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(54) **Title**: NANOPARTICULATE SYSTEMS FOR USE IN GENE TRANSFER OR GENE DELIVERY

(57) **Abstract**: The present invention provides a nanoparticulate system or composition which comprises nanoparticles comprising a core of a sorbitan ester, a surface layer of a cationic substance and a gene construct consisting of plasmid DNA, wherein the gene construct is physically bound to the cationic substance, for use in a method of treatment by gene therapy, in particular for medical applications by using gene therapy in the ophthalmic sector.

NANOPARTICULATE SYSTEMS FOR USE IN GENE TRANSFER OR GENE DELIVERY.

FIELD OF THE INVENTION

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The present invention relates to systems comprising nanoparticles capable of associating genes. More specifically, it relates to nanoparticulate systems comprising sorbitan esters for *in vivo* gene therapy, in particular for applications in the ophthalmic sector.

10

BACKGROUND OF THE INVENTION

Micelles, mixed micelles, emulsions, nanoparticles and liposomes stand out among colloidal systems proposed for gene transport, as an alternative to viral vectors for use
15 in treatment methods by gene therapy.

However, some of systems mentioned above have a serious problem concerning stability. Vesicular systems and emulsions are known to experience aggregation phenomena, and the difficulty in obtaining more stable formulations by means of
20 processes such as lyophilization without significantly changing their initial characteristics is also known. In this sense, a considerable energy input and/or the use of specific combinations of surface-active agents is necessary for the formation of such systems, so that the obtained product is not in an energetically unfavorable situation or unstable. Furthermore, these systems are particularly sensitive to variations in the surrounding
25 environment, such as temperature.

Therefore, there is still a need to provide alternative and efficient systems to the systems referred to above for use in treatment methods by gene therapy, preferably in human subjects.

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SUMMARY OF THE INVENTION

The present invention solves the above mentioned problem by providing, in a first aspect of the invention, a nanoparticulate system or composition which comprises
35 nanoparticles comprising a core of a sorbitan ester, a surface layer of a cationic

substance and a gene construct consisting of plasmid DNA, wherein the gene construct is physically bound to the cationic substance.

This system or composition is particularly suitable for use in a method of treatment by gene transfer or gene delivery in a mammalian subject, preferably in a human subject.

In a preferred embodiment of the first aspect of the invention, the sorbitan ester is selected from the group consisting of sorbitan mono-, di-, tri- or sesquioleate; sorbitan mono-, di-, tri- or sesquilaurate; sorbitan mono-, di-, tri- or sesquipalmitate; sorbitan mono-, di-, tri- or sesquistearate; and sorbitan mono-, di-, tri- or sesquiisostearate or any of their combinations thereof; and the cationic substance is a polyamino acid or oleylamine (OA). Preferably, the cationic substance is polyarginine (PA).

In another preferred embodiment of the first aspect of the invention or of any of its preferred embodiments, the gene construct, physically bound to the cationic substance, comprises a polynucleotide sequence comprising at least a nucleotide sequence encoding a protein with biological activity encoded by any of the genes selected from the list consisting of: *PLA2G5, NMNAT1, RPE65, ABCA4, PRPF3, PRPF31, PRPF8, RD3, EFEMP1, KCNJ13, RGR, RBP4, BEST1, RDH5, RLBP1, CLN3*, or any combinations thereof. Preferably, the selected gene is *PRPF31.* Preferably, the cationic substance is polyarginine and the gene construct, physically bound to the cationic substance, comprises a polynucleotide sequence comprising at least a nucleotide sequence encoding a protein with biological activity encoded by any of the genes selected from the list consisting of: *PLA2G5, NMNAT1, RPE65, ABCA4, PRPF3, PRPF31, PRPF8, RD3, EFEMP1, KCNJ13, RGR, RBP4, BEST1, RDH5, RLBP1, CLN3*, or any combinations thereof. Preferably, the selected gene is *PRPF31.*

In a second aspect of the invention, the nanoparticulate system or composition as defined in the first aspect of the invention, is a pharmaceutical composition which optionally further comprises pharmaceutically acceptable excipients, carriers and/or adjuvants.

In a third aspect of the invention, the nanoparticulate system or composition as defined in the second or third aspect of the invention is use in a method of treatment of abnormalities or dysfunctions of the retinal pigment epithelium. Preferably, the

abnormality or dysfunction of the retinal pigment epithelium is selected from the list consisting of: age related macular degeneration, retinitis pigmentosa, Leber's congenital amaurosis, Stargardt's disease, diabetic retinopathy, macular dystrophy, cataracts, disruption of retinal pigment epithelium cells, deterioration of retinal pigment epithelium cells, death of retinal pigment epithelium cells, drusden, pigment clumping, RPE detachment, geographic atropy, sub-retinal neovascularization and disciform scar, vitelliform macular dystrophy, butterfly macular dystrophy, Sjögren's syndrome, fundus flavimaculatus, dominant foveal dystrophy, central areolar pigment epithelial dystrophy, North Carolina dystrophy, familiar drusen, pattern dystrophy of Marmor and Byers, foveomacular dystrophy (adult type), dominant slowly progressive macular dystrophy of Singerman-Berkow-Patz,macroreticular dystrophy of the RPE, pigment epithelial dystrophy of Noble-Carr-Siegel, benign concentric annular dystrophy, or combinations thereof. More preferably, the abnormality or dysfunction of the retinal pigment epithelium is selected from the list consisting of Retinitis Pigmentosa, Age-Related Macular Degeneration and/or Leber's congenital amaurosis. Still more preferably, the abnormality or dysfunction of the retinal pigment epithelium is Retinitis Pigmentosa.

In a preferred embodiment of the third aspect of the invention, the administration of the system or composition is via subretinal injection, in particular, in the subretinal space between the photoreceptors and the retinal pigment epithelium (RPE).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. A) Retinal sections were obtained 72 hours after the subretinal injection of blank SP-OA nanoparticles (AC) or SP-OA-GFP nanoparticles (DL). Confocal images of a retinal section are shown in images D-F, and images G-L correspond to enlarged images of the pigmented epithelium of the retina (G-I) and ganglion cells (J-L). The cell nuclei were visualized using DAPI stain (left panel; blue) and the GFP signal was enhanced with anti-GFP antibodies (central panel; green). The right panel shows merged images. (RPE = retinal pigment epithelium; ONL = outer nuclear layer; INL = inner nuclear layer; GLC = ganglion cell layer). The scale bars represent 75 (A-F) and 20 m (G-L). B) Western blot using an anti-GFP antibody in three independent samples from each group.

Figure 2. GFP evaluation in mice one month after the subretinal injection of: AAV2-GFP, SP-PA-GFP nanoparticles, SP-OA-GFP nanoparticles, PBS and 5% glucose (image on the left) or blank SP-PA nanoparticles and blank SP-OA nanoparticles (image on the right). Image on the left: *in vivo* scanning laser ophthalmoscopy for ocular fundus (A-E) and GFP signal (F-J); Confocal images of retinal sections showing the characteristic GFP signal (K-O) or GFP signal improved with anti-GFP antibodies (P-T); merged images with the cell nuclei stained with DAPI stain (UY; Blue). Image on the right: Confocal images of retinal sections showing the characteristic GFP signal (AD) or GFP signal improved with anti-GFP antibodies (BE) and merged images with the cell nuclei stained with DAPI stain GFP signal (CF; blue). (RPE = retinal pigment epithelium; ONL = outer nuclear layer; INL = inner nuclear layer; GLC = ganglion cell layer). The scale bars represent 50 micron.

Figure 3. A) Western Blot using the anti-GFP antibody in three independent samples from each group of animals one month after the injection of AAV2-GFP, SP-PA-GFP and SP-OA-GFP nanoparticles. B) Quantification of the expression levels obtained by image analysis. The bars represent the mean ratio between good farming practices and alpha-tubulin ± SEM of three samples. The results were not statistically significant ($p>0.05$). Alpha-tubulin was used for verifying load control.

Figure 4. Electroretinograms of mice injected in dark- and light-adapted conditions one month after the administration of PBS, 5% glucose, AAV2-GFP, SP-PA, SP-OA, SP-PA-GFP and SP-OA-GFP nanoparticles.

Figure 5. Quantification of rods (A, D), joint cone-rod response (B, E) and cone responses (C, F) obtained from electroretinogram. The results are expressed in mean ± SEM and represent the a-wave or b-wave amplitude (mV) of both eyes in 3 mice for each group. The statistical differences between the treated groups and their respective controls were estimated using the t-test. $P = 0.052$ (A), $P = 0.057$ (B) *$P<0.05$ (C, E), **$P<0.01$ (D, F).

Figure 6. Positive optomotor responses observed at a frequency of 0.067 one month after the subretinal injections. The results are expressed in mean ± SEM and represent the percentage of positive responses in 2 mice for each group evaluated at two different times. The statistical differences between the treated groups and their respective

controls were estimated using the t-test. *P<0.05.

Figure 7. In the *in vivo* laser ophthalmoscopy examination for ocular fundus and GFP signal one month after the subretinal injection of a 5% glucose solution in wild-type mice (WT 5% Glu control- -negative) or after the injection of SP-PA-GFP nanoparticles and SP-OA PRPF31 nanoparticles in *Prpf31*$^{A216P/+}$ mice (KI).

Figure 8. Optomotor test establishing 6 frequencies from 0.031 to 0.272 cycles/degree (c/d) at 100% (A), 75% (B) and 50% (C) contrast sensitivity in healthy mice (WT) injected with a 5% glucose negative control solution (WT 5% Glu) (n = 3), and the mouse model of the disease, *Prpf31*$^{A216P/+}$ mice (KI), injected with a control formulation of nanoparticles associating the GFP plasmid (KI SP-PA-GFP) (n = 3) or the developed SP-PA nanoparticles associating the human gene PRPF31 (KI SP-PA-PRPF31) (n = 4). The bars represent ± SEM. *P<0.05, **P<0.01 represents the statistically significant difference between SP-PA-GFP and SP-PA-PRPF31.

Figure 9. A) Optical coherence tomography scanner and retinal map of healthy WT mice treated with a 5% glucose negative control solution (WT 5% Glu), and KI mice, control of the disease, treated with a control formulation of nanoparticles associating the GFP plasmid (KI SP-PA-GFP) or a formulation of SP-PA-based nanoparticles associating the PRPF31 plasmid (KI SP-PA-PRPF31). The color bar represents a retinal thickness scale. B) Graph depicting the mean thickness of the retina in each group. The bars represent ± SEM. *P<0.01, statistical comparisons were made by means of one-way ANOVA with *post hoc* Dunnett comparing the two experimental groups with the control separately.

Figure 10. GFP evaluation in *Prpf31*$^{A216P/+}$ mice retinas four months after the subretinal injection of 1 µL of a viral suspension containing 10^9 pv/ml of AAV2-GFP, the AAV2-PRPF31 viral construct or PBS: Laser ophthalmoscopic scanning for ocular fundus and GFP signal (A, B); Confocal images of retinal sections of the GFP signal with the cell nuclei stained with DAPI stain (C, D; Blue). Image on the right (E): Western Blot in three independent samples from each group. The expression of the PRPF31 protein in RPE cells was visualized through the fluorescence signal of GFP. GAPDH antibodies were used as load control. RPE: retinal pigment epithelium, ONL: outer nuclear layer, INL: inner nuclear layer, GCL: ganglion cell layer. The scale bar represents 50 micron.

Figure 11. Evaluation of retinal function after the injection of AAV2-PRPF31: Electroretinogram (ERG) of *Prpf31*$^{A216P/+}$ mice (KI) was done under dark- and light-adapted conditions 4 months after the subretinal injection of PBS (negative control),
5 AAV2-GFP or AAV2-PRPF31 (4 animals per group). The a-wave and b-wave amplitude and frequency were quantified in different conditions. The amplitude of different ERG waves corresponding to rod responses (B), cone-rod responses (C) or cone responses (D) decreased in injected AAV2-*PRPF31* mice when compared with the group with PBS injection, respectively. Bars in Figures 11B, 11C and 11D represent mean ERG wave
10 amplitudes ± SEM. (*P<0.05, one-way ANOVA). The scale bars in Figure 11A represent 100 ms and 600 mV.

Figure 12. Positive optomotor responses observed at a frequency 0.067 one month after the subretinal injections of a 5% glucose solution (negative control group), SP-OA-
15 based nanoparticles (blank formulation) or SP-OA-based nanoparticles associating the *PRPF31* gene. Positive responses decreased in mice injected with SP-OA-*PRPF31* when compared with the negative control group. The results are expressed in mean ± SEM and represent the percentage of positive responses in 3 mice for each group evaluated at two different times. The statistical differences between the treated groups
20 and their respective controls were estimated using the t-test. *P<0.05.

Figure 13. Test for evaluating the visual function in mutant mice (*Prpf31*$^{A216P/+}$) 8 months of age compared with healthy mice. *Prpf31*$^{A216P/+}$ mice show impairment of spatial vision (visual acuity and contrast perception). Optomotor adjustment test
25 establishing 6 frequencies from 0.031 to 0.272 cycles/degree (c/d) at 100% (A), 75% (B) and 50% (C) contrast sensitivity in wild-type mice (WT) (n = 5) and *Prpf31*$^{A216P/+}$ mice (n = 5). The bars represent ± SEM. *P<0.05, **P<0.01 represents the statistically significant difference between WT and *Prpf31*$^{A216P/+}$

30 **DESCRIPTION OF THE INVENTION**

Definitions

As used herein, the term “nanoparticle” refers to solid colloidal materials, the mean size
35 of which ranges between 1 and 999 nm, having a solid matrix structure and further

characterized by being stable structures having perfectly homogenous, reproducible and modulable characteristics.

As used herein, “Sorbitan esters” are interpreted to mean esterified sorbitan derivatives
5 where the ester groups have a substituent selected from alkyl, alkenyl and alkynyl. Preferably, alkyl, alkenyl and alkynyl have a chain of between 6 and 24 carbon atoms, more preferably between 10 and 16 carbon atoms. Sorbitan esters include derivatives in which one, two, three or four hydroxyl groups are esterified, and they even include esterified derivatives in which one ester molecule is present for every two sorbitan
10 molecules (in which case they are referred to with the “sesqui-” prefix). In that sense, for example, sorbitan monooleate is the sorbitan ester resulting from esterifying a hydroxyl group with oleic acid; sorbitan trioleate is the sorbitan ester resulting from esterifying three sorbitan hydroxyl groups with oleic acid.

15 As used herein, “Alkyl” is interpreted to mean a linear or branched hydrocarbon chain that contains no instauration, of 1 to 24 carbon atoms, optionally substituted with one to three substituents selected from $-OR^b$, $-SR^b$, $-NR^aR^b$, $-C(O)R^b$, $-CO_2R^b$, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aC(O)OR^b$, $-NR^aC(O)NR^aR^b$, $-CF_3$, $-OCF_3$; where R^a and R^b are independently selected from hydrogen, alkyl, alkenyl and alkynyl.

20

As used herein, “Alkenyl” and “alkynyl” in the compounds of the present invention refer to a linear or branched hydrocarbon chain containing at least one instauration, of 2 to 24 carbon atoms, optionally substituted with one to three substituents selected from $-OR^b$, $-SR^b$, $-NR^aR^b$, $-C(O)R^b$, $-CO_2R^b$, $-C(O)NR^aR^b$, $-NR^aC(O)R^b$, $-NR^aC(O)OR^b$,
25 $-NR^aC(O)NR^aR^b$, $-CF_3$, $-OCF_3$; where R^a and R^b are as previously defined.

As used herein, the term “cationic substance” is interpreted to mean that molecule provided with a positive electric charge, for example ammonium salts, cationic polymers and lipophilic or fatty amines.

30

As used herein the term “cationized” refers to the presence of a positively charged group, which may be present naturally or may be introduced by means of a chemical reaction.

35 As used herein, "Gene transfer" or "gene delivery" refers to methods or systems for

reliably introducing a particular nucleotide sequence (e. g., DNA) into targeted cells. The introduced nucleotide sequences may persist in vivo in episomal forms. or integrate into the genome of the target cells. Gene transfer provides a unique approach for the treatment of acquired and inherited diseases, and a number of systems have been
5 developed in the art for gene transfer into mammalian cells. See, e. g., U. S. Pat. No. 5, 399,346.

As used herein the term "expression control element" or "regulatory element" or particularly “plasmid” refers collectively to promoter sequences, polyadenylation signals,
10 transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell. Not all of these control sequences need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated
15 in an appropriate host cell.

A used herein, “Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, control elements operably linked to a coding sequence are capable of effecting the
20 expression of the coding sequence. The control elements need not be contiguous with the coding sequence, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

25

As used herein, the term "promoter" refers to a set of control nucleic acid sequences which are controlling transcription of a target nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription. Also, a promoter may optionally include an enhancer or repressor element. A "constitutive promoter" is a
30 promoter that is continuously active and is not subject to regulation by external signals or molecules. In contrast, the activity of an "inducible promoter" is regulated by an external signal or molecule (such as a transcription factor).

A used herein, the term “active ingredient” refers to an ingredient or cell used in the
35 treatment, cure, prevention or diagnosis of a disease, or used for improving the physical

and mental wellbeing of humans and animals, as well as that ingredient or cell intended for destroying, blocking the action of, counteracting or neutralizing any harmful entity or organism, or any ingredient or cell used as a cosmetic or for hygiene, as well as that ingredient or cell intended for regenerating tissues in tissue engineering or in cell and gene therapy.

As used herein, the term “PRPF31” is as defined in table 1 or as defined as follows by its gene bank sequences. In particular, the Genebank (ncbi) nucleotide sequence of PRPF31 is NM_015629 (SEQ ID NO 1), and the aminoacidic sequence is NP_056444.3 (SEQ ID NO 2). Variant sequences substantially identical to SEQ ID NO 2 are also included within the boundaries of the present invention. The terms "identical" or "identity," in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that are the same. Sequences are "substantially identical" if they have a percentage of amino acid residues or nucleotides that are the same {i.e., about 60% identity, optionally about 65%, about 70%>, about 75%, about 80%), about 85%), about 90%>, or about 95% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection.

SEQ ID NO. 1:

tagtttcctgtttccggcttcgcttcggcccacccccacgtccaccccgaatccctgcttaaaggccttgctttcttgtctaacgc
cgcaaccagtcctctgagttgccaacgtctttcttcttgtctcgacgccccgtcgtccggccacagcgattctctgcttagcag
gatcggtccacagcgggacgtgagtccctttcctcctcgcggcttaccgcctctctccgcctagtgccaggtgctaataaagt
tgttgtttcaaatgcggccaggaacatcgcgagcggggaccaatcagagagtagctttgcctctataacggcgcgagagt
gagacgtcatcggtgagcgactaacgctagaaacagtggtgcgcggagaggagaggcctcgggatgtctctggcagat
gagctcttagctgatctcgaagaggcagcagaagaggaggaaggaggaagctatggggaggaagaagaggagcca
gcgatcgaggatgtgcaggaggagacacagctggatctttccggggattcagtcaagaccatcgccaagctatgggata
gtaagatgtttgctgagattatgatgaagattgaggagtatatcagcaagcaagccaaagcttcagaagtgatgggacca
gtggaggccgcgcctgaataccgcgtcatcgtggatgccaacaacctgaccgtggagatcgaaaacgagctgaacatc
atccataagttcatccgggataagtactcaaagagattccctgaactggagtccttggtccccaatgcactggattacatcc
gcacggtcaaggagctgggcaacagcctggacaagtgcaagaacaatgagaacctgcagcagatcctcaccaatgc
caccatcatggtcgtcagcgtcaccgcctccaccacccaggggcagcagctgtcggaggaggagctggagcggctgg
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atcgcacccaacctgtccatcattatcggggcatccacggccgccaagatcatgggtgtggccggcggcctgaccaacct
ctccaagatgcccgcctgcaacatcatgctgctcggggcccagcgcaagacgctgtcgggcttctcgtctacctcagtgct
gccccacaccggctacatctaccacagtgacatcgtgcagtccctgccaccggatctgcggcggaaagcggcccggct
ggtggccgccaagtgcacactggcagcccgtgtggacagtttccacgagagcacagaagggaaggtgggctacgaac
5 tgaaggatgagatcgagcgcaaattcgacaagtggcaggagccgccgcctgtgaagcaggtgaagccgctgcctgcg
cccctggatggacagcggaagaagcgaggcggccgcaggtaccgcaagatgaaggagcggctggggctgacgga
gatccggaagcaggccaaccgtatgagcttcggagagatcgaggaggacgcctaccaggaggacctgggattcagcc
tgggccacctgggcaagtcgggcagtgggcgtgtgcggcagacacaggtaaacgaggccaccaaggccaggatctc
caagacgctgcagcggaccctgcagaagcagagcgtcgtatatggcgggaagtccaccatccgcgaccgctcctcgg
10 gcacggcctccagcgtggccttcaccccactccagggcctggagattgtgaacccacaggcggcagagaagaaggtg
gctgaggccaaccagaagtatttctccagcatggctgagttcctcaaggtcaagggcgagaagagtggccttatgtccac
ctgaatgactgcgtgtgtccaaggtggcttcccactgaagggacacagaggtccagtccttctgaagggctaggatcgggt
tctggcagggagaacctgccctgccactggccccattgctgggactgcccagggaggaggccttggaagagtccggcct
ggcctcccccaggaccgagatcaccgcccagtatgggctagagcaggtcttcatcatgccttgtctttttaactgagaaag
15 gagatttttgaaaagagtacaattaaaggacattgtcaagatctgtcaaaaaaaaaaaaaaaaa

SEQ ID NO. 2:
MSLADELLADLEEAAEEEEGGSYGEEEEEPAIEDVQEETQLDLSGDSVKTIAKLWDSK
MFAEIMMKIEEYISKQAKASEVMGPVEAAPEYRVIVDANNLTVEIENELNIIHKFIRDKYS
20 KRFPELESLVPNALDYIRTVKELGNSLDKCKNNENLQQILTNATIMVVSVTASTTQGQQ
LSEEELERLEEACDMALELNASKHRIYEYVESRMSFIAPNLSIIIGASTAAKIMGVAGGLT
NLSKMPACNIMLLGAQRKTLSGFSSTSVLPHTGYIYHSDIVQSLPPDLRRKAARLVAAK
CTLAARVDSFHESTEGKVGYELKDEIERKFDKWQEPPPVKQVKPLPAPLDGQRKKRG
GRRYRKMKERLGLTEIRKQANRMSFGEIEEDAYQEDLGFSLGHLGKSGSGRVRQTQV
25 NEATKARISKTLQRTLQKQSVVYGGKSTIRDRSSGTASSVAFTPLQGLEIVNPQAAEKK
VAEANQKYFSSMAEFLKVKGEKSGLMST

Detailed description

30 The present invention provides a nanoparticulate system or composition for use in a method of treatment by gene transfer or gene delivery, which comprises nanoparticles comprising a core of a sorbitan ester (SP), a surface layer of a cationic substance and a gene construct consisting of plasmid DNA, wherein the gene construct is physically bound to the cationic substance.

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As already stated and as used herein, the term “nanoparticle” refers to solid colloidal materials, the mean size of which ranges between 1 and 999 nm, having a solid core or matrix structure and further characterized by being stable structures having perfectly homogenous, reproducible and modulable characteristics. The nanoparticles of the systems of the invention are perfectly distinguishable from other colloidal systems due to their structural characteristics; for example, the nanoparticles of the invention do not have lipid bilayers characteristics of liposomes nor do they have an oily core, which is characteristic of nanoemulsions or nanocapsules. The nanoparticles of the invention do not comprise oils or oily components.

The system or composition of the present invention comprises a core or matrix of sorbitan esters. Sorbitan consists of a mixture of cyclic anhydrides of sorbitol, such as for example and without being limited to, 1,4-anhydrosorbitol, 1,5-anhydrosorbitol and 1,4,3,6-dianhydrosorbitol. In a particular embodiment, the sorbitan ester is selected from the group consisting of sorbitan mono-, di-, tri- or sesquioleate; sorbitan mono-, di-, tri- or sesquilaurate; sorbitan mono-, di-, tri- or sesquipalmitate; sorbitan mono-, di-, tri- or sesquistearate; and sorbitan mono-, di-, tri- or sesquiisostearate; and their combinations. Sorbitan esters are non-ionic surfactants given that they contain two localized regions, a hydrophilic region and another hydrophobic region. These non-ionic surfactants have the advantage of being less irritating than anionic or cationic surfactants. Furthermore, they are generally compatible with both anionic and cationic substances, since they are not ionized in solution.

In a particular embodiment, the invention relates to nanoparticles comprising a core of a sorbitan ester in a proportion by weight (w/w) with respect to the total weight of the nanoparticle components greater than 60%, preferably greater than 80%, more preferably between about 60% and about 100%, still more preferably between about 80% and about 100% characterized by being a solid homogenous matrix and by having a mean size between 1 and 999 nm. More preferably, the nanoparticles of the invention comprise a core of a sorbitan ester in a proportion by weight greater than 90% or between about 90% and about 100%. In a particular embodiment, the nanoparticles of the invention are further characterized by not comprising oils or oily components.

The nanoparticles of the system of the invention have an average particle size of between 1 and 999 nm, preferably between 50 and 600 nm, even more preferably

between 100 and 400 nm. The average particle size is primarily influenced by the composition and the conditions for forming the particles established in the selected production method. The term “average size” is thus interpreted to mean the average diameter of the population of nanoparticles moving together in an aqueous medium.
5 The average size of these systems can be measured using standard methods known to the person skilled in the art. In particular, the average size or mean particle size and the size distribution of the nanoparticles is determined by photon correlation spectroscopy (PCS). For this purpose the sample can be diluted with filtered Milli-Q water at the appropriate concentration, performing the analysis at 25ºC with an angle of detection of
10 173º.

Preferably, the system of the invention comprises a homogenous size distribution and the population of nanoparticles of the system moves together in an aqueous medium having a polydispersity index of less than 0.2, more preferably between 0 and 0.1.

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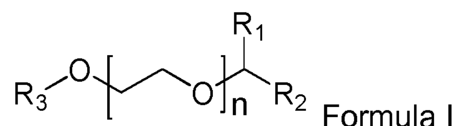
The nanoparticles of the systems of the invention have a core or matrix structure that allows incorporating additional components increasing and improving their stability. In particular, the nanoparticles of the invention comprise a cationic substance, in particular a surface layer of a cationic substance, in a proportion by weight greater than 0% and
20 about 40% with respect to the total weight of the nanoparticle components. In a particular embodiment, the proportion by weight of the cationic substance is greater than 0% and about 20% with respect to the total weight of the nanoparticle components; more particularly greater than 0% and about 10%. The cationic substance allows modulating the characteristics of nanoparticulate systems, such as particle size,
25 electrical surface charge and composition.

In a particular embodiment, the cationic substance is selected from protamine, polyglutamic acid, cationized dextran, polyamino acids and cationized proteins, and their salts. Preferably, the polyamino acids are selected from polylysine and
30 polyarginine. Preferably, the cationized proteins are selected from gelatin, albumin, collagen and atelocollagen, and their cationized derivatives. Preferably, the ammonium salts are selected from cetyltrimethylammonium bromide and benzalkonium chloride. Preferably, the fatty amine is oleylamine (*cis*-1-amino-9-octadecene).

35 The nanoparticles of the invention can optionally comprise an ethylene oxide derivative.

For the purposes of the present invention, "ethylene oxide derivative" is interpreted to mean a compound in which a $-CH_2CH_2O-$ unit is repeated.

5 In a particular embodiment, the ethylene oxide derivative is a compound of formula I



Formula I

where R_1 is a hydrogen or carbonyl group; R_2 is an alkyl, alkenyl or alkynyl group
10 having between 2 to 24 carbon atoms; R_3 is hydrogen or an alkyl group having between 1 to 6 carbon atoms; n is a value between 1 and 100. In a particular embodiment, n has a value of between 1 and 50, more preferably between 1 and 24.
Examples of ethylene oxide derivatives, without being limited to said examples, are polyethylene glycol dodecyl ether (Brij 30), polyethylene glycol hexadecyl ether (Brij 56),
15 polyethylene glycol 2-octadecyl ether (Brij 72), polyethylene glycol 8-octadecyl ether (Brij 78), polyethylene glycol 8-stearate (Myrj 45), 2-hydroxyethyl octadecanoate (Myrj 52), ethylene glycol monostearate, triethylene glycol monostearate.

In addition, the nanoparticles have an electric charge (measured by means of Z
20 potential), the magnitude of which can be modulated by means of a suitable system composition selection. Specifically, said electric charge can have positive or negative values depending on the system components and the proportion existing between them. The zeta potential of the nanoparticles of the systems of the invention can be measured using standard methods known to the person skilled in the art and described, for
25 example, in the experimental part of the present specification.

In a particular embodiment of the invention, the nanoparticles have a charge ranging between –50 mV and +60 mV, even more preferably between –40 mV and +50 mV, depending on the proportion of the components.

30

In addition, the possibility offered by the present invention of modulating the electric charge of nanoparticles has enormous advantages. In that sense, a negative charge is particularly suitable for assuring nanoparticle stability after parenteral administration.

The nanoparticles of the systems of the invention comprises a core or matrix of sorbitan esters, a cationic substance, in particular a surface layer of a cationic substance, and a gene construct consisting of plasmid DNA, wherein the gene construct is physically
5 bound to the cationic substance. Preferably, the gene construct, physically bound to the cationic substance, comprises a plasmid polynucleotide sequence which in turn comprises at least a nucleotide sequence encoding a protein with biological activity encoded by any of the genes selected from table I below.

10 Table 1. Genes and Mapped Loci Causing Retinal Diseases

Gene symbols; OMIM Numbers	Chromosomal localization	Gene ID
NPHP4, SLSN4; 606966, 606996, 607215	1p36.31	261734
MFN2, CMT6, CMT2A2, MARF; 608507, 609260, 601152	1p36.22	9927
NMNAT1, LCA9, PNAT1; 204000, 608553, 608700	1p36.22	64802
PLA2G5; 601192	1p36.13-p36.12	5322
EMC1	1p36.13	23065
DHDDS, RP59; 268000, 608172, 613861	1p36.11	79947
RPE65, LCA2, RP20; 180069, 204000, 204100	1p31.2	6121
ABCA4, ABCR, ARMD2, CORD3, RP19, STGD1; 120970, 153800, 248200, 601691, 601718, 603075, 604116	1p22.1	24
RP32; 609913	1p21.2-p13.3	641433

COL11A1, STL2; 120280, 154780, 604841	1p21.1	1301
GNAT2, ACHM4; 139340	1p13.3	2780
PRPF3, HPRP3, PRP3, RP18; 601414, 607301	1q21.2	9129
SEMA4A, CORD10, SEMAB, RP35; 120970, 607292, 610282, 610283	1q22	64218
CORD8; 120970, 605549	1q23.1-q23.3	54109
HMCN1, ARMD1, FBLN6; 603075, 608548	1q25.3-q31.1	83872
CFH, ARMD4, ARMS1, HF1; 134370, 603075, 609814, 610698	1q31.3	3075
CRB1, LCA8, RP12; 204000, 600105, 604210, 613835	1q31.3	23418
FLVCR1, AXPC1; 609033, 609144	1q32.3	28982
NEK2, NLK1, RP67; 268000, 604043, 615565	1q32.3	4751
RD3, C1orf36, LCA12; 180040, 204000, 610612	1q32.3	343035
USH2A, RP39; 268000, 276901, 608400, 613809	1q41	7399
SDCCAG8, BBS16, CCCAP, NPHP10, SLSN7; 266900, 613524, 613615	1q43	10806
NF513, RP58; 268000, 613598, 613617	2p23.3	130557

C2orf71, RP54; 268000, 613425, 613428	2p23.2	388939
EFEMP1, DHRD, MTLV, FBLN3; 126600, 601548	2p16.1	2202
FAM161A, RP28; 268000, 606068, 613596	2p15	84140
WDPCP, BBS15, FRITZ; 209900, 613580	2p15	51057
ALMS1, ALSS; 203800	2p13.1	7840
ABHD12, PHARC; 613599, 612674	2p11.21	26090
CNGA3, ACHM2, CNCG3, RMCH2; 216900, 600053	2q11.2	1261
CNNM4, ACDP4, LOC619531; 217080, 607805	2q11.2	26504
SNRNP200, ASCC3L1, BRR2, HECIC2, RP33; 601664, 610359	2q11.2	23020
MERTK, RP38; 268000, 604705, 613862	2q13	10461
NPHP1, JBTS4, SLSN1; 256100, 266900, 607100, 609583	2q13	4867
BBS5; 209900, 603650	2q31.1	129880
CERKL, RP26; 608380, 608381	2q31.3	375298
(- - -)	2q33.1-q24.2	
TMEM237, ALS2CR4, JBTS14;	2q33.1	65062

213300, 614423, 614424		
KCNJ13, LCA16, SVD; 204000, 193230, 603208, 614186	2q37.1	3769
SAG, RP47; 181031, 258100, 268000	2q37.1	6295
USH2B; 605472	not 3p24.2-p23	7400
GNAT1, CSNBAD3; 139330, 310500, 610444	3p21.31	2779
LZTFL1, BBS17; 606568	3p21.31	54585
TREX1, AGS1, CHBL, CRV, RVCL; 192315, 225750, 606609, 610448	3p21.31	11277
ATXN7, ADCA2, OPCA3, SCA7; 164500	3p14.1	6314
ARL6, BBS3, RP55; 209900, 268000, 608845, 613575	3q11.2	84100
IMPG2, RP56, SPARCAN; 268000, 607056, 613581	3q12.3	50939
IQCB1, NPHP5, SLSN5; 609237, 609254	3q13.33	9657
NPHP3, SLSN3; 604387, 606995, 608002	3q22.1	27031
RHO, CSNBAD1, OPN2, RP4; 180380, 268000, 310500, 610445	3q22.1	6010
RP5	3q22.1	6010
CLRN1, RP61, USH3, USH3A; 268000, 276902, 606397, 614180	3q25.1	7401
SLC7A14, RP68;	3q26.2	57709

268000, 615720, 615725		
OPA1; 125250, 165500, 605290	3q29	4976
PCYT1A; 123695	3q29	5130
PDE6B, CSNB3, CSNBAD2, RP40; 163500, 180072, 268000, 310500, 613801	4p16.3	5158
WFS1, DFNA38; 222300, 598500	4p16.1	7466
CC2D2A, JBTS9, MKS6; 216360, 612013, 612284, 612285	4p15.33	57545
RAB28; 612994	4p15.33	9364
PROM1, CORD12, MCDR2, PROML1, RP41, STGD4; 120970, 268000, 603786, 604365, 608051, 612095, 612657	4p15.32	8842
GPR125, PGR21, TEM5L; 612303	4p15.2	166647
DTHD1	4p14	401124
WDR19, ATD5, CED4, NPHP13, IFT144, PWDMP; 208500, 218330, 256100, 608151, 614376, 614378	4p14	57728
CNGA1, CNCG, CNCG1, RP49; 123825, 268000, 613756	4p12	1259
WFS2; 604928	4q22-q24	54117
MTTP, ABL, MTP;	4q23	4547

200100, 157147		
LRIT3, FIGLER4; 615004	4q25	345193
BBS7, BBS2L1; 209900, 607590	4q27	55212
BBS12, FLJ35630; 209900, 610683	4q27	166379
RP29; 268000, 612165	4q32-q34	54110
LRAT, LCA14; 204000, 604863, 613341	4q32.1	9227
TLR3; 603029	4q35.1	7098
CYP4V2, BCD; 210370	4q35.2	285440
MCDR3; 608850	5p15.33-p13.1	317668
GPR98, FEB4, MASS1, USH2C, VLGR1; 602851, 604352, 605472	5q14.3	84059
VCAN, CSPG2, ERVR, WGN1; 118661, 143200	5q14.3	1462
NR2F1, EAR3; 132890, 615722	5q15	7025
BSMD; 608970	5q21.2-q33.2	5961
HARS, HRS, USH3B; 614504, 142810	5q31.3	3035
PDE6A, RP43;	5q33.1	5145

180071, 268000, 613810		
GRM6, CSNB1B; 257270, 310500, 604096	5q35.3	2916
MAK, RP62; 154235, 268000, 614181	6p24.2	4117
C2; 217000	6p21.32	717
CFB, BF, BFD; 138470	6p21.32	629
TULP1, LCA15, RP14; 204000, 600132, 602280, 613843	6p21.31	7287
GUCA1A, COD3, CORD14, GCAP1; 120970, 602093, 600364	6p21.1	2978
GUCA1B, GCAP2, RP48; 268000, 602275, 613827	6p21.1	2979
PRPH2, CACD2, RDS, RP7; 169150, 179605, 608133, 608161, 613105	6p21.1	5961
BCAMD, MCDCA; 153870	6p12.3-q16	3617
EYS, RP25, SPAM; 602772	6q12	346007
COL9A1; 120210	6q13	1297
RIMS1, CORD7, RIM1; 120970, 603649, 606629	6q13	22999
MCDR1, NCMD, PBCRA1; 136550, 600790	6q14-q16.2	4167
ELOVL4, STGD3;	6q14.1	6785

600110, 605512		
IMPG1, SPARC; 153870, 600790, 602870	6q14.1	3617
LCA5; 204000, 604537, 611408	6q14.1	167691
RP63; 268000, 614494	6q23	100862681
AHI1, JBTS3; 608629, 608894	6q23.3	54806
PEX7, PTS2R, RCDP1; 215100, 266500, 601757	6q23.3	5191
RCD1; 180020	6q25-q26	5953
MDDC, CYMD; 153880	7p21-p15	7966
KLHL7, RP42; 268000, 611119, 612943	7p15.3	55975
BBS9, PTHB1; 209900, 607968	7p14.3	27241
RP9, PAP1, PIM1K; 180104, 607331	7p14.3	6100
PEX1, IRD; 202370, 214100, 266510, 602136	7q21.2	5189
TSPAN12, NET2; 613138	7q31.31	23554
IMPDH1, LCA11, RP10; 146690, 204000, 180105, 613837	7q32.1	3614
OPN1SW, BCP, CBT; 190900	7q32.1	611

KIAA1549; 613344	7q34	57670
RP1L1; 608581	8p23.1	94137
ADAM9, CORD9, MCMP, MDC9; 120970, 602713, 612775	8p11.23	8754
RP1, ORP1; 180100, 603937	8q12.1	6101
TTPA; 600415	8q12.3	7274
CSPP1; 611654, 213300, 249000	8q13.1-q13.2	79848
OPA6, ROA1; 165500, 258500	8q21-q22	777778
PXMP3, PAF1, PEX2, PMP35; 170993, 214100, 266510	8q21.13	5828
CNGB3, ACHM3, RMCH1; 216900, 248200, 262300, 605080	8q21.3	54714
C8orf37, CORD16, RP64; 120970, 268000, 614477, 614500	8q22.1	157657
GDF6, CDMP2, KFS1, LCA17; 204000, 601147, 613703, 613094, 615360	8q22.1	392255
VMD1; 153840	not 8q24	7438
KCNV2, RCD3B; 607604, 610356	9p24.2	169522
TOPORS, LUN, P53BP3, RP31; 609507, 609923	9p21.1	10210

INVS, NPHP2; 243305, 602088	9q31.1	27130
DFNB31, USH2D, WHRN; 607084, 607928, 611383	9q32	25861
PRPF4; 607795	9q32	9128
TLR4, ARMD10; 603030, 603075, 611488	9q33.1	7099
TRIM32, BBS11, HT2A; 209900, 254110, 602290	9q33.1	22954
RP8, RP21; 500004	not 9q34-qter	8020
INPP5E, CORS1, JBTS1; 213300, 610156	9q34.3	56623
PHYH, PAHX, RDPA; 266500, 600964, 602026	10p13	5264
ACBD5	10p12.1	91452
USH1K	10p11.21-q21.1	101180907
RBP3, IRBP, RP66; 180290, 268000	10q11.22	5949
ERCC6, ARMD5; 133540, 214150, 278800, 603075, 609413	10q11.23	2074
RNANC; 221900	10q21	54719
PCDH15, DFNB23, USH1F; 276900, 601067, 602083, 605514, 609533	10q21.1	65217
CDH23, DFNB12, USH1D;	10q22.1	64072

276900, 601386, 601067, 605516		
CDHR1, CORD15, PCDH21, RP65; 120970, 268000, 609502, 613660	10q23.1	92211
RGR, RP44; 268000, 600342, 613769	10q23.1	5995
KIF11, EG5, HK5P, KNSL1, MCLMR, TRIP5; 148760, 152950	10q23.33	3832
PDE6C, COD4, PDEA2; 600827, 613093	10q23.33	5146
RBP4; 180250	10q23.33	5950
PAX2, ONCR; 120330, 167409	10q24.31	5076
PDZD7, PDZK7; 612971	10q24.31	79955
BBIP1, BBIP10, BBS18; 613605	10q25.2	92482
CORD17	10q26	101409267
ARMS2, ARMD8, LOC387715; 603075, 611313	10q26.13	387715
HTRA1, ARMD7, PRSS11; 602194, 603075, 610149	10q26.13	5654
OAT; 258870	10q26.13	4942
TUB; 601197	11p15.4	7275
TEAD1, AA, TCF13, TEF1; 108985, 189967	11p15.3	7003

USH1C, DFNB18; 276900, 276904, 602092, 605242	11p15.1	10083
EVR3; 133780, 605750	11p13-p12	81864
CORS2, JBTS2; 608091	11p12-q13.3	373067
BEST1, RP50, TU15B, VMD2; 153700, 268000, 607854, 613194	11q12.3	7439
ROM1; 180721	11q12.3	6094
BBS1; 209900, 209901	11q13	582
CABP4, CSNB2B; 310500, 608965, 610427	11q13.1	57010
LRP5, EVR4, HBM, OPPG; 133780, 259770, 601813, 601884, 603506	11q13.2	4041
CAPN5, ADNIV, HTRA3, VRNI; 602537, 193235	11q13.5	726
MYO7A, DFNB2, USH1B; 276900, 276903, 600060	11q13.5	4647
TMEM126A, OPA7; 165500, 612988, 612989	11q14.1	84233
FZD4, EVR1, FEVR; 133780, 604579	11q14.2	8322
C1QTNF5, CTRP5; 605670, 608752	11q23.3	114902
CEP164, NPHP15; 256100, 614845, 614848	11q23.3	22897

MFRP, NNO2; 606227, 609549	11q23.3	83552
CACNA2D4, RCD4; 608171, 610478	12p13.33	93589
PDE6H, RCD3A; 610024, 601190	12p12.3	5149
COL2A1, AOM, STL1; 108300, 120140, 132450, 156550, 183900, 184250, 200610, 609508	12q13.11	1280
CODA1; 611543	12q13.13-q14.3	
RDH5, RDH1; 136880, 601617	12q13.2	5959
BBS10, FLJ23560; 209900, 610148	12q21.2	79738
CEP290, BBS14, JBTS5, LCA10, NPHP6, MKS4, SLSN6; 204000, 610142, 610188, 610189, 611134, 611755	12q21.32	80184
MVK; 251170, 260920, 610377, 175900	12q24.11	4598
C12orf65, COXPD7, SPG55; 613541, 613559, 615035	12q24.31	91574
ITM2B, ABRI; 117300, 176500, 603904	13q14.2	9445
RB1; 180200	13q14.2	5925
GRK1, RHOK, RK; 180381, 258100	13q34	6011
STGD2;	not 13q34	6785

153900		
ACHM1, RMCH; 216900	not 14	6021
RP16	not 14	800750
MCDR4	14q11.2	619370
NRL, RP27; 162080, 613750	14q11.2	4901
RPGRIP1, CORD13, LCA6; 120970, 204000, 605446, 608194, 613826	14q11.2	57096
OTX2; 600037, 610125	14q22.3	5015
RDH12, LCA13, RP53; 204000, 268000, 608830, 612712	14q24.1	145226
TTLL5, STAMP; 612268	14q24.3	23093
SPATA7, HSD3, LCA3; 204000, 268000, 604232, 609868	14q31.3	55812
USH1A, USH1; 276900	not 14q32	7393
TTC8, BBS8, RP51; 209900, 268000, 608132, 613464	14q32.11	123016
FBLN5, ARMD3; 603075, 604580, 608895	14q32.12	10516
TRPM1, CSNB1C, MLSN1; 310500, 603576, 613216	15q13.3	4308
USH1H; 276900, 612632	15q22-q23	100271837
SLC24A1, CSNB1D, NCKX, RODX;	15q22.31	9187

310500, 603617, 613830		
NR2E3, ESCS, PNR, RP37; 268000, 268100, 604485, 611131	15q23	10002
MRST; 602685	15q24	8126
BBS4; 209900, 600374	15q24.1	585
CIB2, DFNB48, KIP2, USH1J; 605564, 609439, 614869	15q25.1	10518
RLBP1, CRALBP; 180090	15q26.1	6017
ARL2BP, BART, BART1	16p13.3	23568
GNPTG; 252605, 607838	16p13.3	84572
IFT140, MZSDS, WDTC2; 266920, 614620	16p13.3	9742
ABCC6, ARA, MRP6, PXE; 177850, 264800, **603234**	16p13.11	368
RP22; 602594	16p12.3-p12.1	6114
CLN3, JNCL; 607042, 204200	16p11.2	1201
ZNF423, NPHP14, JBTS19; 213300, 256100, 604557, 614844	16q12.1	23090
BBS2; 209900, 606151	16q12.2	583
RPGRIP1L, JBTS7, KIAA1005, MKS5, NPHP8; 610937, 611560, 611561	16q12.2	23322

CNGB1, CNCG2, CNCG3L, GAR1, GARP, RP45; 268000, 600724, 613767	16q13	1258
OPA8; 165500	16q21-q22.3	1258
CDH3, CDHP, PCAD; 114021, 601553	16q22.1	1001
DHX38, PRP16; 605584	16q22.2	9785
ADAMTS18, KNO2; 607512, 608454	16q23.1	170692
FHASD; 609218	16q23.2-q24.2	550626
CACD, CACD1; 215500	17p13	772
RCD2; 601777	17p13.1	1322
PRPF8, PRPC8, RP13; 600059, 607300	17p13.3	10594
AIPL1, LCA4; 204000, 604392, 604393	17p13.2	23746
PITPNM3, CORD5, NIR1; 120970, 600977, 608921	17p13.2	83394
GUCY2D, CORD6, LCA1, RETGC, RETGC1; 120970, 204000, 600179, 601777	17p13.1	3000
CORD4	17q	1321
UNC119, HRG4; 604011	17q11.2	9094

GPR179, CSNB1E; 310500, 614515, 614565	17q12	440435
MKS1, BBS13; 209900, 249000, 609883	17q22	54903
CA4, RP17; 600852, 114760	17q23.2	762
RGS9; 604067	17q24.1	8787
PRCD, RP36; 268000, 610598, 610599	17q25.1	768206
USH1G, SANS; 276900, 606943, 607696	17q25.1	124590
FSCN2, RP30; 607643, 607921	17q25.3	25794
PDE6G, RP57; 180073, 268000, 613582	17q25.3	5148
OPA4; 165500, 605293	18q12.2-q12.3	58156
CORD1; 120970, 600624	18q21.1-q21.3	1319
C3, ARMD9, ASP; 120700, 603075, 611378	19p13.3	718
RAX2, ARMD6, CORD11, QRX, RAXL1; 120970, 610362, 610381	19p13.3	84839
RGS9BP, R9AP, RGS9; 607814	19q13.12	388531
MCDR5	19q13.31-q13.32	
CRX, CORD2, LCA7; 120970, 204000, 268000, 602225,	19q13.32	1406

613829		
OPA3, MGA3; 165300, 165500, 258501, 606580	19q13.32	80207
PRPF31, PRP31, RP11; 600138, 606419	19q13.42	26121
IDH3B, RP46; 268000, 604526, 612572	20p13	3420
PANK2, HARP, PKAN; 234200, 606157, 607236	20p13	80025
JAG1, AGS; 118450, 601920	20p12.2	182
MKKS, BBS6; 209900, 236700, 604896	20p12.2	8195
KIZ, RP69; 268000, 615757, 615780	20p11.23	55857
PRPF6, RP60; 268000, 613979, 613983	20q13.33	24148
USH1E; 276900, 602097	21q21	7396
C21orf2; 603191	21q22.3	755
OPA5; 165500, 610708	22q12.1-q13.1	692222
IFT27	22q12.3	11020
TIMP3, SFD; 136900, 188826	22q12.3	7078
VRD1	22q13	
OFD1, RP23; 300170, 300209, 300424, 300804,	Xp22.2	8481

311200		
RS1, XLRS1; 312700	Xp22.13	6247
(- - -)	Xp21-q21	
RP6; 312612	Xp21.3-p21.2	6104
DMD; 310200	Xp21.2-p21.1	1756
OPA2; 165500, 311050	Xp11.4-p11.2	4977
NYX, CSNB1, CSNB1A, CSNB4; 300278, 310500	Xp11.4	60506
COD1; 304020	Xp11.4	347676
RP15; 300029	Xp11.4	6110
RPGR, CORDX1, RP3; 300029, 304020, 312610	Xp11.4	6103
PRD; 312550	Xp11.3-p11.23	5548
NDP, EVR2; 133780, 300658, 305390, 310600	Xp11.3	4693
AIED, OA2; 300600	Xp11.23	198
CACNA1F, CORDX3, CSNB2, CSNB2A, CSNBX2; 300071, 300110, 300476, 300600, 310500	Xp11.23	778
RP2;	Xp11.23	6102

312600		
PGK1; 300653, 311800	Xq21.1	5230
CHM; 303100	Xq21.2	1121
TIMM8A, DDP, DDP2, DFN1; 300356, 304700, 311150	Xq22.1	1678
RP24; 300155	Xq26-q27	6116
COD2, CORDX2; 300085	Xq27	1275
RP34; 300605	Xq28-qter	777642
OPN1LW, BCM, CBP, COD5, RCP; 303700, 303900	Xq28	5956
OPN1MW, CBD, GCP; 303700, 303800	Xq28	2652
KSS; 530000	mitochondrion	3894
LHON; 535000	mitochondrion	3974
MT-TL1, DMDF, TRNL1; 520000, 590050	mitochondrion	4567
MT-ATP6, ATP6, NARP; 516060, 551500	mitochondrion	4508
MT-TH, TRNH; 590040	mitochondrion	4564
MT-TS2, TRNS2; 500004, 590085	mitochondrion	4575

MT-TP, TRNP; 590075	mitochondrion	4571

More preferably, the gene construct, physically bound to the cationic substance, comprises a plasmid polynucleotide sequence which in turn comprises at least a nucleotide sequence encoding a protein with biological activity encoded by any of the genes selected from the list consisting of: *PLA2G5, NMNAT1, MERTK, RPE65, ABCA4, PRPF3, PRPF31, PRPF8, RD3, EFEMP1, KCNJ13, RGR, RBP4, BEST1, RDH5, RLBP1, CLN3*, or any combinations thereof. Preferably, the gene construct encodes a biological active PRPF31 protein.

In one embodiment of the invention, the nanoparticulate system or composition as described throughout the present invention is a pharmaceutical composition which optionally further comprises pharmaceutically acceptable excipients, carriers and/or adjuvants.

In a preferred embodiment of the present invention, the nanoparticulate system or composition as described throughout the present invention is use in a method of treatment of abnormalities or dysfunctions of the retinal pigment epithelium. Preferably, the abnormality or dysfunction of the retinal pigment epithelium is selected from the list consisting of: age related macular degeneration, retinitis pigmentosa, Leber's congenital amaurosis, Stargardt's disease, diabetic retinopathy, macular dystrophy, cataracts, disruption of retinal pigment epithelium cells, deterioration of retinal pigment epithelium cells, death of retinal pigment epithelium cells, drusden, pigment clumping, RPE detachment, geographic atropy, sub-retinal neovascularization and disciform scar, vitelliform macular dystrophy, butterfly macular dystrophy, Sjögren's syndrome, fundus flavimaculatus, dominant foveal dystrophy, central areolar pigment epithelial dystrophy, North Carolina dystrophy, familiar drusen, pattern dystrophy of Marmor and Byers, foveomacular dystrophy (adult type), dominant slowly progressive macular dystrophy of Singerman-Berkow-Patz,macroreticular dystrophy of the RPE, pigment epithelial dystrophy of Noble-Carr-Siegel, benign concentric annular dystrophy, or combinations thereof. More preferably, the abnormality or dysfunction of the retinal pigment epithelium is selected from the list consisting of Retinitis Pigmentosa, Age-Related Macular Degeneration and/or Leber's congenital amaurosis. Still more preferably, the

abnormality or dysfunction of the retinal pigment epithelium is Retinitis Pigmentosa.

In a preferred embodiment, the administration of the system or composition of the invention is via subretinal injection. In particular, the administration of said system or composition is via subretinal injection in the subretinal space between the photoreceptors and the retinal pigment epithelium (RPE).

A further aspect of the invention, refers to a nanoparticulate system or composition, or pharmaceutical compositions comprising said system or composition, which comprises nanoparticles comprising a core of a sorbitan ester, a surface layer of a cationic substance and a gene construct consisting of plasmid DNA, wherein the gene construct is physically bound to the cationic substance, and wherein the gene construct comprises a polynucleotide sequence comprising at least a coding sequence for a protein with biological activity encoded by any of the genes selected from the list consisting of the genes of table 1 or preferably from the list consisting of: PLA2G5, NMNAT1, RPE65, ABCA4, PRPF3, PRPF31, PRPF8, RD3, EFEMP1, KCNJ13, RGR, RBP4, BEST1, RDH5, RLBP1, CLN3, or any combinations thereof. Preferably, the selected gene is PRPF31. More preferably, the cationic substance is polyarginine and the gene construct, physically bound to the cationic substance, comprises a polynucleotide sequence comprising at least a coding sequence for PRPF31.

Another further aspect of the invention refers to an expression control element, such as a DNA plasmid, comprising a coding sequence for the protein PRPF31 and further comprising the appropriate control elements for the coding sequence to be replicated, transcribed and translated in the appropriate host cells for use in a method of treatment of abnormalities or dysfunctions of the retinal pigment epithelium as defined in any of claims 10 to 12.

Finally, the nanoparticulate system or composition as described throughout the present invention may be in a lyophilized form.

To better understand the features and advantages of the present invention, reference will be made below to a series of examples, which, in an explanatory manner, complete the preceding description without meaning in any way that said invention is limited to such examples.

EXAMPLES

5 EXAMPLE 1. Materials and methods

Materials

Sorbitan monooleate (Span® 80) (SP), oleylamine (OA) (purity ≥ 70%) and poly-L-
10 arginine (PA) were acquired from Sigma (Spain). The pEGFP-C3 and pEGFP-C3-PRPF31 plasmids were produced by the research center, CABIMER. The pEGFP-N1 plasmid (Clontech Laboratories, Inc.) with GenBank accession number #U55762 was used to construct the plasmids. pEGFP-N1 encodes a red-shifted variant of wild-type GFP which has been optimized for brighter fluorescence and higher expression in
15 mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm). pEGFP-N1 encodes the GFPmut1 variant containing the double amino acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes, which correspond to human codon usage preferences. Sequences flanking EGFP have been converted to a Kozak consensus
20 translation initiation site to further increase the translation efficiency in eukaryotic cells.

The MCS in pEGFP-N1 is between the immediate early promoter of CMV (P CMV IE) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EGFP if they are in the same reading frame as EGFP and
25 there are no intermediate stop codons. SV40 polyadenylation signals downstream of the EGFP gene suitably and directly process the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance (Neor) cassette, consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and herpes simplex
30 virus thymidine kinase (HSV TK) gene polyadenylation signals, allows stably transfecting eukaryotic cells for being selected using G418. A bacterial promoter upstream of this kanamycin-resistance cassette is expressed in E. coli. The pEGFP-N1 backbone also provides a pUC origin of replication for propagation in E. coli and an f1 origin for single-stranded DNA production.

35

Preparation of DNA-associated Span® 80–based nanoparticles

To produce SP-OA nanoparticles, 6.6 mg/ml of SP and 0.33 mg/ml of OA were dissolved in 30 ml of an organic phase (ethanol) added subsequently under magnetic stirring to 60 ml of an aqueous phase, which leads to the spontaneous formation of nanoparticles. To produce SP-PA nanoparticles, 6.6 mg/ml of SP were dissolved in the organic phase and 0.16 mg/ml of PA were added to the aqueous phase. Ethanol was subsequently eliminated under reduced pressure in a rotary evaporator, and the nanoparticles were concentrated to a final volume of 10 ml. The SP-PA nanoparticles were isolated by filtration-centrifugation (Amicon® Ultra-0.5 Centrifugal Filter Devices, Merck Millipore, Ireland).

The genetic material (pEGFP-C3-GFP or pPRPF31-PRPF31) was associated with the surface of the nanoparticles at a concentration of 0.2 mg/ml or 0.3 mg/ml, respectively, by means of incubation with nanoparticles at a ratio of 1:1 (v/v) under magnetic stirring at room temperature. For the *in vivo* study of the formulations, they were administered in a 5% glucose solution.

Characterization of pDNA nanoparticles

The mean particle size and the nanoparticle size distribution were determined using photon correlation spectroscopy (PCS). To that end, the samples were diluted with filtered Milli-Q water at the suitable concentration. Each analysis was conducted at 25ºC with a detection angle of 173º. The values of the zeta potential of the nanoparticles were obtained using laser Doppler anemometry (LDA), measuring the mean electrophoretic mobility. PCS and LDA analyses were performed with a Zetasizer® 3000HS (Malvern Instruments, UK).

In vivo experiments

Animal handling

All the experiments described herein have been conducted in compliance with the Spanish and European Union laws on care of experimental animals. Animal handling and laboratory experimental methods have been analyzed and approved by the Animal

Experimentation Committee of CABIMER, Seville, Spain. All efforts were made to reduce the number of animals used and their suffering to a minimum. The animals were housed in the Biological Resource Unit of CABIMER and kept in an environment with controlled temperature (21 ± 1ºC), relative humidity of 55 ± 5%, 08h00-20h00 light/dark cycle, and they were given standard food for mice and water *ad libitum*.

Subretinal injection

The surgical procedures were performed under general anesthesia by subcutaneous injection of ketamine hydrochloride and xylazine solution (80/12 mg/Kg of body weight). The eyes were topically anesthetized with 0.1% tetracaine and 0.4% oxybuprocaine. The pupils were dilated with one drop each of 10% phenylephrine and 1% tropicamide. A 32-gauge needle was used for gently opening the choroids 1 mm posterior to the sclerocorneal limbus. A 10 µl syringe (Hamilton, Switzerland) with a 33-gauge needle attached to an UltraMicroPump (World Precision Instruments, Sarasota, FL) was used for slowly injecting 1 µl of nanoparticles (200 ng of the GFP plasmid or 300 ng of the PRPF31 plasmid) in the subretinal space. Finally, a drop of antibiotic (0.3% ciprofloxacin) was put in each eye and the animals were kept on a cushion at 37ºC until they recovered from the anesthesia. Adeno-associated serotype 2 viral vectors (AAV2) carrying the GFP plasmid, PBS or 5% glucose were also injected in the subretinal space and used as controls. The GFP signals were evaluated using a mouse imaging system (Micron III).

Tested groups

Both eyes of the four mice were injected in each group. The mice were sacrificed by cervical dislocation 72 hours or one month after the subretinal injections. The right eyes were collected for immunofluorescence (IF) studies and the left eyes were collected and analyzed by Western blot (WB).

Histology

After cervical dislocation, the right eyes of the injected mice were extirpated and quickly fixed in ice with 4% paraformaldehyde (4% PFA) in PBS overnight at 4ºC. The fixed eyes were cryoprotected for 8 hours at room temperature in 20% sucrose-PBS overnight at 4ºC and in 30% sucrose-PBS for cryotome sections. 18 µm thick serial

sections were mounted in five parallel series and processed for IF, incubating with rabbit anti-GFP antibody (1:100) to improve the GFP signal and prevent the autofluorescence of normal tissue. The sections were mounted with Vectashield mounting medium with DAPI (Vector H-1201). Confocal images were captured in a
5 Leica TCS SP5 confocal microscope with an HCX PL APO Lambda blue 63 x 1.4 OIL lens at 22ºC.

Western Blot

10 For neural retina, the crystalline lens, the vitreous humor and aqueous humor are extracted, and the retina was peeled from the RPE. Tissues were rapidly frozen in liquid nitrogen and stored at -80ºC until use. Proteins were extracted in cold RIPA buffer plus protease and phosphatase inhibitor cocktails. The protein content was measured using the Bradford assay and the samples were stored at -80ºC. 30 g of each extract were
15 separated in a denaturing 10% SDS-PAGE gel and proteins were transferred to a PVDF membrane and it was blocked using 5% NFDM or Superblock (Thermo) for 1 hour. The primary antibody was incubated at 1 mg/ml overnight at 4ºC. The membrane was probed with HRP-conjugated secondary antibody for 1 hour at room temperature. The immunoreactive bands were detected by means of chemiluminescence using ECL plus
20 (Amersham) and registered by film exposure and automatic development.

Statistical analysis

All the experimental measurements were taken in triplicate. The values are expressed
25 as the mean ± standard deviation (SD) or the standard error of the mean (SEM). The statistically significant differences between the treatments were evaluated by means of variance analysis (ANOVA) and Fisher's least significant difference (LSD) was evaluated using Statgraphics X64 software.

30 <u>EXAMPLE 2. RESULTS</u>

Preparation and characterization of Span (SP)-based nanoparticles

Span (SP)-based nanoparticles with positive surface charge were developed using
35 oleylamine (OA) or polyarginine (PA) as cationic residues, as described in

WO2013068625 A1. As shown in Table 2, nanometric sized SP-OA or SP-PA nanoparticles (180 nm and 230 nm, respectively) having a positive surface charge (43 mV and 32 mV, respectively) were obtained. As expected, the surface charge of these nanoparticles changes from positive to negatives values when these nanoparticles were
5 incubated with the GFP plasmid (200 g/ml) or PRPF31 plasmid (300 mg/ml), due to the inherent negative charge of these pDNA molecules. These results indicate an effective association of pDNA which was corroborated by the absence of signal bands characteristic of free DNA when agarose gel electrophoresis assays are performed.

Nanoparticle composition	**Size (nm)**	**PdI**	**ζ Potential (mV)**
SP-OA	181.5 ± 3.2	0.074	+42.3 ± 1.6
SP-PA	228.1 ± 3.2	0.038	+32.5 ± 1.1
SP-OA-GFP	340.1 ± 3.6	0.147	-10.1 ± 2.4
SP-PA- GFP	355.1 ± 1.8	0.067	-7.4 ± 0.2
SP-OA-*PRPF*31	301.4 ± 6,4	0.191	-12.9 ± 0.6
SP-PA -*PRPF*31	257.7 ± 10.6	0.319	-22.1 ± 0.9

10

Table 2. Physicochemical characteristics of Span-oleylamine (SP-OA)- or Span-polyarginine (SP-PA)-based nanoparticles as blank nanoparticles (without associated bioactive molecule) or with the association of a GFP plasmid (200 g/ml) or a PRPF31 plasmid (300 mg/ml). (The data are mean ± SD, n = 3).

15

GFP transfection studies

Blank SP-OA nanoparticles (without associated plasmid) or SP-OA nanoparticles associated with GFP (SP-OA-GFP) were injected subretinally in mice and the
20 expression of GFP was evaluated 72 hours after administration. As shown in Figure 1A, the retinal pigment epithelium and ganglion cells showed positive signals for GFP in both cell layers of the retina. Protein expression in these levels was confirmed using Western blot (Figure 1B). Therefore, the SP-OA-GFP nanoparticles were capable of transfecting the pigmented epithelium of the retina in an efficient manner and also the
25 ganglion cells of the mouse retina 72 hours after injection, whereas the viral vectors require more time to provide *in vivo* transfection (Bennett *et al.,* 1997 *Invest. Ophthalmol. Vis. Sci.* 38, 2857-2863; Bennett *et al.,* 1999. *Proc. Natl. Acad. Sci. U.S.A.*,

96, 9920-9925).

For the purpose of further corroborating the efficiency of the developed nanosystems, their transfection capacity was compared with that provided by the adeno-associated serotype 2 viral vectors (AAV2). For this purpose, the three viral and non-viral vectors associating the GFP plasmid (AAV-GFP, SP-OA-GPF and SP-PA-GFP) and the control formulations (PBS, 5% glucose, blank SP-OA nanoparticles and blank SP-PA nanoparticles) were administered subretinally and GFP expression was evaluated one month after injection. As can be seen in Figure 2 – image on the left- evaluation of the ocular fundus of mice treated with AAV2-GFP, SP-PA-GFP and SP-OA-GFP showed a scar in the area of subretinal injections. The GFP signal was also detected in the same injected area, being more intense in mice injected with SP-OA-GFP nanoparticles. Retinal sections of mice treated with these three vectors show GFP signals both in photoreceptors and in RPE cells. Although GFP expression was higher in the SP-OA-GFP injected group, the number of nuclei in the ONL decreased or nuclei were even completely absent in the same injected zone. Therefore, these results suggest that a toxic event occurs in the case of SP-OA-GFP nanoparticles. Taking into account that mice injected with blank SP-OA nanoparticles (without GFP plasmid) do not show any change in retinal layers, as can also be seen in Figure 2 (image on the right), such toxic events can be due to an intense and unexpected high transfection efficiency of these non-viral vectors and the subsequent overexpression of the plasmid mediated by these formulations.

Western blot analysis and the corresponding quantitative study (Figure 3) also suggest a higher transfection efficacy of the non-viral vectors compared with viral vectors. Specifically, these results suggest the following order in the GFP expression levels provided by the different systems that have been studied: SP-OA-GFP> AAV2-GFP> SP-PA-GFP, although statistically significant differences were not observed. In any case, it must be pointed out that this is the first *in vivo* study comparing transfection levels obtained with viral and non-viral vectors.

To evaluate the developed systems *in vivo*, the mice were subjected to electroretinogram. Figure 4 shows the rod responses and cone-rod responses determined in dark-adapted mice by means of b-wave and a-wave amplitude, respectively, and cone responses were evaluated in light-adapted mice by means of b-

wave amplitude. It can be seen that cone-rod responses were significantly affected by administering these SP-OA-GFP nanoparticles. Specifically, the a-wave and b-wave amplitude decreased considerably in mice treated with SP-OA-GFP nanoparticles. Quantification of the amplitude thereof showed statistically significant differences in rod
5 responses (A, D), cone-rod responses (B, E) and cone responses (C, F) (Figure 5) when compared with the respective control group (treated with a 5% glucose solution), whereas mice injected with blank SP-PA and SP-OA nanoparticles, AAV2-GFP or SP-PA-GFP nanoparticles (associating the GFP plasmid) did not show statistically significant differences. These results can once again be attributed to the intense and
10 unexpected overexpression of GFP after treatment with the SP-OA-GFP non-viral vectors or, in other words, to the high unexpected transfection efficacy resulting in unwanted events.

The conclusions mentioned above concerning the performance of the developed
15 nanoparticles were also supported by the determination of optomotor reflex which involves turning the head in response to moving lines. As shown in Figure 6, although both SP-OA and SP-PA nanoparticles are capable of transfecting mouse retina *in vivo*, positive optomotor test responses decreased significantly in mice injected with SP-OA-GFP nanoparticles, whereas mice injected with AAV2-GFP and SP-PA-GFP did not
20 show statistically significant differences when compared with control groups. This data also support the explanation provided above by correlating the effect of a high transfection efficiency provided by SP-OA-based nanoparticles instead of the nanoparticles themselves as the cause of potential toxic events. In fact, immunofluorescence images and Western blot results demonstrated that SP-OA-GFP
25 nanoparticles had a higher rate of transfection than AAV2-GFP, which could lead to the aforementioned overexpression of GFP, being strong enough to cause toxic effects, also proven by the reduction described in the a-wave and b-wave amplitude of the electroretinogram and positive optomotor responses.

30 *In vivo efficacy in a model of the disease*

Once the transfection efficacy provided by the developed nanoparticles being evaluated has been proven, their *in vivo* potential in a model of the disease is evaluated in a second step. For this purpose, *Prpf31*$^{A216P/+}$ mice (KI) were selected (Bujakowska *et al.*,
35 2009. *Invest. Ophthalmol. Vis. Sci.*, 50, 5927-5933) as a mouse model for hereditary

retinal disease, for the purpose conducting a preclinical study on the therapeutic activity of Span-based nanoparticles associating the plasmid with *PRPF31* as a new nanomedicine for the treatment of haploinsufficiency mentioned above. The animal model used in this study carries the A216P mutation in the human *PRPF31* gene, which
5 is known to cause retinitis pigmentosa (Vithana, *et al.,* 2001. *Mol. Cell,* 8, 375-381). Furthermore, mutations in this gene for producing juvenile macular degeneration are also known (Lu, *et al.,* 2013. PLoS ONE, 8, e78274). Earlier studies conducted by the research group showed that even when the AAV2 vectors are capable of efficiently transfecting RPE cells with the PRPF31 plasmid (Figure 10), no improvement in visual
10 function can be observed by using this viral vector in mutant mice. In fact, the amplitude of different waves of the electroretinogram corresponding to rod responses, cone-rod responses or cone responses decreased in mice injected with AAV2-PRPF31 when compared with the respective negative control group (injected with PBS) (Figure 11). In order to clarify the efficacy of the developed non-viral vectors (SP-OA and SP-PA
15 nanoparticles), the animals were subjected to different evaluation tests one month after subretinal injection. Once again, the aforementioned high transfection efficacy of SP-OA-based nanoparticles and the subsequent overexpression of the gene can explain the significant reduction in positive optomotor responses that are observed (Figure 12). In addition, SP-PA-based nanoparticles provide a suitable rate of transfection (Figure 7)
20 both for PRPF31 or GFP plasmids (used as control).

The optomotor test was used for evaluating visual function in healthy wild-type mice injected with 5% glucose (WT 5% Glu) and *Prpf31*$^{A216P/+}$ mutant mice treated with SP-PA-*PRPF31* nanoparticles (KI SP-PA-*PRPF31*) or SP-PA-GFP control nanoparticles (KI
25 SP-PA-GFP). The graphs of Figure 8 depict the mean positive responses to the optomotor test contrasted at different intensities. The gray line represents the positive response trend of the optomotor tests in mouse models of the disease (untreated mutant KI) used as control (Figure 13). Different spatial frequencies (cycles/degree = c/d) from 0.031-0.272 were used to evaluate visual acuity. A statistically significant
30 improvement in visual acuity (100% contrast in each test frequency, $p = 0.02$ at 0.272 c/d) can be seen in mouse models of the disease after treatment with SP-PA-*PRPF31*. In fact, with a 100% contrast, the mean positive responses in mouse models of the disease treated with SP-PA-*PRPF31* is similar to the control curve of healthy mice injected with a negative control solution (WT 5% Glu). Specifically, a positive optomotor
35 response of 75 ± 2.8% was observed in healthy mice (WT 5% Glu), whereas untreated

mutant mice injected with a control formulation associating the GFP plasmid (KI SP-PA-GFP) showed a positive optomotor response value of only 33.3 ± 4.4%. An unexpected high value of 68.5 ± 8.6% was observed in *Prpf31*$^{A216P/+}$ mutant mice when they were treated with SP-PA-based nanoparticles associating the PRPF31 plasmid. Although the
5 number of positive responses in mice treated with SP-PA-*PRPF31* decreases as the contrast intensity decreases, said response is still more favorable than that observed in mice injected with the SP-PA-GFP control formulation and are even above the trend line of mutant mice models of the disease that were not treated. Therefore, it can be affirm that the spatial vision of KI mouse models of the disease treated with SP-PA-*PRPF31*
10 improved only one month after treatment.

For the in-depth study of the performance of the developed nanomedicines, retinal structure and thickness were also determined by means of optical coherence tomography. As can be seen in Figure 9A, the color scales of healthy WT mice treated
15 with a 5% glucose negative control solution and KI mice, control of the disease, injected with SP-PA-*PRPF31* are similar, whereas the levels cannot be reached in mouse models of the disease treated with the SP-PA-GFP control formulation. Furthermore, after determining retinal thickness (Figure 9B), a mean value of 216.6 ± 6.9 µm was observed for healthy control mice (WT 5% Glu) and 198.5 ± 1.9 µm for untreated
20 mutant mice (KI SP-PA-GFP). However, it must be emphasized that when comparing retinal thickness of these untreated KI mice with that of KI mice treated with SP-PA-*PRPF31* nanoparticles (KI SP-PA-*PRPF31*), it was found that retinal thickness increased significantly ($p = 0.04$) in the treated animals up to a value of 211.9 ± 13.5 microns. In other words, only one month after treatment, the developed nanomedicines
25 not only prevent retinal degeneration, but were also capable of giving rise to a significant and unexpected increase in retinal thickness.

Therefore, the polyarginine-based nanoparticles of the invention were capable of efficiently transfecting *PRPF31* at the retinal level in a mouse model of retinal
30 degeneration without causing adverse effects (i.e., toxicity or immunogenicity). Furthermore, visual acuity and retinal thickness of treated mice increased after treatment.

35

CLAIMS

1. A nanoparticulate system or composition which comprises nanoparticles comprising a core of a sorbitan ester, a surface layer of a cationic substance and
5 a gene construct consisting of plasmid DNA, wherein the gene construct is physically bound to the cationic substance, and wherein the sorbitan ester is selected from the group consisting of sorbitan mono-, di-, tri- or sesquioleate; sorbitan mono-, di-, tri- or sesquilaurate; sorbitan mono-, di-, tri- or sesquipalmitate; sorbitan mono-, di-, tri- or sesquistearate; and sorbitan mono-,
10 di-, tri- or sesquiisostearate or any of their combinations thereof,

for use in a method of treatment by gene transfer or gene delivery in a mammalian subject.

2. The nanoparticulate system or composition of claim 1 for use according to claim
15 1, wherein the cationic substance is a polyamino acid or oleylamine.

3. The nanoparticle system or composition of claim 1 for use according to claim 1, wherein the cationic substance is polyarginine.

20 4. The nanoparticulate system or composition according to any of claims 1 to 3 for use according to claim 1, wherein the gene construct, physically bound to the cationic substance, comprises a polynucleotide sequence comprising at least a coding sequence for a protein with biological activity encoded by a gene selected from the list consisting of: *PLA2G5, NMNAT1, RPE65, ABCA4, PRPF3, PRPF31,*
25 *PRPF8, RD3, EFEMP1, KCNJ13, RGR, RBP4, BEST1, RDH5, RLBP1, CLN3*, or any combinations thereof.

5. The nanoparticulate system or composition according to any of the precedent claims for use according to claim 1, wherein the gene construct comprises a
30 coding sequence for the *PRPF31* protein.

6. The nanoparticulate system or composition according to claim 5 for use according to claim 1, wherein the cationic substance is polyarginine.

35 7. The nanoparticulate system or composition of any of claims 1 to 3 for use

according to claim 1, wherein said composition is a pharmaceutical composition further comprising pharmaceutically acceptable excipients, carriers and/or adjuvants.

5 8. The nanoparticulate system or composition of any of claims 4 to 6 for use according to claim 1, wherein said composition is a pharmaceutical composition further comprising pharmaceutically acceptable excipients, carriers and/or adjuvants.

10 9. The nanoparticulate system or composition as defined in any of claims 4 to 6 or 8, for use in a method of treatment of abnormalities or dysfunctions of the retinal pigment epithelium.

10. The nanoparticulate system or composition of claim 9 for use according to claim
15 9, wherein the abnormality or dysfunction of the retinal pigment epithelium is selected from the list consisting of: age related macular degeneration, retinitis pigmentosa, Leber's congenital amaurosis, Stargardt's disease, diabetic retinopathy, macular dystrophy, cataracts, disruption of retinal pigment epithelium cells, deterioration of retinal pigment epithelium cells, death of retinal pigment
20 epithelium cells, drusden, pigment clumping, RPE detachment, geographic atropy, sub-retinal neovascularization and disciform scar, vitelliform macular dystrophy, butterfly macular dystrophy, Sjögren's syndrome, fundus flavimaculatus, dominant foveal dystrophy, central areolar pigment epithelial dystrophy, North Carolina dystrophy, familiar drusen, pattern dystrophy of
25 Marmor and Byers, foveomacular dystrophy (adult type), dominant slowly progressive macular dystrophy of Singerman-Berkow-Patz,macroreticular dystrophy of the RPE, pigment epithelial dystrophy of Noble-Carr-Siegel, benign concentric annular dystrophy, or combinations thereof.

30 11. The nanoparticulate system or composition of claim 9 for use according to claim 9, wherein the abnormality or dysfunction of the retinal pigment epithelium is selected from the list consisting of Retinitis Pigmentosa, Age-Related Macular Degeneration and/or Leber's congenital amaurosis.

35 12. The nanoparticulate system or composition of claim 9 for use according to claim

9, wherein the abnormality or dysfunction of the retinal pigment epithelium is Retinitis Pigmentosa.

13. The nanoparticulate system or composition of any of claims 9 to 12 for use according to any of claims 9 to 12, wherein the administration of said system or composition is via subretinal injection.

14. The nanoparticulate system or composition of claim 13 for use according to claim 13, wherein the administration of said system or composition is via subretinal injection in the subretinal space between the photoreceptors and the retinal pigment epithelium (RPE).

15. The system or composition as defined in any of claims 1 to 9 for use according to claim 1 or claims 9 to 12, which is in lyophilized form.

16. The nanoparticulate system or composition as defined in any of claims 4 to 8.

17. The nanoparticulate system or composition as defined in claim 6.

18. The nanoparticulate system or composition according to claim 17, wherein said composition is a pharmaceutical composition further comprising pharmaceutically acceptable excipients, carriers and/or adjuvants.

19. An expression control element, such as a DNA plasmid, comprising a coding sequence for the protein PRPF31 and further comprising the appropriate control elements for the coding sequence to be replicated, transcribed and translated in the appropriate host cells for use in a method of treatment of abnormalities or dysfunctions of the retinal pigment epithelium as defined in any of claims 10 to 12.

FIGURES

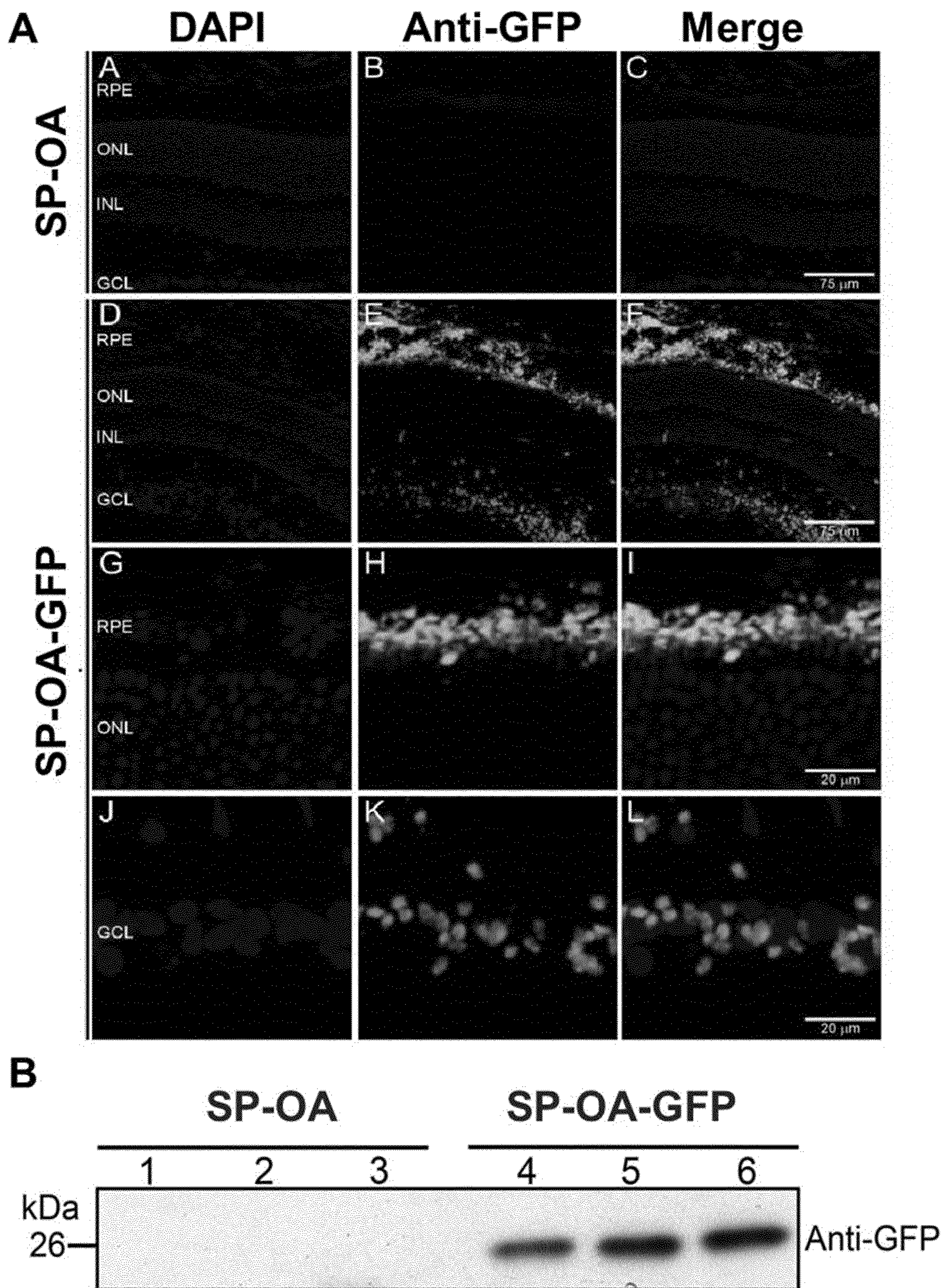


Fig. 1

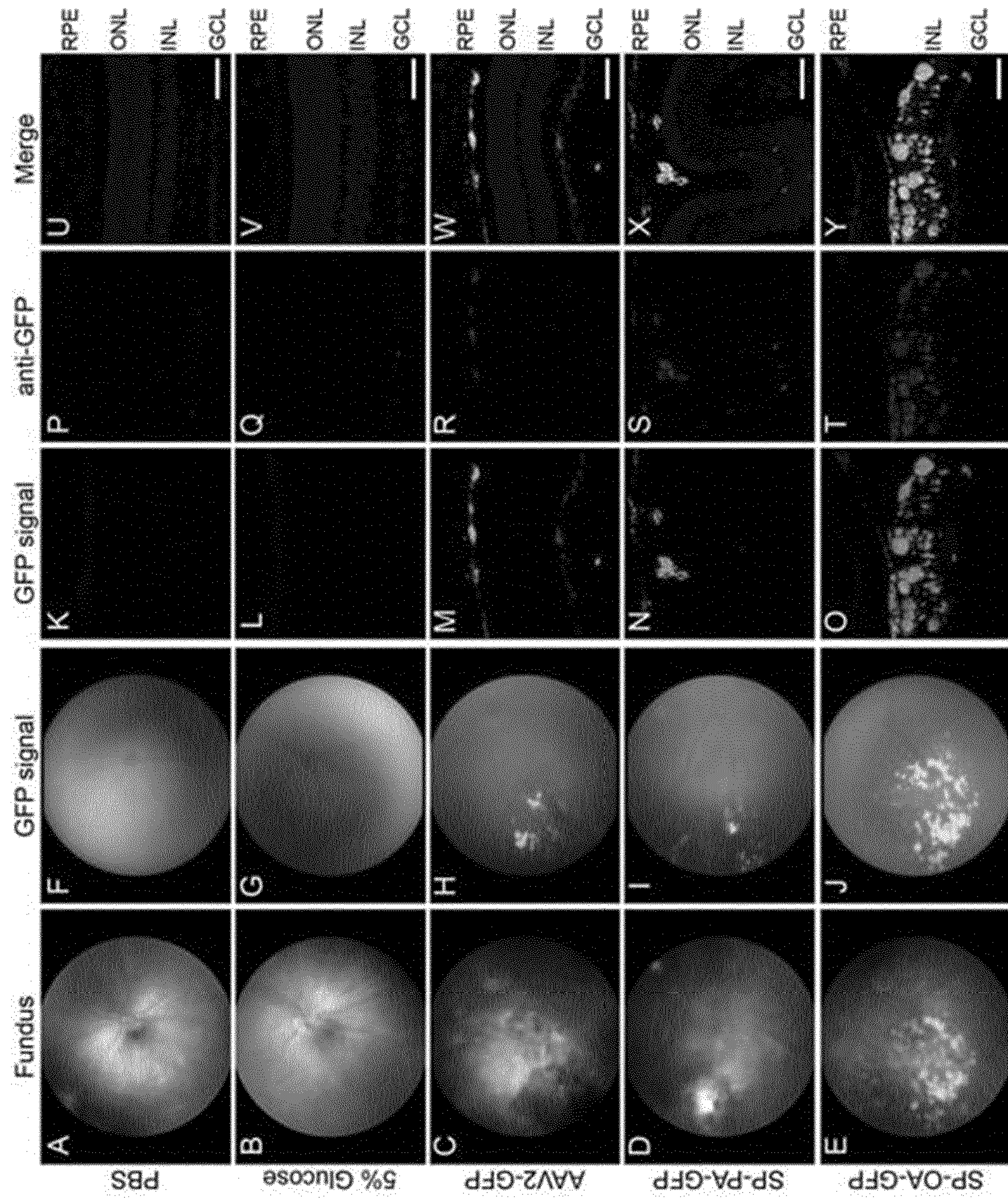


Fig. 2

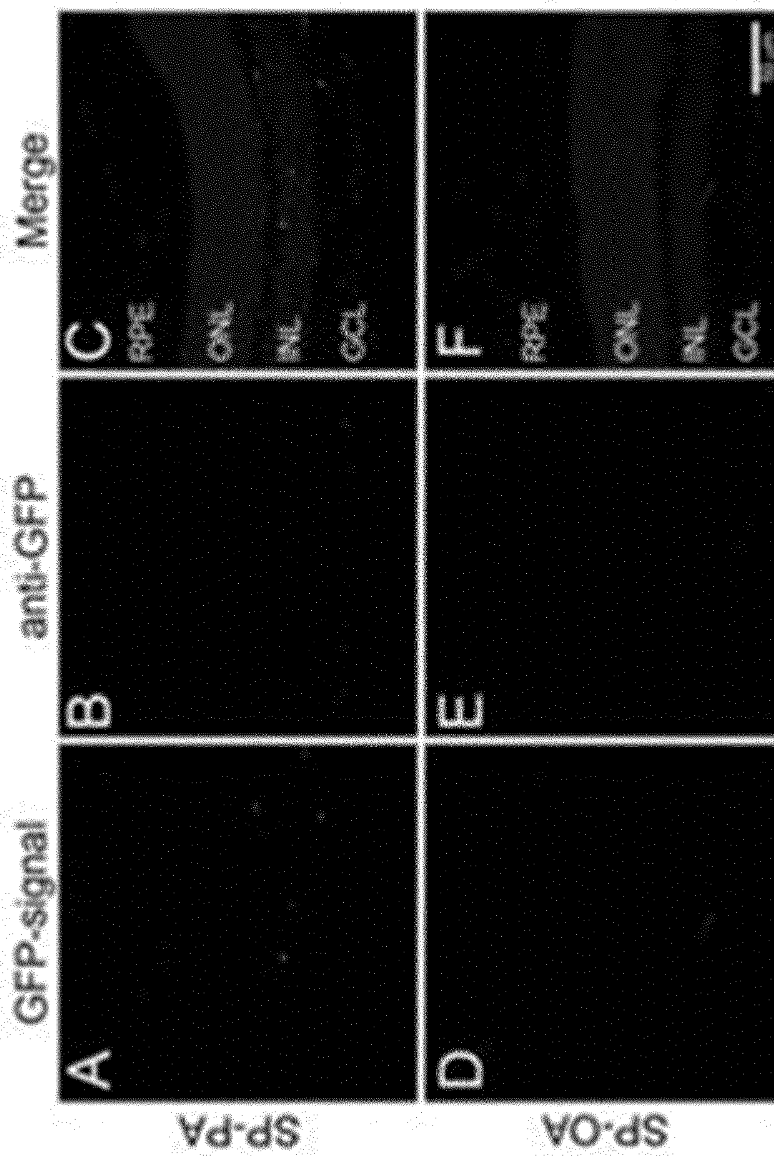


Fig. 2 cont.

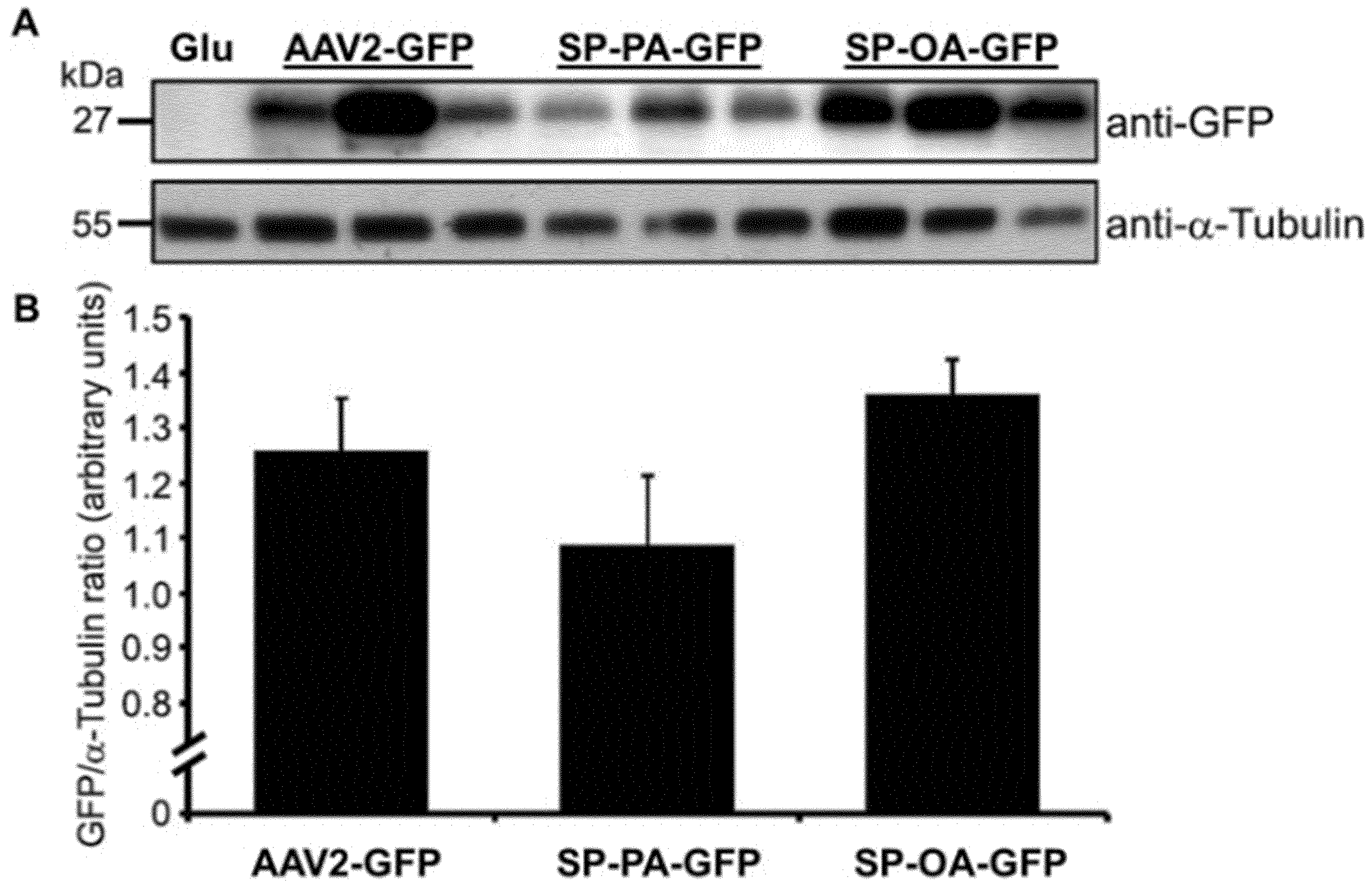


Fig. 3

Rod responses | Cone-rod responses | Cone responses

PBS

5% Glucose

SP-PA

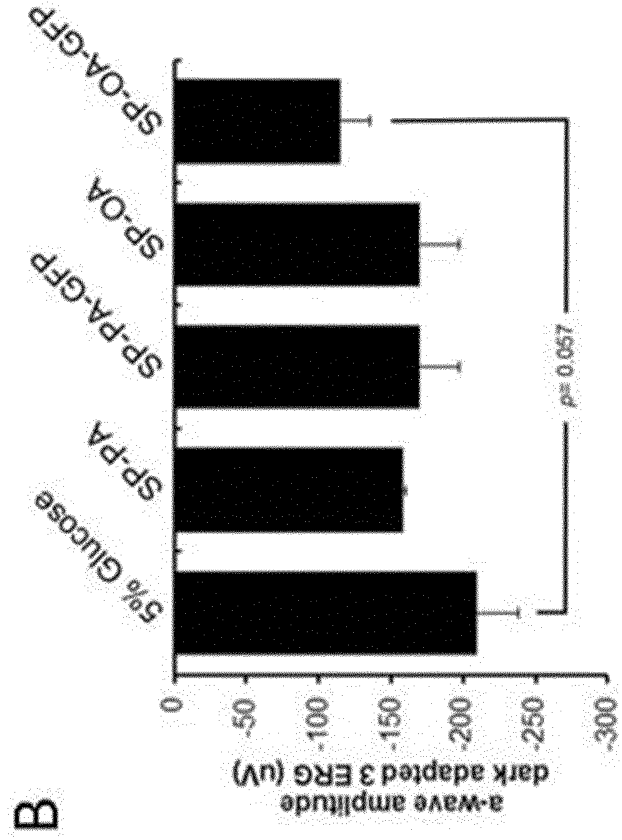
SP-OA

AAV2-GFP

SP-PA-GFP

SP-OA-GFP

Fig. 4



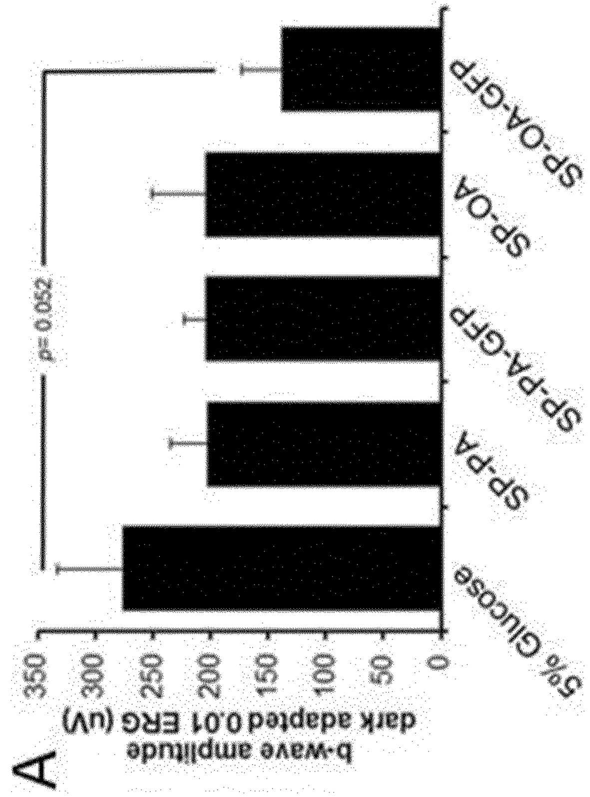


Fig. 5

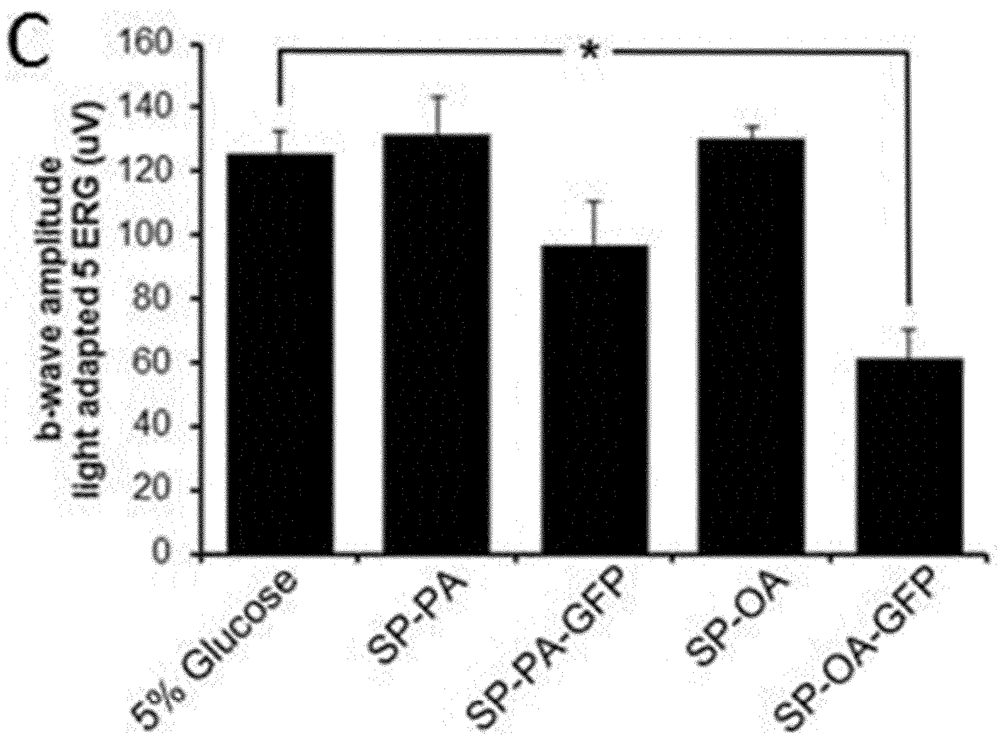
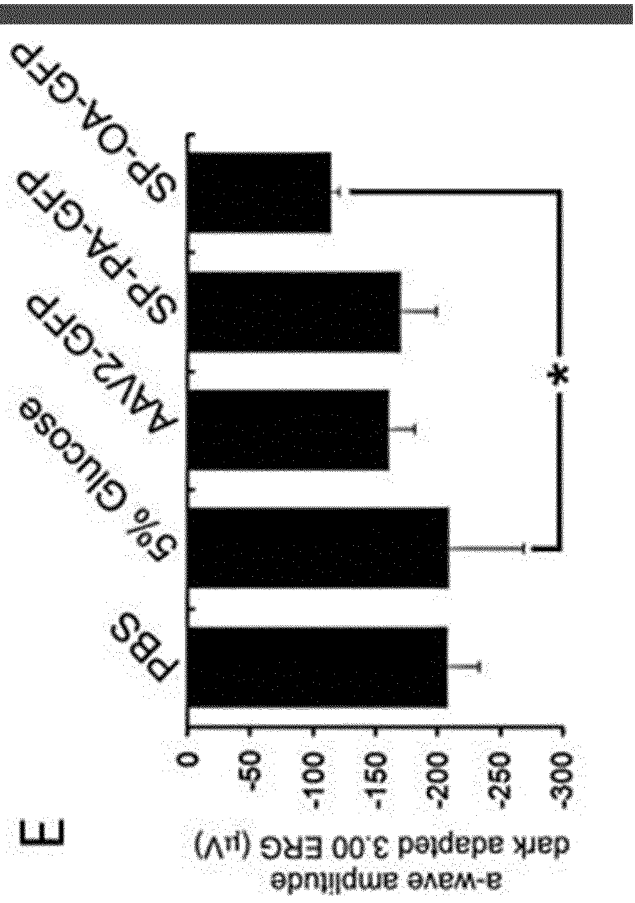


Fig. 5 cont.



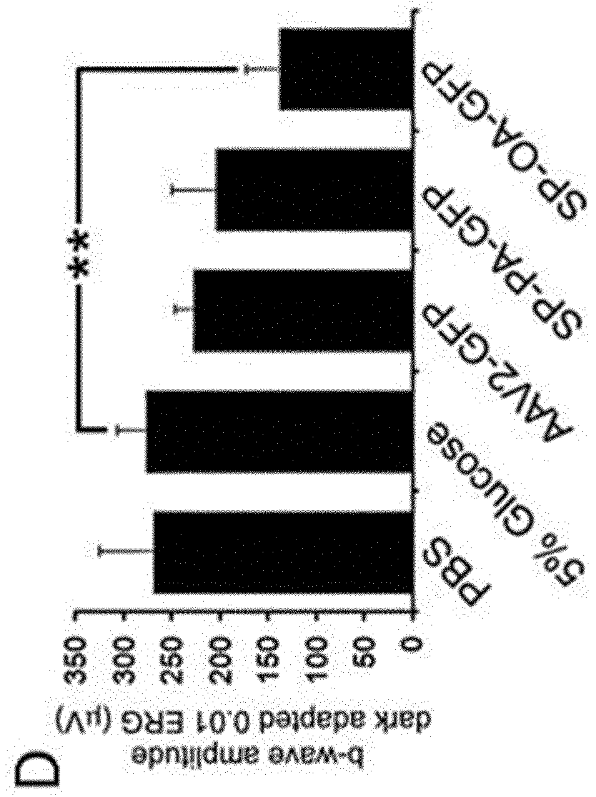


Fig. 5 cont.

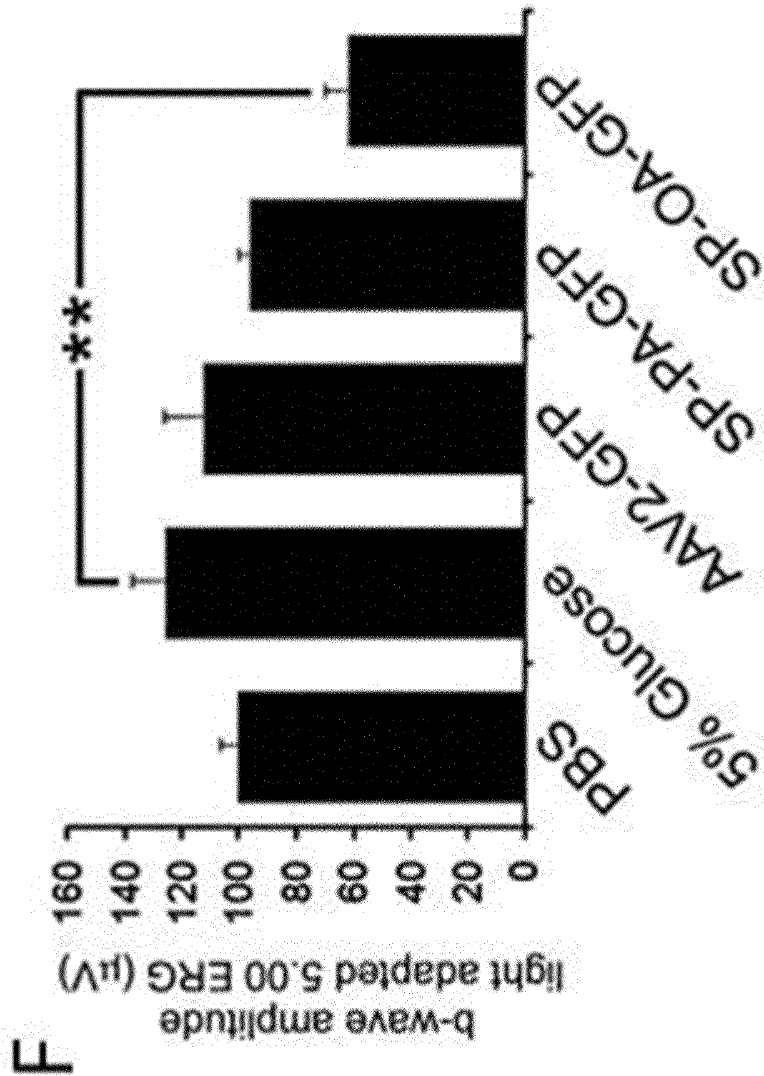


Fig. 5 cont.

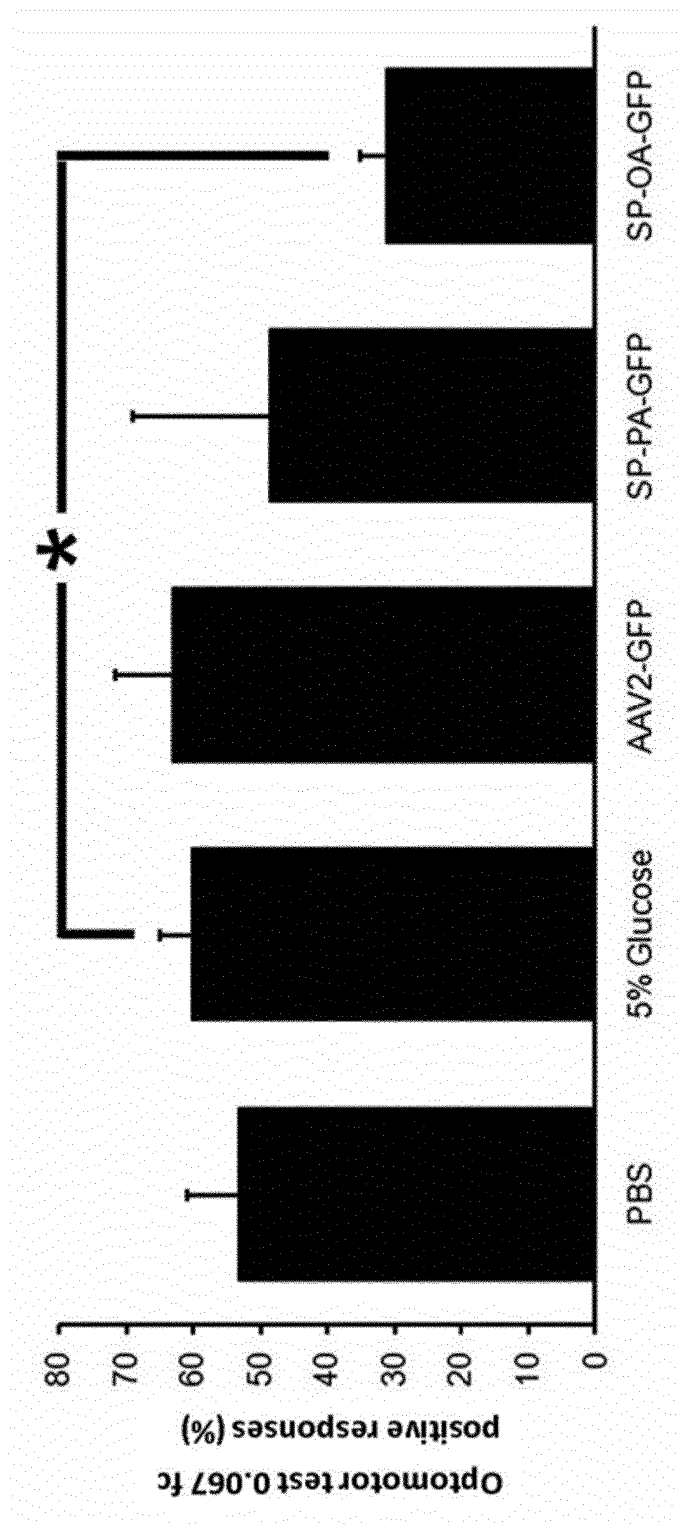


Fig. 6

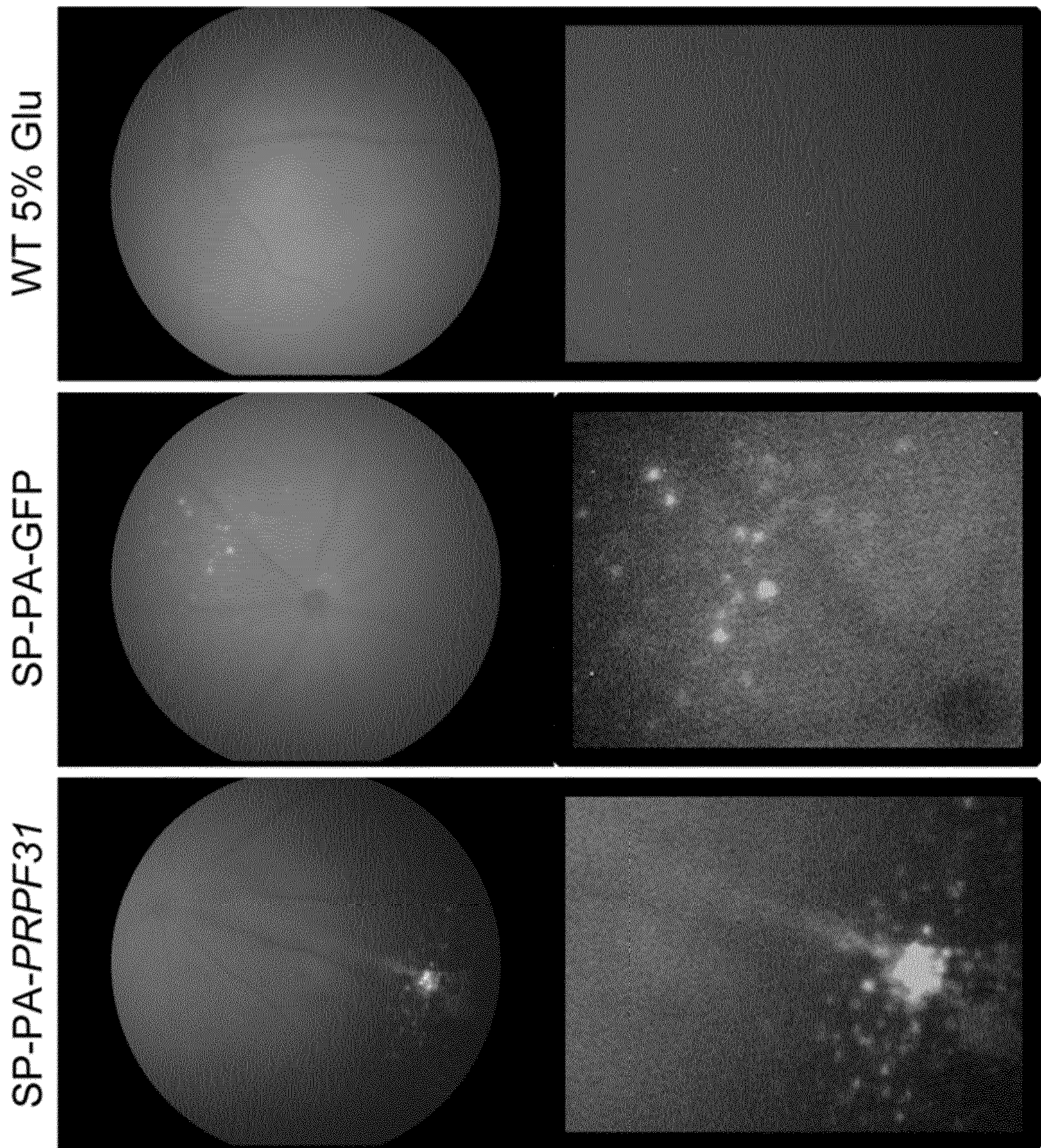
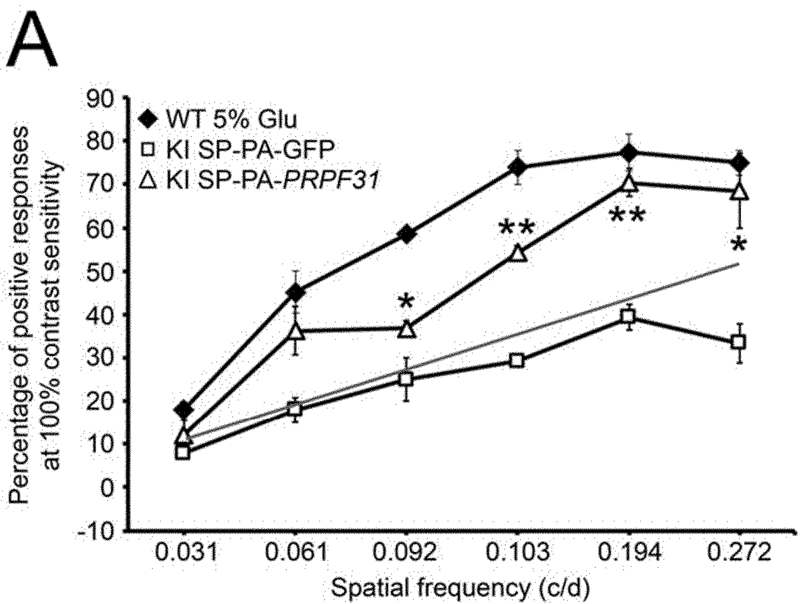


Fig. 7



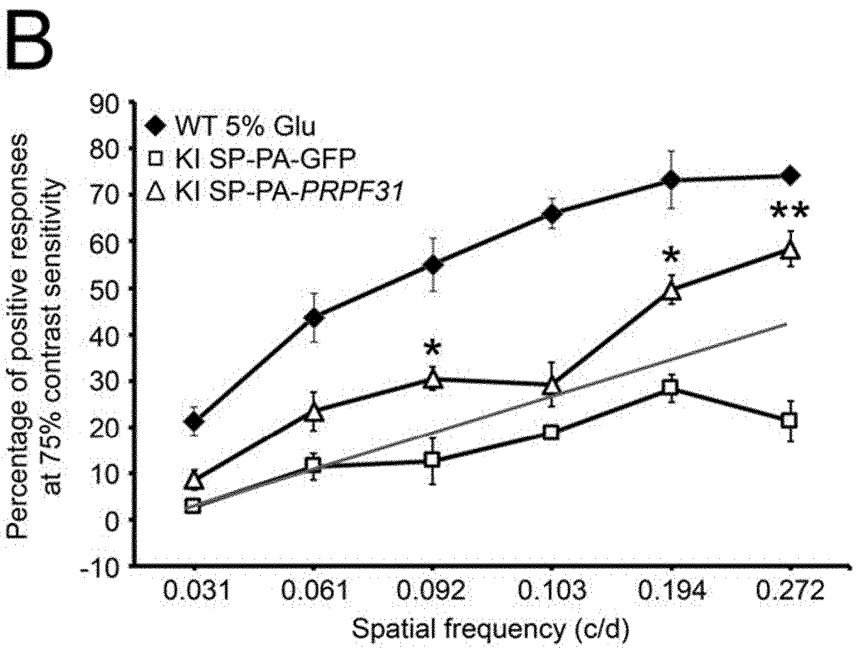


Fig. 8

C

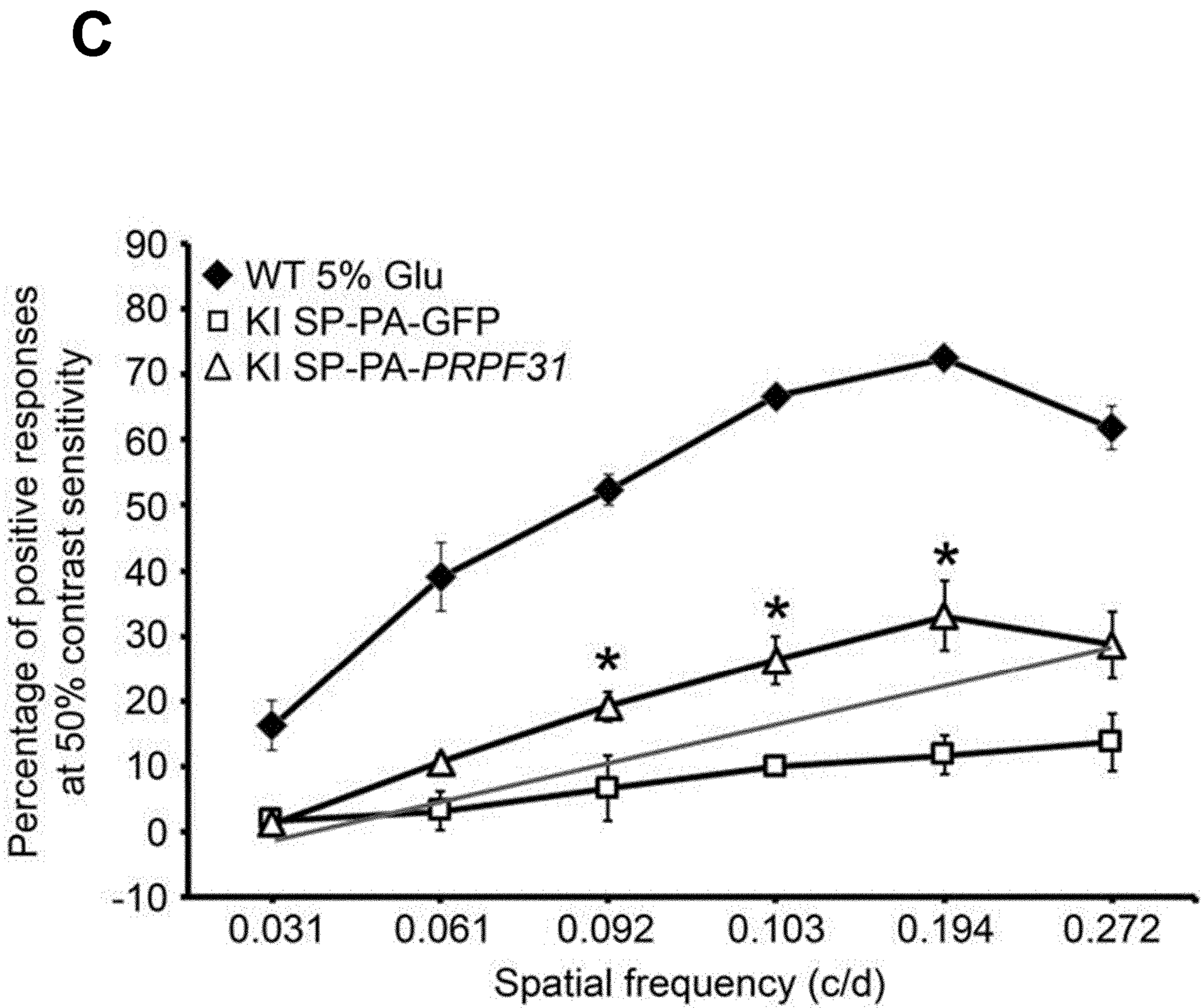


Fig. 8 cont.

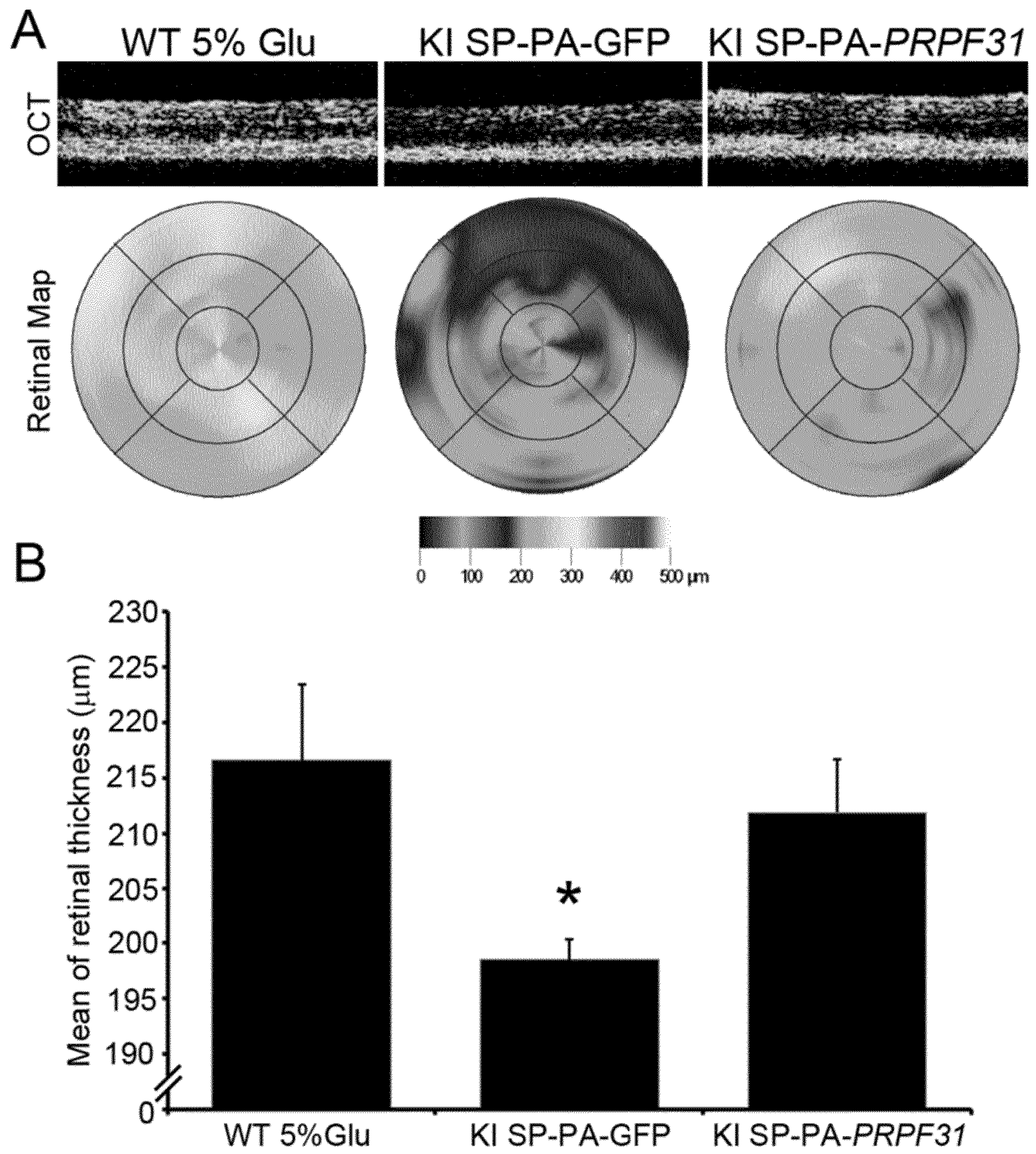
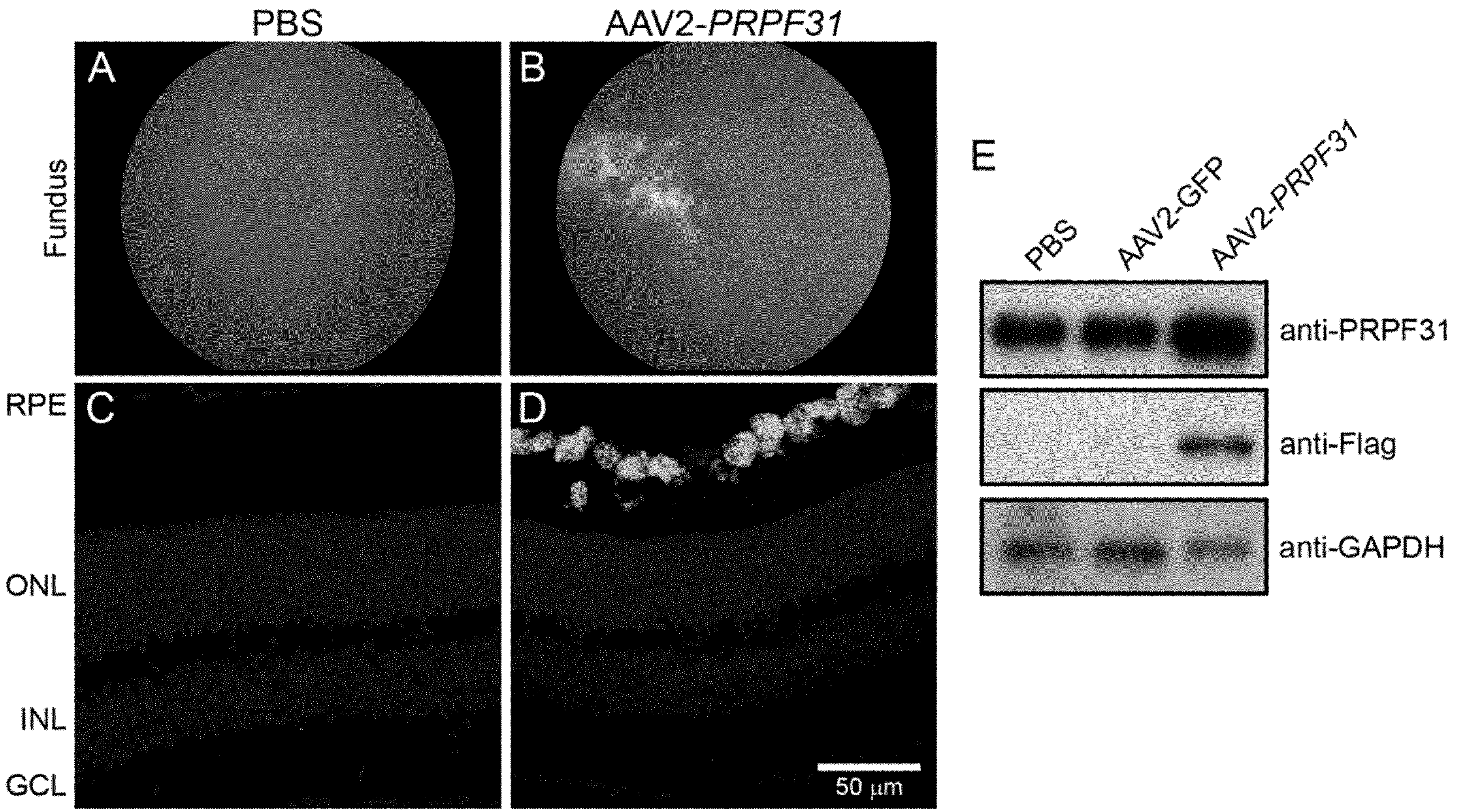


Fig. 9

Fig. 10



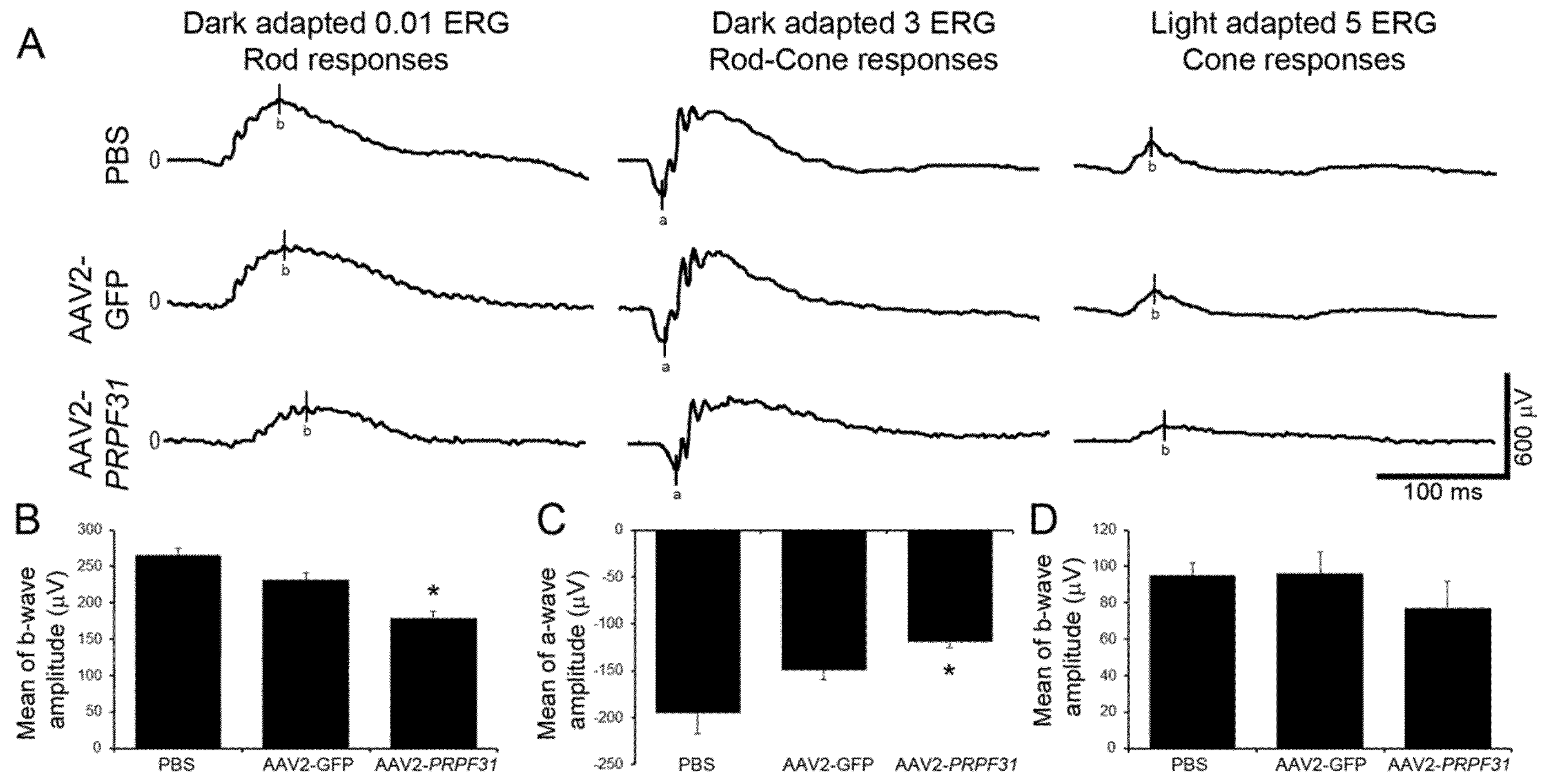


Fig.11

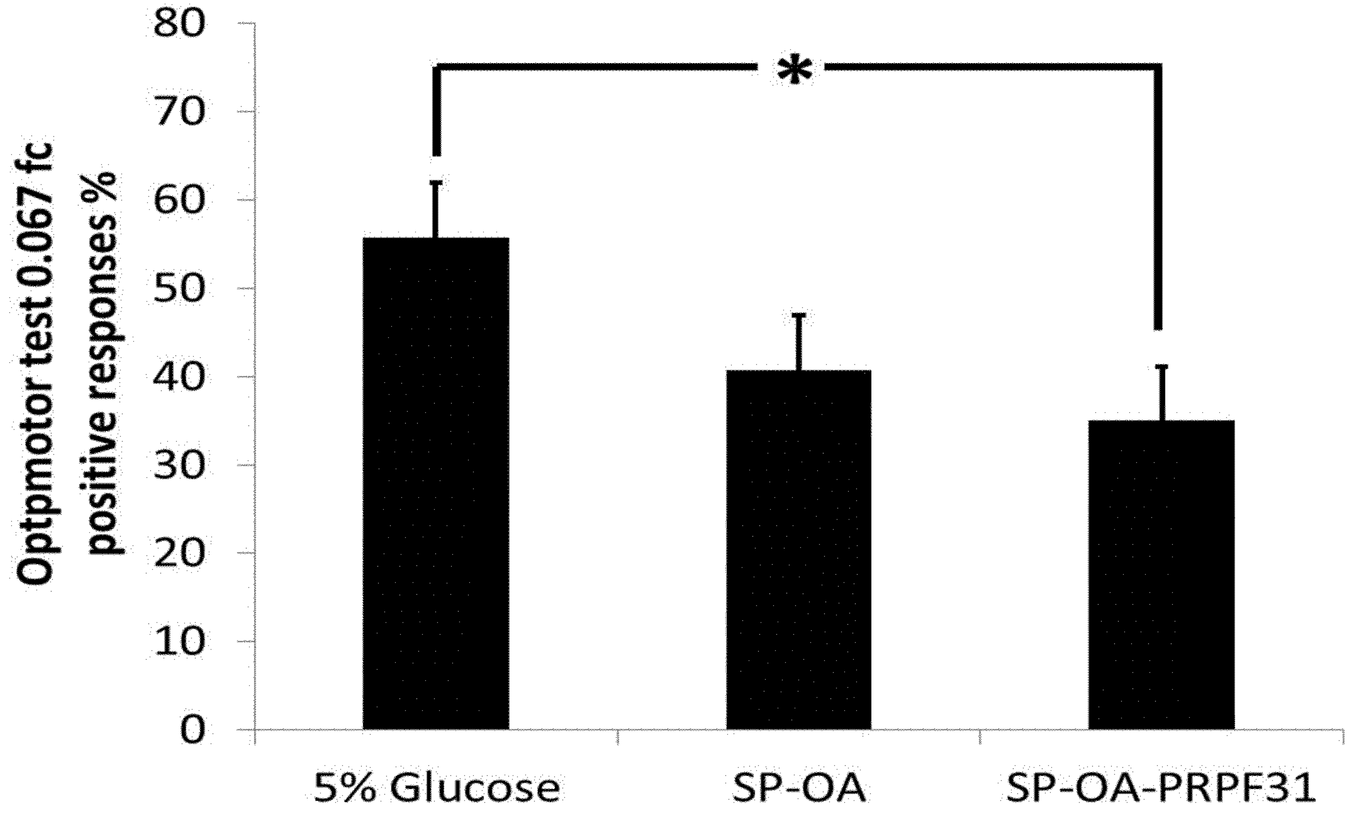


Fig.12

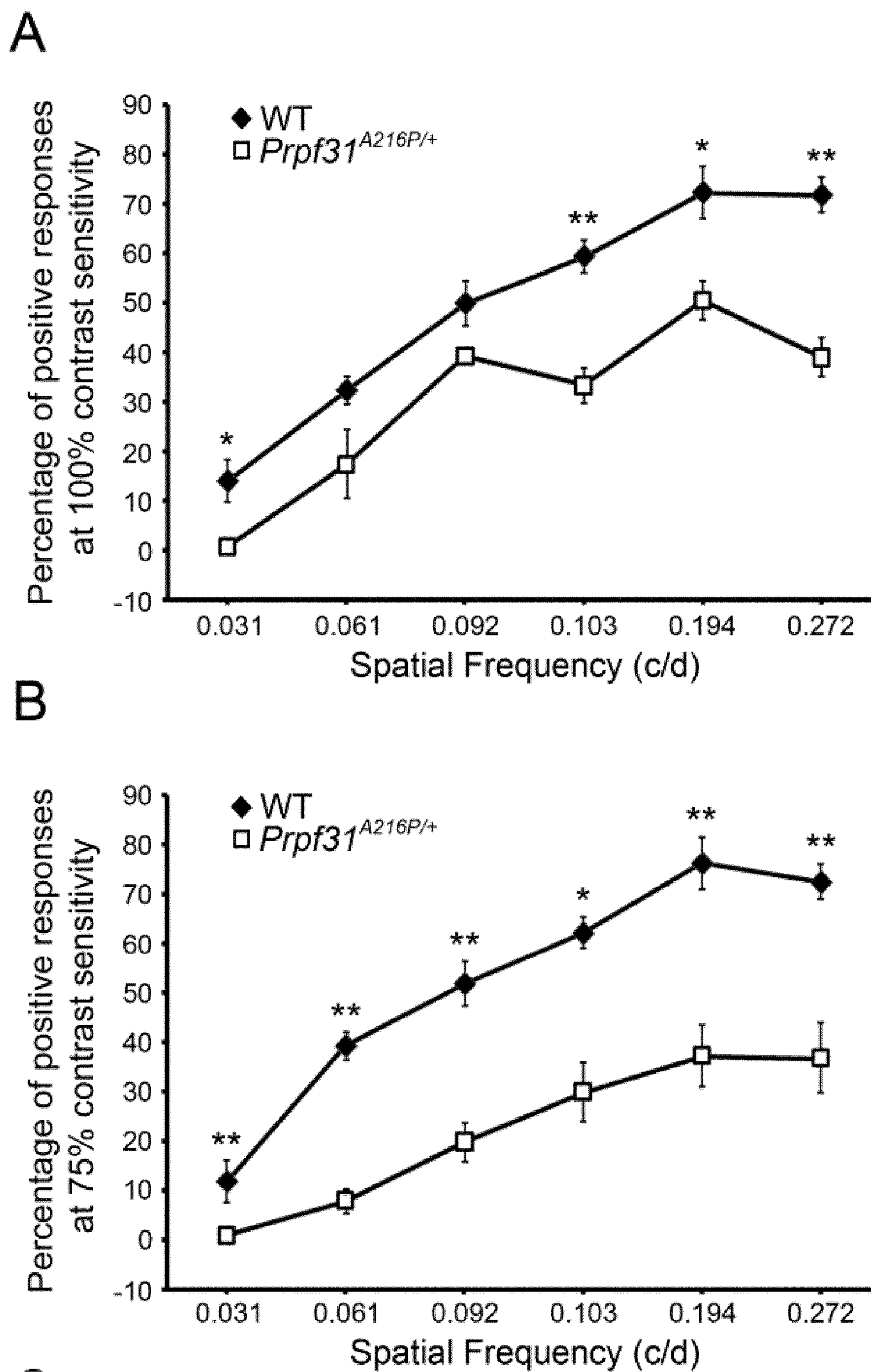


Fig. 13

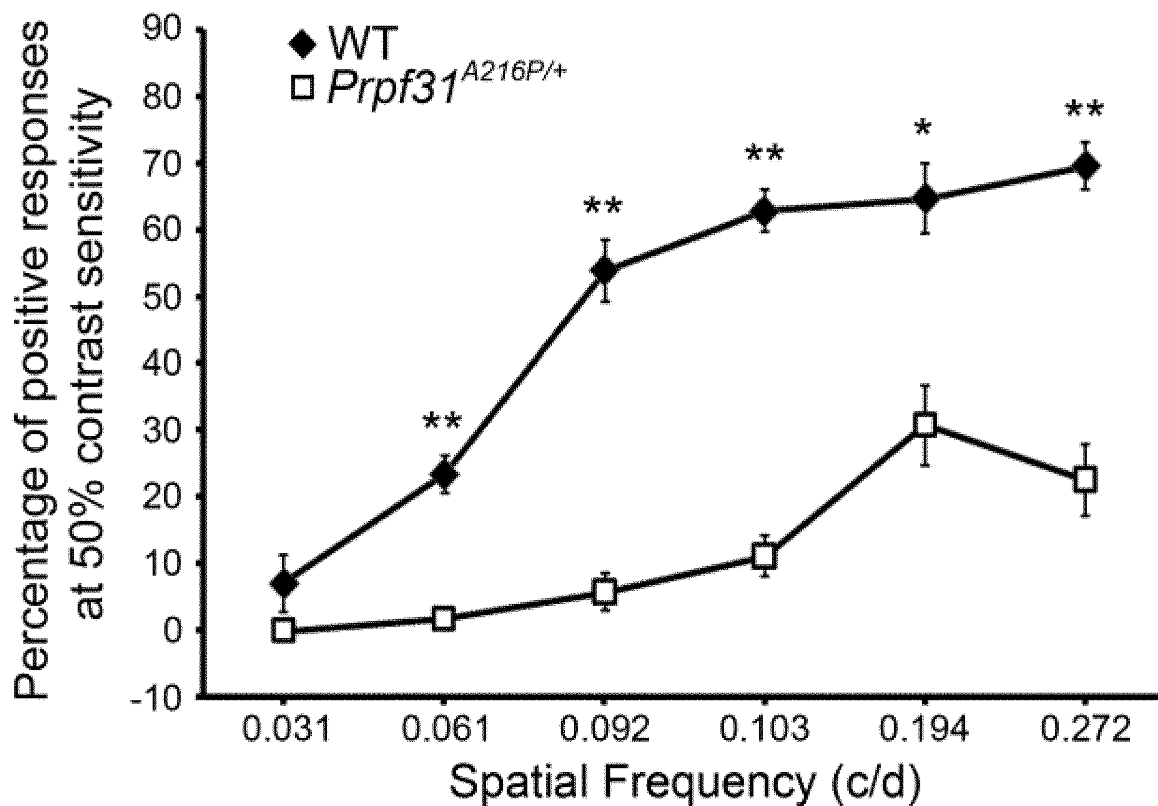


Fig. 13 cont.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/063348

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

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

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

EPO-Internal, BIOSIS, Sequence Search, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/068625 A1 (UNIV SANTIAGO COMPOSTELA [ES]) 16 May 2013 (2013-05-16) cited in the application	1-3
Y	the whole document	4-19
X,P	-& EP 2 792 350 A1 (UNIV SANTIAGO COMPOSTELA [ES]) 22 October 2014 (2014-10-22)	1-3
Y,P	see whole document and in particular Examples 1, 2, 14, 15, paragraphs [0019]-[0023], [0048]-[0061], [0101], [0117] and claims 1-5, 27.	4-19

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2015	26/10/2015

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer
Ury, Alain

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	LIU M M ET AL: "Alternative splicing and retinal degeneration", CLINICAL GENETICS, vol. 84, no. 2, August 2013 (2013-08), pages 142-149, XP002746168, abstract -----	4-19
Y	SUNSHINE JOEL C ET AL: "Poly(beta-Amino Ester)-Nanoparticle Mediated Transfection of Retinal Pigment Epithelial Cells In Vitro and In Vivo", PLOS ONE, vol. 7, no. 5, May 2012 (2012-05), XP002746169, abstract -----	4-19
Y	MITRA RAJENDRA N ET AL: "Synthesis and Characterization of Glycol Chitosan DNA Nanoparticles for Retinal Gene Delivery", CHEMMEDCHEM, vol. 9, no. 1, January 2014 (2014-01), pages 189-196, XP002746170, abstract -----	4-19
A	ELIAS FATTAL ET AL: "Nanotechnologies and controlled release systems for the delivery of antisense oligonucleotides and small interfering RNA", BRITISH JOURNAL OF PHARMACOLOGY, vol. 157, no. 2, 2 April 2009 (2009-04-02), pages 179-194, XP055183700, ISSN: 0007-1188, DOI: 10.1111/j.1476-5381.2009.00148.x -----	1-19
A	SHINTANI K ET AL: "Review and update: Current treatment trends for patients with retinitis pigmentosa", OPTOMETRY - JOURNAL OF THE AMERICAN OPTOMETRIC ASSOCIATION, ELSEVIER, NL, vol. 80, no. 7, 1 July 2009 (2009-07-01), pages 384-401, XP026211569, ISSN: 1529-1839, DOI: 10.1016/J.OPTM.2008.01.026 [retrieved on 2009-06-21] -----	1-19

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/063348

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