or 7 of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA.
11. The photoreceptor rescue cell composition of any one of the preceding claims, wherein
- (i) about 60% to about 95%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, about 65% to about 95%, about 65% to about 90%, about 65% to about 85%, about 65% to about 80%, about 70% to about 95%, about 70% to about 90%, about 70% to about 85%, about 70% to about 80%, about 75% to about 95%, about 75% to about 90%, about 75% to about 85%, or about 75% to about 80% of cells in the composition express STMN2; and/or
 - (ii) at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, or 100% of cells in the composition express STMN2; and/or
 - (iii) about 150 transcripts per million (TPM) to about 600 TPM, about 190 TPM to about 560 TPM, about 400 TPM to about 600 TPM, or about 450 TPM to about 560 TPM of STMN2 transcripts are expressed by the cells of the composition; and/or
 - (iv) at least 150 TPM, 185 TPM, 200 TPM, 250 TPM, 300 TPM, 350 TPM, 400 TPM, 425 TPM, 450 TPM, 475 TPM, 500 TPM, 525 TPM, 550 TPM, 575 TPM, or 600 TPM of STMN2 transcripts are expressed by the cells of the composition.

12. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 65% to about 95%, about 65% to about 85%, about 70% to about 95%, about 70% to about 90%, about 70% to about 89%, about 75% to about 95%, about 75% to about 90%, or about 75% to about 89% of cells in the composition express DCX; and/or

(ii) at least about 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, or 95% of cells in the composition express DCX; and/or

(iii) about 200 transcripts per million (TPM) to about 900 TPM, about 250 TPM to about 900 TPM, about 600 TPM to about 900 TPM, or about 750 TPM to about 850 TPM of DCX transcripts are expressed by the cells of the composition; and/or

(iv) at least 200 TPM, 250 TPM, 350 TPM, 400 TPM, 450 TPM, 500 TPM, 550 TPM, 600 TPM, 650 TPM, 700 TPM, 750 TPM, 800 TPM, 850 TPM, or 900 TPM of DCX transcripts are expressed by the cells of the composition.

13. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 65% to about 98%, about 65% to about 95%, about 70% to about 98%, about 70% to about 95%, about 70% to about 90%, about 75% to about 98%, about 75% to about 90%, or about 80% to about 95% of cells in the composition express LINC00461; and/or

(ii) at least about 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, or 98% of cells in the composition express LINC00461; and/or

(iii) about 50 transcripts per million (TPM) to about 100 TPM, about 50 TPM to about 95 TPM, about 85 TPM to about 95 TPM, or about 87 TPM to about 93 TPM of LINC00461 transcripts are expressed by the cells of the composition; and/or

(iv) at least 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 85 TPM, 87 TPM, 89 TPM, 90 TPM, 92 TPM, 95 TPM, or 100 TPM of LINC00461 transcripts are expressed by the cells of the composition.

14. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 25%, about 1% to about 20%, 1% to about 18%, 1% to about 16%, about 1% to about 14%, about 1% to about 12%, 1% to about 10%, about 1% to about 8%, about 1% to about 7%, about 1% to about 5% or about 2% to about 4% of cells in the composition express NEUROD2; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, or 25% of cells in the composition express NEUROD2; and/or

(iii) about 0 transcripts per million (TPM) to about 10 TPM, about 0.01 TPM to about 9 TPM, about 0.2 TPM to about 2 TPM, or about 0.4 TPM to about 1.2 TPM of NEUROD2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 0.2 TPM, 0.4 TPM, 0.6 TPM, 0.8 TPM, 1.0 TPM, 1.2 TPM, 1.5 TPM, 2 TPM, 4 TPM, 6 TPM, 8 TPM, or 10 TPM of NEUROD2 transcripts are expressed by the cells of the composition.

15. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 35% to about 70%, about 35% to about 68%, about 35% to about 67%, about 35% to about 66%, about 35% to about 65%, about 40% to about 70%, about 40% to about 68%, about 40% to about 67%, about 40% to about 66%, about 40% to about 65%, about 42% to about 70%, about 42% to about 68%, about 42% to about 67%, about 42% to about 66%, or about 42% to about 65% of cells in the composition express GAD1; and/or

(ii) at least about 35%, 40%, 45%, 50%, 55%, 60%, 65%, 66%, 67%, 68%, 69%, or 70% of cells in the composition express GAD1; and/or

(iii) about 10 transcripts per million (TPM) to about 50 TPM, about 12 TPM to about 45 TPM, about 12 TPM to about 40 TPM, or about 25 TPM to about 42 TPM of GAD1 transcripts are expressed by the cells of the composition; and/or

(iv) at least 10 TPM, 12 TPM, 16 TPM, 18 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 35 TPM, 37 TPM, 40 TPM, 42 TPM, 45 TPM, 47 TPM, or 50 TPM of GAD1 transcripts are expressed by the cells of the composition.

16. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 60% to about 95%, about 60% to about 90%, about 60% to about 89%, about 60% to about 88%, about 60% to about 87%, about 60% to about 86%, about 65% to about 95%, about 65% to about 90%, about 65% to about 89%, about 65% to about 88%, about 65% to about 87%, about 65% to about 86%, about 69% to about 90%, about 69% to about 89%, about 69% to about 88%, about 69% to about 87%, or about 69% to about 86% of cells in the composition express NFIA; and/or

(ii) at least about 50%, 55%, 60%, 65%, 67%, 69%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 92%, or 95% of cells in the composition express NFIA; and/or

(iii) about 30 transcripts per million (TPM) to about 120 TPM, about 33 TPM to about 117 TPM, about 60 TPM to about 85 TPM, or about 65 TPM to about 80 TPM of NFIA transcripts are expressed by the cells of the composition; and/or

(iv) at least 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, or 120 TPM of NFIA transcripts are expressed by the cells of the composition.

17. The photoreceptor rescue cell composition of any one of the preceding claims, wherein the plurality of heterogeneous cells cumulatively expresses each of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, and NFIA.

18. The photoreceptor rescue cell composition of any one of the preceding claims, wherein the heterogeneous cells each individually express at least one of markers FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, or NFIA.

19. The photoreceptor rescue cell composition of any one of claims 1-18, wherein the plurality of heterogeneous photoreceptor rescue cells comprises one or more cell types selected from the group consisting of an inhibitory neuron, an excitatory neuron, a progenitor, an astrocyte, and an alternative neuron.

20. The photoreceptor rescue cell composition of claim 19, wherein the plurality of heterogeneous photoreceptor rescue cells comprises each of an inhibitory neuron, an excitatory neuron, a progenitor, an astrocyte, and an alternative neuron.

21. The photoreceptor rescue cell composition of claim 19 or claim 20, wherein the plurality of heterogeneous photoreceptor rescue cells comprises an inhibitory neuron expressing one or more markers selected from the group consisting of DLX5, TUBB3, SCGN, ERBB4, and CALB2.

22. The photoreceptor rescue cell composition of any one of claims 19-21, wherein the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of inhibitory neurons that cumulatively expresses each of markers DLX5, TUBB3, SCGN, ERBB4, and CALB2.

23. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 50% to about 80%, about 50% to about 75%, about 50% to about 70%, about 52% to about 69%, about 52% to about 75%, about 54% to about 69%, about 54% to about 68%, or about 54% to about 66% of cells in the composition express DLX5; and/or

(ii) at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 72%, 74%, 76%, 78%, or 80% of cells in the composition express DLX5; and/or

(iii) about 30 transcripts per million (TPM) to about 150 TPM, about 50 TPM to about 140 TPM, about 80 TPM to about 138 TPM, or about 130 TPM to about 140 TPM of DLX5 transcripts are expressed by the cells of the composition; and/or

(iv) at least 30 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 80 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, 115 TPM, 120 TPM, 130 TPM, 135 TPM, 140 TPM, or 150 TPM of DLX5 transcripts are expressed by the cells of the composition.

24. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 60% to about 95%, about 70% to about 95%, about 72% to about 95%, about 75% to about 95%, about 76% to about 94%, about 77% to about 93%, about 78% to about 93%, about 70% to about 90%, about 72% to about 89%, about 73% to about 88%, about 74% to about 87%, about 75% to about 87%, about 76% to about 86%, about 77% to about 86%, or about 79% to about 86% of cells in the composition express TUBB3; and/or

(ii) at least about 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 91%, 92%, 93%, or 95% of cells in the composition express TUBB3; and/or

(iii) about 150 transcripts per million (TPM) to about 500 TPM, about 160 TPM to about 450 TPM, about 300 TPM to about 450 TPM, about 350 TPM to about 430 TPM, or about 375 TPM to about 430 TPM of TUBB3 transcripts are expressed by the cells of the composition; and/or

(iv) at least 150 TPM, 175 TPM, 200 TPM, 225 TPM, 250 TPM, 275 TPM, 300 TPM, 325 TPM, 350 TPM, 375 TPM, 400 TPM, 425 TPM, 430 TPM, 450 TPM, 475 TPM, or 500 TPM of TUBB3 transcripts are expressed by the cells of the composition.

25. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 45% to about 70%, about 45% to about 65%, about 50% to about 70%, about 50% to about 65%, about 50% to about 64%, about 50% to about 63%, about 50% to about 62%, about 50% to about 61%, about 46% to about 52%, about 47% to about 51%, about 48% to about 51%, or about 48% to about 50% of cells in the composition express SCGN; and/or

(ii) at least about 45%, 47%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 65%, 67%, or 70% of cells in the composition express SCGN; and/or

(iii) about 50 transcripts per million (TPM) to about 200 TPM, about 70 TPM to about 180 TPM, about 75 TPM to about 175 TPM, or about 120 TPM to about 175 TPM of SCGN transcripts are expressed by the cells of the composition; and/or

(iv) at least 50 TPM, 60 TPM, 70 TPM, 80 TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 171 TPM, 173 TPM, 175 TPM, 180 TPM, 185 TPM, 190 TPM, or 200 TPM of SCGN transcripts are expressed by the cells of the composition.

26. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 60% to about 85%, about 60% to about 80%, about 60% to about 79%, about 60% to about 78%, about 60% to about 77%, about 60% to about 76%, about 63% to about 75%, about 63% to about 70%, about 63% to about 79%, about 63% to about 78%, about 63% to about 77%, about 63% to about 75%, about 63% to about 73%, about 63% to about 72%, about 63% to about 71%, about 65% to about 72%, or about 66% to about 71% of cells in the composition express ERBB4; and/or

(ii) at least about 50%, 55%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 75%, 80%, or 85% of cells in the composition express ERBB4; and/or

(iii) about 15 transcripts per million (TPM) to about 120 TPM, about 17 TPM to about 100 TPM, about 18 TPM to about 95 TPM, about 70 TPM to about 95 TPM, or about 73 TPM to about 94 TPM of ERBB4 transcripts are expressed by the cells of the composition; and/or

(iv) at least 15 TPM, 30 TPM, 50 TPM, 70 TPM, 73 TPM, 75 TPM, 80 TPM, 82 TPM, 85 TPM, 88 TPM, 90 TPM, 91 TPM, 93 TPM, 95 TPM, 100 TPM, 110 TPM, or 120 TPM of ERBB4 transcripts are expressed by the cells of the composition.

27. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 35% to about 75%, about 40% to about 70%, about 40% to about 65%, about 41% to about 75%, about 41% to about 70%, about 41% to about 65%, about 41% to about 64%, about 41% to about 63%, about 41% to about 62%, about 35% to about 55%, about 40% to about 52%, or about 41% to about 52% of cells in the composition express CALB2; and/or

(ii) at least about 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 45%, 47%, 50%, 51%, 52%, 55%, 60%, 65%, 70%, or 75% of cells in the composition express CALB2; and/or

(iii) about 30 transcripts per million (TPM) to about 220 TPM, about 50 TPM to about 200 TPM, about 70 TPM to about 200 TPM, or about 75 TPM to about 199 TPM of CALB2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 30 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, 125 TPM, 150 TPM, 175 TPM, 180 TPM, 185 TPM, 190 TPM, 195 TPM, 196 TPM, 220 TPM, 210 TPM, or 220 TPM of CALB2 transcripts are expressed by the cells of the composition.

28. The photoreceptor rescue cell composition of any one of claims 19-27, wherein the plurality of heterogeneous photoreceptor rescue cells comprises an excitatory neuron expressing one or more markers selected from the group consisting of NEUROD2, NEUROD6, SLA, NELL2, and SATB2.

29. The photoreceptor rescue cell composition of any one of claims 19-28, wherein the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of excitatory neurons that cumulatively expresses each of markers NEUROD2, NEUROD6, SLA, NELL2, and SATB2.

30. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 0.1% to about 30%, about 0.1% to about 25%, about 0.1% to about 24%, about 0.5% to about 30%, about 0.5% to about 25%, about 0.5% to about 24%, about 0.8% to about 30%, about 0.8% to about 25%, about 0.8% to about 24%, about 1% to about 5%, about 1% to about 4.5%, about 2% to about 5%, about 2% to about 4.5%, or about 2% to about 4% of cells in the composition express NEUROD6; and/or

(ii) at least about 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 1%, 2%, 4%, 5%, 7%, 10%, 12%, 15%, 17%, 20%, 22%, 23%, 24%, 26%, 28%, or 30% of cells in the composition express NEUROD6; and/or

(iii) about 1 transcripts per million (TPM) to about 210 TPM, about 1 TPM to about 205 TPM, about 1 TPM to about 25 TPM, or about 10 TPM to about 20 TPM of NEUROD6 transcripts are expressed by the cells of the composition; and/or

(iv) at least 1 TPM, 5 TPM, 10 TPM, 12 TPM, 15 TPM, 19 TPM, 50 TPM, 100 TPM, 150 TPM, 175 TPM, 200 TPM, 203 TPM, or 210 TPM of NEUROD6 transcripts are expressed by the cells of the composition.

31. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 0.5% to about 20%, about 0.5% to about 15%, about 0.5% to about 10%, about 0.5% to about 5%, about 0.5% to about 4%, about 0.5% to about 3%, about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 5%, about 1% to about 4%, or about 1% to about 3% of cells in the composition express SLA; and/or

(ii) at least about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 15%, 16%, 18%, or 20% of cells in the composition express SLA; and/or

(iii) about 0.1 transcripts per million (TPM) to about 60 TPM, about 0.1 TPM to about 50 TPM, about 1 TPM to about 10 TPM, about 2 TPM to about 8 TPM, or about 3 TPM to about 6 TPM, of SLA transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 10 TPM, 20 TPM, 30 TPM, 40 TPM, 50 TPM, or 60 TPM of SLA transcripts are expressed by the cells of the composition.

32. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 15% to about 30%, about 15% to about 25%, about 20% to about 30%, or about 20% to about 28% of cells in the composition express NELL2; and/or

(ii) at least about 10%, 12%, 15%, 17%, 20%, 21%, 24%, 25%, 27%, 30%, 32%, 35%, 40%, or 45% of cells in the composition express NELL2; and/or

(iii) about 1 transcripts per million (TPM) to about 150 TPM, about 4 TPM to about 130 TPM, about 4 TPM to about 35 TPM, about 20 TPM to about 30 TPM, or about 25 TPM to about 28 TPM of NELL2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 1 TPM, 5 TPM, 15 TPM, 20 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, 120 TPM, or 150 TPM of NELL2 transcripts are expressed by the cells of the composition.

33. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 20%, about 1% to about 15%, about 1% to about 12%, about 1% to about 11%, about 2% to about 20%, about 2% to about 15%, about 2% to about 12%, about 2% to about 11%, about 3% to about 20%, about 3% to about 15%, about 3% to about 12%, about 3% to about 11%, about 2% to about 6%, about 2% to about 5%, or about 3% to about 4% of cells in the composition express SATB2; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 15%, 17%, or 20% of cells in the composition express SATB2; and/or

(iii) about 0.1 transcripts per million (TPM) to about 30 TPM, about 0.5 TPM to about 20 TPM, about 1 TPM to about 5 TPM, or about 2 TPM to about 3 TPM of SATB2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 12 TPM, 15 TPM, 20 TPM, 25 TPM, or 30 TPM of SATB2 transcripts are expressed by the cells of the composition.

34. The photoreceptor rescue cell composition of any one of claims 19-33, wherein the plurality of heterogeneous photoreceptor rescue cells comprises a progenitor expressing one or more markers selected from the group consisting of VIM, MKI67, CLU, and GLI3.

35. The photoreceptor rescue cell composition of any one of claims 19-34, wherein the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of progenitors that cumulatively expresses each of markers VIM, MKI67, CLU, and GLI3.

36. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 30% to about 80%, about 30% to about 75%, about 30% to about 70%, about 40% to about 75%, about 40% to about 70%, about 40% to about 69%, about 40% to about 60%, or about 42% to about 47% of cells in the composition express VIM; and/or

(ii) at least about 30%, 35%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 50%, 52%, 55%, 57%, 60%, 62%, 65%, 67%, 69%, 72%, 75%, or 80% of cells in the composition express VIM; and/or

(iii) about 250 transcripts per million (TPM) to about 900 TPM, about 250 TPM to about 865 TPM, about 200 TPM to about 350 TPM, or about 250 TPM to about 340 TPM of VIM transcripts are expressed by the cells of the composition; and/or

(iv) at least 250 TPM, 260 TPM, 270 TPM, 300 TPM, 320 TPM, 350 TPM, 370 TPM, 400 TPM, 500 TPM, 600 TPM, 700 TPM, 800 TPM, or 900 TPM of VIM transcripts are expressed by the cells of the composition.

37. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 5% to about 20%, about 5% to about 15%, about 5% to about 12%, about 6% to about 15%, about 6% to about 12%, about 7% to about 15%, about 7% to about 12%, or about 6% to about 8% of cells in the composition express MKI67; and/or

(ii) at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% of cells in the composition express MKI67; and/or

(iii) about 5 transcripts per million (TPM) to about 40 TPM, about 10 TPM to about 35 TPM, about 15 TPM to about 25 TPM, or about 18 TPM to about 22 TPM of MKI67 transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 10 TPM, 12 TPM, 15 TPM, 17 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 32 TPM, 33 TPM, 35 TPM, 37 TPM, or 40 TPM of MKI67 transcripts are expressed by the cells of the composition.

38. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 10% to about 60%, about 15% to about 55%, about 20% to about 60%, about 20% to about 55%, about 20% to about 50%, about 20% to about 40%, about 20% to about 35%, or about 25% to about 32% of cells in the composition express CLU; and/or

(ii) at least about 10%, 15%, 17%, 20%, 22%, 25%, 27%, 30%, 32%, 35%, 37%, 40%, 42%, 45%, 47%, 50%, 55%, or 60% of cells in the composition express CLU; and/or

(iii) about 30 transcripts per million (TPM) to about 400 TPM, about 40 TPM to about 150 TPM, about 60 TPM to about 150 TPM, or about 60 TPM to about 105 TPM of CLU transcripts are expressed by the cells of the composition; and/or

(iv) at least 30 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 80 TPM, 90 TPM, 100 TPM, 125 TPM, 150 TPM, 175 TPM, 200 TPM, 225 TPM, 250 TPM, 275 TPM, 300 TPM, 325 TPM, 350 TPM, 365 TPM, 375 TPM, or 400 TPM of CLU transcripts are expressed by the cells of the composition.

39. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 10% to about 50%, about 10% to about 40%, about 10% to about 30%, about 12% to about 50%, about 12% to about 35%, about 12% to about 29%, about 15% to about 29%, about 15% to about 29%, or about 15% to about 17% of cells in the composition express GLI3; and/or

(ii) at least about 10%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 35%, 40%, 45%, or 50% of cells in the composition express GLI3; and/or

(iii) about 5 transcripts per million (TPM) to about 60 TPM, about 10 TPM to about 45 TPM, about 15 TPM to about 30 TPM, or about 20 TPM to about 25 TPM of GLI3 transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 10 TPM, 12 TPM, 15 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 25 TPM, 30 TPM, 35 TPM, 37 TPM, 40 TPM, 42 TPM, 45 TPM, 50 TPM, 55 TPM, or 60 TPM of GLI3 transcripts are expressed by the cells of the composition.

40. The photoreceptor rescue cell composition of any one of claims 19-39, wherein the plurality of heterogeneous photoreceptor rescue cells comprises an astrocyte expressing one or more markers selected from the group consisting of GFAP, LUCAT1, MIR99AHG, and FBXL7.

41. The photoreceptor rescue cell composition of any one of claims 19-40, wherein the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of astrocytes that cumulatively expresses each of markers GFAP, LUCAT1, MIR99AHG, and FBXL7.

42. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 50%, about 1% to about 20%, about 1% to about 15%, about 1% to about 13%, about 1% to about 10%, about 1% to about 7%, about 1% to about 5%, about 1% to about 4%, or about 1% to about 3% of cells in the composition express GFAP; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of cells in the composition express GFAP; and/or

(iii) about 0.1 transcripts per million (TPM) to about 150 TPM, about 0.1 TPM to about 125 TPM, about 1 TPM to about 20 TPM, or about 3 TPM to about 15 TPM of GFAP transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 0.2 TPM, 0.5 TPM, 1 TPM, 5 TPM, 7 TPM, 10 TPM, 12 TPM, 14 TPM, 16 TPM, 30 TPM, 40 TPM, 50 TPM, 80 TPM, 100 TPM, 110 TPM, 115 TPM, 120 TPM, 130 TPM, 140 TPM, or 150 TPM of GFAP transcripts are expressed by the cells of the composition.

43. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 5% to about 20%, about 5% to about 17%, about 5% to about 15%, about 5% to about 13%, about 7% to about 20%, about 7% to about 17%, about 7% to about 15%, about 7% to about 13%, about 5% to about 12%, or about 7% to about 10% of cells in the composition express LUCAT1; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 17%, or 20% of cells in the composition express LUCAT1.

44. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 50% to about 100%, about 50% to about 90%, about 50% to about 88%, about 60% to about 100%, about 60% to about 90%, about 60% to about 88%, about 70% to about 90%, about 70% to about 88%, or about 75% to about 82% of cells in the composition express MIR99AHG; and/or

(ii) at least about 50%, 60%, 65%, 70%, 72%, 75%, 77%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 92%, 94%, 96%, 98%, or 100% of cells in the composition express MIR99AHG; and/or

(iii) about 5 transcripts per million (TPM) to about 40 TPM, about 5 TPM to about 30 TPM, about 6 TPM to about 25 TPM, or about 10 TPM to about 15 TPM of MIR99AHG transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 16 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM,

28 TPM, 30 TPM, 34 TPM, 38 TPM, or 40 TPM of MIR99AHG transcripts are expressed by the cells of the composition.

45. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 20% to about 70%, about 20% to about 60%, about 25% to about 70%, about 25% to about 65%, about 30% to about 60%, about 30% to about 55%, about 30% to about 40%, about 32% to about 39%, or about 34% to about 39% of cells in the composition express FBXL7; and/or

(ii) at least about 20%, 22%, 25%, 27%, 30%, 32%, 34%, 35%, 37%, 38%, 39%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 60%, 65%, or 70% of cells in the composition express FBXL7; and/or

(iii) about 5 transcripts per million (TPM) to about 40 TPM, about 5 TPM to about 30 TPM, about 7 TPM to about 25 TPM, about 10 TPM to about 15 TPM, or about 11 TPM to about 14 TPM of FBXL7 transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 16 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 28 TPM, 30 TPM, 34 TPM, 38 TPM, or 40 TPM of FBXL7 transcripts are expressed by the cells of the composition.

46. The photoreceptor rescue cell composition of any one of claims 19-45, wherein the plurality of heterogeneous photoreceptor rescue cells comprises an alternative neuron expressing one or more markers selected from the group consisting of MEIS2, PBX3, GRIA2, and CACNA1C.

47. The photoreceptor rescue cell composition of any one of claims 19-46, wherein the plurality of heterogeneous photoreceptor rescue cells comprises a plurality of alternative neurons that cumulatively expresses each of markers MEIS2, PBX3, GRIA2, and CACNA1C.

48. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 30% to about 80%, about 30% to about 90%, about 40% to about 90%, about 45% to about 85%, about 45% to about 80%, about 49% to about 79%, or about 50% to about 78% of cells in the composition express MEIS2; and/or

(ii) at least about 30%, 35%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 57%, 60%, 62%, 65%, 67%, 70%, 72%, 75%, 77%, 80%, 81%, or 82% of cells in the composition express MEIS2; and/or

(iii) about 5 transcripts per million (TPM) to about 200 TPM, about 10 TPM to about 180 TPM, about 50 TPM to about 180 TPM, or about 60 TPM to about 173 TPM of MEIS2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 10 TPM, 15 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 80 TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 135 TPM, 136 TPM, 138 TPM, 140 TPM, 145 TPM, 150 TPM, 160 TPM, 170 TPM, 180 TPM, 190 TPM, or 200 TPM of MEIS2 transcripts are expressed by the cells of the composition.

49. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 30% to about 90%, about 35% to about 85%, about 40% to about 85%, about 40% to about 80%, about 45% to about 75%, or about 49% to about 75% of cells in the composition express PBX3; and/or

(ii) at least about 30%, 35%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 57%, 60%, 62%, 65%, 67%, 70%, 72%, 75%, 80%, 85%, or 90% of cells in the composition express PBX3; and/or

(iii) about 5 transcripts per million (TPM) to about 100 TPM, about 5 TPM to about 90 TPM, about 25 TPM to about 90 TPM, or about 29 TPM to about 88 TPM of PBX3 transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 15 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, or 100 TPM of PBX3 transcripts are expressed by the cells of the composition.

50. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 20% to about 60%, about 20% to about 50%, about 20% to about 50%, about 20% to about 48%, about 22% to about 55%, about 22% to about 50%, about 22% to about 47%, or about 23% to about 47% of cells in the composition express GRIA2; and/or

(ii) at least about 20%, 22%, 25%, 27%, 30%, 32%, 34%, 35%, 37%, 38%, 39%, 40%, 42%, 45%, 46%, 47%, 50%, 52%, 54%, 56%, 58%, or 60% of cells in the composition express GRIA2; and/or

(iii) about 2 transcripts per million (TPM) to about 40 TPM, about 2 TPM to about 30 TPM, about 4 TPM to about 35 TPM, or about 10 TPM to about 30 TPM of GRIA2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 15 TPM, 17 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 25 TPM, 26 TPM, 27 TPM, 28 TPM, 29 TPM, 30 TPM, 35 TPM, or 40 TPM of GRIA2 transcripts are expressed by the cells of the composition.

51. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 20% to about 70%, about 30% to about 60%, about 35% to about 70%, about 35% to about 60%, about 33% to about 60%, about 35% to about 60%, or about 39% to about 60% of cells in the composition express CACNA1C; and/or

(ii) at least about 20%, 25%, 30%, 32%, 34%, 35%, 37%, 38%, 39%, 40%, 42%, 45%, 47%, 50%, 52%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 65%, or 70% of cells in the composition express CACNA1C; and/or

(iii) about 1 transcripts per million (TPM) to about 15 TPM, about 1 TPM to about 10 TPM, about 1 TPM to about 7 TPM, or about 3 TPM to about 7 TPM of CACNA1C transcripts are expressed by the cells of the composition; and/or

(iv) at least 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 10 TPM, 12 TPM, or 15 TPM of CACNA1C transcripts are expressed by the cells of the composition.

52. The photoreceptor rescue cell composition of any one of claims 1-51, wherein the plurality of heterogeneous photoreceptor rescue cells comprises:

(i) an inhibitory neuron expressing one or more markers selected from the group consisting of DLX5, TUBB3, SCGN, ERBB4, and CALB2;

(ii) an excitatory neuron expressing one or more markers selected from the group consisting of NEUROD2, NEUROD6, SLA, NELL2, and SATB2;

(iii) a progenitor expressing one or more markers selected from the group consisting of VIM, MKI67, CLU, and GLI3;

(iv) an astrocyte expressing one or more markers selected from the group consisting of GFAP, LUCAT1, MIR99AHG, and FBXL7; and

(v) an alternative neuron expressing one or more markers selected from the group consisting of MEIS2, PBX3, GRIA2, and CACNA1C.

53. The photoreceptor rescue cell composition of any one of claims 1-52, wherein the plurality of heterogeneous photoreceptor rescue cells comprises:

(i) a plurality of inhibitory neurons that cumulatively expresses each of markers DLX5, TUBB3, SCGN, ERBB4, and CALB2;

(ii) a plurality of excitatory neurons that cumulatively expresses each of markers NEUROD2, NEUROD6, SLA, NELL2, and SATB2;

(iii) a plurality of progenitors that cumulatively expresses each of markers VIM, MKI67, CLU, and GLI3;

(iv) a plurality of astrocytes that cumulatively expresses each of markers GFAP, LUCAT1, MIR99AHG, and FBXL7; and

(v) a plurality of alternative neurons that cumulatively expresses each of markers MEIS2, PBX3, GRIA2, and CACNA1C.

54. The photoreceptor rescue cell composition of any one of claims 1-53, wherein the composition comprises:

(i) about 25% to about 55%, about 25% to about 50%, about 30% to about 55%, about 30% to about 50%, about 35% to about 55%, about 35% to about 50%, or about 38% to about 49% inhibitory neurons; and/or

(ii) about 0% to about 15%, about 0% to about 12%, about 0% to about 10%, about 0% to about 8%, or about 0.5% to about 9% excitatory neurons; and/or

(iii) about 10% to about 45%, about 10% to about 40%, about 10% to about 35%, about 15% to about 45%, about 15% to about 40%, about 15% to about 35%, about 17% to about 45%, about 17% to about 40%, about 17% to about 35%, or about 20% to about 35% progenitors; and/or

(iv) about 0% to about 6%, about 0% to about 5%, about 0% to about 4%, about 0% to about 3%, about 0% to about 2%, about 0.5% to about 6%, about 0.5% to about 5%, about 0.5% to about 4%, about 0.5% to about 4%, about 0.5% to about 3%, about 0.5% to about 2%, or about 0.5% to about 1.5% astrocytes; and/or

(v) about 10% to about 50%, about 10% to about 45%, about 10% to about 40%, about 12% to about 50%, about 12% to about 45%, about 12% to about 40%, about 15% to about 50%, about 15% to about 45%, about 15% to about 40%, or about 17% to about 37% mixed neurons.

55. The photoreceptor rescue cell composition of any one of claims 1-54, wherein cells in the composition further express one or more eye field progenitor markers, rod/cone photoreceptor markers, and/or neuron markers.

56. The photoreceptor rescue cell composition of claim 55, wherein the eye field progenitor markers are selected from the group consisting of PAX6, LHX2, SIX3, NES, and SOX2.

57. The photoreceptor rescue cell composition of claim 55 or claim 56, wherein the plurality of heterogeneous cells cumulatively expresses at least 1, 2, 3 or 4 of the eye field progenitor markers PAX6, LHX2, SIX3, NES, or SOX2.

58. The photoreceptor rescue cell composition of any one of claims 55-57, wherein the plurality of heterogeneous cells cumulatively expresses at least each of the eye field progenitor markers PAX6, LHX2, SIX3, NES, and SOX2.

59. The photoreceptor rescue cell composition of any one of claims 55-58, wherein the plurality of heterogeneous cells cumulatively expresses SOX2.

60. The photoreceptor rescue cell composition of any one of claims 55-59, wherein the composition is substantially free of cells that express eye field progenitor markers RAX, SIX6, and/or TBX3.

61. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 25% to about 60%, about 25% to about 55%, about 25% to about 52%, about 25% to about 45%, about 30% to about 60%, about 30% to about 55%, about 30% to about 45%, , or about 30% to about 42% of cells in the composition express PAX6; and/or

(ii) at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, or 60% of cells in the composition express PAX6; and/or

(iii) about 20 transcripts per million (TPM) to about 125 TPM, about 30 TPM to about 110 TPM, about 35 TPM to about 100 TPM, about 70 TPM to about 80 TPM, or about 73 TPM to about 78 TPM of PAX6 transcripts are expressed by the cells of the composition; and/or

(iv) at least 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 50 TPM, 60 TPM, 70 TPM, 75 TPM, 78 TPM, 80 TPM, 82 TPM, 85 TPM, 87 TPM, 90 TPM, 92 TPM, 95 TPM, 97 TPM, 100 TPM, 105 TPM, 110 TPM, 115 TPM, 120 TPM, or 125 TPM of PAX6 transcripts are expressed by the cells of the composition.

62. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 3% to about 35%, about 5% to about 35%, about 5% to about 35%, about 6% to about 35%, about 7% to about 35%, about 3% to about 30%, about 5% to about 30%, about 6% to about 30%, about 7% to about 30%, about 3% to about 25%, about 5% to about 25%, about 6% to about 25%, or about 7% to about 9% of cells in the composition express LHX2; and/or

(ii) at least about 3%, 4%, 6%, 8%, 10%, 15%, 20%, 25%, 26%, 27%, 28%, 29%, 30%, 32%, or 35% of cells in the composition express LHX2 ; and/or

(iii) about 5 transcripts per million (TPM) to about 40 TPM, about 5 TPM to about 36 TPM, about 5 TPM to about 10 TPM, or about 6 TPM to about 8 TPM of LHX2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 17 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 32 TPM, 36 TPM, 38 TPM, or 40 TPM of LHX2 transcripts are expressed by the cells of the composition.

63. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 25%, about 1% to about 20%, 1% to about 18%, 1% to about 16%, about 1% to about 14%, about 1% to about 12%, 1% to about 10%, about 2% to about 25%, about 2% to about 20%, 2% to about 18%, 2% to about 16%, about 2% to about 14%, about 2% to about 12%, or 2% to about 10%, of cells in the composition express SIX3; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, or 25% of cells in the composition express SIX3 ; and/or

(iii) about 1 transcripts per million (TPM) to about 50 TPM, about 2 TPM to about 30 TPM, about 1 TPM to about 25 TPM, or about 5 TPM to about 20 TPM of SIX3 transcripts are expressed by the cells of the composition; and/or

(iv) at least 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM 9 TPM, 10 TPM, 12 TPM, 15 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, or 50 TPM of SIX3 transcripts are expressed by the cells of the composition.

64. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 15% to about 40%, about 15% to about 35%, about 15% to about 34%, about 15% to about 33%, about 15% to about 32% about 15% to about 31%, about 18% to about 40%, about 18% to about 35%, about 18% to about 34%, about 18% to about 33%, about 18% to about 32%, or about 18% to about 31% of cells in the composition express NES; and/or

(ii) at least about 15%, 17%, 20%, 25%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, or 40% of cells in the composition express NES ; and/or

(iii) about 5 transcripts per million (TPM) to about 35 TPM, about 5 TPM to about 28 TPM, about 7 TPM to about 15 TPM, about 10 TPM to about 15 TPM, or about 11 TPM to about 13 TPM of NES transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 13 TPM, 14 TPM, 15 TPM, 17 TPM, 19 TPM, 20 TPM, 22 TPM, 24 TPM, 25 TPM, 26 TPM, 27 TPM, 30 TPM, or 35 TPM of NES transcripts are expressed by the cells of the composition.

65. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 55% to about 90%, about 55% to about 85%, about 55% to about 80%, about 55% to about 75%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, or about 60% to about 75% of cells in the composition express SOX2; and/or

(ii) at least about 50%, 55%, 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, or 90% of cells in the composition express SOX2 ; and/or

(iii) about 50 transcripts per million (TPM) to about 250 TPM, about 90 TPM to about 200 TPM, about 125 TPM to about 190 TPM, or about 155 TPM to about 175 TPM of CACNA1C transcripts are expressed by the cells of the composition; and/or

(iv) at least 50 TPM, 60 TPM, 70 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, 120 TPM, 130 TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 180 TPM, 190 TPM, 200 TPM, 210 TPM, 220 TPM, 230 TPM, 240 TPM, or 250 TPM of CACNA1C transcripts are expressed by the cells of the composition.

66. The photoreceptor rescue cell composition of any one of claims 55-65, wherein the rod/cone photoreceptor markers are selected from the group consisting of ASCL1, RORB, NR2E3, and NRL.

67. The photoreceptor rescue cell composition of claim 66, wherein the plurality of heterogeneous cells cumulatively expresses each of the rod/cone photoreceptor markers ASCL1, RORB, NR2E3, and NRL.

68. The photoreceptor rescue cell composition of any one of claims 55-67, wherein the composition is substantially free of cells that express rod/cone photoreceptor markers CRX, RHO, OPN1SW, PDE6B, RCVRN, ARR3, CNGB1, GNAT1, and GNAT2.

69. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 10% to about 60%, about 20% to about 60%, about 20% to about 50%, about 20% to about 45%, about 22% to about 45%, about 22% to about 43%, about 25% to about 420%, or about 28% to about 30% of cells in the composition express ASCL1; and/or

(ii) at least about 10%, 15%, 20%, 22%, 25%, 27%, 30%, 32%, 35%, 37%, 40%, 41%, 42%, 45%, 50%, 55%, or 60% of cells in the composition express ASCL1; and/or

(iii) about 50 transcripts per million (TPM) to about 150 TPM, about 60 TPM to about 140 TPM, about 65 TPM to about 130 TPM, or about 95 TPM to about 130 TPM of ASCL1 transcripts are expressed by the cells of the composition; and/or

(iv) at least 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, 120 TPM, 125 TPM, 130 TPM, 140 TPM, or 150 TPM of ASCL1 transcripts are expressed by the cells of the composition.

70. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 22%, about 10% to about 25%, or about 11% to about 22% of cells in the composition express RORB; and/or

(ii) at least about 5%, 7%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 25%, 30%, 35%, 40%, 45%, or 50% of cells in the composition express RORB; and/or

(iii) about 0.1 transcripts per million (TPM) to about 20 TPM, about 0.1 TPM to about 10 TPM, about 2 TPM to about 8 TPM, or about 1 TPM to about 6 TPM of RORB transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 1 TPM, 2 TPM, 3 TPM, 4 TPM, 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 12 TPM, 14 TPM, 16 TPM, 18 TPM, or 20 TPM of RORB transcripts are expressed by the cells of the composition.

71. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 25%, about 1% to about 20%, 1% to about 18%, 1% to about 16%, about 1% to about 14%, about 1% to about 12%, 1% to about 10%, about 2% to about 25%, about 2% to about 20%, 2% to about 18%, 2% to about 16%, about 2% to about 14%, about 2% to about 12%, or about 2% to about 5% of cells in the composition express NR2E3; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, or 20% of cells in the composition express NR2E3; and/or

(iii) about 0.1 transcripts per million (TPM) to about 10 TPM, about 0.1 TPM to about 9 TPM, about 0.1 TPM to about 3 TPM, or about 0.1 TPM to about 1 TPM of NR2E3 transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 0.4 TPM, 0.5 TPM, 0.6 TPM, 0.7 TPM, 0.8 TPM, 0.9 TPM, 1 TPM, 2 TPM, 5 TPM, 7 TPM, 9 TPM, or 10 TPM of NR2E3 transcripts are expressed by the cells of the composition.

72. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 2% to about 20%, about 2% to about 15%, about 2% to about 10%, about 2% to about 9%, or about 2% to about 8% of cells in the composition express NRL; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, or 20% of cells in the composition express NRL; and/or

(iii) about 0.1 transcripts per million (TPM) to about 10 TPM, about 0.1 TPM to about 9 TPM, about 0.1 TPM to about 7 TPM, or about 0.1 TPM to about 2 TPM of NRL transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 0.4 TPM, 0.5 TPM, 0.6 TPM, 0.7 TPM, 0.8 TPM, 0.9 TPM, 1 TPM, 1.5 TPM, 2 TPM, 4 TPM, 6 TPM, 8 TPM, or 10 TPM of NRL transcripts are expressed by the cells of the composition.

73. The photoreceptor rescue cell composition of any one of claims 55-72, wherein the neuron markers are selected from the group consisting of TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

74. The photoreceptor rescue cell composition of claim 73, wherein the plurality of heterogeneous cells cumulatively expresses each of the neuron markers TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

75. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 50% to about 100%, about 60% to about 100%, about 60% to about 95%, about 70% to about 100%, about 70% to about 95%, about 80% to about 100%, about 80% to about 95%, or about 80% to about 90% of cells in the composition express NFIB; and/or

(ii) at least about 50%, 55%, 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 98%, or 100% of cells in the composition express NFIB; and/or

(iii) about 150 transcripts per million (TPM) to about 650 TPM, about 180 TPM to about 610 TPM, about 400 TPM to about 500 TPM, or about 400 TPM to about 480 TPM of NFIB transcripts are expressed by the cells of the composition; and/or

(iv) at least 150 TPM, 175 TPM, 180 TPM, 185 TPM, 190 TPM, 200 TPM, 225 TPM, 250 TPM, 275 TPM, 300 TPM, 325 TPM, 350 TPM, 375 TPM, 400 TPM, 425 TPM, 450 TPM, 475 TPM, 500 TPM, 550 TPM, 600 TPM, 625 TPM, or 650 TPM of NFIB transcripts are expressed by the cells of the composition.

76. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 20%, about 1% to about 15%, about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 2% to about 20%, about 2% to about 15%, about 2% to about 10%, about 2% to about 9%, about 2% to about 8%, or about 2% to about 6% of cells in the composition express OTX2; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 14%, 16%, 18%, or 20% of cells in the composition express OTX2; and/or

(iii) about 5 transcripts per million (TPM) to about 50 TPM, about 8 TPM to about 40 TPM, about 8 TPM to about 25 TPM, or about 12 TPM to about 15 TPM of OTX2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 5 TPM, 6 TPM, 7 TPM, 8 TPM, 9 TPM, 10 TPM, 11 TPM, 12 TPM, 15 TPM, 16 TPM, 17 TPM, 18 TPM, 19 TPM, 20 TPM, 21 TPM, 22 TPM, 23 TPM, 24 TPM, 26 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, or 50 TPM of OTX2 transcripts are expressed by the cells of the composition.

77. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 78%, about 55% to about 90%, about 55% to about 85%, about 55% to about 80%, about 55% to about 78%, about 60% to about 90%, about 60% to about 85%, about 60% to about 80%, or about 60% to about 78% of cells in the composition express ELAVL3; and/or

(ii) at least about 50%, 55%, 60%, 65%, 70%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, or 90% of cells in the composition express ELAVL3; and/or

(iii) about 10 transcripts per million (TPM) to about 120 TPM, about 15 TPM to about 100 TPM, about 20 TPM to about 90 TPM, about 60 TPM to about 90 TPM, or about 70 TPM to about 80 TPM of ELAVL3 transcripts are expressed by the cells of the composition; and/or

(iv) at least 10 TPM, 15 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 71 TPM, 72 TPM, 73 TPM, 74 TPM, 75 TPM, 76 TPM, 77 TPM, 78 TPM, 80 TPM, 85 TPM, 90 TPM, 95 TPM, 100 TPM, 110 TPM, or 120 TPM of ELAVL3 transcripts are expressed by the cells of the composition.

78. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 30% to about 90%, about 30% to about 85%, about 30% to about 80%, about 30% to about 78%, about 35% to about 90%, about 35% to about 85%, about 35% to about 80%, about 35% to about 78%, about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, or about 50% to about 65% of cells in the composition express ELAVL4; and/or

(ii) at least about 30%, 40%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 75%, 80%, 85%, or 90% of cells in the composition express ELAVL4; and/or

(iii) about 10 transcripts per million (TPM) to about 120 TPM, about 15 TPM to about 100 TPM, about 20 TPM to about 90 TPM, about 50 TPM to about 90 TPM, about 70 TPM to about 90

TPM, or about 79 TPM to about 85 TPM of ELAVL4 transcripts are expressed by the cells of the composition; and/or

(iv) at least 10 TPM, 15 TPM, 20 TPM, 25 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM, 65 TPM, 70 TPM, 71 TPM, 72 TPM, 73 TPM, 74 TPM, 75 TPM, 76 TPM, 77 TPM, 78 TPM, 80 TPM, 82 TPM, 84 TPM, 86 TPM, 90 TPM, 100 TPM, 110 TPM, or 120 TPM of ELAVL4 transcripts are expressed by the cells of the composition.

79. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 40% to about 90%, about 40% to about 85%, about 40% to about 80%, about 40% to about 75%, about 50% to about 90%, about 50% to about 85%, about 50% to about 80%, about 50% to about 75%, about 50% to about 73%, about 51% to about 73%, or about 52% to about 66% of cells in the composition express SLC1A2; and/or

(ii) at least about 40%, 45%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 75%, 80%, 85%, or 90% of cells in the composition express SLC1A2; and/or

(iii) about 10 transcripts per million (TPM) to about 120 TPM, about 10 TPM to about 90 TPM, about 20 TPM to about 90 TPM, about 40 TPM to about 70 TPM, about 50 TPM to about 70 TPM, or about 58 TPM to about 63 TPM of SLC1A2 transcripts are expressed by the cells of the composition; and/or

(iv) at least 10 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 35 TPM, 40 TPM, 45 TPM, 50 TPM, 60 TPM, 65 TPM, 70 TPM, 75 TPM, 80 TPM, 90 TPM, 100 TPM, or 120 TPM of SLC1A2 transcripts are expressed by the cells of the composition.

80. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 10% to about 40%, about 10% to about 35%, about 10% to about 30%, about 10% to about 25%, about 11% to about 19%, or about 10% to about 12% of cells in the composition express SLC1A3; and/or

(ii) at least about 5%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 25%, 30%, 35%, or 40% of cells in the composition express SLC1A3; and/or

(iii) about 50 transcripts per million (TPM) to about 200 TPM, about 70 TPM to about 190 TPM, about 60 TPM to about 100 TPM, about 60 TPM to about 80 TPM, or about 72 TPM to about 79 TPM of SLC1A3 transcripts are expressed by the cells of the composition; and/or

(iv) at least 50 TPM, 60 TPM, 65 TPM, 70 TPM, 71 TPM, 72 TPM, 73 TPM, 74 TPM, 75 TPM, 76 TPM, 77 TPM, 78 TPM, 79 TPM, 80 TPM, 90 TPM, 100 TPM, 110 TPM, 120 TPM, 130

TPM, 140 TPM, 150 TPM, 160 TPM, 170 TPM, 175 TPM, 180 TPM, or 200 TPM of SLC1A3 transcripts are expressed by the cells of the composition.

81. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 1% to about 10%, about 1% to about 9%, about 1% to about 8%, about 1% to about 7%, about 1% to about 6%, about 1% to about 5%, about 1% to about 4%, or about 3% to about 4% of cells in the composition express HCN1; and/or

(ii) at least about 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, or 10% of cells in the composition express HCN1; and/or

(iii) about 0.1 transcripts per million (TPM) to about 10 TPM, about 0.1 TPM to about 5 TPM, about 0.1 TPM to about 1 TPM, or about 0.2 TPM to about 0.6 TPM of HCN1 transcripts are expressed by the cells of the composition; and/or

(iv) at least 0.1 TPM, 0.2 TPM, 0.3 TPM, 0.4 TPM, 0.5 TPM, 0.6 TPM, 0.7 TPM, 0.8 TPM, 0.9 TPM, 1.0 TPM, 2 TPM, 4 TPM, 6 TPM, 8 TPM, or 10 TPM of HCN1 transcripts are expressed by the cells of the composition.

82. The photoreceptor rescue cell composition of any one of the preceding claims, wherein

(i) about 5% to about 30%, about 5% to about 25%, about 1% to about 30%, about 1% to about 25%, about 5% to about 25%, about 10% to about 25%, or about 10% to about 15% of cells in the composition express HES5; and/or

(ii) at least about 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, or 30% of cells in the composition express HES5; and/or

(iii) about 10 transcripts per million (TPM) to about 60 TPM, about 5 TPM to about 50 TPM, about 12 TPM to about 46 TPM, or about 15 TPM to about 39 TPM of HES5 transcripts are expressed by the cells of the composition; and/or

(iv) at least 10 TPM, 15 TPM, 17 TPM, 20 TPM, 22 TPM, 25 TPM, 27 TPM, 30 TPM, 32 TPM, 35 TPM, 37 TPM, 40 TPM, 42 TPM, 45 TPM, 50 TPM, 55 TPM, 60 TPM of HES5 transcripts are expressed by the cells of the composition.

83. The photoreceptor rescue cell composition of any one of claims 1-82, wherein the plurality of heterogeneous cells cumulatively expresses:

(i) one or more of the eye field progenitor markers PAX6, LHX2, SIX3, NES, and SOX2;

(ii) one or more of the rod/cone photoreceptor markers ASCL1, RORB, NR2E3, and NRL;

and

(iii) one or more of the neuron markers TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

84. The photoreceptor rescue cell composition of any one of claims 1-83, wherein the plurality of heterogeneous cells cumulatively expresses:

- (i) each of the eye field progenitor markers PAX6, LHX2, SIX3, NES, and SOX2;
- (ii) each of the rod/cone photoreceptor markers ASCL1, RORB, NR2E3, and NRL; and
- (iii) each of the neuron markers TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, and HES5.

85. The photoreceptor rescue cell composition of any one of the preceding claims, wherein the composition is substantially free of at least one cell type selected from the group consisting of pluripotent stem cells, retinal ganglion cells, photoreceptors and amacrine cells.

86. The photoreceptor rescue cell composition of any one of the preceding claims, wherein the composition is substantially free of pluripotent stem cells, retinal ganglion cells, photoreceptors and amacrine cells.

87. The photoreceptor rescue cell composition of any one of the preceding claims, wherein the composition is substantially free of retinal progenitors expressing VSX2 and/or POU5F1.

88. The photoreceptor rescue cell composition of any one of preceding claims, wherein the composition has less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, or less than 0.5% of cells expressing SSEA4, optionally, determined by flow cytometry, or wherein the composition is free of cells expressing SSEA4, optionally, determined by flow cytometry.

89. The photoreceptor rescue cell composition of any one of preceding claims, wherein the cells in the composition have phagocytic activity, optionally the ability to phagocytose isolated photoreceptor outer segments, dye conjugates or both.

90. The photoreceptor rescue cell composition of any one of preceding claims, wherein the cells in the composition secrete one or more neuroprotective factors.

91. The photoreceptor rescue cell composition of claim 90, wherein the neuroprotective factors are selected from the group consisting of CNTF, MIF, S100B, GFAP, TAU, NCAM1, and TNC.

92. The photoreceptor rescue cell composition of any one of preceding claims, wherein at least 50%, at least 55%, at least 60%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 75%, or at least 80% of the cells in the composition are viable.

93. The photoreceptor rescue cell composition of any one of preceding claims, wherein about 50% to about 80%, about 55% to about 80%, about 60% to about 80%, about 65% to about 80%, about 70% to about 80%, about 75% to about 80%, about 50% to about 75%, about 55% to about 75%, about 60% to about 75%, about 65% to about 75%, about 70% to about 75%, about 50% to about 70%, about 55% to about 70%, about 60% to about 70%, about 65% to about 70%, about 50% to about 65%, about 55% to about 65%, about 60% to about 65%, about 50% to about 60%, about 55% to about 60%, or about 50% to about 55% of the cells in the composition are viable.

94. The photoreceptor rescue cell composition of any one of preceding claims, wherein at least 50% of the cells in the composition are viable.

95. The photoreceptor rescue cell composition of any one of preceding claims, wherein at least 55% of the cells in the composition are viable.

96. The photoreceptor rescue cell composition of any one of preceding claims, wherein at least 60% of the cells in the composition are viable.

97. The photoreceptor rescue cell composition of any one of preceding claims, wherein at least 65% of the cells in the composition are viable.

98. The photoreceptor rescue cell composition of any one of preceding claims, wherein at least 68% of the cells in the composition are viable.

99. The photoreceptor rescue cell composition of any one of claims 1-98, wherein the composition is produced according to a method comprising:

- 1) culturing pluripotent stem cells in Rescue Induction Medium (RIM) and Noggin;
- 2) culturing the cells from step 1 in Neural Differentiation Medium (NDM) and Noggin;
- 3) expanding the cells from step 2 in NDM in the absence of Noggin, comprising a) culturing the cells in low-adherence or non-adherent conditions in NDM without Noggin, and b) culturing the cells in adherent conditions in NDM without Noggin.

100. The photoreceptor rescue cell composition of claim 99, wherein the pluripotent stem cells are embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).

101. The photoreceptor rescue cell composition of claim 99 or 100, wherein step 3 is performed at least 1 time, at least 2 times, at least 3 times, at least 4 times, at least 5 times, or at least 6 times.

102. The photoreceptor rescue cell composition of any one of claims 99-101, wherein the cells in the composition are harvested after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3.

103. The photoreceptor rescue cell composition of 102, wherein the cells in the composition are harvested after the fifth repeat of step 3.

104. The photoreceptor rescue cell composition of 102 or 103, wherein the harvested cells in the composition are cryopreserved.

105. The photoreceptor rescue cell composition of any one of claims 99-104, wherein the composition is cryopreserved between the first and second repeat of step 3, between the second and third repeat of step 3, between the third and fourth repeat of step 3, or between the fourth and fifth repeat of step 3.

106. The photoreceptor rescue cell composition of any one of claims 99-104, wherein the composition is cryopreserved after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3.

107. The photoreceptor rescue cell composition of claim 106, wherein step 3 is repeated five times and wherein the composition is cryopreserved after the fifth repeat of step 3.

108. The photoreceptor rescue cell composition of any one of claims 99-107, wherein at least 50%, at least 55%, at least 60%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 75%, or at least 80% of the cells are viable after cryopreservation and thawing.

109. The photoreceptor rescue cell composition of any one of claims 99-108, wherein about 50% to about 80%, about 55% to about 80%, about 60% to about 80%, about 65% to about 80%, about 70% to about 80%, about 75% to about 80%, about 50% to about 75%, about 55% to about 75%, about 60% to about 75%, about 65% to about 75%, about 70% to about 75%, about 50% to about 70%, about 55% to about 70%, about 60% to about 70%, about 65% to about 70%, about

50% to about 65%, about 55% to about 65%, about 60% to about 65%, about 50% to about 60%, about 55% to about 60%, or about 50% to about 55% viable after cryopreservation and thawing.

110. The photoreceptor rescue cell composition of any one of claims 99-109, wherein at least about 50% of the cells in the composition are viable after cryopreservation and thawing.

111. The photoreceptor rescue cell composition of any one of claims 99-110, wherein at least about 55% of the cells in the composition are viable after cryopreservation and thawing.

112. The photoreceptor rescue cell composition of any one of claims 99-111, wherein at least about 60% of the cells in the composition are viable after cryopreservation and thawing.

113. The photoreceptor rescue cell composition of any one of claims 99-112, wherein at least about 65% of the cells in the composition are viable after cryopreservation and thawing.

114. The photoreceptor rescue cell composition of any one of claims 99-113, wherein at least 68% of the cells in the composition are viable after cryopreservation and thawing.

115. The photoreceptor rescue cell composition of any one of claims 99-114, wherein the composition comprises cell spheres.

116. The photoreceptor rescue cell composition of claim 115, wherein about 50% to about 100%, about 60% to about 100%, about 70% to about 100%, about 80% to about 100%, about 50% to about 90%, about 50% to about 80%, about 50% to about 70%, about 60% to about 90%, about 60% to about 80%, or about 70% to about 90% of the cells are cell spheres.

117. The photoreceptor rescue cell composition of any one of claims 99-116, wherein during steps 1, 2 and/or 3 the cells of the composition are cultured in a cell culture vessel selected from the group consisting of a culture dish, a culture flask, and a culture chamber.

118. The photoreceptor rescue cell composition of claim 117, wherein the cell culture vessel has about 50 cm² to about 800 cm², about 60 cm² to about 800 cm², about 100 cm² to about 800 cm², about 150 cm² to about 800 cm², about 175 cm² to about 800 cm², about 200 cm² to about 800 cm², about 250 cm² to about 800 cm², about 300 cm² to about 800 cm², about 400 cm² to about 800 cm², about 500 cm² to about 800 cm², about 600 cm² to about 800 cm², about 700 cm² to about 800 cm², about 30 cm² to about 100 cm², about 50 cm² to about 100 cm², about 100 cm² to about 300 cm², about 150 cm² to about 250 cm², or about 150 cm² to about 200 cm² cell growth area.

119. The photoreceptor rescue cell composition of claim 117 or 118, wherein the cell culture vessel has at least about 60 cm², about 175 cm², or about 636 cm² cell growth area.
120. The photoreceptor rescue cell composition of any one of claims 117-119, wherein the culture chamber is a stackable rectangular chamber.
121. The photoreceptor rescue cell composition of any one of claims 117-120, wherein step 3 has 1 to 40, 2 to 40, 5 to 40, 10 to 40, 20 to 40, 1 to 20, 2 to 20, 5 to 20, 10 to 20, 1 to 10, 2 to 10, 5 to 10, 1 to 5, or 1 to 2 culture chambers.
122. The photoreceptor rescue cell composition of claim 121, wherein step 3 has at least 1, 2, 5, 10, or 40 culture chambers.
123. The photoreceptor rescue cell composition of any one of claims 117-122, wherein the cell culture vessel is coated for low-adherence or non-adherent cell culture for step 3a, and/or adherent cell culture for step 3b.
124. The photoreceptor rescue cell composition of any one of claims 99-123, wherein the cells are enzymatically disassociated from the plate into cell suspension.
125. The photoreceptor rescue cell composition of claim 124, wherein the enzymatic disassociation utilizes an enzyme selected from the group consisting of thermolysin, liberase, accutase and a combination thereof.
126. The photoreceptor rescue cell composition of claim 125, wherein the enzyme used to disassociate cells is accutase.
127. The photoreceptor rescue cell composition of any one of claims 99-126, wherein disassociation of the cells from the plate does not involve manual scraping.
128. A pharmaceutical preparation comprising the photoreceptor rescue cell composition of any one of claims 1-127 and a pharmaceutically acceptable excipient.
129. The pharmaceutical preparation of claim 128, wherein the pharmaceutically acceptable excipient is suitable for ocular delivery.

130. A formulation comprising:
- a) the composition of any one of claims 1-127, or the pharmaceutical preparation of claim 128 or claim 129; and
 - b) about 4-10% (v/v) cryoprotectant, about 2-3% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer.
131. The formulation of claim 130, wherein the cryoprotectant is selected from DMSO, glycerol, and ethylene glycol.
132. The formulation of claim 130 or 131, wherein the cryoprotectant is DMSO.
133. The formulation of any one of claims 130-132, wherein the formulation comprises about 4-6% (v/v) cryoprotectant.
134. The formulation of any one of claims 130-133, wherein the formulation comprises about 0.08-0.10% (w/v) glucose.
135. The formulation of any one of claims 130-133, wherein the formulation comprises about 5% (v/v) DMSO, about 2.5% (w/v) albumin, about 0.09% (w/v) glucose, and a buffer.
136. The formulation of any one of claims 130-134, wherein the formulation comprises about 0.6% (w/v) glucose.
137. The formulation of any one of claims 130-134 or 136, wherein the formulation comprises about 5% DMSO, about 2.5% albumin, about 0.6% glucose, and a buffer.
138. The formulation of any one of claims 130-137, wherein albumin is human albumin.
139. The formulation of any one of claims 130-138, wherein albumin is recombinant human albumin.
140. The formulation of any one of claims 130-139, wherein the buffer is buffered saline.
141. The formulation of any one of claims 130-140, wherein the buffer is phosphate-buffered saline (PBS).

142. The formulation of claim 140 or 141, wherein the buffered saline comprises Ca^{2+} and Mg^{2+} .
143. The formulation of claim 140 or 141, wherein the buffered saline does not comprise Ca^{2+} and Mg^{2+} .
144. The formulation of any one of claims 130-143, wherein the formulation is cryopreserved.
145. A formulation comprising:
- a) the composition of any one of claims 1-127, or the pharmaceutical preparation of claim 128 or claim 129; and
 - b) a solution comprising
 - (1) a buffer, maintaining the solution at a physiological pH; and
 - (2) at least 2 mM or at least 0.05% (w/v) glucose; and
 - (3) an osmotically active agent maintaining the solution at a physiological osmolarity.
146. The formulation of claim 145, wherein the solution comprises at least 5 mM or at least 0.1% (w/v) glucose; or at least 7.5 mM or at least 0.14% (w/v) glucose; or at least 10 mM or at least 0.2% (w/v) glucose; or at least 15 mM or at least 0.25% (w/v) glucose; or at least 20 mM or at least 0.4% (w/v) glucose; or at least 25 mM or at least 0.5% (w/v) glucose.
147. The formulation of claim 145 or 146, wherein the solution further comprises
- (4) a source of divalent cations, optionally wherein the source of divalent cations comprises a calcium and/or a magnesium source, and/or
 - (5) an acetate buffer and/or a citrate buffer.
148. A formulation comprising:
- a) the composition of any one of claims 1-127, or the pharmaceutical preparation of claim 128 or claim 129; and
 - b) a solution comprising
 - (1) a buffer, maintaining the solution at a physiological pH, wherein the buffer is not a dicarbonate buffer; and
 - (2) glucose; and
 - (3) an osmotically active agent maintaining the solution at a physiological osmolarity; and

(4) a source of divalent cations,

optionally wherein the source of divalent cations comprises a calcium source and/or a magnesium source, and/or wherein the buffer comprises an acetate buffer and/or a citrate buffer.

149. The formulation of claim 147 or 148, wherein the calcium source comprises a pharmaceutically acceptable calcium salt, and/or the magnesium source comprises a pharmaceutically acceptable magnesium salt.

150. The formulation of claim 149, wherein the pharmaceutically acceptable calcium and/or the pharmaceutically acceptable magnesium salt is selected from the group of calcium and/or magnesium salts formed with an acid selected from the group comprising acetic acid, ascorbic acid, citric acid, hydrochloric acid, maleic acid, oxalic acid, phosphoric acid, stearic acid, succinic acid, and sulfuric acid.

151. The formulation of any one of claims 147-150, wherein the calcium source comprises calcium chloride, optionally wherein the calcium source comprises calcium chloride dihydrate.

152. The formulation of any one of claims 147-151, wherein the magnesium source comprises magnesium chloride, optionally wherein the magnesium source comprises magnesium chloride hexahydrate.

153. The formulation of any one of claims 147-152, wherein the citrate buffer is provided as sodium citrate.

154. The formulation of any one of claims 145-153, wherein the glucose is D-glucose (Dextrose).

155. The formulation of any one of claims 145-154, wherein the osmotically active agent is a salt, optionally wherein the osmotically active agent is a sodium salt, further optionally wherein the osmotically active agent is sodium chloride.

156. The formulation of any one of claims 145-155, wherein the solution comprises calcium chloride, magnesium chloride, sodium citrate, sodium chloride, and glucose.

157. The formulation of any one of claims 145-156, wherein the pH of the solution is 6.8-7.8, or 7.4-7.5, or about 7.5.

158. The formulation of any one of claims 145-157, wherein the solution is isotonic or hypertonic.

159. The formulation of any one of claims 145-157, wherein the solution exhibits an osmolality of about 270-345 mOsm/l or about 315 mOsm/l.

160. The formulation of any one of claims 145-159, wherein the concentration of the calcium source is

- (a) 0.25-0.75mM, or 0.4-0.65mM, or 0.5-0.6mM, or about 0.6 mM; or
- (b) 0.5-0.9 mM, or 0.6-0.8 mM, or about 0.7 mM.

161. The formulation of any one of claims 145-160, wherein the concentration of the magnesium source is 0.05-5 mM, or 0.1-0.3 mM, or about 0.3 mM.

162. The formulation of any one of claims 145-161, wherein the concentration of the glucose is 5-50 mM, or 10-25 mM, or 10-20 mM, or about 16 mM.

163. The formulation of any one of claims 145-162, wherein the concentration of the osmotically active agent is about 100-200 mM, or about 125-175 mM, or about 150 mM.

164. The formulation of any one of claims 145-163, wherein the concentration of citrate or acetate is 0.1-5 mM, or 0.5-2mM, or about 1mM.

165. The formulation of any one of claims 145-164, wherein the solution further comprises a potassium salt, optionally wherein the potassium salt is potassium chloride, further optionally wherein the concentration of KCl is 0.2-5 mM, or 1-2.5 mM, or about 2mM.

166. The formulation of any one of claims 145-165, wherein the solution comprises

(a) about 0.7 mM CaCl_2 (calcium chloride), about 0.3 mM MgCl_2 (magnesium chloride), about 1 mM sodium citrate, about 16 mM dextrose, and about 145 mM NaCl, or

(b) about 0.5-0.9 mM CaCl_2 (calcium chloride), about 0.2-.4 mM MgCl_2 (magnesium chloride), about 0.8-1.2 mM sodium citrate, about 13-19 mM dextrose, and about 116-174 mM NaCl, or

(c) about 0.85% NaCl, about 0.01% CaCl_2 dihydrate (calcium chloride dihydrate), about 0.006% MgCl_2 hexahydrate (magnesium chloride hexahydrate), about 0.035% sodium citrate dihydrate, and about 0.29% dextrose, or

(d) about 0.68-1.02 % NaCl, about 0.008-0.012% CaCl₂ dihydrate (calcium chloride dihydrate), about 0.0048-0.0072% MgCl₂ hexahydrate (magnesium chloride hexahydrate), about 0.028-0.042% sodium citrate dihydrate, and about 0.23-0.35% dextrose.

167. The formulation of any one of claims 145-166, wherein the solution further comprises

(a) about 2 mM KCl, and/or

(b) a viscoelastic polymer, optionally wherein the polymer is hyaluronic acid or a salt or solvate thereof, further optionally wherein the polymer is sodium hyaluronate.

168. The formulation of claim 167, wherein the polymer is present at a concentration effective to reduce the exposure of cells in the solution to shear stress, optionally wherein the concentration of the polymer is 0.005-5% w/v or about 0.05% w/v.

169. The formulation of any one of claims 145-168, wherein the solution comprises

(a) about 0.7 mM CaCl₂ (calcium chloride), about 0.3 mM MgCl₂ (magnesium chloride), about 2 mM KCl, about 1 mM sodium citrate, about 16 mM dextrose, about 145 mM NaCl, and about 0.05% hyaluronic acid, or

(b) about 0.5-0.8 mM CaCl₂ (calcium chloride), about 0.2-4 mM MgCl₂ (magnesium chloride), about 1.6-2.4 mM KCl, about 0.8-1.2 mM sodium citrate, about 13-19 mM dextrose, about 116-174 mM NaCl, and about 0.04-0.06% hyaluronic acid.

170. The formulation of any one of claims 145-169, wherein the solution does not comprise

(a) a carbonate buffer, and/or

(b) glutathione, or glutathione disulfide (GSSG), and/or

(c) a zwitterionic organic buffer.

171. The formulation of any one of claims 145-170, wherein the solution can be

(a) stored for at least 48 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 144 hours, at least one week, at least two weeks, at least three weeks, or at least one month at 25 °C without measurable precipitation of solutes and/or measurable loss of the capability of the solution to support survival and viability of cells stored in the solution, and/or

(b) stored for at least 48 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 144 hours, at least one week, at least two weeks, at least three weeks, or at least one month at 2-8 °C without measurable precipitation of solutes and/or measurable loss of the capability of the solution to support survival and viability of cells stored in the solution.

172. The formulation of any one of claims 145-171, wherein the solution is (a) suitable for administration to a subject, suitable for administration to the eye of a subject, and/or suitable for transplanting cells into the eye of a subject, and/or

(b) essentially pyrogen-free, and/or

(c) sterile, and/or

(d) for irrigation, cell reconstitution, cell storage, cell transport, and/or administration to a subject.

173. A method of producing the composition of any one of claims 1-127, comprising

1) culturing pluripotent stem cells in Rescue Induction Medium (RIM) and Noggin;

2) culturing the cells from step 1 in Neural Differentiation Medium (NDM) and Noggin;

3) expanding the cells from step 2 in NDM without Noggin, comprising a) culturing the cells in low-adherence or non-adherent conditions in NDM without Noggin, and b) and culturing the cells in non-adherent conditions in NDM without Noggin, thereby differentiating the pluripotent stem cells into photoreceptor rescue cells.

174. A method of producing a photoreceptor rescue cell composition comprising a plurality of heterogeneous cells, the method comprising:

1) culturing pluripotent stem cells in Rescue Induction Medium (RIM) and Noggin;

2) culturing the cells in Neural Differentiation Medium (NDM) and Noggin;

3) expanding the cells in NDM without Noggin, comprising a) culturing the cells in low-adherence or non-adherent conditions in NDM without Noggin, and b) and culturing the cells in adherent conditions in NDM without Noggin, thereby differentiating the pluripotent stem cells into the plurality of cells of the photoreceptor rescue cell composition.

175. The method of claim 173 or claim 174, wherein the pluripotent stem cells are embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs).

176. The method of any one of claims 173-175, wherein step 3 is performed at least 1 times, at least 2 times, at least 3 times, at least 4 times, at least 5 times, or at least 6 times.

177. The method of any one of claims 173-176, wherein the cells in the composition are harvested after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3.

178. The method of claim 177, wherein the composition are harvested after the fifth repeat of step 3.

179. The method of claim 177 or 178, wherein the harvested cells in the composition are cryopreserved.

180. The method of any one of claims 173-179, wherein the composition is cryopreserved between the first and second repeat of step 3, between the second and third repeat of step 3, between the third and fourth repeat of step 3, or between the fourth and fifth repeat of step 3.

181. The method of any one of claims 173-180, wherein the composition is cryopreserved after the third repeat of step 3, after the fourth repeat of step 3, or after the fifth repeat of step 3.

182. The method of claim 180, wherein step 3 is repeated five times and wherein the composition is cryopreserved after the fifth repeat of step 3.

183. The method any one of claims 173-182, wherein at least 50%, at least 55%, at least 60%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 75%, or at least 80% of the cells in the composition are viable after cryopreservation and thawing.

184. The method any one of claims 173-183, wherein about 50% to about 80%, about 55% to about 80%, about 60% to about 80%, about 65% to about 80%, about 70% to about 80%, about 75% to about 80%, about 50% to about 75%, about 55% to about 75%, about 60% to about 75%, about 65% to about 75%, about 70% to about 75%, about 50% to about 70%, about 55% to about 70%, about 60% to about 70%, about 65% to about 70%, about 50% to about 65%, about 55% to about 65%, about 60% to about 65%, about 50% to about 60%, about 55% to about 60%, or about 50% to about 55% of the cells in the composition are viable after cryopreservation and thawing.

185. The method any one of claims 173-184, wherein less than about 50% of the cells in the composition are viable after cryopreservation and thawing.

186. The method any one of claims 173-185, wherein less than about 55% of the cells in the composition are viable after cryopreservation and thawing.

187. The method any one of claims 173-186, wherein less than about 60% of the cells in the composition are viable after cryopreservation and thawing.

188. The method any one of claims 173-187, wherein less than about 65% of the cells in the composition are viable after cryopreservation and thawing.

189. The method any one of claims 173-188, wherein less than about 68% of the cells in the composition are viable after cryopreservation and thawing.

190. The method of any one of claims 173-189, wherein the composition comprises cell spheres.

191. The method of claim 190, wherein about 50% to about 100%, about 60% to about 100%, about 70% to about 100%, about 80% to about 100%, about 50% to about 90%, about 50% to about 80%, about 50% to about 70%, about 60% to about 90%, about 60% to about 80%, or about 70% to about 90% of the cells are cell spheres.

192. The method of any one of claims 173-191, wherein during steps 1, 2 and/or 3 the cells of the composition are cultured in a cell culture vessel selected from the group consisting of a culture dish, a culture flask, and a culture chamber.

193. The method of claim 192, wherein the cell culture vessel has about 50 cm² to about 800 cm², about 60 cm² to about 800 cm², about 100 cm² to about 800 cm², about 150 cm² to about 800 cm², about 175 cm² to about 800 cm², about 200 cm² to about 800 cm², about 250 cm² to about 800 cm², about 300 cm² to about 800 cm², about 400 cm² to about 800 cm², about 500 cm² to about 800 cm², about 600 cm² to about 800 cm², about 700 cm² to about 800 cm², about 30 cm² to about 100 cm², about 50 cm² to about 100 cm², about 100 cm² to about 300 cm², about 150 cm² to about 250 cm², or about 150 cm² to about 200 cm² cell growth area.

194. The method of claim 192 or 193, wherein the cell culture vessel has at least about 60 cm², about 175 cm², or about 636 cm² cell growth area.

195. The method of claim 192 or 193, wherein the culture chamber is a stackable rectangular chamber.

196. The method of claim 195, wherein step 3 has 1 to 40, 2 to 40, 5 to 40, 10 to 40, 20 to 40, 1 to 20, 2 to 20, 5 to 20, 10 to 20, 1 to 10, 2 to 10, 5 to 10, 1 to 5, or 1 to 2 culture chambers.

197. The method of claim 196, wherein step 3 has at least 1, 2, 5, 10, or 40 culture chambers.

198. The method of any one of claims 192-197, wherein the cell culture vessel is coated for low-adherence or non-adherent cell culture for step 3a, and/or adherent cell culture for step 3b.

199. The method of any one of claims 173-198, wherein the cells are enzymatically disassociated from the plate into cell suspension.

200. The method of claim 199, wherein the enzyme used to disassociate cells is thermolysin, liberase, and/or accutase.

201. The method of claim 200, wherein the enzyme used to disassociate cells is accutase.

202. The method of any one of claims 173-201, wherein disassociation of the cells from the plate does not involve manual scraping.

203. A photoreceptor rescue cell composition produced by the method of any one of claims 173-202.

204. A method of treating an eye disease or disorder in a subject, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

205. A method of increasing secretion of neuroprotective factors in an eye of a subject having an eye disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

206. The method of claim 205, wherein the method increases secretion of neuroprotective factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to secretion of neuroprotective factors prior to administration.

207. A method of improving visual acuity in a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

208. The method of claim 207, wherein the method increases visual acuity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to visual acuity prior to administration.

209. The method of claim 207 or 208, wherein visual acuity is measured by optometer response (OMR) and/or electroretinogram (ERG).

210. The method of claim 209, wherein the treated eye has increased spatial frequency threshold measured by OMR of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to spatial frequency threshold prior to administration.

211. The method of claim 209 or 210, wherein the treated eye has increased scotopic b-wave amplitude measured by ERG of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to scotopic b-wave amplitude prior to administration.

212. A method of preventing or slowing loss of photoreceptor cells in a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

213. The method of claim 212, wherein preventing loss of photoreceptor cells is measured by the expression of CNFT.

214. The method of claim 213, wherein the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% as compared to expression of CNFT in the eye prior to administration.

215. A method of increasing phagocytic activity in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

216. The method of claim 215, wherein the method increases phagocytic activity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least

60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to phagocytic activity prior to administration.

217. A method of inhibiting microglial activation in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

218. The method of claim 217, wherein inhibiting microglial activation is measured by the expression of CNFT and/or MIF.

219. The method of claim 218, wherein the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration.

220. The method of claim 217 or claim 218, wherein the method increases expression of MIF by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of MIF prior to administration.

221. A method of decreasing oxidative stress in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

222. The method of claim 221, wherein decreasing oxidative stress is measured by the expression of CNFT.

223. The method of claim 222, wherein the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration.

224. A method of increasing expression of anti-apoptotic factors in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue

cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

225. The method of claim 224, wherein the method increases expression of anti-apoptotic factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of anti-apoptotic factors prior to administration.

226. The method of claim 224 or 225, wherein the anti-apoptotic factor is S100B.

227. A method of preventing degeneration of outer nuclear layer (ONL) in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the photoreceptor rescue cell composition of any one of claims 1-127 or 203, the pharmaceutical preparation of any one of claims 128 or 129, or the formulation of any one of claims 130-172.

228. The method of any one of claims 204-227, wherein the disease or disorder is rod or cone dystrophy, retinal degeneration, retinitis pigmentosa, diabetic retinopathy, macular degeneration, geographic atrophy secondary to macular degeneration, intermediate dry age-related macular degeneration (AMD), Leber congenital amaurosis or Stargardt disease.

229. The method of claim 228, wherein the eye disease is macular degeneration or retinitis pigmentosa.

230. The method of any one of claims 204-229, wherein the disease is a retinal degenerative disease.

231. The method of any one of claims 204-230, wherein the disease is associated with loss of photoreceptor cells.

232. The method of claim 231, wherein the disease is associated with loss of photoreceptor cells in the outer nuclear layer of the retina.

233. The method of any one of claims 204-232, wherein the photoreceptor rescue cell composition, formulation or pharmaceutical preparation is administered to the subretinal space, the suprachoroidal space, by depot to the eye, or by systemic delivery to other part of the body of the subject.

234. The method of any one of claims 204-233, wherein the cell preparation or formulation is administered by injection or implantation.

235. The method of claim 234, wherein the injection is administered intraocularly.

236. The method of claim 234 or 235, wherein the intraocular administration comprises injection of an aqueous solution, optionally an isotonic solution and/or a saline solution, into the subretinal space, thereby forming a pre-bleb, and removal of said aqueous solution, prior to administration of said photoreceptor rescue cell composition into the same subretinal space as said aqueous solution.

237. The method of any one of claims 204-237, wherein the cell preparation or formulation is administered within at least 1 week, at least 1 month, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years of onset of symptoms.

238. The method of any one of claims 204-237, wherein

- (i) the subject has intermediate or near end-stage disease;
- (ii) the subject has a Best Corrected Visual Acuity (BCVA) ranging from 20/50 to 20/200;
- (iii) the subject has a BCVA worse than 20/200 but maintains light perception; or
- (iv) is diagnosed as having retinitis pigmentosa by genotyping.

239. The method of any one of claims 204-238, further wherein one or more anti-inflammatory agents are administered to the subject.

240. The method of claim 239, wherein the one or more anti-inflammatory agents are administered concurrently.

241. The method of claim 239, wherein the one or more anti-inflammatory agents are administered separately.

242. The method of claim 241, wherein the one or more anti-inflammatory agents are administered prior to the cell preparation or formulation, or the cell preparation or formulation is administered prior to the one or more anti-inflammatory agents.

243. The method of any one of claims 239, 241, and 242, wherein the one or more anti-inflammatory agents are administered before and after administration of the cell preparation or formulation.

244. The method of any one of claims 204-238, wherein the administration of the cell preparation or formulation is without one or more anti-inflammatory agents.

245. The method of any one of claims 243, wherein the one or more anti-inflammatory agents comprises dexamethasone and/or cyclosporine.

246. A population of extracellular vesicles (EVs) secreted from the photoreceptor rescue cell composition of any one of claims 1-127 and 203.

247. The population of EVs of claim 246, wherein the EVs secreted from the photoreceptor rescue cell express at least one of the proteins selected from FOXG1, MAP2, STMN2, DCX, LINC00461, NEUROD2, GAD1, NFIA, DLX5, TUBB3, SCGN, ERBB4, CALB2, NEUROD2, NEUROD6, SLA, NELL2, SATB2, VIM, MKI67, CLU, GLI3, GFAP, LUCAT1, MIR99AHG, FBXL7, MEIS2, PBX3, GRIA2, CACNA1C, PAX6, LHX2, SIX3, NES, SOX2, ASCL1, RORB, NR2E3, NRL, TUBB3, NFIA, NFIB, OTX2, ELAVL3, ELAVL4, SLC1A2, SLC1A3, HCN1, HES5, AGT, ACBLN2, CDH7, DNAH11, EGR1, FAM216B, FOS, KCNC2, LGI2, LOC221946, LRRC4C, MAP3k19, OLFM3, PRND, PTGER3, RELN, TCERGIL, TSHR, UNC13C, TRb2, PDE6B, CNGB1, Tuj1, CHX10, Nestin, TRbeta2, MASH1, RORbeta, MAP2, ELAVL3, NFIA, DCX, LHX2, SLC1A2, ELAVL4, PAX6, EMX2, ASCL1, DLL1, NFIB, ENOX1, TUBB3, MAP2, DCLK1/2, DCX, KALRN, LINC00461, C1orf61, NCAM1, SETBP1, PAK3, AKAP6, RTN1, CRMP1, FOXG1, TRIM2, BACH2, Recoverin, Opsin, Rhodopsin, rod and cone cGMP Phosphodiesterase.

248. A pharmaceutical composition comprising the population of EVs of claim 246 or claim 247 and a pharmaceutically acceptable carrier.

249. A formulation comprising:

a) the population of EVs of claim 192 or 193, or the pharmaceutical composition of claim 194; and

b) about 4-10% (v/v) cryoprotectant, about 2-3% (w/v) albumin, about 0-1.5% (w/v) glucose, and a buffer.

250. The formulation of claim 249, wherein the cryoprotectant is selected from DMSO, glycerol, and ethylene glycol.

251. The formulation of claim 249 or 250, wherein the cryoprotectant is DMSO.

252. The formulation of any one of claims 248-251, wherein the formulation comprises about 4-6% (v/v) cryoprotectant.

253. The formulation of any one of claims 248-252, wherein the formulation comprises about 0.08-0.10% (w/v) glucose.

254. The formulation of any one of claims 248-253, wherein the formulation comprises about 5% (v/v) DMSO, about 2.5% (w/v) albumin, about 0.09% (w/v) glucose, and a buffer.

255. The formulation of any one of claims 248-252, wherein the formulation comprises about 0.6% (w/v) glucose.

256. The formulation of any one of claims 248-252 or 255, wherein the formulation comprises about 5% DMSO, about 2.5% albumin, about 0.6% glucose, and a buffer.

257. The formulation of any one of claims 248-256, wherein albumin is human albumin.

258. The formulation of any one of claims 248-257, wherein albumin is recombinant human albumin.

259. The formulation of any one of claims 248-258, wherein the buffer is buffered saline.

260. The formulation of any one of claims 248-259, wherein the buffer is phosphate-buffered saline (PBS).

261. The formulation of claim 259 or 260, wherein the buffered saline comprises Ca^{2+} and Mg^{2+} .

262. The formulation of claim 259 or 260, wherein the buffered saline does not comprise Ca^{2+} and Mg^{2+} .

263. The formulation of any one of claims 249-262, wherein the formulation is cryopreserved.

264. A method of treating an eye disease in a subject, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the

population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

265. A method of increasing secretion of neuroprotective factors in an eye of a subject, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

266. The method of claim 265, wherein the method increases secretion of neuroprotective factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to secretion of neuroprotective factors prior to administration.

267. A method of improving visual acuity in a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

268. The method of claim 267, wherein the method increases visual acuity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to visual acuity prior to administration.

269. The method of claim 267 or 268, wherein visual acuity is measured by optometer response (OMR) and/or electroretinogram (ERG).

270. The method of claim 269, wherein the treated eye has increased spatial frequency threshold measured by OMR of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to spatial frequency threshold prior to administration.

271. The method of claim 269 or 270, wherein the treated eye has increased scotopic b-wave amplitude measured by ERG of at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to scotopic b-wave amplitude prior to administration.

272. A method of preventing or slowing loss of photoreceptor cells in a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

273. The method of claim 272, wherein preventing or slowing loss of photoreceptor cells is measured by the expression of CNFT.

274. The method of claim 273, wherein the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% as compared to expression of CNFT in the eye prior to administration.

275. A method of increasing phagocytic activity in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

276. The method of claim 275, wherein the method increases phagocytic activity by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to phagocytic activity prior to administration.

277. A method of inhibiting microglial activation in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

278. The method of claim 277, wherein inhibiting microglial activation is measured by the expression of CNFT and/or MIF.

279. The method of claim 278, wherein the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration.

280. The method of claim 277 or claim 278, wherein the method increases expression of MIF by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%,

at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of MIF prior to administration.

281. A method of decreasing oxidative stress in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

282. The method of claim 281, wherein decreasing oxidative stress is measured by the expression of CNFT.

283. The method of claim 282, wherein the method increases expression of CNFT by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of CNFT prior to administration.

284. A method of increasing expression of anti-apoptotic factors in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

285. The method of claim 284, wherein the method increases expression of anti-apoptotic factors by at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, or at least 90% as compared to expression of anti-apoptotic factors prior to administration.

286. The method of claim 284 or 285, wherein the anti-apoptotic factor is S100B.

287. A method of preventing degeneration of outer nuclear layer (ONL) in an eye of a subject having a retinal disease or disorder, comprising administering to the subject the population of EVs of claims 246 or 247, the pharmaceutical composition comprising the population of EVs of claim 248, or the formulation comprising the population of EVs of any one of claims 249-263.

288. The method of any one of claims 264-287, wherein the disease or disorder is rod or cone dystrophy, retinal degeneration, retinitis pigmentosa, diabetic retinopathy, macular degeneration, geographic atrophy secondary to macular degeneration, intermediate dry age-related macular degeneration (AMD), Leber congenital amaurosis or Stargardt disease.

289. The method of claim 288, wherein the eye disease is macular degeneration or retinitis pigmentosa.

290. The method of any one of claims 264-289, wherein the disease is a retinal degenerative disease.

291. The method of any one of claims 264-290, wherein the disease is associated with loss of photoreceptor cells.

292. The method of claim 291, wherein the disease is associated with loss of photoreceptor cells in the outer nuclear layer of the retina.

293. The method of any one of claims 264-292, wherein the population of EVs, the pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered to the subretinal space or to the suprachoroidal space of the subject.

294. The method of any one of claims 264-293, wherein the population of EVs, the pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered by injection or implantation.

295. The method of claim 294, wherein the injection is administered intraocularly.

296. The method of claim 294 or 295, wherein the intraocular administration comprises injection of an aqueous solution, optionally an isotonic solution and/or a saline solution, into the subretinal space, thereby forming a pre-bleb, and removal of said aqueous solution, prior to administration of said photoreceptor rescue cell composition into the same subretinal space as said aqueous solution.

297. The method of any one of claims 287-296, wherein the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered within at least 1 week, at least 1 month, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, or at least 5 years of onset of symptoms.

298. The method of any one of claims 287-297, further wherein one or more anti-inflammatory agents are administered to the subject.

299. The method of claim 298, wherein the one or more anti-inflammatory agents and the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs are administered concurrently.

300. The method of claim 298, wherein the one or more anti-inflammatory agents and the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs are administered separately.

301. The method of claim 300, wherein

1) the one or more anti-inflammatory agents are administered prior to the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs, or

2) the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is administered prior to the one or more anti-inflammatory agents.

302. The method of any one of claims 298, 300, and 301, wherein the one or more anti-inflammatory agents are administered before and after administration of the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs.

303. The method of any one of claims 287-297, wherein the administration of the population of EVs, pharmaceutical composition comprising the population of EVs, or the formulation comprising the population of EVs is without one or more anti-inflammatory agents.

304. The method of claim 303, wherein the one or more anti-inflammatory agents comprises dexamethasone and/or cyclosporine.