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(54) **Title:** LIGHT-INDUCIBLE PROTEIN AGGREGATION SYSTEM FOR MODELING PROTEINOPATHIES AND NEURODEGENERATIVE DISORDERS

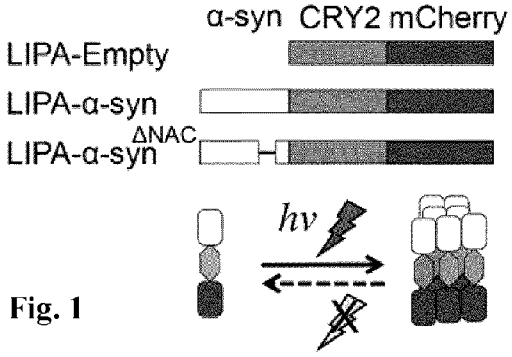

Fig. 1

(57) **Abstract:** A light-inducible intracellular protein aggregation system is described herein, which provides invaluable tools to study the role of protein aggregates in proteinopathies and to screen for novel therapeutic compounds. The system generally comprises a cell expressing an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide. Illumination of the photoactivatable polypeptide with light having a wavelength sufficient for photoactivation triggers irreversible accumulation of intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide. The intracellular protein aggregates can be made to accumulate in real-time during the illumination, thereby enabling spatiotemporal control of protein aggregation. In some embodiments, the intracellular protein aggregates may exhibit pathologically-relevant properties of those found in disease-associated proteinopathies, such as irreversibility, auto-perpetuation (seeding) activity, and comprising misfolded proteins rich in beta-sheet conformation.

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LIGHT-INDUCIBLE PROTEIN AGGREGATION SYSTEM FOR MODELING PROTEINOPATHIES AND NEURODEGENERATIVE DISORDERS

The present description relates to optobiology. More specifically, the present description relates to a light-inducible protein aggregation system for modeling proteinopathies and neurodegenerative disorders.

BACKGROUND

Protein aggregation is a process by which misfolded proteins generally adopt an organized and structurally well-defined fibrillar conformation, often leading to the formation of proteinaceous amyloid deposits. A typical example is the accumulation of proteinaceous intraneuronal inclusions, called Lewy bodies (LB), in the brain of patients suffering from alpha-synucleinopathies, a group of neurological disorders which encompasses Parkinson's disease (PD), PD with dementia (PDD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA).

Since their initial description more than a century ago, a causal link between LB formation and neurotoxicity in PD and other proteopathic disorders has been suggested. However, the exact role of LB in the pathogenesis and progression of disease and how these aggregates precipitate neuronal death remains uncertain. This is in part due to the fact that current cellular and animal models of PD do not allow to monitor the aggregation process in living cells and do not result in the formation of intraneuronal inclusions resembling authentic LB observed in the brains of PD disease patients. Thus, there remains a need for improved systems for modeling proteinopathies and neurodegenerative disorders.

SUMMARY

The present invention generally relates to a versatile light-inducible intracellular protein aggregation system (LIPA) useful for modeling proteinopathies and neurodegenerative disorders, such as in real-time in living cells, in a spatiotemporal controlled manner.

In some aspects, the LIPA system may comprise a cell expressing an alpha-synuclein (α-syn) polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide, wherein illumination (light stimulation) of the photoactivatable polypeptide with light having a wavelength sufficient for photoactivation triggers irreversible accumulation of intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide.

In some embodiments, the intracellular protein aggregates accumulate rapidly and in real-time during the illumination, thereby enabling spatiotemporal control of protein aggregation. Furthermore, in some embodiments, the intracellular protein aggregates comprise misfolded proteins in beta-sheet

conformation that may remain stable for days following cessation of light stimulation. In some embodiments, the intracellular protein aggregates may have a morphology different from that of intracellular inclusions formed following photoactivation of the photoactivatable polypeptide in the absence of the alpha-synuclein polypeptide or other proteopathic polypeptide.

In some embodiments, the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide may be comprised in a fusion protein, and/or further fused to a detectable marker enabling real-time detection *in vivo* (e.g., a fluorescent protein).

In some embodiments, the proteopathic polypeptide is or comprises a polypeptide that, upon self-aggregation, adopts a misfolded conformation rich in beta-sheet structure and/or exhibits seeding activity. In some embodiments, the proteopathic polypeptide is, comprises, or is from: an Aβ precursor protein, an Aβ peptide, Tau, a prion, an Ig light chain, serum amyloid A, transthyretin, cystatin C, beta-2-microglobulin, apolipoprotein AI, apolipoprotein AII, gelsolin, amylin, calcitonin, atrial natriuretic factor, lysozyme, insulin, fibrinogen α-A chain, superoxide dismutase 1, huntingtin, an androgen receptor, an ataxin, a TATA box-binding protein, or TDP-43.

In some embodiments, the proteopathic polypeptide is associated with a proteinopathy comprising: a neurodegenerative disease, a proliferative disease, an inflammatory disease, a cardiovascular disease, diabetes, a synucleinopathy, Parkinson's disease (PD), dementia with Lewy bodies, diffuse Lewy body disease, multiple system atrophy, a tauopathy, Alzheimer's disease (AD), Creutzfeldt-Jakob disease or other prion disease, spinocerebellar ataxias, spinal or bulbar muscular atrophy, amyotrophic lateral sclerosis (ALS), hereditary renal amyloidosis, medullary carcinoma of the thyroid, familial amyloid polyneuropathy, amyloidosis, or frontotemporal degeneration (FTD).

In some embodiments, the alpha-synuclein polypeptide or other proteopathic polypeptide is incapable of self-aggregation in the cell in the absence of the light stimulation (illumination).

In some embodiments, the photoactivatable polypeptide, upon photoactivation, brings molecules of the alpha-synuclein polypeptide or other proteopathic polypeptide into sufficient physical proximity to enable their irreversible self-aggregation.

In some embodiments, the photoactivatable polypeptide is incapable of self-clustering in the cell in the absence of said illumination.

In some embodiments, the cell is comprised in, or is obtained from, an animal (e.g., a transgenic animal engineered to express the alpha-synuclein polypeptide or other proteopathic polypeptide, and the photoactivatable polypeptide).

In some embodiments, the cell expresses an alpha-synuclein polypeptide operably linked to the photoactivatable polypeptide, and wherein the intracellular protein aggregates comprise Lewy body-like

aggregates (e.g., aggregates positive for alpha-synuclein phosphorylated at Ser129, ubiquitination, staining with thioflavin S or another amyloid-specific dye, p62, HSP70, or any combination thereof).

In some embodiments, the present description relates to a method for inducing protein aggregation in a cell or animal, the method comprising providing the system as defined herein, and illuminating the cell or animal with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering irreversible accumulation of the intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide.

In some aspects, the present description relates to a method for selecting a candidate compound for the treatment of a proteinopathy or neurodegenerative disease, the method comprising: (a) providing the system as defined herein; (b) illuminating the cell, or animal comprising said cell, with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering accumulation of the intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide; (c) administering a compound of interest to the cell or animal; and (d) determining the effect of the compound of interest on the intracellular protein aggregates. In some embodiments, the method may further comprise: (e) selecting the compound of interest as a candidate compound when a decrease in the number and/or size of intracellular protein aggregates is observed.

In some aspects, the present description relates to a method for treating a subject having or susceptible for developing a proteinopathy, the method comprising: (a) providing the system as defined herein, wherein the proteopathic polypeptide is selected based on the subject's proteinopathy; (b) illuminating the cell or animal with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering accumulation of the intracellular protein aggregates comprising the proteopathic polypeptide; (c) administering a compound of interest to the cell or animal comprising said cell; (d) determining the effect of the compound of interest on the intracellular protein aggregates; and (e) treating the subject with the compound of interest when a decrease in the intracellular protein aggregates is observed (i.e., in step (d)).

In some aspects, the present description relates to an expression cassette comprising a polynucleotide encoding an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide. In some embodiments, the alpha-synuclein polypeptide or other proteopathic polypeptide may be fused to the proteopathic polypeptide, and/or to a detectable marker as defined herein.

In some aspects, the present description relates to a vector comprising the expression cassette as defined herein, or a cell comprising a genome-integrated expression cassette or vector as defined herein.

In some aspects, the present description relates to a transgenic animal model comprising an expression cassette, a vector, or a cell as defined herein. In some embodiments, the animal may be a mouse, a rat, or other rodent, a non-human primate, fish, nematode, yeast, or a fly.

In some aspects, the present description relates to a protein aggregate comprising an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide. In some embodiments, the protein aggregate may comprise an alpha-synuclein polypeptide operably linked to the photoactivatable polypeptide, wherein the protein aggregate comprises a Lewy body-like aggregate (e.g., positive for: alpha-synuclein phosphorylated at Ser129, ubiquitination, staining with thioflavin S or another amyloid-specific dye, p62, HSP70, or any combination thereof).

In some aspects, the present description relates to a variant alpha-synuclein polypeptide for use in preventing aggregation of a proteopathic polypeptide other than alpha-synuclein, or for use in treating a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide.

In some aspects, the present description relates to the use of a variant alpha-synuclein polypeptide for the manufacture of a medicament for the treatment of a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of a proteopathic polypeptide associated with said proteinopathy.

In some aspects, the present description relates to an expression cassette encoding a variant alpha-synuclein polypeptide for use in preventing aggregation of a proteopathic polypeptide other than alpha-synuclein, or for use in treating a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide.

In some aspects, the present description relates to the use of an expression cassette encoding a variant alpha-synuclein polypeptide in the manufacture of a medicament for the treatment of a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide.

General Definitions

Headings, and other identifiers, e.g. (a), (b), (i), (ii), etc., are presented merely for ease of reading the specification and claims. The use of headings or other identifiers in the specification or claims does not necessarily require the steps or elements be performed in alphabetical or numerical order or the order in which they are presented.

The use of the word "**a**" or "**an**" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one" but it is also consistent with the meaning of "one or more", "at least one", and "one or more than one".

As used in this specification and claim(s), the words "**comprising**" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

As used herein, "**subject**" generally refers to a mammal, including primates, and particularly to a human.

Other objects, advantages and features of the present description will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

SEQUENCE LISTING

This specification contains a Sequence Listing in computer readable form created June 28, 2019, having a size of about 56 kb. The computer readable form is incorporated herein by reference.

SEQ ID NO: 1	pAAV-LIPA-Empty
SEQ ID NO: 2	pAAV-LIPA-α-syn
SEQ ID NO: 3	pAAV-LIPA-αsynΔNAC
SEQ ID NO: 4	pmCherryN1-LIPA-Empty
SEQ ID NO: 5	pmCherry-LIPA-α-syn
SEQ ID NO: 6	pmCherry-LIPA-α-synΔNAC

BRIEF DESCRIPTION OF THE FIGURES

In the appended figures:

Fig. 1 shows a schematic representation of an embodiment of the light-inducible protein aggregation system described herein.

Fig. 2 shows a time-lapse live imaging illustration of representative HEK-293T cells overexpressing the LIPA system.

Fig. 3 shows an analysis of the number of LIPA system-induced aggregates per volume of cells after 0 to 30 min stimulation with a confocal 488 nm laser (1% intensity = 15.5 μW).

Fig. 4 shows a time-lapse live imaging illustration of representative region of interest (ROI) light stimulation in HEK-293T cells overexpressing LIPA-α-syn.

Fig. 5 shows an analysis of the total number of LIPA-α-syn aggregates per cell after ROI stimulation.

Fig. 6 shows the results of a cell toxicity assay in which cell death was assessed by quantifying the extracellular release of cytosolic lactate dehydrogenase (LDH) spectrophotometrically using a colorimetric assay. The results indicate that neither overexpression of LIPA constructs, nor the exposure to the blue light, were toxic in the experimental design employed herein.

Fig. 7 shows representative confocal images of HEK-293T cells overexpressing each of the LIPA system constructs (LIPA-α-syn$^{\Delta NAC}$, LIPA-α-syn, LIPA-Empty) and exposed to blue light for 0 to 24 h.

Fig. 8 shows an analysis of the percentage of mCherry-positive cells exhibiting LIPA aggregates (n=5).

Fig. 9 shows a 3D reconstitution of LIPA-Empty and LIPA-α-syn aggregates (white arrows) in HEK-293T cells exposed 24 h to the blue light (scale bar = 2 μm).

Figs. 10 and 11 show the results of a filter retardation assay and dot blot analysis showing the time course of LIPA-Empty and LIPA-α-syn aggregate formation (n=3).

Figs. 12 and 13 show the results of a semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) exhibiting the time-dependent formation of high-molecular LIPA-Empty and LIPA-α-syn aggregates after 0, 6, or 12 h exposure to blue light (n=3).

Fig. 14 shows a schematic representation of the experimental paradigm used in **Figs. 15-21.**

Fig. 15 shows an analysis of the time course of LIPA-Empty or LIPA-α-syn aggregates dissociation/disappearance, after 12 h pre-exposure to the blue light, by assessing the proportion of mCherry-positive cells exhibiting inclusions (n=5).

Fig. 16 shows the effect of treatment with the small molecule inhibitors of α-syn aggregation, baicalein and myricetin, on the stability of LIPA-α-syn aggregates (n=5).

Fig. 17 shows the effect of treatment with baicalein and myricetin on the stability of LIPA-Empty aggregates (n=5).

Fig. 18 shows the effect of treatment with inactive small molecules, naringenin and diadzein, on LIPA-α-syn aggregates stability (n=5).

Fig. 19 shows the effect of beta-synuclein (β-syn) and mouse α-syn overexpression on the stability of LIPA-α-syn aggregates (n=5).

Fig. 20 shows the effect of β-syn and mouse α-syn overexpression on the stability of LIPA-Empty aggregates (n=5).

Fig. 21 shows the effect of GFP overexpression on the stability of LIPA-α-syn aggregates (n=5).

Fig. 22 shows the results of co-immunoprecipitation (IP) of LIPA-α-syn aggregates with monomeric α-syn-GFP (n=3).

Fig. 23 shows the experimental design (left panel), confocal microscopy images (middle panels; scale bar = 10 μm), and 3D reconstitution (right panel; scale bar = 0.5 μm) illustrating the seeding of monomeric α-syn-GFP by LIPA-α-syn aggregates (n=3).

Fig. 24 shows the experimental design (left panel), confocal microscopy images (middle panels; scale bar = 10 μm), and 3D reconstitution (right panel; scale bar = 0.7 μm) illustrating the seeding of monomeric α-syn Pffs-488 by LIPA-α-syn aggregates (n=3).

Figs. 25-29 show representative confocal images of HEK-293T cells overexpressing LIPA-α-syn and exposed to light for 12 h and stained with authentic LBs markers: Phosphorylated α-syn at S129 (pS129; **Fig. 25**), ubiquitin (**Fig. 26**), thioflavin S (**Fig. 27**), p62 (**Fig. 28**), and HSP70 (**Fig. 29**) (n=5). Scale bar = 10 μm.

Fig. 30 shows transmission electron microscope images of representative HEK-293T cells exhibiting LIPA-Empty and LIPA-α-syn aggregates, after 12 h of light stimulation. Scale bar = 500 nm.

Fig. 31 shows a magnified transmission electron microscope image (black box in **Fig. 30**) showing the presence of mitochondria (mito) and endoplasmic reticulum (ER) within a LIPA-α-syn aggregate. Scale bar = 500 nm.

Fig. 32 shows the experimental design of the overexpression and the induction of LIPA-α-syn aggregation in the brain of wild type mice.

Figs. 33-38 shows confocal microscope images of representative striatal neurons with LIPA-α-syn aggregates exhibiting authentic LBs markers: α-syn (BDlab and FL140; **Figs. 33 and 34**), Phosphorylated α-syn at S129 (pS129; **Fig. 35**), ubiquitin (**Fig. 36**), thioflavin S (**Fig. 37**), and p62 (**Fig. 38**) (n=5 mice). Scale bar = 5 μm.

Figs. 39-44 are plasmid maps of plasmid used for mammalian expression, including pmCherryN1-LIPA-Empty (**Fig. 42**), pmCherry-LIPA-α-syn (**Fig. 43**), and pmCherry-LIPA-α-synΔNAC (**Fig. 44**).

Fig. 45 shows a schematic representation of the *in vivo* experimental paradigm for **Figs. 46-53**.

Figs. 46-49 shows the results of assessments of behavioral impairment induced after overexpression of LIPA constructs, with and without optogenetic stimulation, using the cylinder test (**Fig. 46**), the grip strength test (**Fig. 47**), the rotarod test (**Fig. 48**), and the gait test (**Fig. 49**) (n = 5-9 mice per experimental condition).

Fig. 50 shows confocal microscopy images illustrating the expression of LIPA-constructs in the dopaminergic midbrain neurons and the presence of pathological (pS129-positive) α-syn aggregates in LIPA-α-syn-positive and optogenetically stimulated neurons.

Fig. 51 shows microscope images illustrating the dopaminergic neuronal loss in the midbrain of mice overexpressing LIPA constructs and exposed to the blue light stimulation (* injected side; scale bar = 1 mm).

Figs. 52 and 53 show stereological quantification of the TH-positive dopaminergic neurons (**Fig. 52**) and total neuronal marker (Nissl; **Fig. 53**) in the midbrain of mice overexpressing LIPA constructs and exposed or not to the optogenetic stimulation. Results are expressed as % of contralateral non-injected side (n = 5-9 mice per experimental condition).

Figs. 54A-54F shows that LIPA-α-syn co-aggregates with and promotes prion-like propagation of mutant huntingtin (mHtt). **Fig. 54A**: Low resolution confocal image illustrating the presence of LIPA-α-syn and mHtt-GFP in the injected striatum of wild type C57/Bl6 mice. **Fig. 54B**: high resolution confocal images illustrating the co-localization of LIPA-α-syn and mHtt-GFP in the striatal neurons. Low (**Fig. 54C**) and high (**Fig. 54D**) resolution confocal images illustrating the presence of LIPA-α-syn and mHtt-GFP in the *globus pallidus*. Low (**Fig. 54E**) and high (**Fig. 54F**) resolution confocal image illustrating the presence of LIPA-α-syn and mHtt-GFP in entopeduncular nucleus.

DETAILED DESCRIPTION

The present description relates, at least in part, to the discovery that proteopathic polypeptides (e.g., alpha-synuclein or other proteopathic polypeptides, particularly those that self-aggregate under pathogenic conditions) when forced into physical proximity within cell systems using optobiological systems, may trigger the formation of irreversible intracellular protein aggregates that may more closely resemble those found in subjects having proteopathic diseases, thereby providing an improved system for modeling proteinopathies and neurodegenerative disorders. Advantageously, the implementation of optobiological approaches enables the rapid formation of the intracellular protein aggregates in real-time (e.g., in living cells), and in a spatiotemporally-controlled manner.

In some aspects, the present description relates to a light-inducible intracellular protein aggregation (LIPA) system, and methods employing same, useful for the study of proteinopathies or other neurodegenerative disorders. The system generally comprises a cell expressing an alpha-synuclein polypeptide or another proteopathic polypeptide that is known to self-aggregate under pathogenic conditions, operably linked to a photoactivatable polypeptide, wherein illumination of the photoactivatable polypeptide with light having a wavelength sufficient for photoactivation triggers accumulation (e.g., irreversible accumulation) of intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide.

As used herein, the expression "**alpha-synuclein polypeptide**" refers to alpha-synuclein (e.g., human alpha-synuclein), as well as variants and/or fragments thereof (e.g., associated with alpha-

synucleinopathies such as Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy). In some embodiments, the alpha-synuclein polypeptide may comprise variants and/or fragments of alpha-synuclein that possess the ability to self-aggregate into Lewy-bodies or Lewy-body-like structures as defined herein. As used herein, the expressions "**proteopathic polypeptide**", "another proteopathic polypeptide", or "other proteopathic polypeptide" refer to polypeptides associated with proteinopathies (also known as proteopathies, protein conformational disorders, or protein misfolding diseases) other than those encompassed by "alpha-synuclein polypeptide". In some embodiments, the proteopathic polypeptides described herein may possess, or comprise variants and/or fragments that possess, self-aggregating activity and/or seeding activity under pathogenic conditions, which may be the result of protein misfolding.

In some embodiments, the alpha-synuclein polypeptide or proteopathic polypeptide described herein may comprise variants and/or fragments of unknown self-aggregating and/or seeding activity, wherein the LIPA system described herein may be used to study the variants and/or fragments. In some embodiments, the LIPA system described herein may comprise or further comprise an alpha-synuclein polypeptide or proteopathic polypeptide that lacks self-aggregating and/or seeding activity, or that has a dominant negative effect with respect to self-aggregating and/or seeding activity. Such variants and/or fragments of alpha-synuclein may be useful, for example, as experimental controls for the system described herein.

In some embodiments, the alpha-synuclein polypeptide or other proteopathic polypeptide described herein may be or comprise a polypeptide that, upon self-aggregation (e.g., under pathogenic conditions), adopts a misfolded conformation (e.g., a filamentous confirmation) that may be rich in beta-sheets (e.g., having an increase in beta-sheets as compared to a corresponding non-pathogenic native alpha-synuclein polypeptide or other proteopathic polypeptide).

In some embodiments, the alpha-synuclein polypeptide or other proteopathic polypeptide described herein may be or comprise a polypeptide that, upon self-aggregation (e.g., under pathogenic conditions), exhibits seeding activity. As used herein, the expression "**seeding activity**" refers to the ability of an alpha-synuclein polypeptide or other proteopathic polypeptide described herein to nucleate (i.e., induce or trigger) self-aggregation or aggregation of proteins having similar aggregation domains, whereby small oligomers, or seeds, provide a template for the assembly of soluble monomer proteins into highly ordered protein aggregates defined by their insolubility and beta-sheet structure.

In some embodiments, the proteopathic polypeptide described herein, may be, comprise, or be derived from: an Aβ precursor protein, an Aβ peptide, Tau, a prion, an Ig light chain, serum amyloid A, transthyretin, cystatin C, beta-2-microglobulin, apolipoprotein AI, apolipoprotein AII, gelsolin, amylin, calcitonin, atrial natriuretic factor, lysozyme, insulin, fibrinogen α-A chain, superoxide dismutase 1,

huntingtin, an androgen receptor, an ataxin, a TATA box-binding protein, TDP-43, or another protein associated with a proteinopathy (Stefani and Dobson, 2003). In some embodiments, the proteopathic polypeptide described herein may be associated with a proteinopathy comprising: a neurodegenerative disease, a proliferative disease, an inflammatory disease, a cardiovascular disease, diabetes, a synucleinopathy, Parkinson's disease, dementia with Lewy bodies, diffuse Lewy body disease, multiple system atrophy, a tauopathy, Alzheimer's disease, Creutzfeldt-Jakob disease or other prion disease, spinocerebellar ataxias, spinal or bulbar muscular atrophy, amyotrophic lateral sclerosis, hereditary renal amyloidosis, medullary carcinoma of the thyroid, familial amyloid polyneuropathy, amyloidosis, or frontotemporal degeneration.

In some embodiments, the alpha-synuclein polypeptide or other proteopathic polypeptide described herein is incapable of self-aggregation in the cell in the absence of said illumination, within the context of the LIPA system. Such a characteristic may reduce undesirable "background" introduced into the LIPA system.

In some embodiments, the "**photoactivatable polypeptide**" described herein generally refers to a protein useful as an optobiological tool, more particularly proteins that respond to illumination with light (e.g., visible or infrared light) by changing their conformations (Kim and Lin, 2013; Khamo et al., 2017). Such light-triggered conformational changes can induce, for example, inter- or intra-molecular interactions, which can then be exploited as taught herein to bring molecules of the alpha-synuclein polypeptide or other proteopathic polypeptide (operably linked to the photoactivatable polypeptide) into sufficient physical proximity to enable or trigger their self-aggregation and/or seeding activity. Some examples of photoactivatable proteins and systems that may be adapted for use in the LIPA systems and methods described herein, in view of the present disclosure, are reviewed in Khamo et al. (2017).

In some embodiments, the photoactivatable polypeptides of the present description, upon photoactivation, bring molecules of the alpha-synuclein polypeptide or other proteopathic polypeptide into sufficient physical proximity to enable their self-aggregation (e.g., irreversible self-aggregation). In some embodiments, the molecules of the alpha-synuclein polypeptide or other proteopathic polypeptide may be brought into physical proximity via: light-induced homo- or hetero-protein association, light-induced conformation change, or light-induced inter- or intra-molecular interactions of the photoactivatable polypeptide. In some embodiments, the homo- or hetero- protein association, conformation change, or inter- or intra-molecular interactions of the photoactivatable polypeptide may be reversible following cessation of the illumination. In some embodiments, the photoactivatable polypeptide is preferably incapable of self-clustering in the cell in the absence of the illumination within the LIPA system. Indeed, photoinactivation-independent self-clustering of the photoactivatable polypeptide may negatively affect the sensitivity and the LIPA system described herein.

In some embodiments, the photoactivatable polypeptide may be, comprise, or be derived from: a photoreceptor, a cryptochrome, a phytochrome, cryptochrome 2 (CRY2), light-oxygen-voltage (LOV) domains, CRY2-CIB1 (calcium and integrin-binding protein 1) variants, CRY2E490G, CRY2clust, iLID nano and iLID micro, LOVTRAP, Magnets, cobalamin binding domain CBD, VfAU1-LOV, CPH1S, BphP1-PpsR2 (see Table 1 of Khamo et al., 2017), or another photoactivatable protein or system employed in optobiology.

In some embodiments, the LIPA system comprises the replacement of one photoactivatable polypeptide within another photoactivatable polypeptide that is photoactivatable by a light of a different color or wavelength (Zhang and Cui, 2015), providing a further degree of modularity to the LIPA system.

In some embodiments, the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide described herein are operably linked. In such a context, the term "**operably linked**" refers to the joining of the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide via a covalent or non-covalent interaction within the cell. For example, in some embodiments, the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide may be comprised in a fusion protein expressed from a single polynucleotide. In other embodiments, the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide may be each engineered (e.g., as fusion proteins) to comprise protein interaction domains that bind directly or indirectly to one another, to the effect that the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide are expressed in the cell as separate proteins, which then come together via their protein interaction domains within the intracellular environment (e.g., the cytosol).

In some embodiments, the LIPA system comprises non-naturally-occurring polypeptides and/or cells. In some embodiments, at least one of the alpha-synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide is heterologous with respect to the cell and/or to each other. In some embodiments, the expression of at least one of the alpha-synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide may be engineered to be under the control of a constitutively active promoter or an inducible promoter.

In some embodiments, the LIPA system comprises cells such as neuronal cells, brain cells, induced pluripotent stem cells, mammalian cells, neuroblastomas, immune cells, or blood cells. Such cells may serve as models for studying various proteinopathies. In some embodiments, the cells may be comprised in an animal (e.g., a transgenic animal engineered to expresses the alpha-synuclein polypeptide or other proteopathic polypeptide, and the photoactivatable polypeptide). In some embodiments, the cells may be obtained from such an animal (or a human subject having or suspected of having a proteinopathy) in *ex vivo* implementations of the present LIPA system.

In particular embodiments, illumination of the photoactivatable polypeptide with light having a wavelength sufficient for photoactivation triggers accumulation (e.g., irreversible accumulation) of intracellular protein aggregates (e.g., insoluble aggregates) comprising the alpha-synuclein polypeptide or proteopathic polypeptide. In some embodiments, the LIPA system and methods described herein enable the intracellular protein aggregates to accumulate in real-time during following the illumination, thereby enabling spatiotemporal control of protein aggregation. In some embodiments, the intracellular protein aggregates accumulate rapidly, such as within 8 h, 7 h, 6 h, 5 h, 4 h, 3 h, 2 h, 1 h, 30 min, 15 min, 10 min, or 5 min following the illumination of the photoactivatable polypeptide.

In some embodiments, the intracellular protein aggregates described herein may be, at least in part, irreversible following cessation of the illumination. As used herein, the term "**irreversible**" refers to intracellular protein aggregates induced by the LIPA system described herein having the ability to remain relatively more stable following cessation of illumination, as compared to corresponding control cells lacking the alpha-synuclein or other proteopathic polypeptide and/or control cells comprising the photoactivatable peptide alone. In particular embodiments, the intracellular protein aggregates may remain stable for at least 5 h, 10 h, 15 h, 20 h, 25 h, 30 h, 35 h, 40 h, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 20 days, or 30 days post-illumination.

In some embodiments, the intracellular protein aggregates induced by the present LIPA system may comprise misfolded proteins (e.g., misfolded alpha-synuclein or other proteopathic polypeptides described herein) in beta-sheet conformation. In some embodiments, the intracellular protein aggregates induced by the present LIPA system may comprise the misfolded proteins adopting a conformation (e.g., a filamentous conformation) that may be rich in beta-sheets (e.g., having an increase in beta-sheets as compared to a corresponding non-pathogenic native alpha-synuclein polypeptide or other proteopathic polypeptide). In some embodiments, the intracellular protein aggregates induced by the present LIPA system may comprise misfolded proteins (e.g., misfolded alpha-synuclein or other proteopathic polypeptides described herein) having an increase in phosphorylation (e.g., serine phosphorylation) as compared to a corresponding non-pathogenic native alpha-synuclein polypeptide or other proteopathic polypeptide. In some embodiments, the intracellular protein aggregates induced by the present LIPA system may comprise misfolded proteins (e.g., misfolded alpha-synuclein or other proteopathic polypeptides described herein) having an increase in ubiquitination as compared to a corresponding non-pathogenic native alpha-synuclein polypeptide or other proteopathic polypeptide.

In some embodiments, the present LIPA system may comprise a cell expressing an alpha-synuclein polypeptide operably linked to the photoactivatable polypeptide, and wherein the intracellular protein aggregates comprise Lewy body-like aggregates. As used herein, "**Lewy body-like aggregates**" refer to protein aggregates comprising alpha-synuclein polypeptides which having properties resembling

those of Lewy bodies found in the brains of Parkinson's disease subjects. In some embodiments, the Lewy body-like aggregates described herein may be positive for: alpha-synuclein phosphorylated at Ser129, ubiquitination, staining with thioflavin S or another amyloid-specific dye, p62, HSP70, or any combination thereof.

In some embodiments, the photoactivatable polypeptide and/or the alpha-synuclein or other proteopathic polypeptide may be fused to a detectable marker (e.g., a fluorescent protein, a reporter enzyme, a transcription factor, a radioisotope binding protein, or a bioluminescent protein). In some embodiments, the fluorescent protein is a green fluorescent protein, a cyan fluorescent protein, a blue fluorescent protein, a yellow fluorescent protein, a red fluorescent protein (e.g., mCherry), or any combination thereof. As used herein in the context of florescent proteins, the terms "green", "cyan", "blue", "yellow", and "red" relate to proteins that fluoresce in that color spectra when excited.

In some embodiments, the LIPA system may comprise cells stably transfected or infected with, or genome-edited to comprise, one or more polynucleotides encoding the photoactivatable polypeptide and/or the alpha-synuclein polypeptide or other proteopathic polypeptide.

In some aspects, the present description relates to a method for inducing protein aggregation in a cell or animal, the method comprising providing a LIPA system as described herein, and illuminating the cell or animal with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering accumulation (e.g. irreversible accumulation) of the intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide.

In some aspects, the present description relates to a method for selecting a candidate compound for the control or treatment of a proteinopathy or neurodegenerative disease (e.g., a proteinopathy or neurodegenerative disease as described herein), the method comprising: (a) providing a LIPA system as described herein; (b) illuminating the cell, or animal comprising said cell, with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering accumulation of the intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide; (c) administering a compound of interest to the cell or animal; and (d) determining the effect of the compound of interest on the intracellular protein aggregates. In some embodiments, the method may further comprise: (e) selecting the compound of interest as a candidate compound when a decrease in the number and/or size of intracellular protein aggregates is observed.

In some embodiments, the compound of interest or candidate compound may be, or comprise, a small molecule or a biologic.

In some aspects, the present description relates to pharmacological, genetic, or biomarker-based approaches for treating a subject having developed or being susceptible to developing a proteinopathy (e.g., a proteinopathy described herein). In some embodiments, the method may comprise: (a) providing a

LIPA system as described herein, wherein the proteopathic polypeptide is selected based on the subject's proteinopathy; (b) illuminating the cell or animal with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering accumulation of the intracellular protein aggregates comprising the proteopathic polypeptide; (c) administering a compound of interest to the cell or animal comprising said cell; (d) determining the effect of the compound of interest on the intracellular protein aggregates; and (e) treating the subject with the compound of interest when a decrease in the intracellular protein aggregates is observed (i.e., in step (d)).

In some embodiments, the methods described herein may be an *in vitro*, *ex vivo*, or *in vivo* method.

In some aspects, the present description relates to an expression cassette comprising a polynucleotide encoding an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide. In some embodiments, the alpha-synuclein polypeptide or other proteopathic polypeptide may be fused to the proteopathic polypeptide, and/or to a detectable marker (e.g. a detectable marker as described herein). In some embodiments, the expression cassette may comprise an alpha-synuclein polypeptide or other proteopathic polypeptide placed under control of a constitutively active promoter, an inducible promoter, or a tissue-specific promoter (e.g., neuronal cell-specific promoter).

In some aspects, the present description relates to a vector comprising the expression cassette as described herein.

In some aspects, the present description relates to a cell comprising a genome-integrated expression cassette or vector as described herein. In some embodiments, the cell may be a neuronal cell, a brain cell, an induced pluripotent stem cell, a mammalian cell, a neuroblastoma, an immune cell, or a blood cell.

In some aspects, the present description relates to a transgenic animal model comprising the expression cassette, a vector, or a cell as described herein. In some embodiments, the animal may be a mouse, rat or other rodent, a non-human primate, fish, nematode, yeast, or a fly.

In some aspects, the present description relates to a protein aggregate comprising an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide. In some embodiments, (a) the protein aggregate is as defined herein; (b) the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide are comprised in a fusion protein; (c) the photoactivatable polypeptide and/or the alpha-synuclein or other proteopathic polypeptide are fused to a detectable marker or a detectable marker as defined herein; (d) the alpha-synuclein polypeptide or the proteopathic polypeptide is as defined herein; (e) the photoactivatable polypeptide is as defined herein; (f) at least one of the alpha-

synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide is heterologous with respect to the cell and/or to each other; (g) at least one of the alpha-synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide is under the control of a constitutively active promoter, an inducible promoter, or a tissue-specific promoter; (h) the protein aggregate is comprised in a neuronal cell, a brain cell, an induced pluripotent stem cell, a mammalian cell, a neuroblastoma, an immune cell, or a blood cell; (g) the protein aggregate is comprised in a cell in an animal or a transgenic animal engineered to express the alpha-synuclein polypeptide or other proteopathic polypeptide, and the photoactivatable polypeptide; (h) the protein aggregates comprise misfolded proteins in beta-sheet conformation; or (i) any combination of (a) to (h).

In some embodiments, the protein aggregate may comprise an alpha-synuclein polypeptide operably linked to the photoactivatable polypeptide, wherein the protein aggregate comprises a Lewy body-like aggregate. In some embodiments, the Lewy body-like aggregate is positive for: alpha-synuclein phosphorylated at Ser129, ubiquitination, staining with thioflavin S or another amyloid-specific dye, p62, HSP70, or any combination thereof.

In some embodiments, the protein aggregates as defined herein may be for use in a light-inducible intracellular protein aggregation system, in a method for inducing protein aggregation in a cell or animal, in a method for selecting a candidate compound for the treatment of a proteinopathy or neurodegenerative disease, or in the method for treating a subject (e.g., an animal or cell) having or susceptible for developing a proteinopathy.

In some embodiments, the present description relates to the surprising discovery that a variant of alpha-synuclein incapable of self-aggregating (e.g., lacking the NAC region) was able to preclude, prevent, or inhibit the self-aggregation of a polypeptide other than alpha-synuclein (e.g., CRY2). Thus, in some aspects, the present description relates to a variant alpha-synuclein polypeptide for use in preventing aggregation of a proteopathic polypeptide other than alpha-synuclein, or for use in treating a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide.

In some aspects, the present description also relates to the use of a variant alpha-synuclein polypeptide for the manufacture of a medicament for the treatment of a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of a proteopathic polypeptide associated with said proteinopathy. In some embodiments, the variant alpha-synuclein polypeptide referred to above is, comprises, or is from beta-synuclein.

In some aspects, the present description relates to an expression cassette encoding a variant alpha-synuclein polypeptide for use in preventing aggregation of a proteopathic polypeptide other than alpha-

synuclein, or for use in treating a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide. In some embodiments, the present description relates to the use of an expression cassette encoding a variant alpha-synuclein polypeptide in the manufacture of a medicament for the treatment of a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide. In some embodiments, the proteopathic polypeptide other than alpha-synuclein is, comprises, or is from: an Aβ precursor protein, an Aβ peptide, Tau, a prion, an Ig light chain, serum amyloid A, transthyretin, cystatin C, beta-2-microglobulin, apolipoprotein AI, apolipoprotein AII, gelsolin, amylin, calcitonin, atrial natriuretic factor, lysozyme, insulin, fibrinogen α-A chain, superoxide dismutase 1, huntingtin, an androgen receptor, an ataxin, a TATA box-binding protein, or TDP-43. In some embodiments, the proteinopathy other than an alpha-synucleinopathy is: a neurodegenerative disease, a proliferative disease, an inflammatory disease, a cardiovascular disease, diabetes, Parkinson's disease, dementia with Lewy bodies, diffuse Lewy body disease, multiple system atrophy, a tauopathy, Alzheimer's disease, Creutzfeldt-Jakob disease or other prion disease, spinocerebellar ataxias, spinal or bulbar muscular atrophy, amyotrophic lateral sclerosis, hereditary renal amyloidosis, medullary carcinoma of the thyroid, familial amyloid polyneuropathy, amyloidosis, or frontotemporal degeneration.

In some embodiments, the present description may relate to the use of genome-editing (e.g., a CRISPR-based genomic editing system) to generate variant alpha-synuclein polypeptide as defined herein in a subject (e.g., in the brain of a subject).

EXAMPLES

Example 1

Materials and methods

1.1 Construction of plasmids and production of recombinant adeno-associated 2/6 viral vectors

CRY2olig-mCherry plasmid was kindly provided by Dr. Chandra Tucker (Addgene plasmid #60032), pCDNA-human α-syn plasmid was kindly provided by Dr. Hilal Lashuel (EPFL, Switzerland), pCDNA-human α-syn$^{\Delta NAC}$, missing the NAC region (aa71-82) plasmid was kindly provided by Dr. Benoit Giasson (University of Florida, USA), and AAV-CMV-MCS plasmid was kindly provided by Dr. Bernard Schneider (EPFL, Switzerland).

To generate LIPA constructs, the cDNAs encoding human α-syn or human α-syn$^{\Delta NAC}$ were subcloned in CRY2olig-mCherry and verified by sequencing. To generate plasmids for the production of

adeno-associated viral vectors, the cDNA encoding LIPA-α-syn (α-syn-CRY2olig-mCherry) was subcloned in the AAV-CMV-MCS shuttle plasmid, using standard cloning procedures, and verified by sequencing.

The production of the recombinant pseudotyped AAV2/6 vectors (serotype 2 genome/serotype 6 capsid) and relative infectivity titers were performed by the Canadian Neurophotonics Platform (CERVO, Québec City). The final viral titers of AAV-LIPA-α-syn viral vector was $1x10^{13}$ genome copies (GC)/mL.

Plasmids used for adeno-associated viral production (AAV2) include pAAV-LIPA-Empty (**Fig. 39**; **SEQ ID NO: 1**), pAAV-LIPA-α-syn (**Fig. 40; SEQ ID NO: 2**), and pAAV-LIPA-αsynΔNAC (**Fig. 41; SEQ ID NO: 3**).

1.2 Cell culture and DNA transient transfection

Human HEK-293T cells were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% Fetal Bovine Serum (FBS, Sigma) and 1% penicillin/streptomycin (Gibco/Life technologies) at 37°C and 5% CO_2.

HEK-293T cells were transfected using calcium phosphate, FastFect™ transfection (Feldan, 9K-010-0001) or Lipofectamine™ 2000 (Thermo Fisher Scientific) according to a standard protocol and leading to more than 95% of transfection efficiency.

Plasmids used for mammalian expression include pmCherryN1-LIPA-Empty (**Fig. 42; SEQ ID NO: 4**), pmCherry-LIPA-α-syn (**Fig. 43; SEQ ID NO: 5**), and pmCherry-LIPA-α-synΔNAC (**Fig. 44; SEQ ID NO: 6**).

1.3 Live-cell imaging and 3D animation

HEK-293T cells were transfected at 30-50% confluence using Lipofectamine™ 2000. 24 h later, live imaging was performed making use of a Zeiss LSM 700 confocal microscope and the heating insert P Lab-Tek™ S1 systems. LIPA photoactivation was induced by laser illumination (488 nm) at 1% (15.5 µW). Time-lapse acquisitions were performed every 35 sec with photoactivation of a small ROI in the high magnification images (40X) or the whole field in the low magnification images (20X) with the 488 nm laser wavelength just prior to each frame recording. During the entire recording time, conditions were controlled and cells were maintained at 37°C, 5% CO_2.

Time lapse microscopy data was analysed using Imaris™ software version 7.6.1 (Bitplane, Zurich, Switzerland). Images were segmented using the isocontour feature in the Surpass module. Highly expressing mCherry-positive aggregates were detected based on the local contrast, their shape and their volume. Cell volume was detected as a low-intensity mCherry expression volume and used to normalise

the quantity of detected aggregates. Animation rendering was performed with the Imaris software and video were exported using QuickTime™ Pro 7 (Apple, Cupertino, CA).

1.4 Induction of protein aggregation, immunocytochemistry and cell quantification in culture

HEK-293T cells were seeded on 0.1% gelatin-coated glass coverslips in 24-well plates at a density of 10^4 cells per well (50% confluence) and transiently transfected, 24 h later, with 0.5 µg of LIPA-Empty, 0.6 µg LIPA-α-syn, and 1 µg LIPA-α-syn$^{\Delta NAC}$ per 4 wells, to obtain similar protein expression levels in all conditions. 24 h post-transfection, cells were exposed to blue light (λ=456 nm) using UHP-T-DI-LED series Ultra High-Power LEDs (Prizmatix), at an intensity of 0.8 mW/cm^2, as measured using LPM-100 light power meter (Amuza). Cells were collected at different time points and fixed in 4% paraformaldehyde for 15 min at room temperature (RT), then washed three times (5 min) with PBS and permeabilized with PBS containing 0.25% Triton X-100, for 30 min at RT. Cells were incubated in blocking buffer solution (PBS containing 5% BSA and 0.1% Triton X-100), for 1 h at RT. Coverslips were incubated with primary antibodies (see **Table 1**) diluted in blocking buffer solution for 2 h at RT. Coverslips were washed three times in blocking buffer solution (10 min) and appropriate Alexa Fluor® secondary antibodies (Life Technologies, see **Table 1**) were applied for 1 h at RT. Coverslips were washed twice with PBS (10 min) and counterstained with the fluorescent nuclear stain diluted in 4,6-diamidino-2-phenylindole (DAPI) (1:5000) for 5 min. Coverslips were then washed twice with PBS (10 min), mounted on Prolong® Gold Antifade (Molecular Probes), and imaged using a Zeiss LSM800 confocal microscope. For thioflavin S (ThS) staining, fixed and permeabilized cells were incubated with 0.05% ThS for 15 min, washed three times with 70% ethanol for 10 min, and then cells were directly counterstained with DAPI. All immunocytochemistry experiments were made with gentle rocking and protected from light.

To evaluate the number of mCherry-positive cells with aggregates, a total of 8-10 images per condition were collected using a fluorescence microscope (Nikon Eclipse 80i; 20X objective) and a total of 220-250 cells were quantified by two experimenters, in a blind manner, using ImageJ.

1.5 Semi-Denaturing Detergent-Agarose Gel Electrophoresis (SDD-AGE)

HEK-293T cells transiently overexpressing LIPA constructs were collected at different time points post-stimulation with blue light (0.8 mW/cm^2). Cells were lysed with a Dremel tissue homogenizer at low intensity for 10 sec (BioSpec Products, Inc., Bartlesville, OK) in lysis buffer (PBS-Tween 0.05% + Protease inhibitor, Phosphatase inhibitor II and Phosphatase inhibitor III [Sigma] and PMSF). Samples were centrifuged at 4°C (2000 x *g* for 10 min). Supernatant was collected and samples were treated at RT for 7 min in sample buffer (20% glycerol, 0.01% bromophenol blue, and 0.08% SDS). Samples were immediately electrophoresed through a 1.8% agarose gel prepared in buffer G (20 mM Tris and 0.2 M

glycine), containing 0.02% SDS. The SDD-AGE was run in buffer G containing 0.01% SDS at 120 V until the dye reached the end of the gel. The gels were then transferred to nitrocellulose membranes using the Trans-Blot® Turbo™ Transfer (Bio-Rad). The membranes were then dried for 1 h at RT then incubated in Odyssey blocking buffer (LI-COR Biosciences) at RT for 1 h prior to overnight incubation with primary antibodies in the blocking solution. Membranes were then washed 3 times with PBS-Tween 0.1% (PBST) (10 min), and incubated with the appropriate secondary antibodies; either IRDye® 680RD-conjugated or IRDye® 800W-conjugated secondary antibodies (LI-COR Biosciences). Membranes were washed 3 times with PBS-T (10 min). Visualisation and quantification were carried out with the LI-COR Odyssey scanner and software (LI-COR Biosciences). Blots were imaged using the Odyssey Infrared Imaging System Scan resolution of the instrument ranges from 21 to 339 μm, and in this study membrane blots were imaged at 169 μm. Quantification was performed on single channels with the analysis software provided with the LI-COR imaging system. Molecular weight markers were not used because nondenatured protein aggregates were being analyzed. At least three independent experiments were analysed for SDD-AGE. The data is presented the means ± s.e.m.

1.6 Filter retardation blotting assay

Cells were processed similarly to SDD-AGE, except that after cell lysis and centrifugation, the samples were diluted with lysis buffer containing 1% SDS, and samples were incubated at RT for 10 min. The vacuum manifold (Core LifeScience) was prepared by using thin filter paper pre-soaked in water and placed on the manifold. A cellulose acetate membrane (pore size 0.2 μm; SterliTech) was soaked in PBS containing 1% SDS and placed on top of the filter paper on the manifold. The manifold was tightly closed and samples were loaded in triplicates into the wells. Then, the samples then were filtered through the membrane by applying a vacuum. After filtration, the membrane was washed 2 times with PBS containing 0.1% SDS. The acetate membrane was then used for immunoblotting as described herein. At least three independent experiments were analysed for retardation assays. The data is presented as mean ± s.e.m.

1.7 Pull-down assay

Whole cell lysates from HEK-293T cells overexpressing LIPA constructs and exposed to blue light for 12 h, were extracted in lysis buffer containing (PBS-Tween 0.05% + protease inhibitor, phosphatase inhibitor II, Phosphatase inhibitor III [Sigma] and PMSF). Samples were then lysed with a Dremel tissue homogenizer, at low intensity for 10 sec (BioSpec Products, Inc., Bartlesville, OK). Samples were sequentially centrifuged at 4°C (500 x *g* for 5 min, and 1000 x *g* for 10 min). The supernatant was collected and 10% of the volume was kept as input sample and diluted in 4X Laemmli lysis buffer. The remaining supernatant incubated with 5 μg of anti-mCherry antibody for 30 min at 4°C

with rotation. During this incubation time, Dynabeads Protein G (Life technologies) were resuspended by tilting the vial several times for proper mixing of the beads with the solution. A volume of 70 µL (per 300 µL sample) of Dynabeads was transferred to clean microcentrifuge tubes, equilibrated 3 times (10 min) in 300 µL of PBS + 0.05% Tween + protease inhibitors. Dynabeads were then resuspended with the antibody-sample mixture, and this mix was then incubated with rotation at 4°C for 2 h. After incubation, tubes were placed on the magnet and the supernatant was discarded. Beads were then washed with the PBS + 0.05% Tween + Protease inhibitors mixture 3 times (10 min) at 4°C with rotation, removing the supernatant between washes. At the last wash, samples were transferred to a new clean microcentrifuge tube to avoid elution of proteins bound to the tube wall. Dynabeads were then eluted by resuspending beads in 50 µL of 2X Laemmli lysis buffer. Pull-down and input samples were heated at 95°C for 10 min and samples were then subjected to Western blot analysis. At least three independent experiments were analysed for Pull-down assays.

1.8 Animals

Three-month-old C57/BL6 mice were obtained from Charles River Laboratories and remained seven days in habituation period, from their arrival to the CHUL of Québec City animal facility, before any handling or experiment. Mice were housed with a 12h light/dark cycle and had food and water *ad libitum*. All animal experiments were approved by the Animal Welfare Committee of Université Laval, in accordance with the Canadian Council on Animal Care policy.

1.9 Transmission electron microscopy

HEK-293T cells overexpressing LIPA-Empty and LIPA-α-syn and exposed to blue light were fixed in 0.5 mL of 3.5% acrolein and 4% paraformaldehyde (PFA) in 0.05 M phosphate-buffered saline (PBS; pH 7.4), overnight at 4°C. Then, cells were washed three times in PBS to remove excess fixative. Cell pellets were mixed gently with 125 µL of 4% agarose, kept at 4°C until solid, and then cut into 50 µm sections using a Leica VT1000S vibratome (Leica Biosystems, Concord, ON, Canada). Sections were washed 3 times in PBS for 10 min and were incubated in 1.5% potassium ferrocyanide and 2% aqueous osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 1 hour at RT. After 5 washes with ddH_2O for 3 minutes, sections were incubated 20 min in a fresh solution of thiocarbohydrazide (1% w/v) at room temperature then washed again 5 times with ddH_2O for 3 minutes, incubated 30 min in 2% aqueous osmium tetroxide, and washed 5 times with ddH_2O for 3 minutes. Sections were dehydrated using sequential alcohol baths followed by propylene oxide and embedded in Durcupa™ resin, infiltrated between ACLAR sheets overnight at room temperature, then polymerized in the oven at 55°C for 3 days. Ultrathin sections (~65 nm) were generated using a Leica UC7 ultramicrotome. Images of 12-13 cells per experimental condition

were randomly acquired at 9300X using a FEI Tecnai Spirit G2 transmission electron microscope (Thermo Fisher Scientific Company, Hillsboro, OR) operating at 80kV and equipped with a Hamamatsu ORCA-HR digital camera (10 MP).

1.10 Stereotaxic injections of AAV viral particles and implantation of wireless optogenetic devices

Three-month-old C57/BL6 mice were obtained from Charles River Laboratories and remained seven days in habituation period before any handling. Mice were housed with a 12 h light/dark cycle and had food and water *ad libitum*. All animal experiments were approved by the Animal Welfare Committee of Université Laval, in accordance with the Canadian Council on Animal Care policy.

Mice were anesthetized with 2% isoflurane-O_2 and placed in a stereotaxic frame (David Kopf Instruments). The top of the skull was incised with a scalpel and tissues were cleared to visualize the interaural point and the bregma. After piercing the skull with a drill, mice received a unilateral injection of 2 µL of viral suspension at the rate of 0.2 µL/minute, which corresponds to a total viral load of $2x10^{10}$ GC, using automatic pumps (David Kopf Instruments). Injections were performed in the substantia nigra and in the neostriatum according the following coordinates, respectively: (-3.08 mm posterior, -1.5 mm lateral, -4.25 mm ventral) and (+0.2 mm posterior, -2 mm lateral, -3 mm ventral). Injections were made using a 10-µL syringe (Hamilton) and 30-gauge needle. Needle was placed to injection site 3 minutes before starting the injection and left for additional 5 min post-injection before it was slowly withdrawn. During the same surgery session, mice were implanted with wireless optogenetic devices (Eicom, USA) in the SNc or the neostriatum (Jeong et al., 2015), using the following coordinates, respectively: (-3.08 mm posterior, -1.5 mm lateral, -4.20 mm ventral) and (+0.2 mm posterior, -2 mm lateral, -3 mm ventral).

1.11 Immunohistochemistry/immunofluorescence

Animals were sacrificed by ketamine/xylazine overdose (100 mg/mL, injected 0.1 mL/20 g mice) and brains were removed after transcardial perfusion with PBS then 4% PFA-PBS (50 mL each). Brains were post-fixed 4 h in 10 mL of 4% PFA, then incubated in 25% sucrose-PBS for 24 h. After post-fixation, brains were cut in coronal sections (30 µm thick) with a microtome (SM2000R; Leica) and slices were stored at -20 °C in cryoprotection medium.

For immunohistochemistry, slices were washed twice beforehand with PBS (10 min) and then incubated for 1 h in blocking buffer (Bb : 3% bovine serum albumin [BSA], 0.1% Triton™ X- 100-PBS). After blocking, slices were incubated overnight at 4°C with primary antibody in Bb: anti-tyrosine hydroxylase (TH; 1:1000; AB152 and AB318; Millipore), anti-human α-syn (1:1000; Syn1; BD Laboratories), anti-α-syn S129 phosphorylated (1:2000; pSyn ; Wako), anti-ubiquitin (1:1000; Ubi; Dako),

anti-p62 (1:1000; SQSTM1; Santa Cruz Biotechnology) or anti-HSP70 (1:1000; HSP 70/HSC 70 [H-300]; Santa Cruz Biotechnology). For immunofluorescence, slices were washed three times with Bb (10 min) and then incubated for 2 h at RT, with appropriate secondary antibodies conjugated to Alexa™ Fluor-488 or Alexa™ Fluor-633 (Invitrogen; diluted 1:1,000 in PBS containing 0.1% Triton™ X-100). After incubation with the secondary antibodies, slices were washed three times with PBS containing 0.1% Triton™ X-100, then incubated in DAPI-PBS (1:5000 for 7 min), and finally washed twice in PBS. For microscopic observations, slices were mounted on Superfrost™ Plus microscope slides (Fisherbrand) in aqueous mounting media (Fluoromount-G T, EMS) and allowed to dry in the dark for at least 2 days.

For enzymatic revelation, slices were incubated for 2h at room temperature with biotinylated secondary antibody (1:200; Vector Laboratories), before exposure to 300 µL of 3,3'-diaminobenzidine tetrahydrochloride (DAB, Pierce) for 1 min, and subsequently counterstained with Nissl stain. Slides were then mounted on glass coverslips.

For the quantification of TH+ cell numbers in SN, slices were washed twice beforehand with PBS (10 min), then incubated at RT for 15 minutes in 0.1% H_2O_2-PBS. After 15 minutes, slices were washed three times with PBS (10 min), and then incubated at RT for 1 h in Bb. After blocking, slices were incubated overnight at 4°C with primary antibody in Bb (see immunofluorescence above). Subsequently, slices were washed three times with Bb (10 min) and then incubated at RT for 2 h with appropriate biotinylated secondary antibody (1:500; Vector Laboratories) for DAB revelation reagent (Pierce). After incubation with secondary antibodies, slices were washed three times with PBS and then incubated with the VECTASTAIN™ ABC kit (PK-6100; Vector Laboratories) for 1 h. After this incubation, slices were washed twice with PBS and then once with PBS containing 0.05 M Tris. Subsequently, slices were incubated in DAB solution (one DAB tablet in 20 mL of Tris 0.05 M , pH 7.6) about 1 min, until the appearance of staining, then slices were washed three times with PBS. For stereology experiments, slices were mounted on Superfrost™ Plus microscope slides (Fisherbrand), dried, and finally mounted in DTX Mounting Medium (13512, EMS) after dehydration of slices with 90% and 100% ethanol and immersion in CitriSolv™ solution (DECON).

1.12 Behavioural tests

All behavioural tests were completed during the light phase of the light-dark cycle, *i. e.* between 8 am and 4 pm. Mice were habituated to the experimenter and the testing room for several days before starting the tests. The experimenter was blind to viral vector treatment during testing and blind to both vector group and illumination group during scoring and all group assignments were randomized.

The cylinder test: The cylinder test was performed to evaluate the motor impairment induced after a dopaminergic neuronal loss, by quantifying the deficits in using the contralateral forelimb

(akinesia; Schallert et al., 2000; Oueslati et al., 2013; Oueslati et al., 2015). Briefly, mice were placed in a transparent Plexiglas cylinder (15 cm diameter, 12 cm high) surrounded by a mirror to monitor the mouse from all directions and videotaped using a camera (Microsoft LifeCam Cinema; H5D-00018). A total number of 30 forepaw contacts made on the cylinder wall by the ipsilateral or the contralateral (impaired) forelimbs were scored and the results were expressed as the ratio of contralateral contacts relative to the total contacts made by both forelimbs. Analysis was performed in a blinded fashion.

The Grip Strength test: Grip Strength (CHATILLON® DFE Series, USA) was used to measure the muscle strength of forelimbs as previously described (Meyer et al., 1979). During testing, the mouse is placed horizontally on the grid to allow gripping of the grid with the forepaws while being supported by the tail. Once the grid is gripped, the mouse is pulled back until the grip on the grid is released and then the value on the apparatus is recorded. Mice underwent three trials per testing session and analysis was performed on the average for the three trials.

The rotarod test: The rotarod test was used to measure motor coordination, endurance, and balance (Rozas et al., 1997). All mice were pre-trained on the rotarod (LE 8200, Panlab Harvard apparatus, USA) at baseline to reach a stable performance. At testing, mice were placed on the rotarod for three consecutive 3 min trials, at fixed speed (12 and 8 RPM) and accelerating speed (4-40 RPM in 2 minutes). Mice rested for 1 min between each trial at each speed. The latency to fall was recorded for each trial and the mean value from each speed and was used for analysis.

The gait test: To test for gait abnormalities, footprint gait analysis was performed as previously described (Fernagut et al., 2002) with some modifications. Briefly, the hind- and forefeet of the mice were painted with blue (right paws) and orange (left paws) nontoxic paint (Liquid tempera, SCHOLA) immediately prior to placement in a 45 cm long and 15 cm wide runway coated in newsprint paper. Stride length was measured as the distance of forward movement between paw prints for. The mean value of each set of three values measuring stride length was used in the analysis.

1.13 Unbiased stereological estimation of dopaminergic neurons in the SNc

Dopaminergic neuronal number was estimated using unbiased stereology, according to the optical fractionator principle described by West and colleagues (West et al., 1991). Briefly, the number of TH-immunoreactive neurons and Nissl-positive cells were determined every fourth coronal section (1/4) covering the entire SNc structure. The SNc was delineated at low magnification (20X) and then the dopaminergic neurons were counted under an oil immersion objective (60X). DA neurons were counted in a blinded fashion and the results are expressed as the mean ± standard error of the total number of TH+ neurons in the injected side. Analysis was performed using the MBF Stereo Investigator software (MBF Bioscience). The parameters used for the stereological analysis were as follows: grid size, 150 x 150 μm;

counting frame, 75 x 75 μm; and 2 μm guard zones. Tissue thickness was determined at each counting field. The coefficient of error was < 0.1.

1.14 Statistical analysis

All cell-based assays were performed in at least three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. For analysis of behavioural data, statistics were performed using two-way ANOVAs followed by Tukey's post-hoc tests. Across time data was analyzed with a linear mixed-effects model of viral vector and implant. Within-subjects variance was controlled for by including random effects of intercept and slope for each mouse. The model was estimated using maximum likelihood and contrast comparisons were performed to determine the effect of viral vector and implants at each time point. Analyses were performed using RStudio™ version 3.4.1 with nlme version 3.1-131. All values were expressed as the means ± s.e.m. and the software used for the statistical analysis was Prism v.6 (GraphPad, La Jolla, CA, USA).

Table 1. Antibodies used in this study

Antigen	Antibody name/catalog number	Epitope	Source
α-syn	Syn1 / 610787	15-123	BD
Human α-syn	α-synuclein (211) / sc-12767	121-125	Santa Cruz Biotechnology
α/β/γ-synuclein	Syn FL-140 / sc-10717		Santa Cruz Biotechnology
pS129 α-Syn	Wako / pSyn #64	124-134	Wako
Ubiquitin	Ubiquitin / Z0458		Dako
P62	SQSTM1(D-3) / sc-28359	151-440	Santa Cruz Biotechnology
HSP70	HSP 70/HSC 70 (H-300) sc-33575	342-641	Santa Cruz Biotechnology
mCherry	mCherry / ab167453		Abcam
GFP	GFP Antibody (4B10B2) [HRP] / NBP2-22111H		Novus Biologicals
Beta-actin	β-actin / G043		abm (Applied Biological Materials)
Secondary Antibodies			
	680RD-conjugated goat anti-rabbit		LI-COR Biosciences
	800RD-conjugated goat anti-rabbit		LI-COR Biosciences
	680RD-conjugated goat anti-mouse		LI-COR Biosciences
	800RD-conjugated goat anti-mouse		LI-COR Biosciences

Example 2:

Engineering of light-inducible protein aggregation (LIPA) system

Protein aggregation is a process by which misfolded proteins adopt an organized and structurally well-defined fibrillar conformation, leading to the formation of proteinaceous amyloid deposits. This process occurs via a nucleation-propagation polymerization mechanism, also called "seeding effect", whereby small oligomers, or seeds, provide a template for the assembly of soluble monomer proteins into highly ordered protein aggregates defined by their insolubility and β-sheet structure (Oueslati et al., 2014). One example is the accumulation of proteinaceous intraneuronal inclusions, called Lewy bodies (LB), in the brain of patients suffering from alpha-synucleinopathies (Knowles et al., 2014; Lashuel et al., 2013), a group of neurological disorders which encompasses Parkinson's disease (PD), PD with dementia (PDD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA) (Fares et al., 2016; McCann et al., 2014). These inclusions are predominantly composed of aggregated alpha-synuclein (α-syn), a small protein ubiquitously and abundantly expressed in the brain (Lashuel et al., 2013; Spillantini et al., 1997).

Since their initial description more than a century ago (Goedert et al., 2013), a causal link between LB formation and neurotoxicity in PD and related disorders has been suggested. However, the exact role of LB in the pathogenesis and progression of PD and how these α-syn-rich inclusions precipitate neuronal death remains hypothetical. This in part due to the fact that current cellular and animal models of PD and α-syn overexpression do not allow to monitor the α-syn aggregation process in living cells and do not result in the formation of intraneuronal inclusions that more closely resemble actual LB observed in the brains of PD-disease patients. Accordingly, there is need for improved cellular and animal models enabling the study of protein aggregation in living cells, particularly models in which the protein aggregates more closely resemble those found *in vivo* in patients suffering from proteinopathies.

In the present study, a versatile system employing optogenetic methodologies was engineered to permit the spatiotemporal control of protein aggregation (e.g., α-syn aggregation and LB formation) in living cells *in vitro* and *in vivo*. This system, herein referred to as light-inducible protein aggregation (LIPA), is based on the use of engineered photoreceptors that change their conformation in response to light stimuli, and thereby influence the functional output of proteins when fused together, notably the induction of protein clustering. More specifically, the approach is based on the use of a mutant form of the *Arabidopsis thaliana* photoreceptor cryptochrome 2 (CRY2), also referred to as CRY2olig (Taslimi et al., 2014). When stimulated with blue light, mutant CRY2 undergoes rapid, reversible, and robust protein clustering.

A construct was engineered in which the human α-syn gene was fused to the CRY2 and mCherry (LIPA-α-syn) coding regions (**Fig. 1**). The constructs expressing CRY2-mCherry (LIPA-Empty) and CRY2-mCherry fused to a non-aggregatable form of α-syn missing the NAC region (residues 71 to 82) (Giasson et al., 2001) referred to herein as LIPA-α-syn$^{\Delta NAC}$, were used as controls (**Fig. 1**).

Live imaging analysis revealed that cells overexpressing LIPA-Empty and LIPA-α-syn, but not those expressing LIPA-α-syn$^{\Delta NAC}$, undergo a rapid and robust formation of protein inclusions following exposure to laser illumination (488 nm; 15.5 μW), suggesting that the presence of non-aggregatable form of α-syn precludes light-induced LIPA clustering (**Figs. 2 and 3**). Remarkably, the presence of α-syn enhanced LIPA aggregation and LIPA-α-syn inclusions appeared within seconds of a light pulse, compared to LIPA-Empty inclusions which occurred 10-12 min after light stimulation, suggesting the active role of α-syn in prompting CRY2 light-induced responses (**Figs. 2 and 3**). Furthermore, the number of aggregates per cell volume, as well as the volume of aggregates *per se*, increased dramatically in cells overexpressing LIPA-α-syn, compared to the LIPA-Empty condition, suggesting that the presence of α-syn may prompt CRY2 light-induced responses.

Moreover, the system allowed focal induction of LIPA-α-syn aggregation within the cytoplasm of a single cell, reflecting the high spatial resolution of the LIPA system (**Figs. 4 and 5**). Local light stimulation of LIPA-α-syn in the cytosol of HEK-293T cells was induced and live imaging analysis showed the formation of LIPA-α-syn inclusions exclusively under the stimulated region of interest (ROI) (**Figs. 4 and 5**). These inclusions increased in number and volume over time and spread from the stimulation site to the rest of the cytosol.

Example 3:

LIPA system induces long-term protein inclusions upon prolonged light stimulation

In pathological conditions, α-syn aggregates and LBs are present in the brain for long periods of time. Consequently, the long-term LIPA system-induced aggregation in HEK-293T cells was monitored by assessing the proportion of mCherry-positive cells with aggregates at different time points over 24 h of illumination. Cells were stimulated with blue LED light at the intensity of 0.8 mW/cm^2, a condition that was not observed to induce cell toxicity (**Fig. 6**).

Quantification revealed that the proportion of mCherry-positive cells overexpressing LIPA-Empty and exhibiting intracellular inclusions increased with time upon light stimulation and reached a maximum of 50% of cells with aggregates after 8 h of illumination, which remained stable (plateau) for at least 24 h (**Figs. 7 and 8**). Notably, cells overexpressing the LIPA-α-syn construct exhibited a more rapid formation of mCherry inclusions, as the number of cells positive for LIPA inclusions reached 60% in less

than 30 min, plateauing (80%) after 6 h, where they remained stable after 24 h of light stimulation (**Figs. 7 and 8**). Cells overexpressing LIPA-α-syn$^{\Delta NAC}$ did not exhibit apparent inclusions, despite prolonged light stimulation. These results suggest that the non-aggregatable form of α-syn either precludes the LIPA aggregation process or induces small and discrete inclusions undetectable (e.g., by confocal microscopy) (**Figs. 7 and 8**).

Example 4:

α-syn dictates morphology of LIPA system-induced protein inclusions

A closer look at the morphology of the inclusions revealed that LIPA-α-syn aggregates are relatively round-shaped, whereas LIPA-Empty inclusions adopted a needle-like shape, suggesting that the presence of α-syn dictates morphological aspects of the inclusions (**Fig. 9**).

At the biochemical level, filter retardation assay confirmed that light stimulation of LIPA-Empty and LIPA-α-syn resulted in an apparent time-dependent accumulation of insoluble aggregates (**Figs. 10 and 11**). This observation was corroborated using semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) (**Figs. 12 and 13**).

Example 5:

LIPA system generates stable α-syn aggregates after transient light stimulation

The presence of α-syn fused to the LIPA system was associated with a dramatic increase in aggregation rate and a distinctive inclusion morphology (see **Examples 2-4**), suggesting that α-syn may play a leading role in driving protein aggregation. Given that light-induced aggregation of LIPA-Empty is reversible (with inclusions rapidly dissociating in the dark; Taslimi et al., 2014), the experiments described below were performed to investigate whether α-syn fused to CRY2 could maintain protein aggregation and inclusion formation after the cessation of light stimulation.

The stability of LIPA-α-syn inclusions in the absence of light stimulation was evaluated by assessing the proportion of mCherry-positive cells having inclusions. LIPA aggregates were allowed to form over a 12 h period under light stimulation and cells were collected at different time points up to 48 h post-illumination (**Fig. 14**).

In accordance with previous observations (Taslimi et al., 2014), the percentage of cells with LIPA-Empty aggregates dropped consistently, and virtually no cells exhibited aggregates 48 h post-light exposure (**Fig. 15**). However, the percentage of mCherry-positive cells with LIPA-α-syn aggregates only

partially decreased (from 80% to 50%) 3 h following light stimulation, and then stabilized, reaching a plateau of 40% between 6 h and 48 h (**Fig. 15**).

These results suggest that the presence of α-syn is associated with the lingering LIPA-α-syn aggregates once light-stimulation has ceased, implying that a transient exposure to the blue light is sufficient to trigger the formation of stable and self-perpetuating α-syn aggregates.

Example 6:

Inhibitors of α-syn aggregation reduce the proportion of cells with LIPA-α-syn inclusions

The impact of treatment with potent small molecule inhibitors of α-syn aggregation, namely baicalein and myrecitin (Masuda et al., 2006; Zhu et al., 2004), on the stability of LIPA-α-syn inclusions was next evaluated. Quantification revealed a 50% reduction in the number of cells exhibiting stable LIPA-α-syn inclusions, demonstrating that inhibition of α-syn aggregation reduces inclusion stability (**Fig. 16**). Treatment with baicalein or myrecitin had no effect on LIPA-Empty dissociation, confirming the specificity of these inhibitors (**Fig. 17**). Moreover, treatment with inactive small molecules, naringenin and daidzein (Masuda et al., 2006), did not affect the proportion of cells with mCherry inclusions (**Fig. 18**), suggesting a role of α-syn in maintaining LIPA inclusions in the dark.

Previous studies have reported that both β-synuclein (β-syn) and mouse α-syn (mα-syn) overexpression inhibit human α-syn aggregation in cell culture and animal models. To test the impact of these two proteins on LIPA-α-syn inclusion formation, LIPA-α-syn was overexpressed with β-syn or mα-syn in HEK-293T cells, then transiently exposed to blue light for 12 h. Post-stimulation analysis showed a significant reduction of the proportion of cells exhibiting mCherry aggregates in the presence of β-syn or mα-syn (**Fig. 19**) for LIPA-α-syn inclusions, but not for LIPA-empty inclusions (**Fig. 20**) or when LIPA-α-syn was overexpressed with GFP (**Fig. 21**).

Collectively, the above results show that treatment with pharmacological or genetic inhibitors of α-syn aggregation results in a reduction in the proportion of cells with LIPA-α-syn inclusions, further suggesting a central role of α-syn in maintaining aggregation self-perpetuation.

Example 7:

LIPA system initiates self-perpetuating α-syn aggregates after transient light stimulation

The maintenance and the auto-perpetuation of α-syn aggregates depends on self-amplification through the recruitment of soluble proteins, a process known as seeding (Oueslati et al., 2014). To investigate if LIPA-α-syn is able to recruit and seed the aggregation of endogenous monomeric α-syn, a

pull-down assay was performed for LIPA-α-syn inclusions overexpressed in the presence of monomeric α-syn-GFP.

Western blot analysis revealed the presence of an α-syn-GFP band that co-immunoprecipitated with LIPA-α-syn aggregates under the illuminated condition (+light), in contrast to the weak α-syn-GFP signal detected in the non-illuminated condition (-light; **Fig. 22**), suggesting that LIPA-α-syn aggregates can recruit monomeric α-syn-GFP. No α-syn-GFP signal was observed after LIPA-Empty pull-down (+/-light), suggesting that the seeding effect is specific to LIPA-α-syn (**Fig. 22**). Moreover, immunocytochemistry analysis confirmed the seeding effect of LIPA-α-syn aggregates and revealed co-localization of LIPA-α-syn and α-syn-GFP signals, where LIPA-α-syn forms the core of the aggregates surrounded by recruited α-syn-GFP monomers (**Fig. 23**).

Additionally, the seeding capacity of LIPA-α-syn was confirmed in living cells using exogenous synthetic α-syn preformed fibrils (Pffs) tagged with Alexa™ 488 and assimilated by cells overexpressing LIPA-α-syn. Twelve hours after light exposure, co-localization of LIPA-α-syn aggregates with α-syn Pffs could be observed (**Fig. 24**), demonstrating that light-induced α-syn aggregates recruited synthetic α-syn fibrils.

Example 8:

LIPA-α-syn-induced inclusions exhibit features of authentic Lewy bodies in cell culture

It was next investigated whether LIPA-α-syn inclusions reproduced key features of authentic LBs as observed post-mortem in α-synucleinopathy-diseased brains (Shults et al., 2006). Immunostaining of HEK-293T cells overexpressing LIPA-α-syn and exposed to light for 12 h revealed a complete co-localization of mCherry and α-syn signals within the LIPA-α-syn inclusions, demonstrating that α-syn is the main constituent of these deposits (data not shown). Moreover, LIPA-α-syn inclusions were positive for phosphorylated α-syn at Ser129 (pS129; **Fig. 25**) and ubiquitin (**Fig. 26**), two neuropathological hallmarks of authentic LBs. Staining with thioflavin S (ThS), an amyloid-specific dye, revealed that LIPA-α-syn inclusions contained β-sheets (**Fig. 27**), a structural characteristic of authentic LBs. Finally, LIPA-α-syn inclusions were also positive for p62 (**Fig. 28**) and HSP70 (**Fig. 29**), two chaperone proteins commonly observed within LBs in α-synucleinopathy-diseased brains. Neither inclusions nor LB-associated markers were observed in non-illuminated cells (data not shown).

The ultrastructure of LIPA aggregates was examined by transmission electron microscopy (TEM) analysis which revealed that LIPA-Empty aggregates appear as small electron dense inclusions, whereas LIPA-α-syn inclusions exhibit larger electron-dense core inclusions (**Fig. 30**). TEM analyses also revealed the presence of different organelles exclusively within the LIPA-α-syn aggregates

(mitochondria, endoplasmic reticulum) suggesting that these aggregates interact with and sequester intracellular organelles (**Fig. 31**).

Example 9:

LIPA-α-syn-induced inclusions exhibit features of authentic Lewy bodies *in vivo*

The ability of LIPA-α-syn system to induce LB-like inclusion formation *in vivo* was evaluated. An adeno-associated virus (AAV) gene delivery system was used to overexpress LIPA-α-syn directly in the *substantia nigra* of wild-type mice. Fifteen days post-injection, intracerebral light stimulation was performed using an implantable wireless optogenetic device (Eicom), for 30 min every other day, for a period of 7 days (**Fig. 5B**), using non-toxic illumination parameters that did not induce any cell loss (data not shown). Fifteen days post-injection, intracerebral light stimulation was performed with an implantable wireless optofluidic device (Eicom; Jeong et al., 2015), for 30 min every other day, for a period of 7 days (**Fig. 32**).

Post mortem-analysis revealed the presence of mCherry-positive inclusions within striatal neurons after light stimulation, whereas the brains of non-stimulated animals were completely devoid of such inclusions. Immunohistochemistry using anti-α-syn antibodies (Syn1 and FL140) demonstrated that α-syn is a major constituent of these aggregates (**Figs. 33 and 34**). LIPA-α-syn inclusions were phosphorylated at the residue Ser129 (**Fig. 35**), ubiquitinated (**Fig. 36**), and exhibited β-pleated sheet content that was positive for ThS staining (**Fig. 37**). Finally, LIPA-α-syn inclusions were positive for p62 (**Fig. 38**).

To verify the possibility of inducing LBs-like inclusions in other brain regions using the present LIPA system, LIPA-α-syn was overexpressed in midbrain neurons and protein aggregation was stimulated with the same parameters used for the striatal stimulation. Immunohistochemistry analysis revealed the formation of LIPA-α-syn inclusions that exhibited many of the principal features of authentic LBs in midbrain neurons, including phosphorylation at residue Ser129, ubiquitination, the presence of β-pleated sheets positive for ThS staining, and LIPA-α-syn inclusion positive for p62 (data not shown).

Example 10:

LIPA-induced α-syn inclusions precipitate dopaminergic neuronal loss and induce parkinsonian-like motor impairment *in vivo*

The behavioral and cellular effects of long-term induction of LIPA-α-syn aggregation in the midbrain of mice overexpressing LIPA constructs was evaluated to determine whether α-syn aggregation and LBs-like formation could impact the integrity of dopaminergic (DA) neurons. LIPA constructs were

delivered in the substantia nigra using AAV gene delivery system. Two weeks post-injection, α-syn aggregation was stimulated for 1 h every other day, during 8 weeks, using a wireless implantable optogenetic device (**Fig. 45**). LIPA constructs were overexpressed at non-toxic levels, to specifically assess the toxicity induced by α-syn aggregation and to rule out any DA toxicity caused by overexpression of the constructs per se.

A battery of motor tests revealed that overexpression of the three LIPA constructs, in absence of light stimulation, did not induce any motor impairment (**Fig. 46-49**). Interestingly, when aggregation was induced by the blue light, only animals overexpressing LIPA-α-syn showed behavioral impairment, including a significant asymmetry in the use of contralateral forepaw in the cylinder test (**Fig. 46**), a significant reduction of grip strength test (**Fig. 47**) and a problem of coordination, reflected by the reduction of the latency time on the rotarod (**Fig. 48**) and gait abnormalities (**Fig. 49**).

At the cellular level, the presence of protein aggregates was confirmed in the midbrain of mice overexpressing LIPA-empty and LIPA-α-syn, but not in LIPA-α-syn$^{\Delta NAC}$ (**Fig. 50**). Moreover, only DA neurons overexpressing LIPA-α-syn exhibited pathogenic pS129-α-syn aggregates (**Fig. 50**). Unbiased stereological quantification revealed that overexpression of LIPA constructs did not affect DA neuronal viability (**Figs. 51 and 52**). However, after optogenetic stimulation, only mice overexpressing LIPA-α-syn exhibited a significant loss of the DA neurons, compared to non-stimulated LIPA-α-syn and to stimulated LIPA-Empty and LIPA-α-syn$^{\Delta NAC}$ (**Figs. 51 and 52**). In LIPA-α-syn illuminated condition, Nissl positive midbrain neuronal quantification confirmed that TH-positive neuronal loss was indeed due to neurodegeneration rather than a loss of the DA phenotype (**Fig. 53**).

Collectively, these results suggest that the induction of LIPA-α-syn aggregation precipitates the DA neuronal loss and induces parkinsonian-like motor impairment.

Example 11:

LIPA-α-syn co-aggregates with mutant Huntingtin and promotes its propagation in the central nervous system

It was next investigated whether if LIPA-α-syn can co-aggregate with the pathological form of the protein Htt (mHtt) (harboring 75 poly-glutamine [poly-Q] repetition) and propagate in the mouse brain in a prion-like manner. This experiment aimed at studying the potential role α-syn in the pathogenesis of different proteinopathies or neurodegenerative diseases by potentiating the aggregation and the toxicity of different proteins implicated in these disorders, such as huntingtin (Htt) in Huntington's disease.

Briefly, LIPA-α-syn-mCherry and mHtt-GFP were co-overexpressed in HEK-293T cells and exposed to the blue light for 24h, to allow protein co-aggregation. Light-induced aggregates were then extracted as described by Sanders and colleagues (Sanders et al., 2014) and injected stereotaxically in the striatum of 3-month-old wild type C57/Bl6 mice (n=3). One-month post-injection, animals were sacrificed and the expression of LIPA-α-syn and mHtt-GFP were analyzed in the brain.

Fig. 54A shows that LIPA-α-syn (red; mCherry) and mHtt-GFP (Green; Alexa 488) aggregates are present in the injected striatum, one-month post-injection. Moreover, high resolution confocal imaging revealed that the two proteins co-localize within the majority of the striatal neurons, demonstrating that the two proteins co-aggregate *in vivo* and the aggregates are stable for at least one month *in vivo* (**Fig. 54B**).

Analysis of other brain regions revealed the presence of LIPA-α-syn and mHtt-GFP co-aggregates in the *globus pallidus* (**Fig. 54C and 54D**) and the entopendocular nucleus (**Fig. 54E and 54F**), two brain nuclei synaptically connected to the striatum. These results demonstrate that LIPA-α-syn and mHtt aggregates can propagate from the injection site, in the striatum, to other brain regions.

Collectively, data presented herein demonstrate that α-syn can co-aggregate with mHtt and promote its propagation in the central nervous system, thereby providing direct experimental evidence on how α-syn can participate in Huntington's disease pathogenesis. Moreover, the results presented herein suggest the utility of the LIPA system described herein to study α-syn co-aggregation with other pathological proteins, as well as to better understand and to decorticate the pathophysiology of different proteinopathies or neurodegenerative disorders.

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CLAIMS:

1. A light-inducible intracellular protein aggregation system, the system comprising a cell expressing an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide, wherein illumination of the photoactivatable polypeptide with light having a wavelength sufficient for photoactivation triggers irreversible accumulation of intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide.

2. The system of claim 1, wherein the intracellular protein aggregates accumulate in real-time during said illumination, thereby enabling spatiotemporal control of protein aggregation.

3. The system of claim 2, wherein the intracellular protein aggregates accumulate within 8 h, 7 h, 6 h, 5 h, 4 h, 3 h, 2 h, 1 h, 30 min, 15 min, 10 min, or 5 min following said illumination of the photoactivatable polypeptide.

4. The system of any one of claims 1 to 3, wherein the intracellular protein aggregates remain stable for at least 5 h, 10 h, 15 h, 20 h, 25 h, 30 h, 35 h, 40 h, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 20 days, or 30 days post-illumination.

5. The system of any one of claims 1 to 4, wherein the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide are comprised in a fusion protein.

6. The system of any one of claims 1 to 5, wherein the photoactivatable polypeptide and/or the alpha-synuclein or other proteopathic polypeptide are fused to a detectable marker.

7. The system of claim 6, wherein the detectable marker is a fluorescent protein, a reporter enzyme, a transcription factor, a radioisotope binding protein, or a bioluminescent protein.

8. The system of claim 7, wherein the fluorescent protein is a green fluorescent protein, a cyan fluorescent protein, a blue fluorescent protein, a yellow fluorescent protein, a red fluorescent protein, or any combination thereof.

9. The system of any one of claims 1 to 8, wherein the cell is stably transfected or infected with one or more polynucleotides encoding the photoactivatable polypeptide and/or the alpha-synuclein polypeptide or other proteopathic polypeptide.

10. The system of any one of claims 1 to 9, wherein said proteopathic polypeptide is or comprises a polypeptide that, upon self-aggregation, adopts a misfolded conformation rich in beta-sheets.

11. The system of any one of claims 1 to 10, wherein said proteopathic polypeptide is or comprises a polypeptide that, upon self-aggregation, exhibits seeding activity.

12. The system of any one of claims 1 to 11, wherein said proteopathic polypeptide is, comprises, or is from: an Aβ precursor protein, an Aβ peptide, Tau, a prion, an Ig light chain, serum amyloid A, transthyretin, cystatin C, beta-2-microglobulin, apolipoprotein AI, apolipoprotein AII, gelsolin, amylin, calcitonin, atrial natriuretic factor, lysozyme, insulin, fibrinogen α-A chain, superoxide dismutase 1, huntingtin, an androgen receptor, an ataxin, a TATA box-binding protein, or TDP-43.

13. The system of any one of claims 1 to 12, wherein said proteopathic polypeptide is associated with a proteinopathy comprising: a neurodegenerative disease, a proliferative disease, an inflammatory disease, a cardiovascular disease, diabetes, a synucleinopathy, Parkinson's disease, dementia with Lewy bodies, diffuse Lewy body disease, multiple system atrophy, a tauopathy, Alzheimer's disease, Creutzfeldt-Jakob disease or other prion disease, spinocerebellar ataxias, spinal or bulbar muscular atrophy, amyotrophic lateral sclerosis, hereditary renal amyloidosis, medullary carcinoma of the thyroid, familial amyloid polyneuropathy, amyloidosis, or frontotemporal degeneration.

14. The system of any one of claims 1 to 13, wherein the alpha-synuclein polypeptide or other proteopathic polypeptide is incapable of self-aggregation in the cell in the absence of said illumination.

15. The system of any one of claims 1 to 14, wherein said photoactivatable polypeptide, upon photoactivation, brings molecules of the alpha-synuclein polypeptide or other proteopathic polypeptide into sufficient physical proximity to enable their irreversible self-aggregation.

16. The system of claim 15, wherein the molecules of the alpha-synuclein polypeptide or other proteopathic polypeptide are brought into physical proximity via: light-induced homo- or hetero- protein

association, light-induced conformation change, or light-induced inter- or intra-molecular interactions of the photoactivatable polypeptide.

17. The system of claim 16, wherein the homo- or hetero- protein association, conformation change, or inter- or intra-molecular interactions of the photoactivatable polypeptide is reversible following cessation of the illumination.

18. The system of any one of claims 1 to 17, wherein the photoactivatable polypeptide is incapable of self-clustering in the cell in the absence of said illumination.

19. The system of any one of claims 1 to 18, wherein the photoactivatable polypeptide is, comprises, or is from: a photoreceptor, a cryptochrome, a phytochrome, cryptochrome 2 (CRY2), light-oxygen-voltage (LOV) domains, CRY2- CIB1 (calcium and integrin-binding protein 1) variants, CRY2E490G, CRY2clust, iLID nano and iLID micro, LOVTRAP, Magnets, cobalamin binding domain CBD, VfAU1-LOV, CPH1S, or BphP1-PpsR2.

20. The system of any one of claims 1 to 19, wherein at least one of the alpha-synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide is heterologous with respect to the cell and/or to each other.

21. The system of any one of claims 1 to 20, wherein expression of at least one of the alpha-synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide is under the control of a constitutively active promoter, an inducible promoter, or a tissue-specific promoter.

22. The system of any one of claims 1 to 21, wherein the cell is a neuronal cell, a brain cell, an induced pluripotent stem cell, a mammalian cell, a neuroblastoma, an immune cell, or a blood cell.

23. The system of any one of claims 1 to 22, wherein the cell is comprised in, or is obtained from, an animal.

24. The system of claim 23, wherein the animal is a transgenic animal engineered to express the alpha-synuclein polypeptide or other proteopathic polypeptide, and the photoactivatable polypeptide.

25. The system of any one of claims 1 to 24, wherein said intracellular protein aggregates comprise misfolded proteins in beta-sheet conformation, and/or wherein the intracellular protein aggregates have a

morphology different from that of intracellular inclusions formed following photoactivation of the photoactivatable polypeptide in the absence of the alpha-synuclein polypeptide or other proteopathic polypeptide.

26. The system of any one of claims 1 to 25, wherein the cell expresses an alpha-synuclein polypeptide operably linked to the photoactivatable polypeptide, and wherein the intracellular protein aggregates comprise Lewy body-like aggregates.

27. The system of claim 26, wherein the Lewy body-like aggregates are positive for: alpha-synuclein phosphorylated at Ser129, ubiquitination, staining with thioflavin S or another amyloid-specific dye, p62, HSP70, or any combination thereof.

28. A method for inducing protein aggregation in a cell or animal, the method comprising providing the system as defined in any one of claims 1 to 27, and illuminating the cell or animal with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering irreversible accumulation of the intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide.

29. A method for selecting a candidate compound for the control or treatment of a proteinopathy or neurodegenerative disease, the method comprising:

(a) providing the system as defined in any one of claims 1 to 27;

(b) illuminating the cell, or animal comprising said cell, with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering accumulation of the intracellular protein aggregates comprising the alpha-synuclein polypeptide or proteopathic polypeptide;

(c) administering a compound of interest to the cell or animal; and

(d) determining the effect of the compound of interest on the intracellular protein aggregates.

30. The method of claim 29, further comprising:

(e) selecting the compound of interest as a candidate compound when a decrease in the number and/or size of intracellular protein aggregates is observed.

31. The method of claim 29 or 30, wherein the proteinopathy or neurodegenerative disease is as defined in claim 13.

32. A method for treating a subject having or susceptible for developing a proteinopathy, the method comprising:

(a) providing the system as defined in any one of claims 1 to 27, wherein the proteopathic polypeptide is selected based on the subject's proteinopathy;

(b) illuminating the cell or animal with light having a wavelength sufficient for photoactivation of the photoactivatable polypeptide, thereby triggering accumulation of the intracellular protein aggregates comprising the proteopathic polypeptide;

(c) administering a compound of interest to the cell or animal comprising said cell;

(d) determining the effect of the compound of interest on the intracellular protein aggregates; and

(e) treating the subject with the compound of interest when a decrease in the intracellular protein aggregates is observed.

33. The method of any one of claims 28 to 32, which is an *in vitro*, *ex vivo*, or *in vivo* method.

34. An expression cassette comprising a polynucleotide encoding an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide.

35. The expression cassette of claim 34, wherein the alpha-synuclein polypeptide or other proteopathic polypeptide is fused to the proteopathic polypeptide, and/or to a detectable marker.

36. The expression cassette of claim 30 or 31, wherein the detectable marker is as defined in claim 7 or 8.

37. The expression cassette of any one of claims 34 to 36, wherein expression of the alpha-synuclein polypeptide or other proteopathic polypeptide is placed under control of a constitutively active promoter, an inducible promoter, or tissue-specific promoter.

38. The expression cassette of any one of claims 34 to 37, wherein the proteopathic polypeptide is as defined in any one of claims 10 to 14 or 20, and/or the photoactivatable polypeptide is as defined in any one of claims 15 to 20.

39. A vector comprising the expression cassette as defined in any one of claims 34 to 38.

40. A cell comprising a genome-integrated expression cassette as defined in any one of claims 34 to 38, or the vector of claim 39.

41. The cell of claim 40, which is a neuronal cell, a brain cell, an induced pluripotent stem cell, a mammalian cell, a neuroblastoma, an immune cell, or a blood cell.

42. A transgenic animal model comprising the expression cassette as defined in any one of claims 34 to 38, the vector of claim 39, or the cell as defined in claim 40 or 41.

43. The transgenic animal model of claim 42, wherein the animal is a mouse, a rat, or other rodent, a non-human primate, fish, nematode, yeast, or a fly.

44. A protein aggregate comprising an alpha-synuclein polypeptide or another proteopathic polypeptide that self-aggregates under pathogenic conditions, operably linked to a photoactivatable polypeptide.

45. The protein aggregate of claim 44, wherein:

(a) the protein aggregate is as defined in any one of claims 2 to 4 or 25;

(b) the photoactivatable polypeptide and the alpha-synuclein or other proteopathic polypeptide are comprised in a fusion protein;

(c) the photoactivatable polypeptide and/or the alpha-synuclein or other proteopathic polypeptide are fused to a detectable marker or a detectable marker as defined in claim 7 or 8;

(d) the alpha-synuclein polypeptide or the proteopathic polypeptide is as defined in any one of claims 10 to 14;

(e) the photoactivatable polypeptide is as defined in any one of claims 15 to 19;

(f) at least one of the alpha-synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide is heterologous with respect to the cell and/or to each other;

(g) at least one of the alpha-synuclein polypeptide, other proteopathic polypeptide, or photoactivatable polypeptide is under the control of a constitutively active promoter, an inducible promoter, or a tissue-specific promoter;

(h) the protein aggregate is comprised in a neuronal cell, a brain cell, an induced pluripotent stem cell, a mammalian cell, a neuroblastoma, an immune cell, or a blood cell;

(g) the protein aggregate is comprised in a cell in an animal or a transgenic animal engineered to express the alpha-synuclein polypeptide or other proteopathic polypeptide, and the photoactivatable polypeptide;

(h) the protein aggregates comprise misfolded proteins in beta-sheet conformation; or

(i) any combination of (a) to (h).

46. The protein aggregate of claim 44 or 45 comprising an alpha-synuclein polypeptide operably linked to the photoactivatable polypeptide, wherein the protein aggregate comprises a Lewy body-like aggregate.

47. The protein aggregate of claim 46, wherein the Lewy body-like aggregate is positive for: alpha-synuclein phosphorylated at Ser129, ubiquitination, staining with thioflavin S or another amyloid-specific dye, p62, HSP70, or any combination thereof.

48. The protein aggregate as defined in any one of claims 44 to 47 for use in a light-inducible intracellular protein aggregation system, in a method for inducing protein aggregation in a cell or animal, in a method for selecting a compound for the treatment of a proteinopathy or neurodegenerative disease, or in the method for treating a subject having or susceptible for developing a proteinopathy.

49. A variant alpha-synuclein polypeptide for use in preventing aggregation of a proteopathic polypeptide other than alpha-synuclein, or for use in treating a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide.

50. Use of a variant alpha-synuclein polypeptide for the manufacture of a medicament for the treatment of a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of a proteopathic polypeptide associated with said proteinopathy.

51. An expression cassette encoding a variant alpha-synuclein polypeptide for use in preventing aggregation of a proteopathic polypeptide other than alpha-synuclein, or for use in treating a

proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide.

52. Use of an expression cassette encoding a variant alpha-synuclein polypeptide in the manufacture of a medicament for the treatment of a proteinopathy other than an alpha-synucleinopathy, wherein the variant alpha-synuclein polypeptide lacks self-aggregating activity and inhibits aggregation of the proteopathic polypeptide.

53. The variant alpha-synuclein polypeptide for the use of claim 49, the use of claim 50 or 52, or the expression cassette of claim 51, wherein the variant alpha-synuclein polypeptide is, comprises, or is from beta-synuclein.

54. The variant alpha-synuclein polypeptide for the use of claim 49 or 53, the use of claim 50, 52 or 53, or the expression cassette for the use of claim 51 or 53, wherein:

(i) the proteopathic polypeptide other than alpha-synuclein is, comprises, or is from: an Aβ precursor protein, an Aβ peptide, Tau, a prion, an Ig light chain, serum amyloid A, transthyretin, cystatin C, beta-2-microglobulin, apolipoprotein AI, apolipoprotein AII, gelsolin, amylin, calcitonin, atrial natriuretic factor, lysozyme, insulin, fibrinogen α-A chain, superoxide dismutase 1, huntingtin, an androgen receptor, an ataxin, a TATA box-binding protein, or TDP-43; or

(ii) the proteinopathy other than an alpha-synucleinopathy is: a neurodegenerative disease, a proliferative disease, an inflammatory disease, a cardiovascular disease, diabetes, Parkinson's disease, dementia with Lewy bodies, diffuse Lewy body disease, multiple system atrophy, a tauopathy, Alzheimer's disease, Creutzfeldt-Jakob disease or other prion disease, spinocerebellar ataxias, spinal or bulbar muscular atrophy, amyotrophic lateral sclerosis, hereditary renal amyloidosis, medullary carcinoma of the thyroid, familial amyloid polyneuropathy, amyloidosis, or frontotemporal degeneration.

Fig. 1

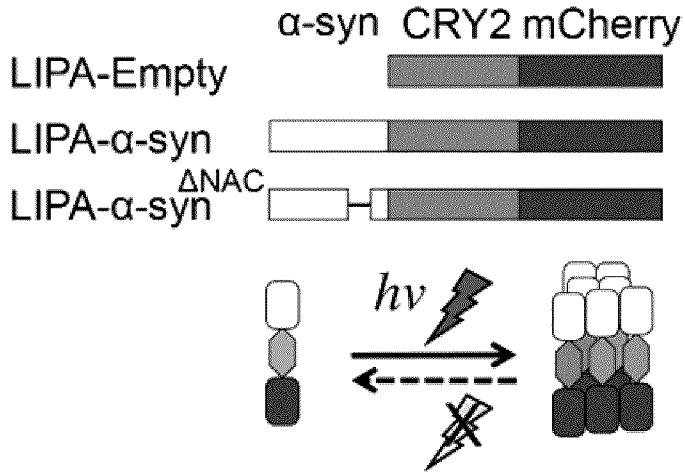

α-syn
CRY2
mCherry
LIPA-Empty
LIPA-α-syn
LIPA-α-syn ΔNAC
hv

Fig. 2

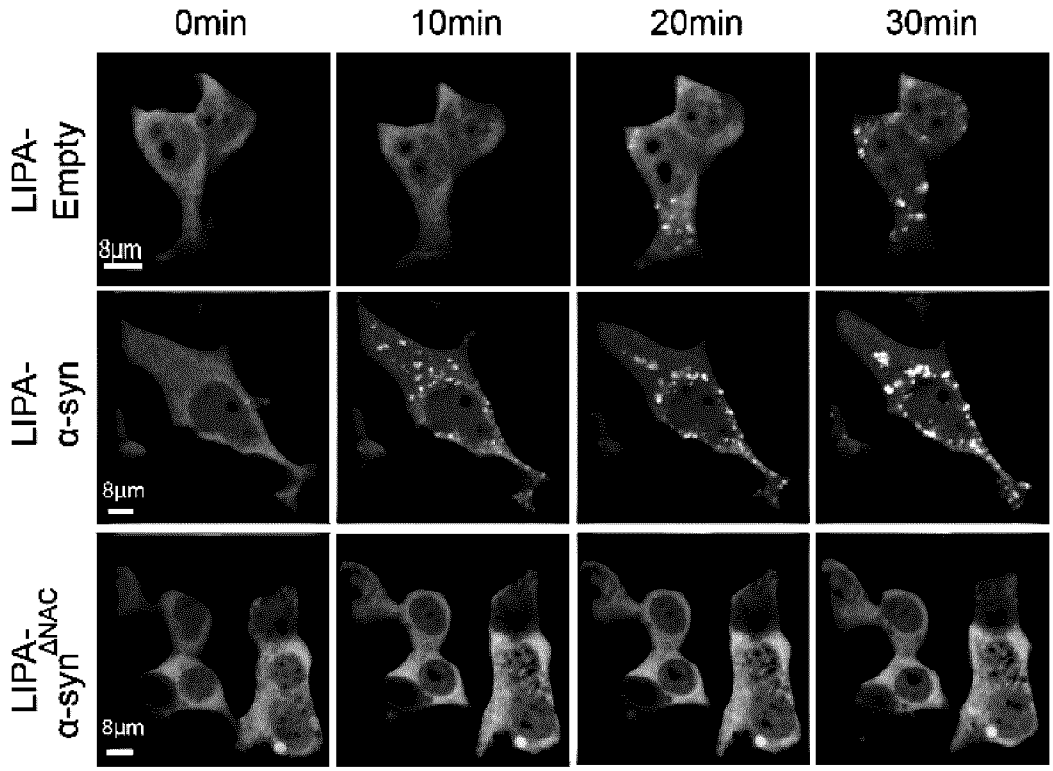

0min
10min
20min
30min
LIPA-Empty
LIPA-α-syn
LIPA-α-syn ΔNAC
8μm
8μm
8μm

Fig. 3

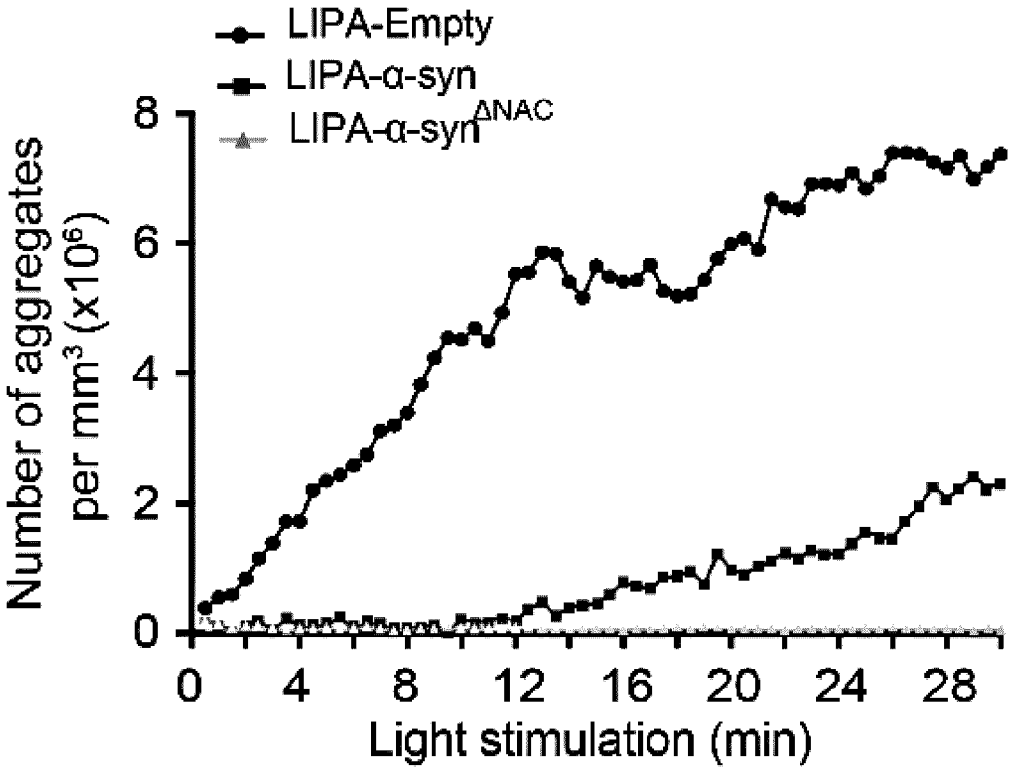

LIPA-Empty
LIPA-α-syn
LIPA-α-syn^ΔNAC
Number of aggregates per mm³ (x10⁶)
0
2
4
6
8
0
4
8
12
16
20
24
28
Light stimulation (min)

Fig. 4

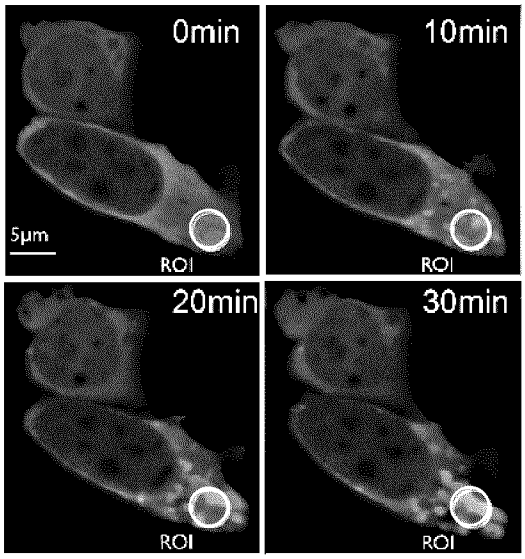

0min
10min
5µm
ROI
ROI
20min
30min
ROI
ROI

Fig. 5

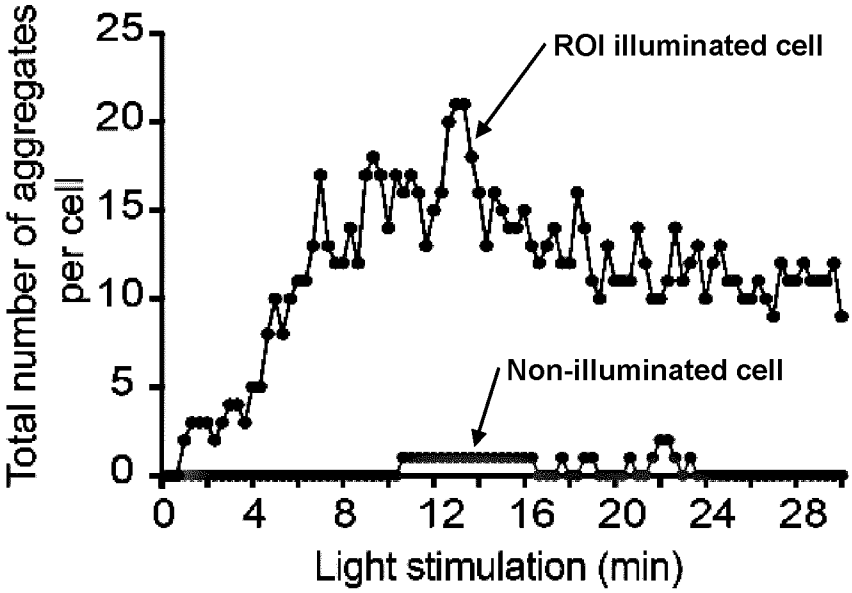

Total number of aggregates per cell
25
20
15
10
5
0
0
4
8
12
16
20
24
28
Light stimulation (min)
ROI illuminated cell
Non-illuminated cell

Fig. 6

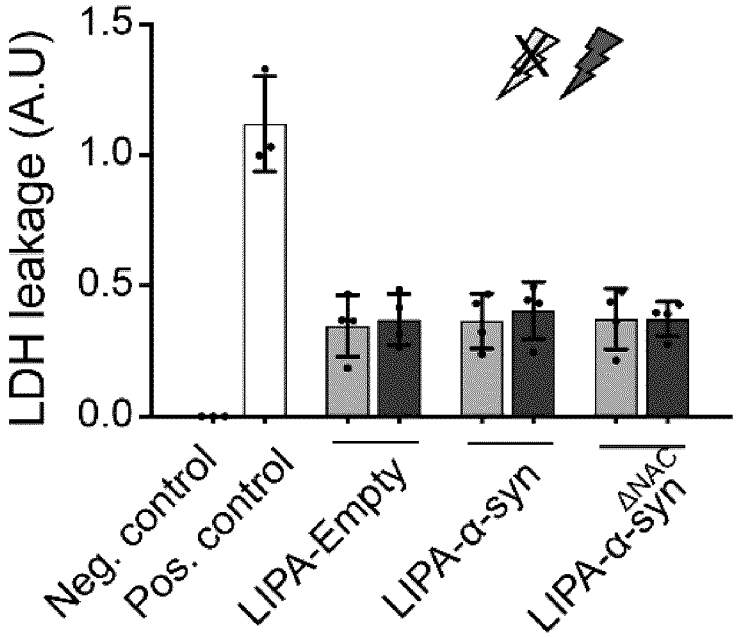

LDH leakage (A.U)
1.5
1.0
0.5
0.0
Neg. control
Pos. control
LIPA-Empty
LIPA-α-syn
LIPA-α-syn ΔNAC

Fig. 7

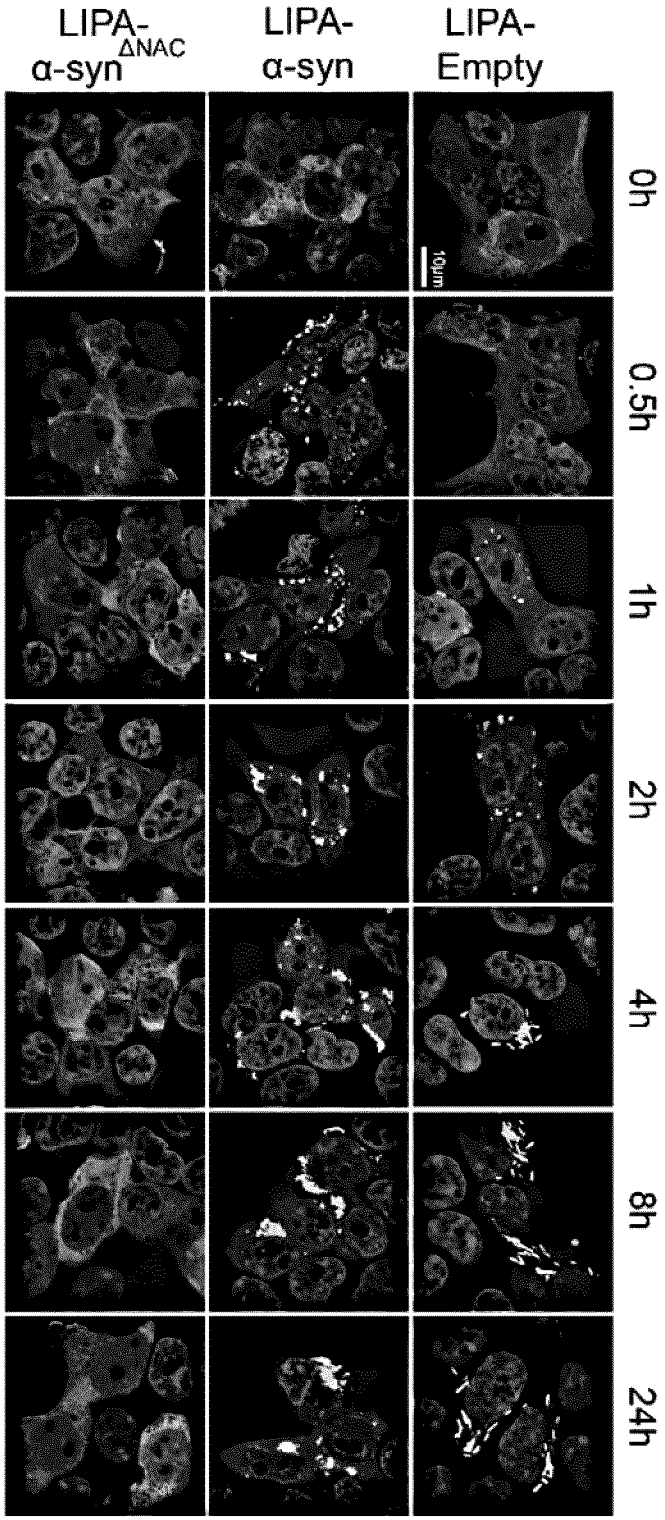


Fig. 8

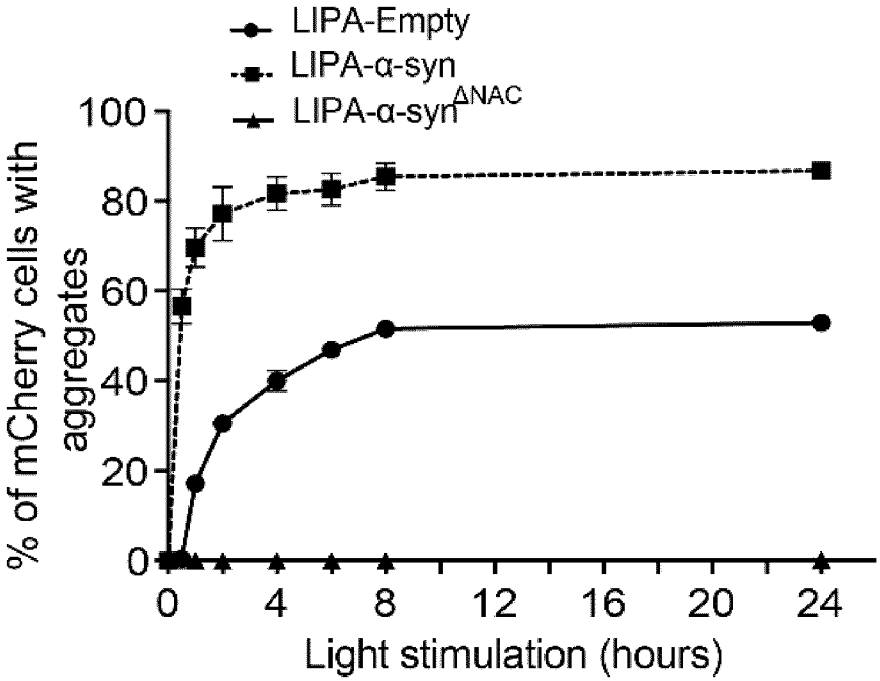

LIPA-Empty
LIPA-α-syn
LIPA-α-syn$^{\Delta NAC}$
% of mCherry cells with aggregates
100
80
60
40
20
0
0
4
8
12
16
20
24
Light stimulation (hours)

Fig. 9

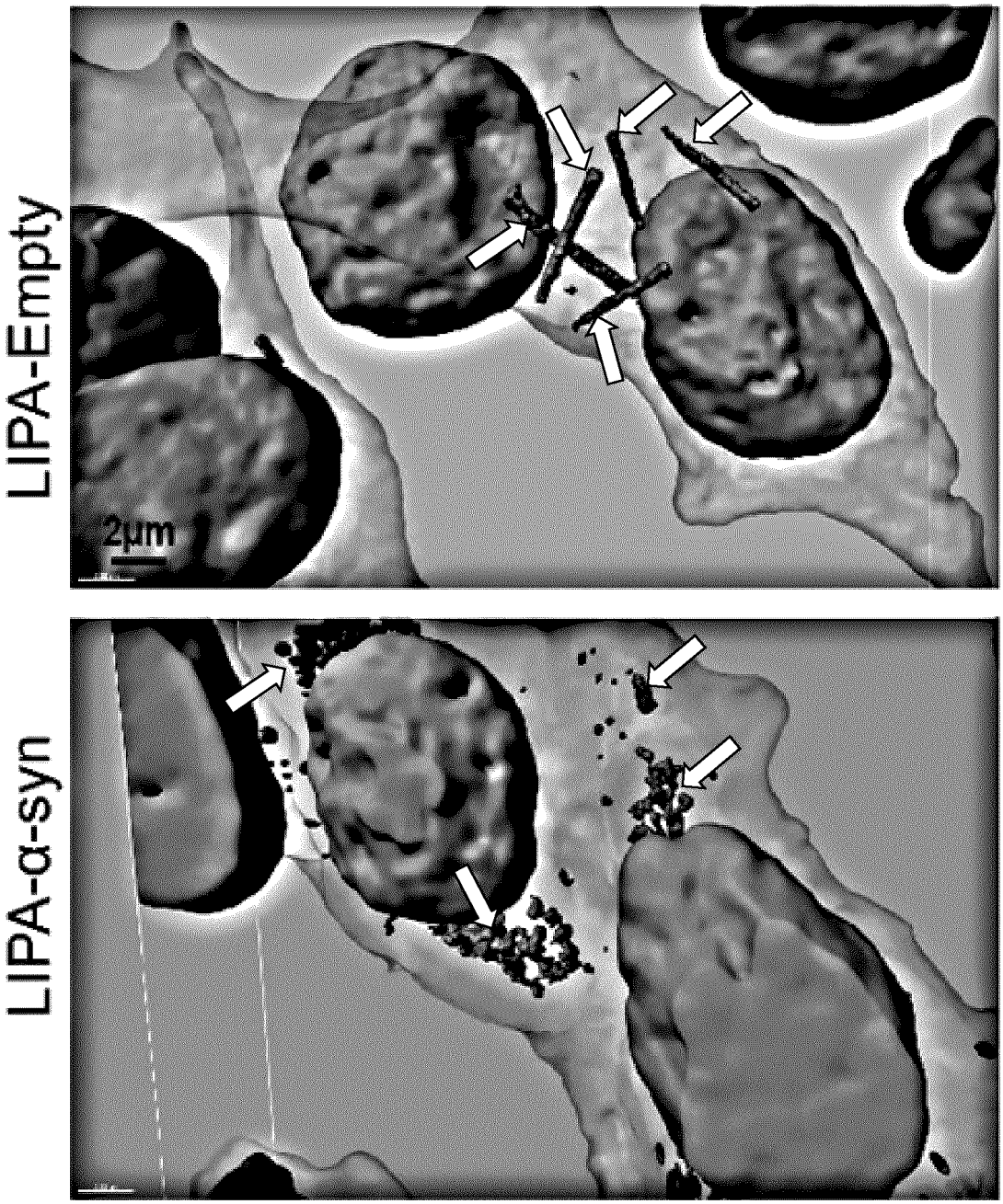


Fig. 10

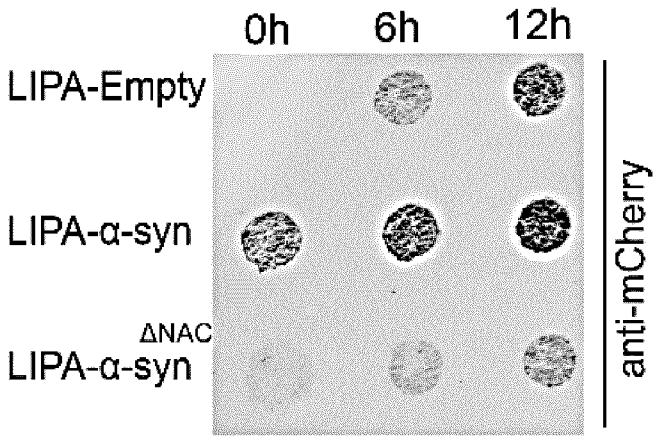

0h
6h
12h
LIPA-Empty
LIPA-α-syn
ΔNAC
LIPA-α-syn
anti-mCherry

Fig. 11

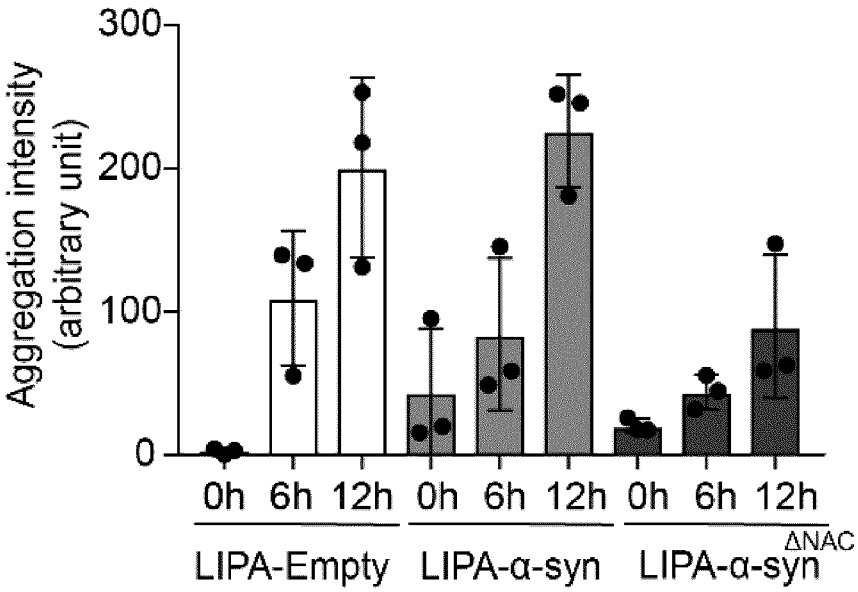

Aggregation intensity
(arbitrary unit)
300
200
100
0
0h 6h 12h 0h 6h 12h 0h 6h 12h
LIPA-Empty
LIPA-α-syn
ΔNAC
LIPA-α-syn

Fig. 12

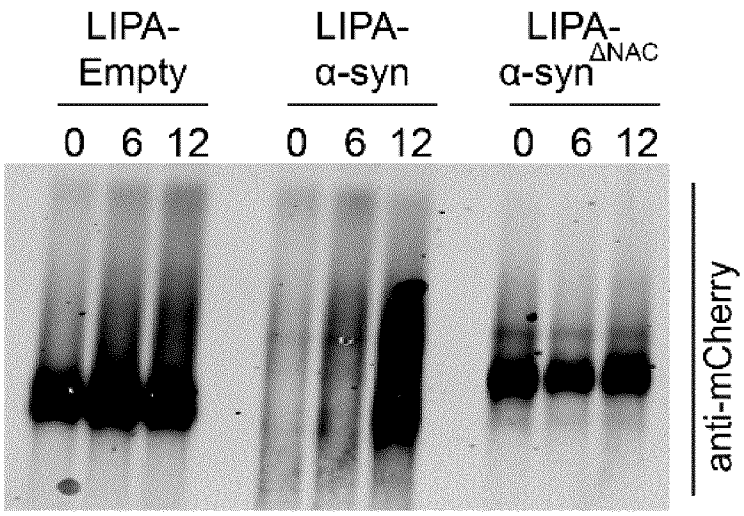

LIPA-Empty
LIPA-α-syn
LIPA-α-synΔNAC
0 6 12 0 6 12 0 6 12
anti-mCherry

Fig. 13

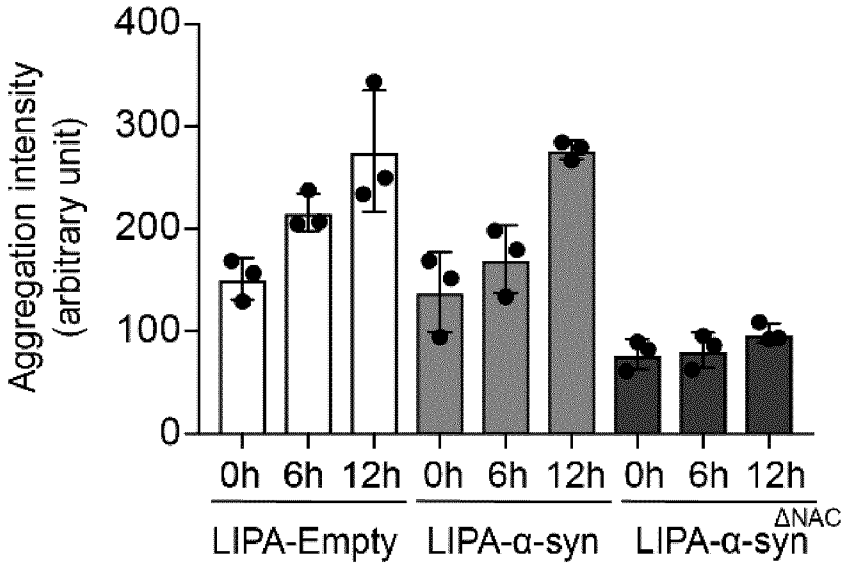

Aggregation intensity (arbitrary unit)
400
300
200
100
0
0h 6h 12h 0h 6h 12h 0h 6h 12h
LIPA-Empty
LIPA-α-syn
LIPA-α-synΔNAC

Fig. 14

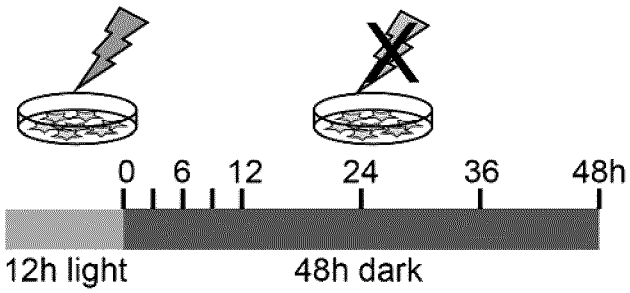

0
6
12
24
36
48h
12h light
48h dark

Fig. 15

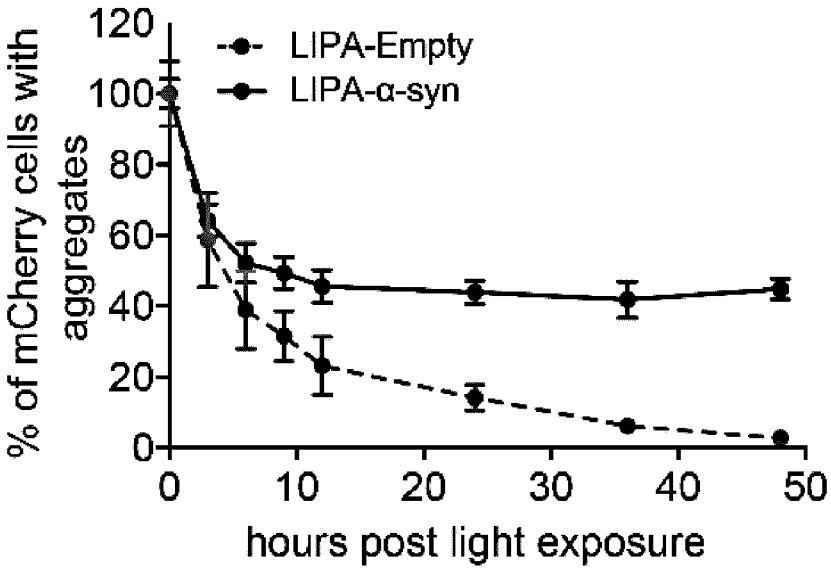

LIPA-Empty
LIPA-α-syn
% of mCherry cells with aggregates
120
100
80
60
40
20
0
0
10
20
30
40
50
hours post light exposure

Fig. 16

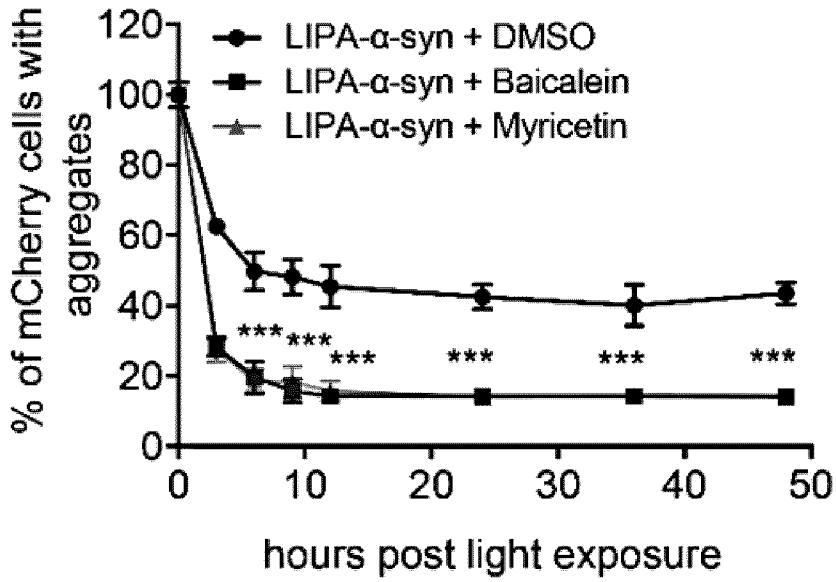

LIPA-α-syn + DMSO
LIPA-α-syn + Baicalein
LIPA-α-syn + Myricetin
% of mCherry cells with aggregates
120
100
80
60
40
20
0
0
10
20
30
40
50
hours post light exposure
***
***
***
***
***
***

Fig. 17

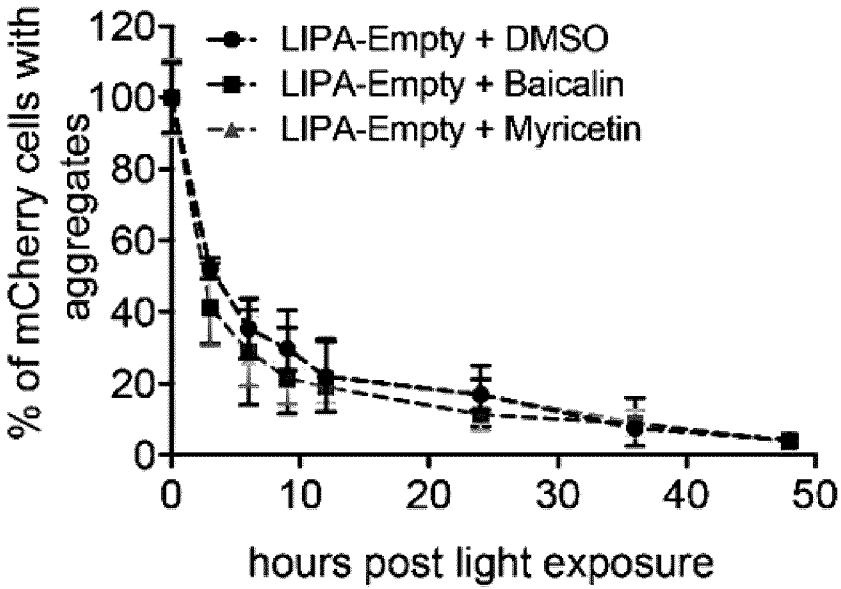

LIPA-Empty + DMSO
LIPA-Empty + Baicalin
LIPA-Empty + Myricetin
% of mCherry cells with aggregates
120
100
80
60
40
20
0
0
10
20
30
40
50
hours post light exposure

Fig. 18

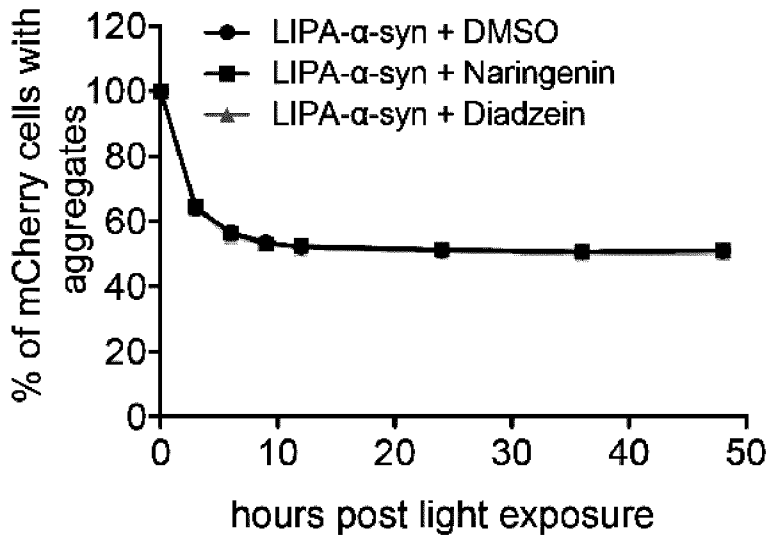

LIPA-α-syn + DMSO
LIPA-α-syn + Naringenin
LIPA-α-syn + Diadzein
% of mCherry cells with aggregates
120
100
80
60
40
20
0
0
10
20
30
40
50
hours post light exposure

Fig. 19

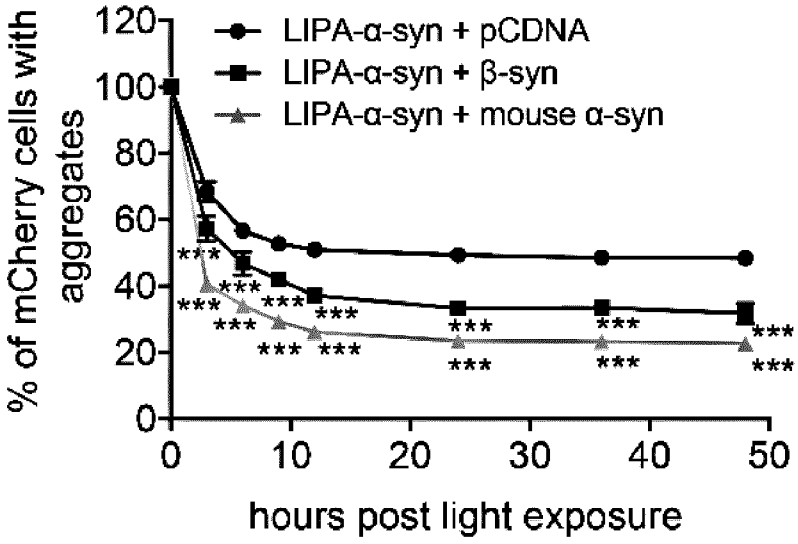

LIPA-α-syn + pCDNA
LIPA-α-syn + β-syn
LIPA-α-syn + mouse α-syn
% of mCherry cells with aggregates
120
100
80
60
40
20
0
0
10
20
30
40
50
hours post light exposure
***

Fig. 20

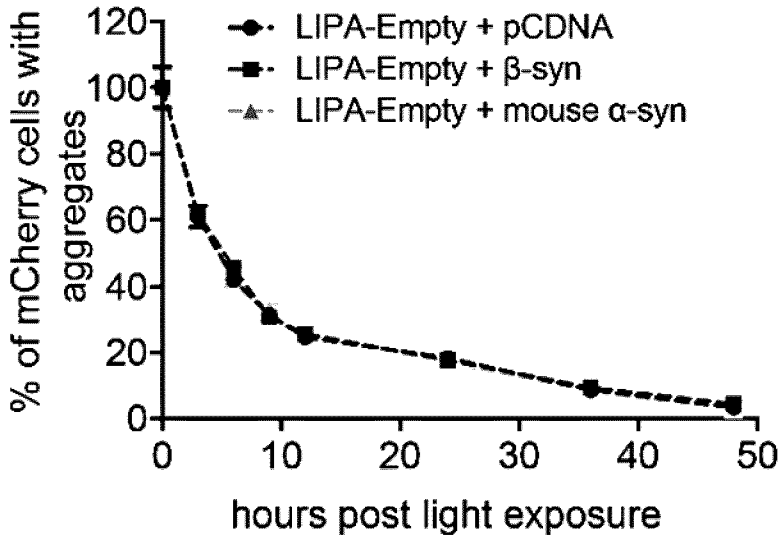

LIPA-Empty + pCDNA
LIPA-Empty + β-syn
LIPA-Empty + mouse α-syn
% of mCherry cells with aggregates
120
100
80
60
40
20
0
0
10
20
30
40
50
hours post light exposure

Fig. 21

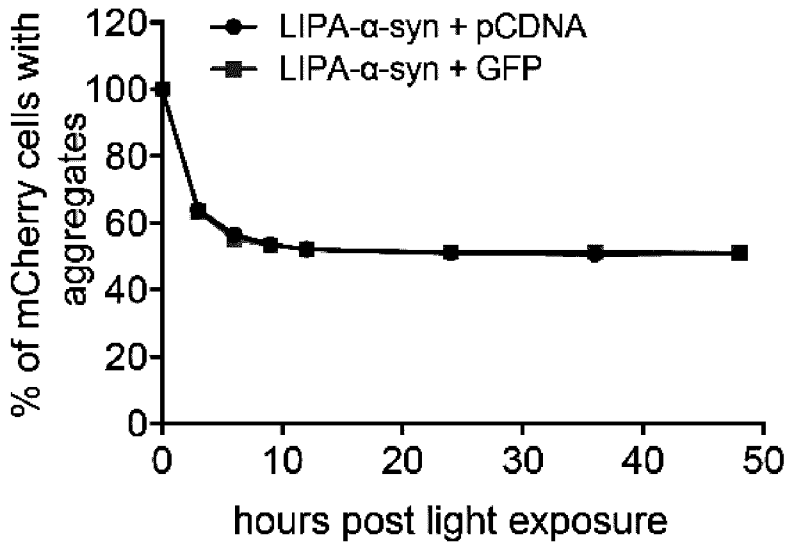

LIPA-α-syn + pCDNA
LIPA-α-syn + GFP
% of mCherry cells with aggregates
120
100
80
60
40
20
0
0
10
20
30
40
50
hours post light exposure

Fig. 22

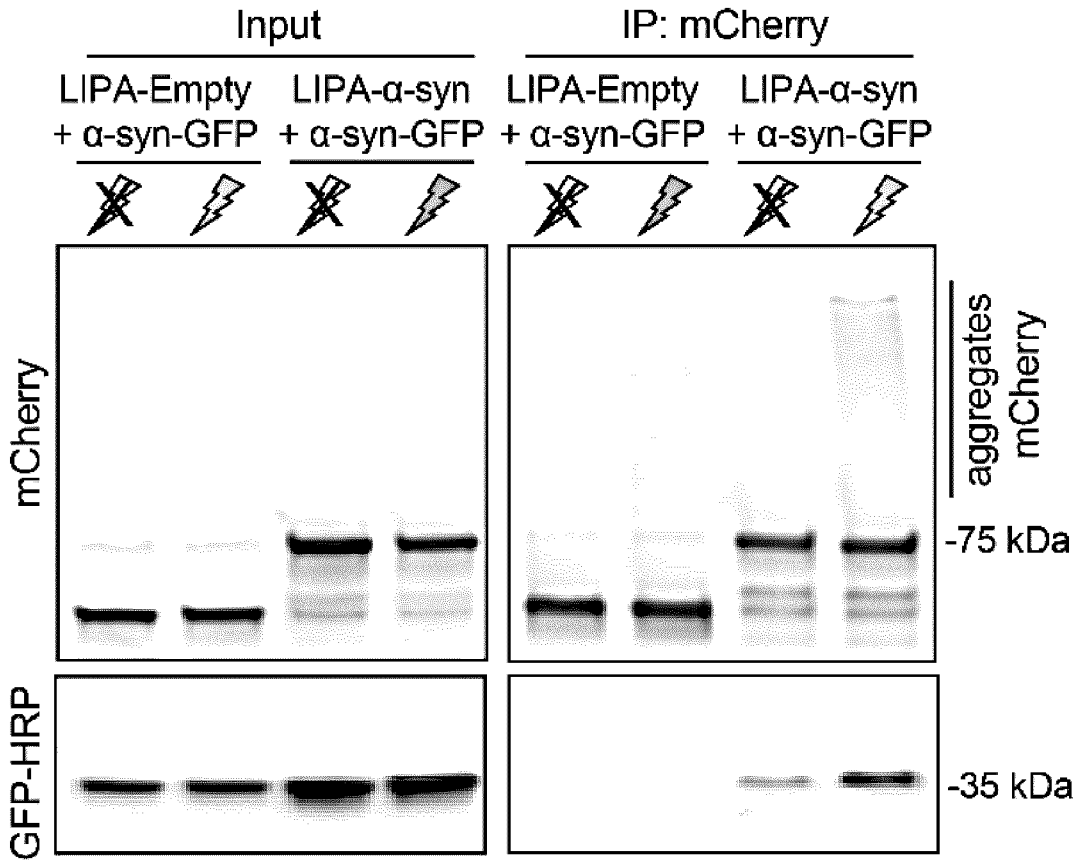

Input
IP: mCherry
LIPA-Empty + α-syn-GFP
LIPA-α-syn + α-syn-GFP
LIPA-Empty + α-syn-GFP
LIPA-α-syn + α-syn-GFP
mCherry
aggregates mCherry
-75 kDa
GFP-HRP
-35 kDa

Fig. 23

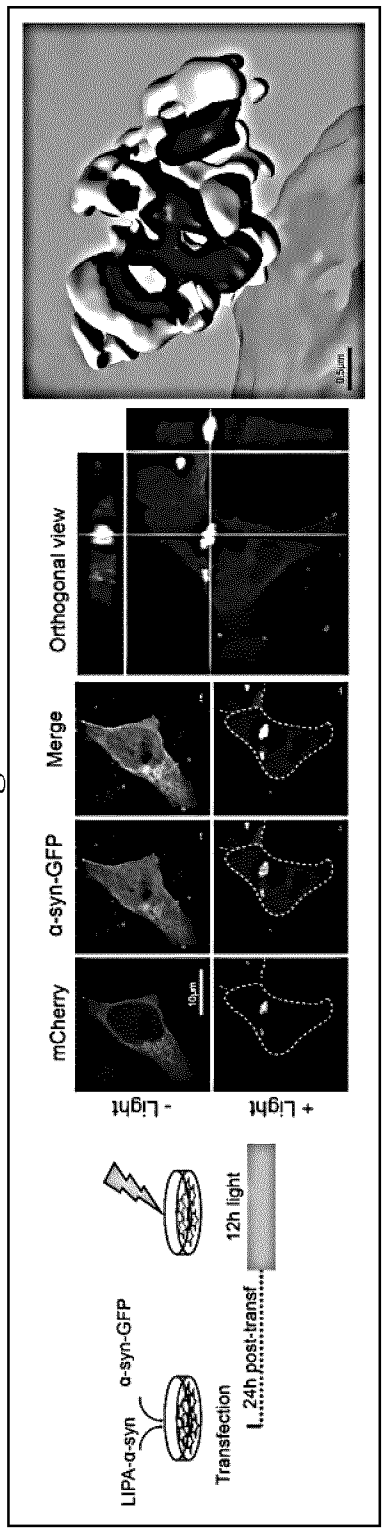

LIPA-α-syn
α-syn-GFP
Transfection
24h post-transf
12h light
- Light
+ Light
mCherry
α-syn-GFP
Merge
Orthogonal view
10µm
0.5µm

Fig. 24

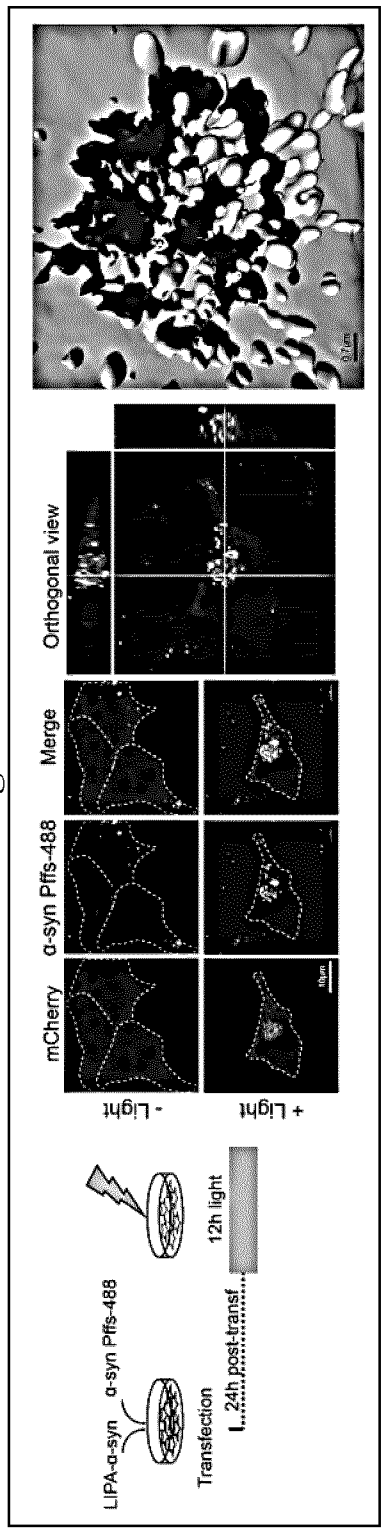

LIPA-α-syn
α-syn Pffs-488
Transfection
24h post-transf
12h light
- Light
+ Light
mCherry
α-syn Pffs-488
Merge
Orthogonal view
10µm
0.5µm

Fig. 25

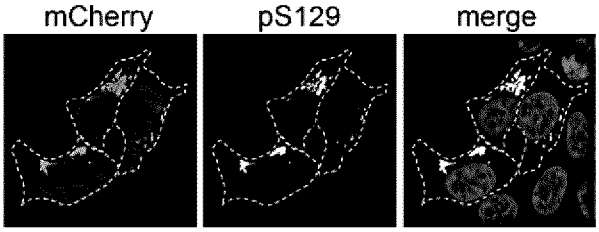


Fig. 26

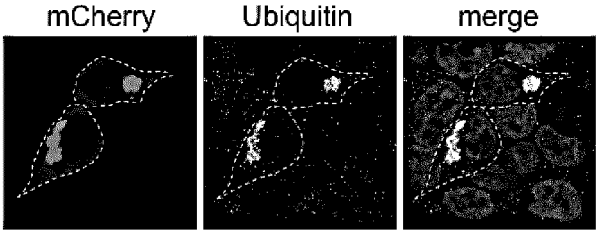


Fig. 27

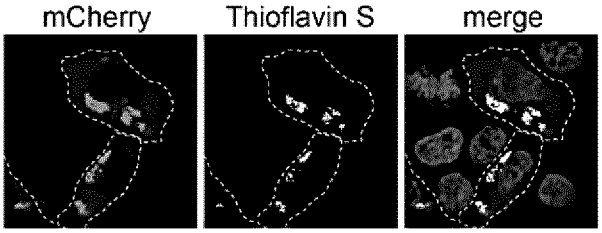


Fig. 28

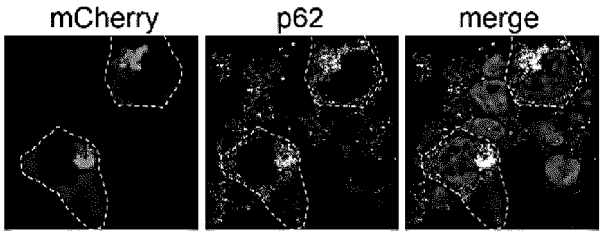


Fig. 29

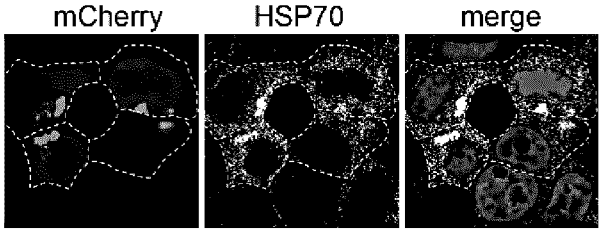


Fig. 30

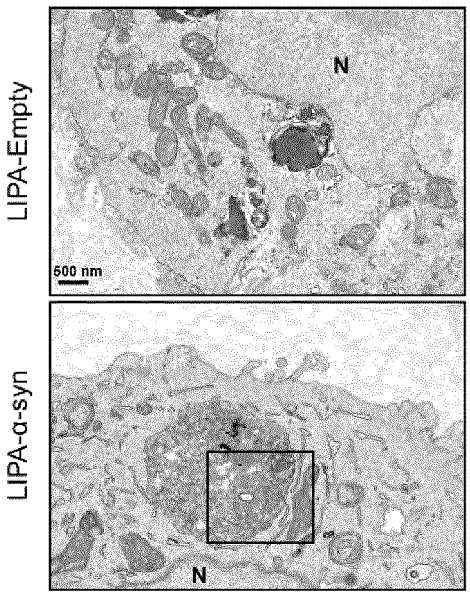


Fig. 31

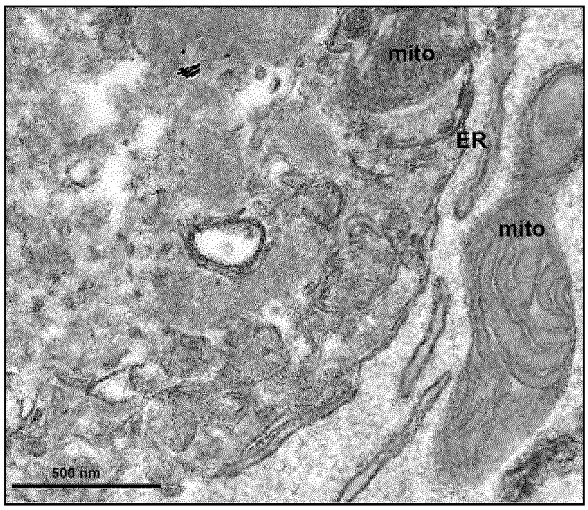


Fig. 32

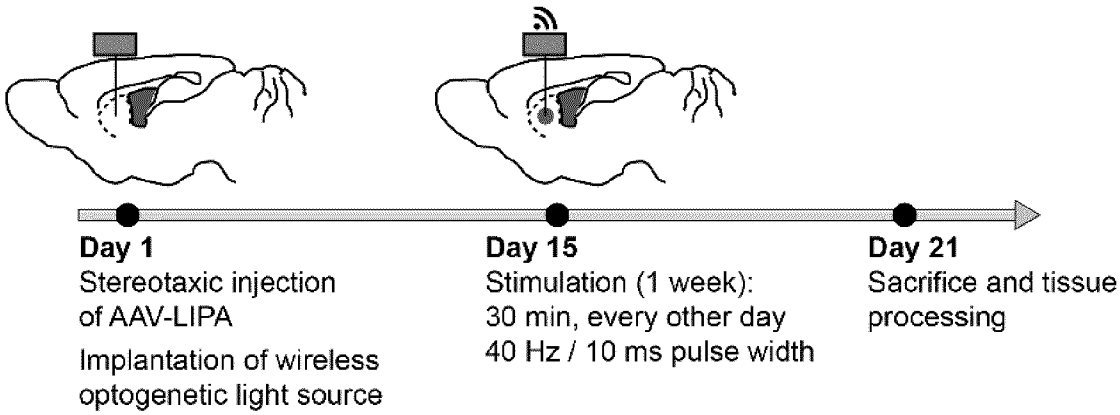


Fig. 33

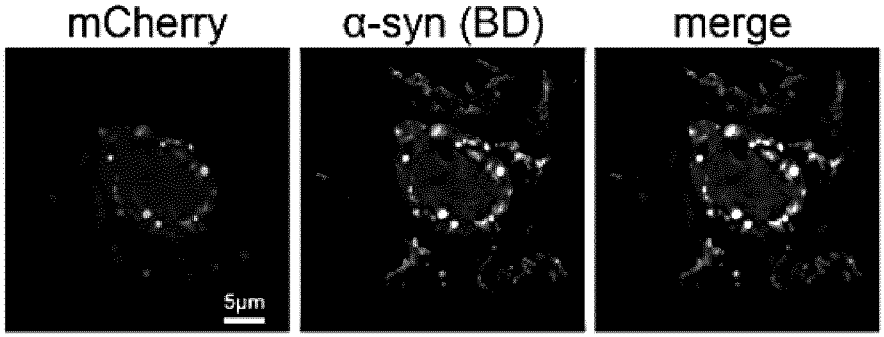


Fig. 34

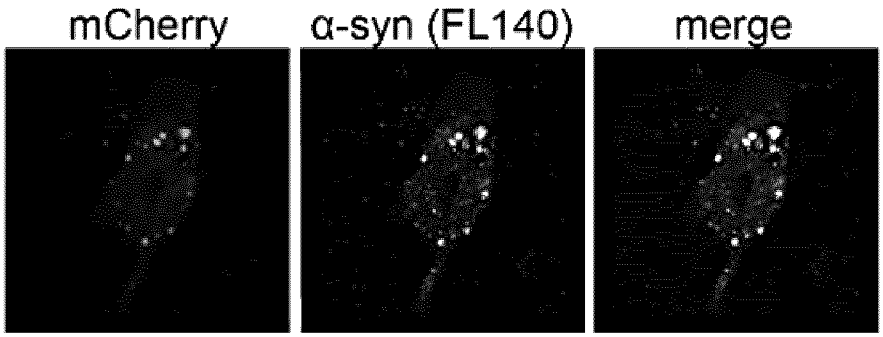


Fig. 35

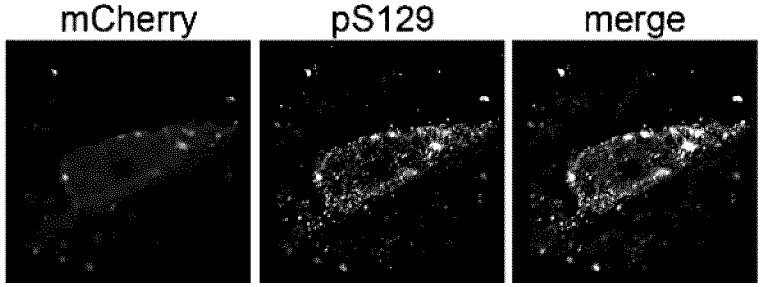


Fig. 36

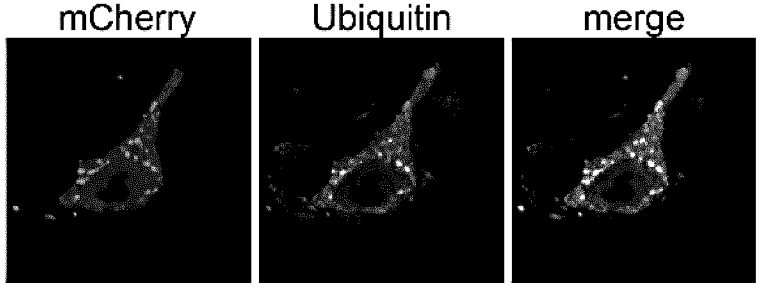


Fig. 37

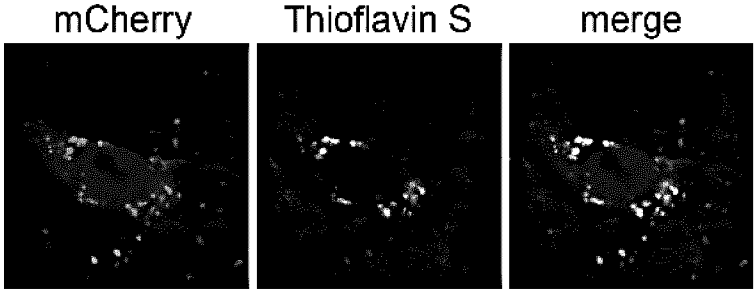


Fig. 38

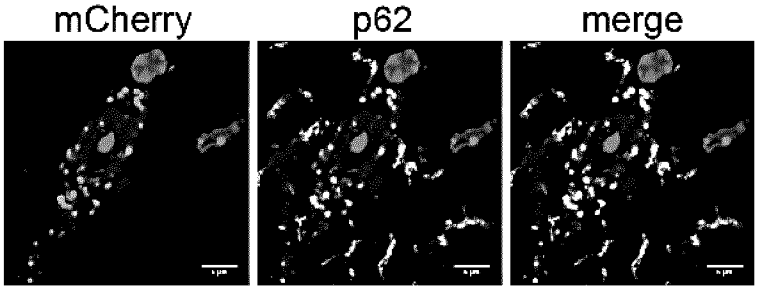


Fig. 39

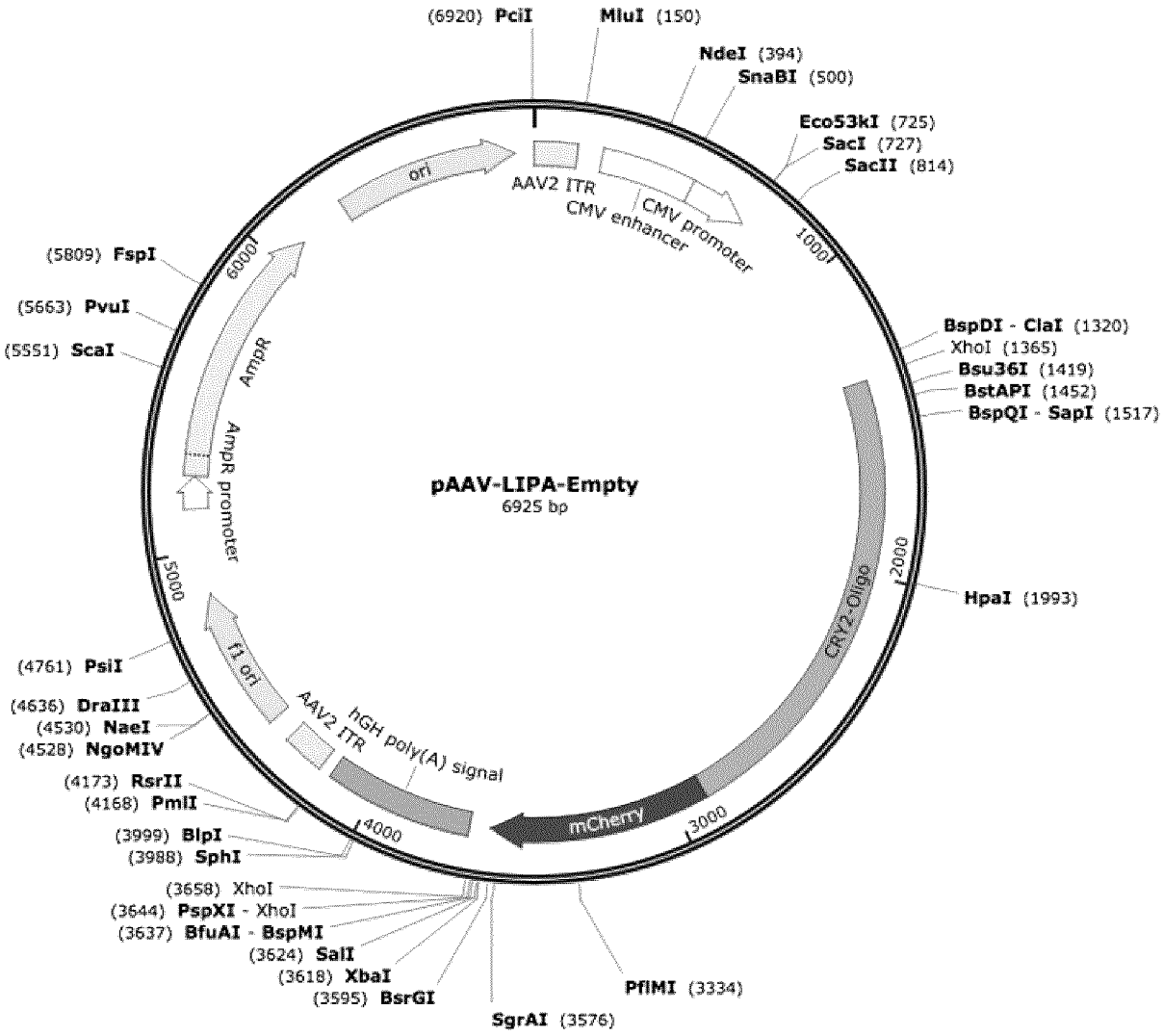

(6920) PciI
MluI (150)
NdeI (394)
SnaBI (500)
Eco53kI (725)
SacI (727)
SacII (814)
1000
BspDI - ClaI (1320)
XhoI (1365)
Bsu36I (1419)
BstAPI (1452)
BspQI - SapI (1517)
2000
HpaI (1993)
CR22-Oligo
3000
mCherry
4000
hGH poly(A) signal
AAV2 ITR
f1 ori
5000
AmpR promoter
AmpR
6000
(5809) FspI
(5663) PvuI
(5551) ScaI
(4761) PsiI
(4636) DraIII
(4530) NaeI
(4528) NgoMIV
(4173) RsrII
(4168) PmlI
(3999) BlpI
(3988) SphI
(3658) XhoI
(3644) PspXI - XhoI
(3637) BfuAI - BspMI
(3624) SalI
(3618) XbaI
(3595) BsrGI
SgrAI (3576)
PflMI (3334)
ori
AAV2 ITR
CMV enhancer
CMV promoter
pAAV-LIPA-Empty
6925 bp

Fig. 40

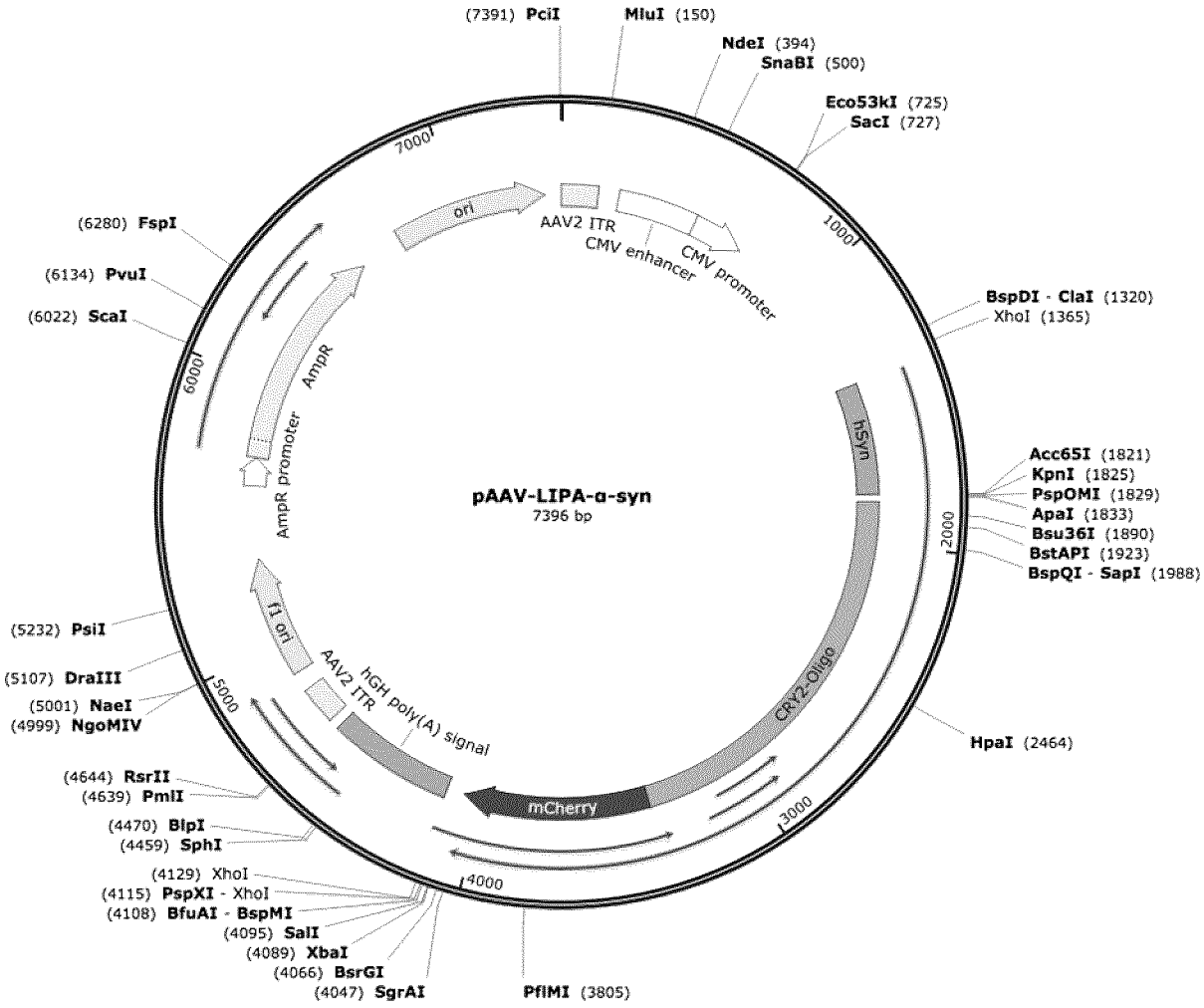

(7391) PciI
MluI (150)
NdeI (394)
SnaBI (500)
Eco53kI (725)
SacI (727)
1000
BspDI - ClaI (1320)
XhoI (1365)
Acc65I (1821)
KpnI (1825)
PspOMI (1829)
ApaI (1833)
Bsu36I (1890)
BstAPI (1923)
BspQI - SapI (1988)
2000
HpaI (2464)
3000
PflMI (3805)
4000
(4047) SgrAI
(4066) BsrGI
(4089) XbaI
(4095) SalI
(4108) BfuAI - BspMI
(4115) PspXI - XhoI
(4129) XhoI
(4459) SphI
(4470) BlpI
(4639) PmlI
(4644) RsrII
5000
(4999) NgoMIV
(5001) NaeI
(5107) DraIII
(5232) PsiI
6000
(6022) ScaI
(6134) PvuI
(6280) FspI
7000
ori
AAV2 ITR
CMV enhancer
CMV promoter
hSyn
CRY2-Olige
mCherry
hGH poly(A) signal
AAV2 ITR
f1 ori
AmpR promoter
AmpR
pAAV-LIPA-α-syn
7396 bp

Fig. 41

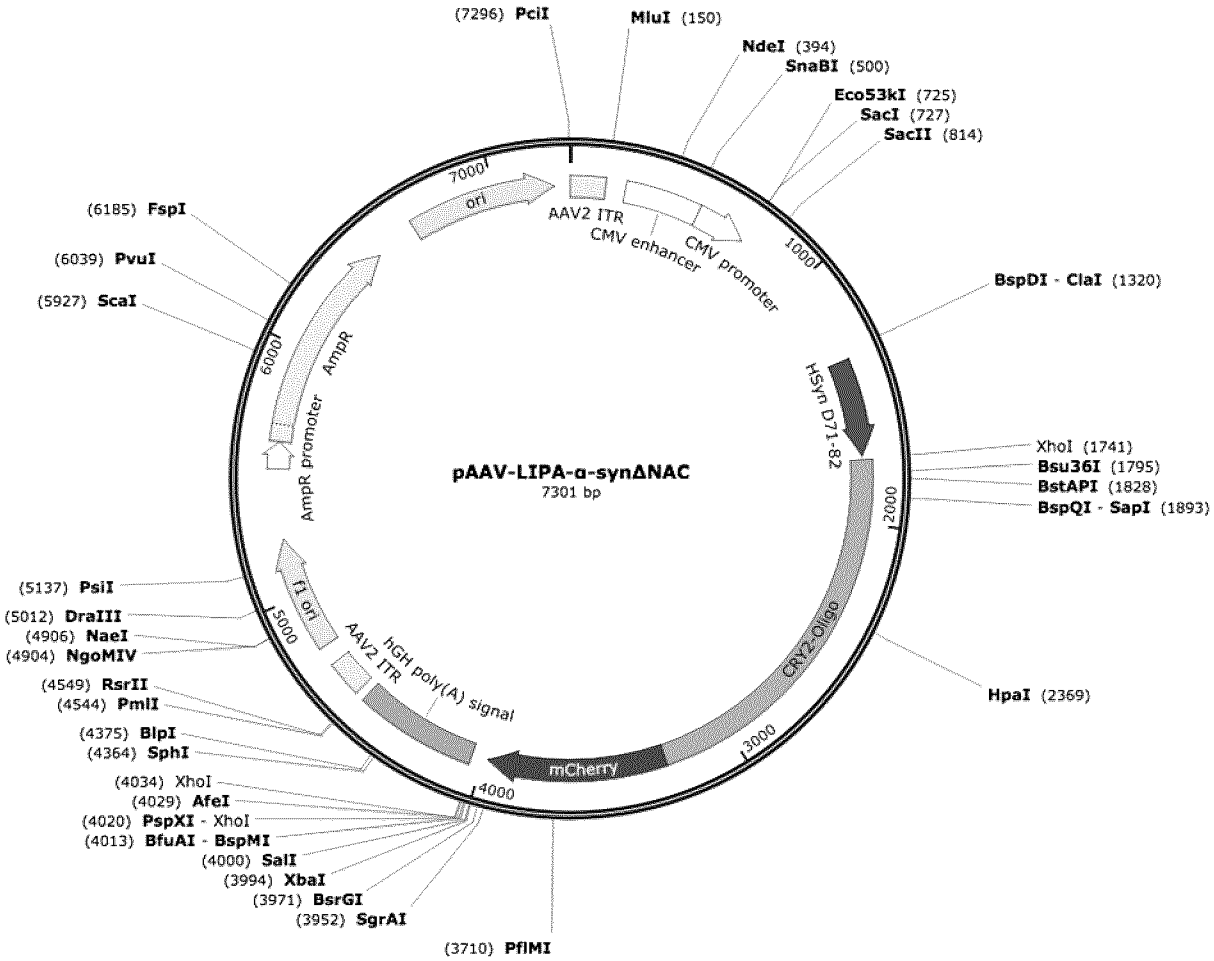

pAAV-LIPA-α-synΔNAC
7301 bp
(7296) PciI
MluI (150)
NdeI (394)
SnaBI (500)
Eco53kI (725)
SacI (727)
SacII (814)
BspDI - ClaI (1320)
XhoI (1741)
Bsu36I (1795)
BstAPI (1828)
BspQI - SapI (1893)
HpaI (2369)
(3710) PflMI
(3952) SgrAI
(3971) BsrGI
(3994) XbaI
(4000) SalI
(4013) BfuAI - BspMI
(4020) PspXI - XhoI
(4029) AfeI
(4034) XhoI
(4364) SphI
(4375) BlpI
(4544) PmlI
(4549) RsrII
(4904) NgoMIV
(4906) NaeI
(5012) DraIII
(5137) PsiI
(5927) ScaI
(6039) PvuI
(6185) FspI
1000
2000
3000
4000
5000
6000
7000
ori
AAV2 ITR
CMV enhancer
CMV promoter
HSyn D71-82
CRY2-Olig6
mCherry
hGH poly(A) signal
AAV2 ITR
f1 ori
AmpR promoter
AmpR

Fig. 42

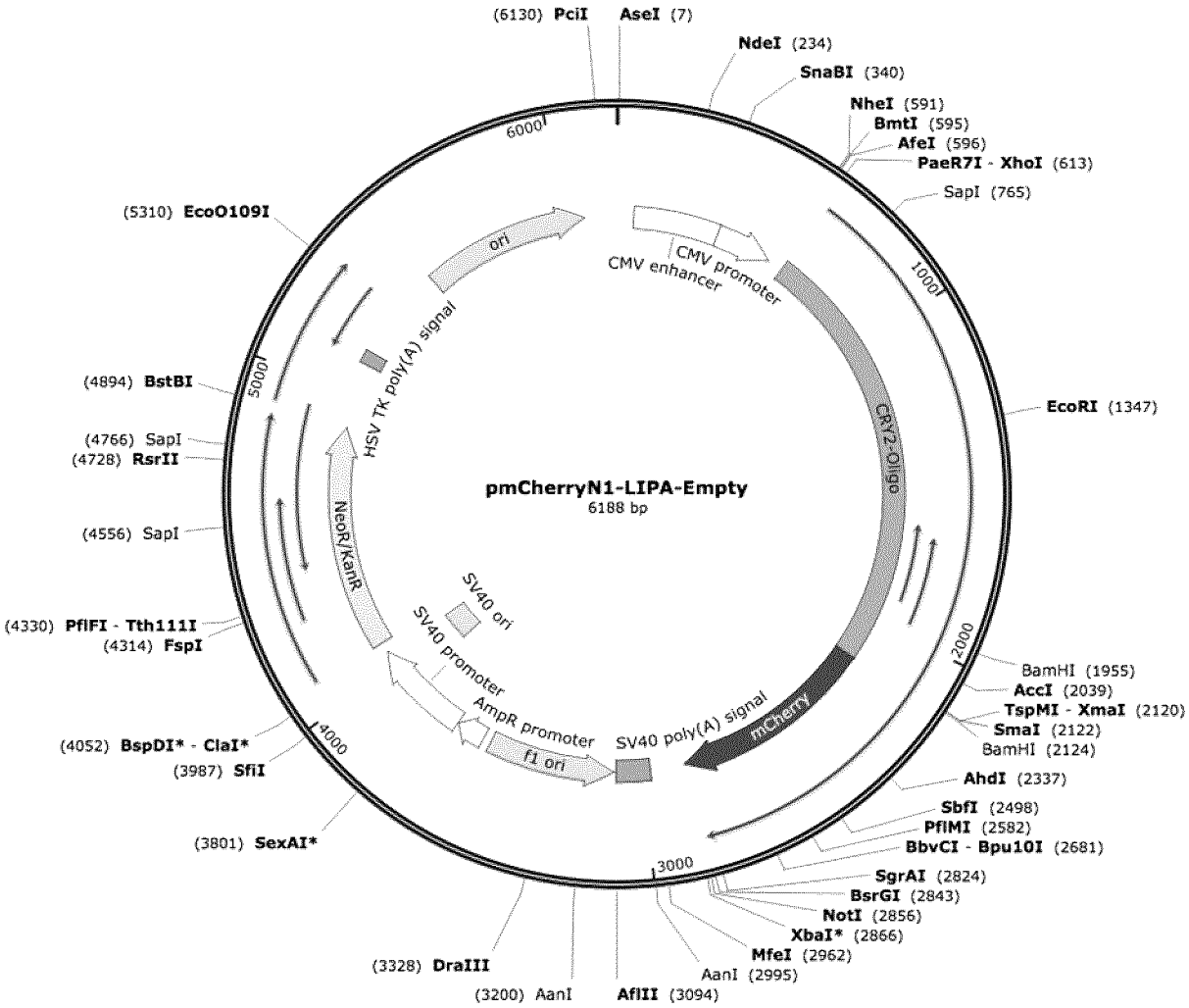

pmCherryN1-LIPA-Empty
6188 bp
(6130) PciI
AseI (7)
NdeI (234)
SnaBI (340)
NheI (591)
BmtI (595)
AfeI (596)
PaeR7I - XhoI (613)
SapI (765)
EcoRI (1347)
BamHI (1955)
AccI (2039)
TspMI - XmaI (2120)
SmaI (2122)
BamHI (2124)
AhdI (2337)
SbfI (2498)
PflMI (2582)
BbvCI - Bpu10I (2681)
SgrAI (2824)
BsrGI (2843)
NotI (2856)
XbaI* (2866)
MfeI (2962)
AanI (2995)
AflII (3094)
(3200) AanI
(3328) DraIII
(3801) SexAI*
(3987) SfiI
(4052) BspDI* - ClaI*
(4314) FspI
(4330) PflFI - Tth111I
(4556) SapI
(4728) RsrII
(4766) SapI
(4894) BstBI
(5310) EcoO109I
1000
2000
3000
4000
5000
6000
ori
CMV enhancer
CMV promoter
CR1-2-Oligo
mCherry
SV40 poly(A) signal
f1 ori
AmpR promoter
SV40 promoter
SV40 ori
NeoR/KanR
HSV TK poly(A) signal

Fig. 43

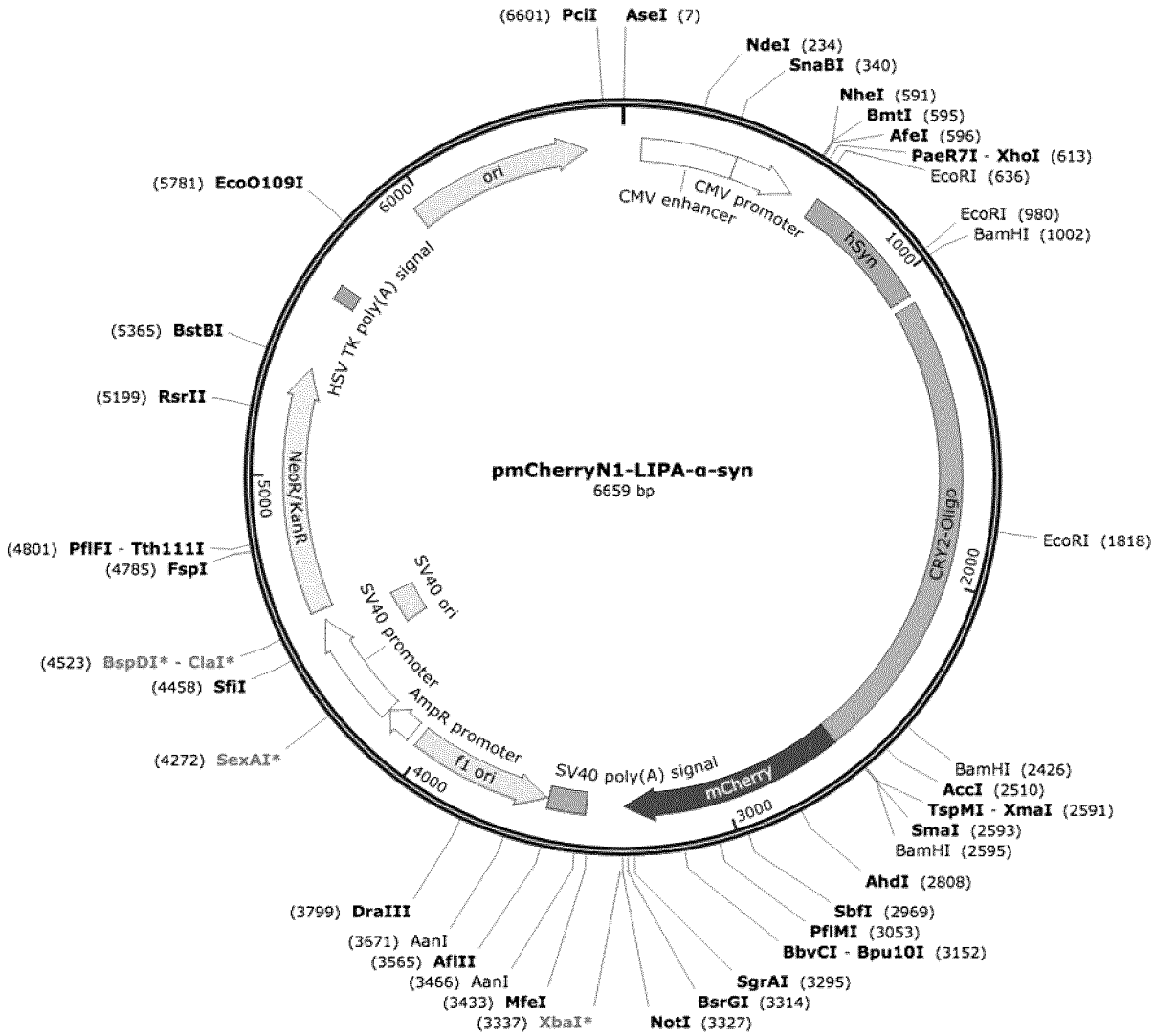

pmCherryN1-LIPA-α-syn
6659 bp
(6601) PciI
AseI (7)
NdeI (234)
SnaBI (340)
NheI (591)
BmtI (595)
AfeI (596)
PaeR7I - XhoI (613)
EcoRI (636)
EcoRI (980)
BamHI (1002)
EcoRI (1818)
BamHI (2426)
AccI (2510)
TspMI - XmaI (2591)
SmaI (2593)
BamHI (2595)
AhdI (2808)
SbfI (2969)
PflMI (3053)
BbvCI - Bpu10I (3152)
SgrAI (3295)
BsrGI (3314)
NotI (3327)
(3337) XbaI*
(3433) MfeI
(3466) AanI
(3565) AflII
(3671) AanI
(3799) DraIII
(4272) SexAI*
(4458) SfiI
(4523) BspDI* - ClaI*
(4785) FspI
(4801) PflFI - Tth111I
(5199) RsrII
(5365) BstBI
(5781) EcoO109I
ori
CMV enhancer
CMV promoter
hSyn
CRY2-Oligo
mCherry
SV40 poly(A) signal
f1 ori
AmpR promoter
SV40 promoter
SV40 ori
NeoR/KanR
HSV TK poly(A) signal
1000
2000
3000
4000
5000
6000

Fig. 44

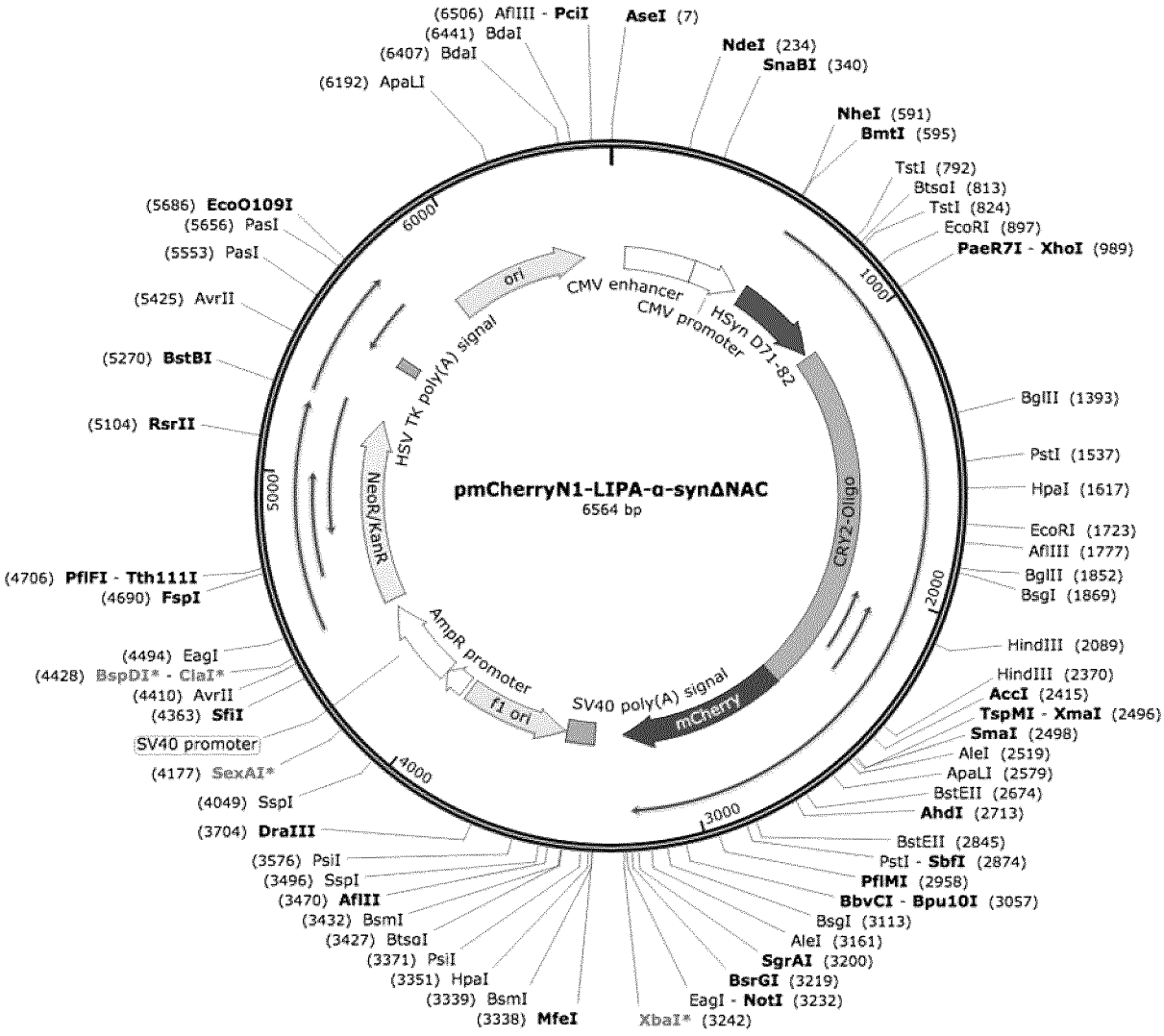

pmCherryN1-LIPA-α-synΔNAC
6564 bp
ori
CMV enhancer
CMV promoter
HSyn D71-82
CRY2-Oligo
mCherry
SV40 poly(A) signal
f1 ori
AmpR promoter
NeoR/KanR
HSV TK poly(A) signal
1000
2000
3000
4000
5000
6000
(6506) AflIII - PciI
AseI (7)
(6441) BdaI
(6407) BdaI
(6192) ApaLI
NdeI (234)
SnaBI (340)
NheI (591)
BmtI (595)
TstI (792)
BtsaI (813)
TstI (824)
EcoRI (897)
PaeR7I - XhoI (989)
(5686) EcoO109I
(5656) PasI
(5553) PasI
(5425) AvrII
(5270) BstBI
(5104) RsrII
BglII (1393)
PstI (1537)
HpaI (1617)
EcoRI (1723)
AflIII (1777)
BglII (1852)
BsgI (1869)
(4706) PflFI - Tth111I
(4690) FspI
(4494) EagI
(4428) BspDI* - ClaI*
(4410) AvrII
(4363) SfiI
SV40 promoter
(4177) SexAI*
(4049) SspI
(3704) DraIII
HindIII (2089)
HindIII (2370)
AccI (2415)
TspMI - XmaI (2496)
SmaI (2498)
AleI (2519)
ApaLI (2579)
BstEII (2674)
AhdI (2713)
BstEII (2845)
PstI - SbfI (2874)
PflMI (2958)
BbvCI - Bpu10I (3057)
BsgI (3113)
AleI (3161)
SgrAI (3200)
BsrGI (3219)
EagI - NotI (3232)
XbaI* (3242)
(3576) PsiI
(3496) SspI
(3470) AflII
(3432) BsmI
(3427) BtsaI
(3371) PsiI
(3351) HpaI
(3339) BsmI
(3338) MfeI

Fig. 45

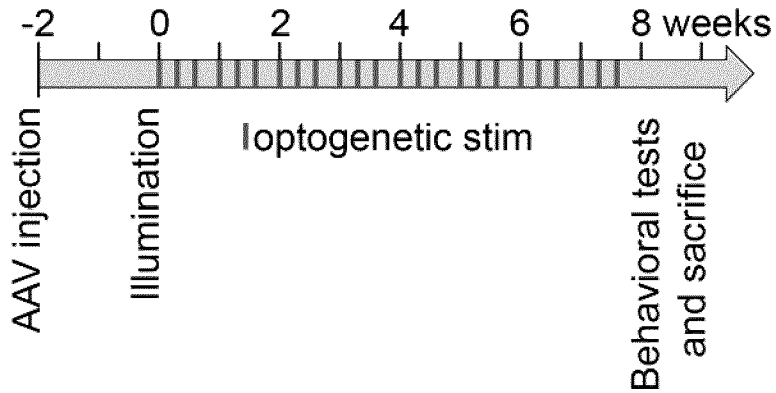

-2
0
2
4
6
8 weeks
AAV injection
Illumination
Optogenetic stim
Behavioral tests and sacrifice

Fig. 46

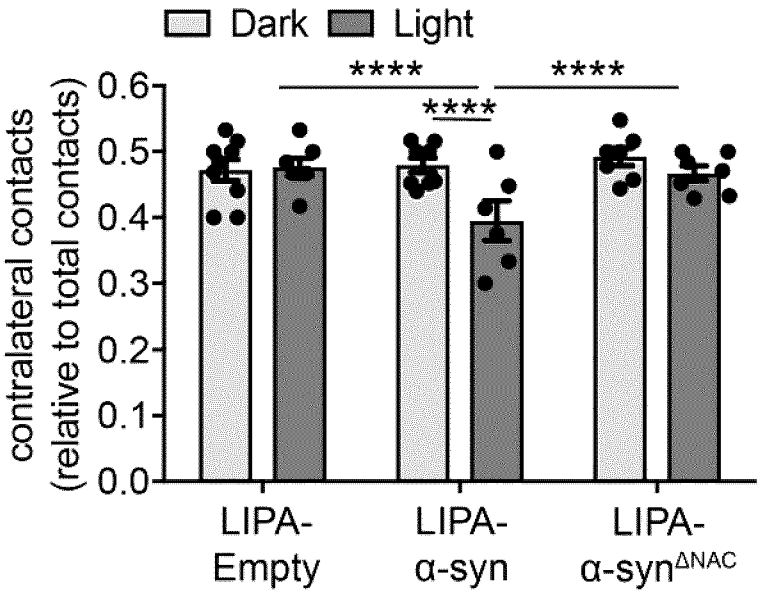

Dark
Light
****
****
****
contralateral contacts (relative to total contacts)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
LIPA-Empty
LIPA-α-syn
LIPA-α-synΔNAC

Fig. 47

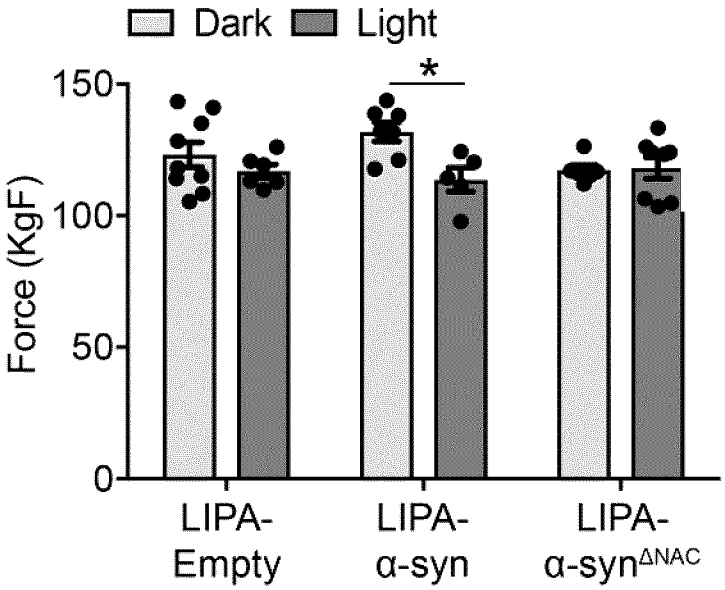

Dark
Light
*
150
100
50
0
Force (KgF)
LIPA-
Empty
LIPA-
α-syn
LIPA-
α-syn^ΔNAC

Fig. 48

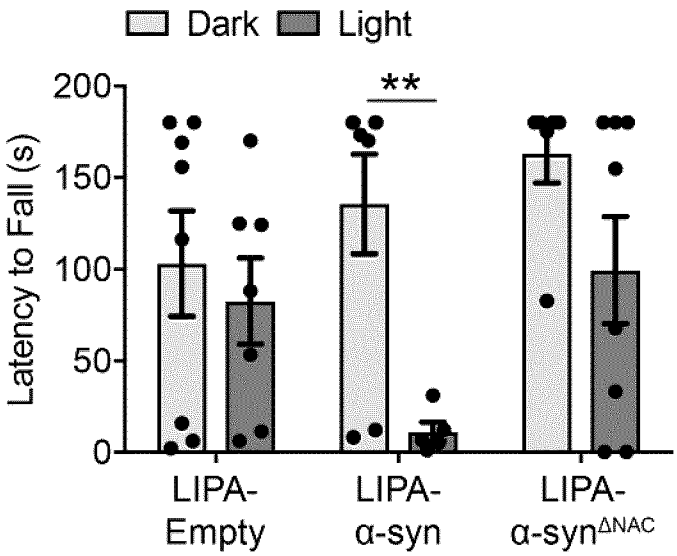

Dark
Light
**
200
150
100
50
0
Latency to Fall (s)
LIPA-
Empty
LIPA-
α-syn
LIPA-
α-syn^ΔNAC

Fig. 49

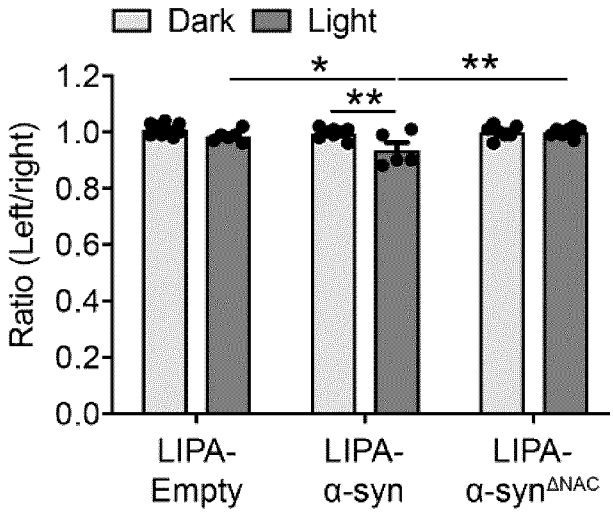

Dark
Light
*
**
**
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ratio (Left/right)
LIPA-Empty
LIPA-α-syn
LIPA-α-synΔNAC

Fig. 50

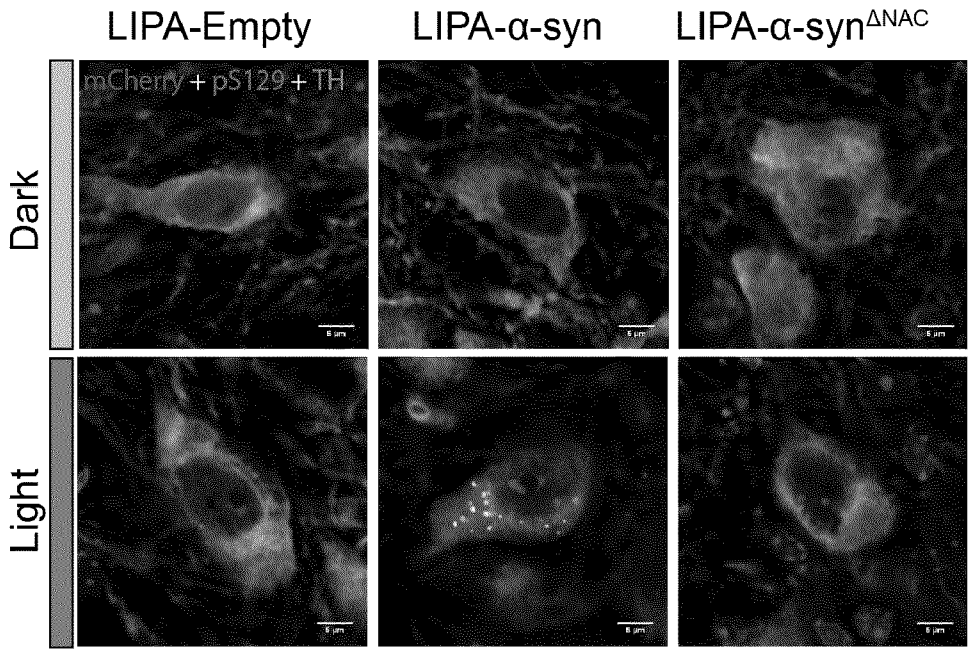

LIPA-Empty
LIPA-α-syn
LIPA-α-synΔNAC
mCherry + pS129 + TH
Dark
Light

Fig. 51

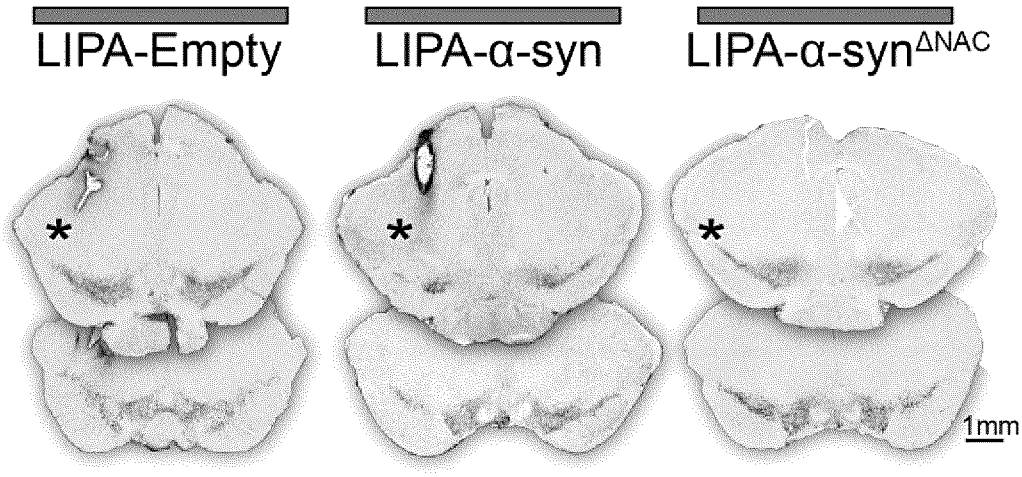

LIPA-Empty
LIPA-α-syn
LIPA-α-synΔNAC
*
*
*
1mm

Fig. 52

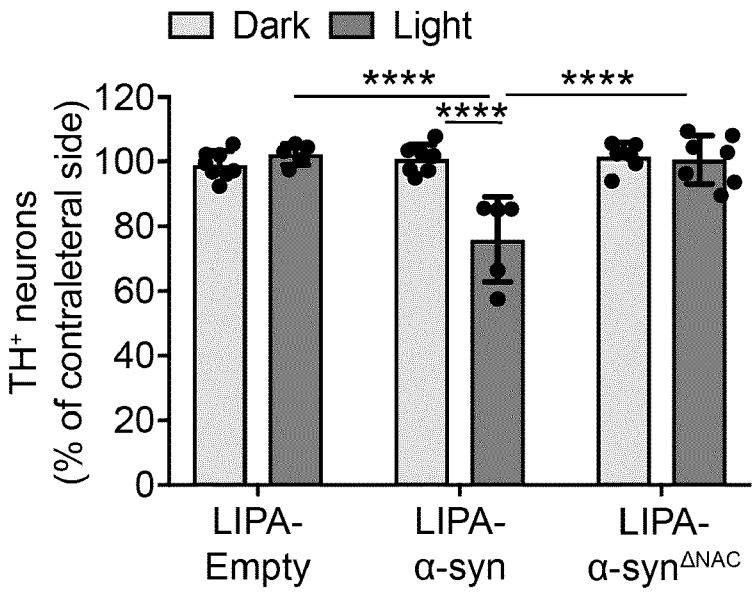

Dark
Light
****
****
****
TH+ neurons
(% of contraleteral side)
120
100
80
60
40
20
0
LIPA-
Empty
LIPA-
α-syn
LIPA-
α-synΔNAC

Fig. 53

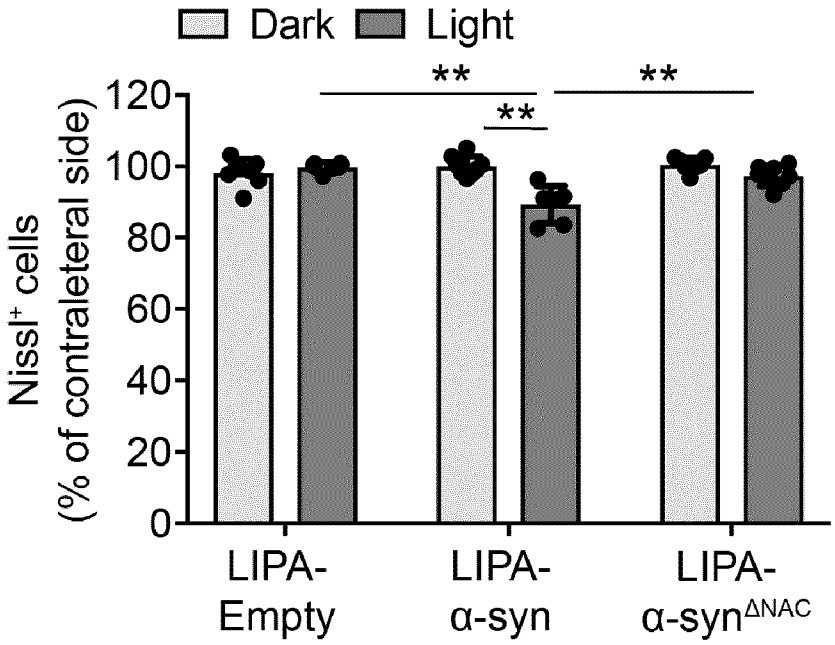

Dark
Light
**
**
**
Nissl+ cells
(% of contraleteral side)
120
100
80
60
40
20
0
LIPA-
Empty
LIPA-
α-syn
LIPA-
α-synΔNAC

Fig. 54A

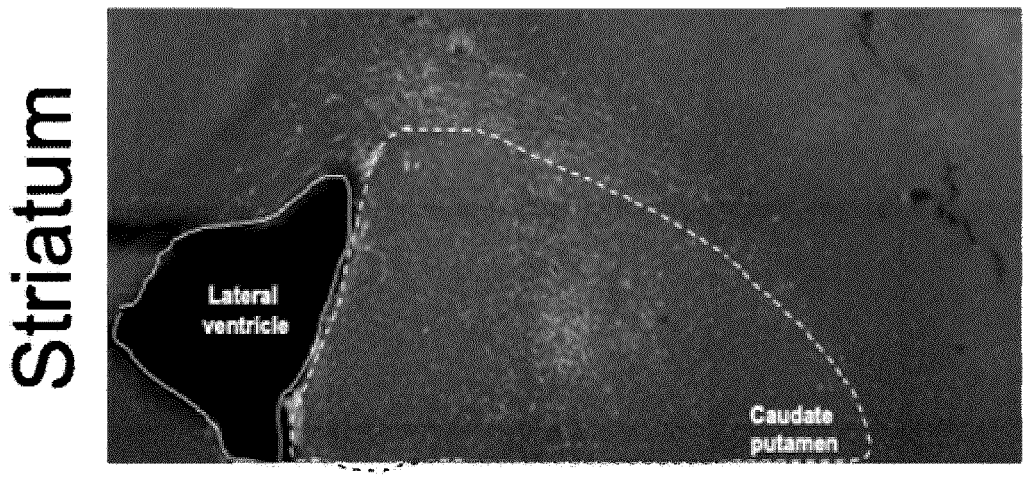


Fig. 54B

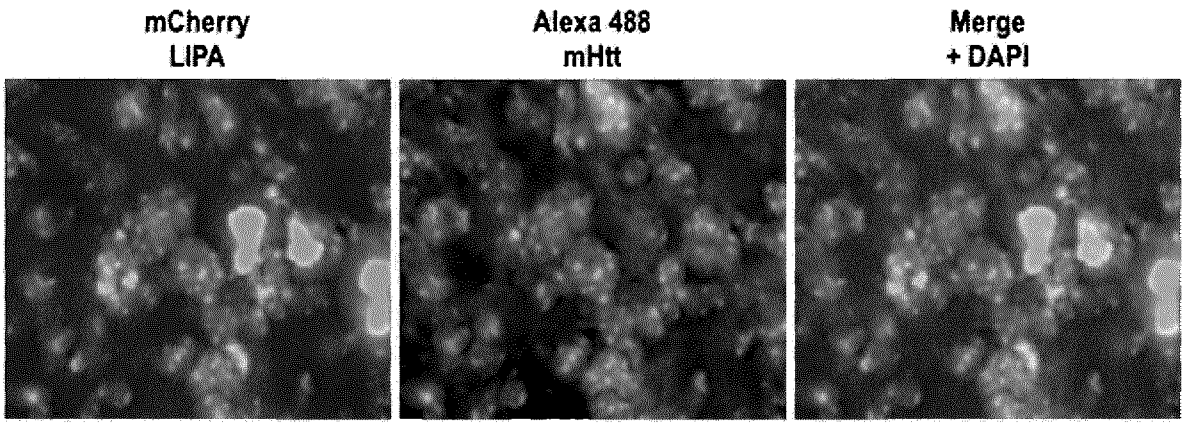


Fig. 54C

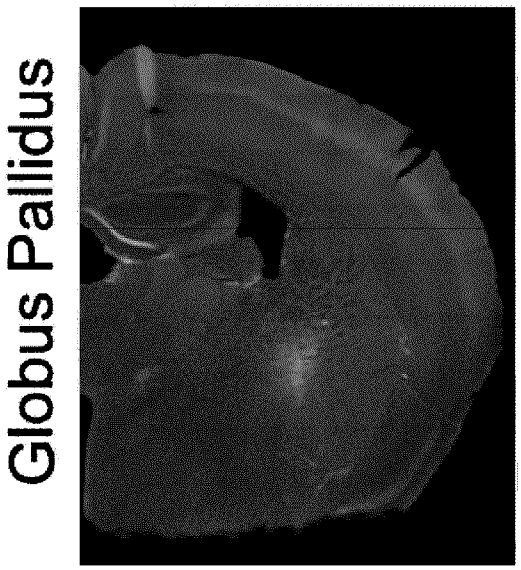


Fig. 54D

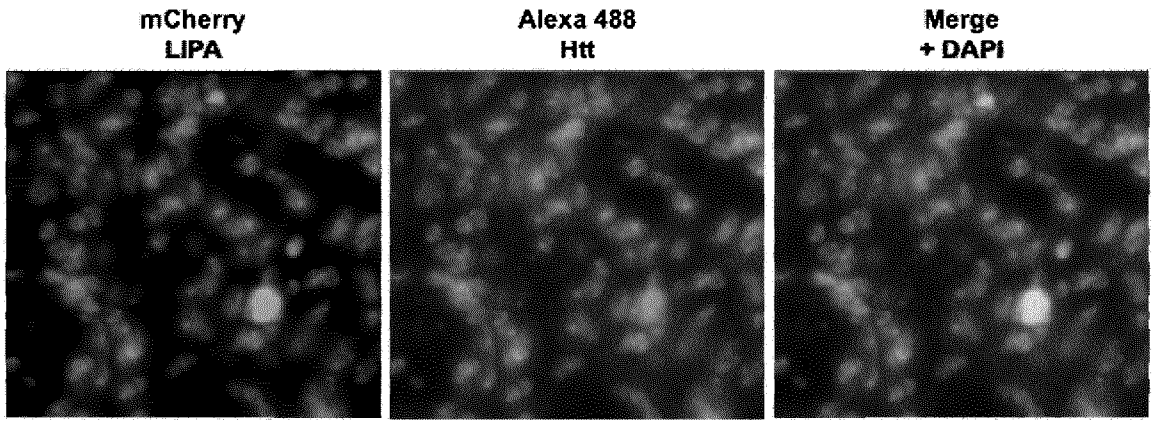


Fig. 54E

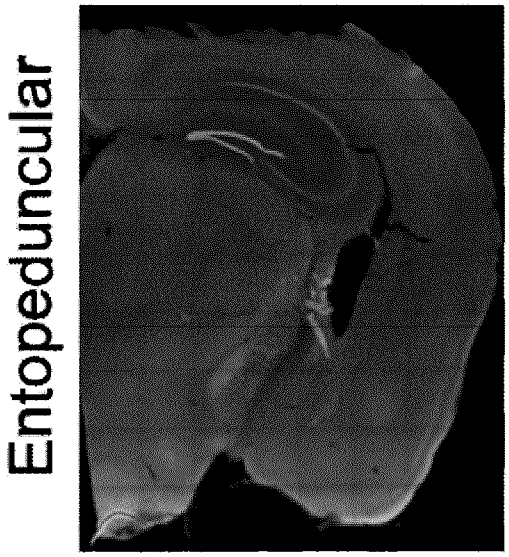
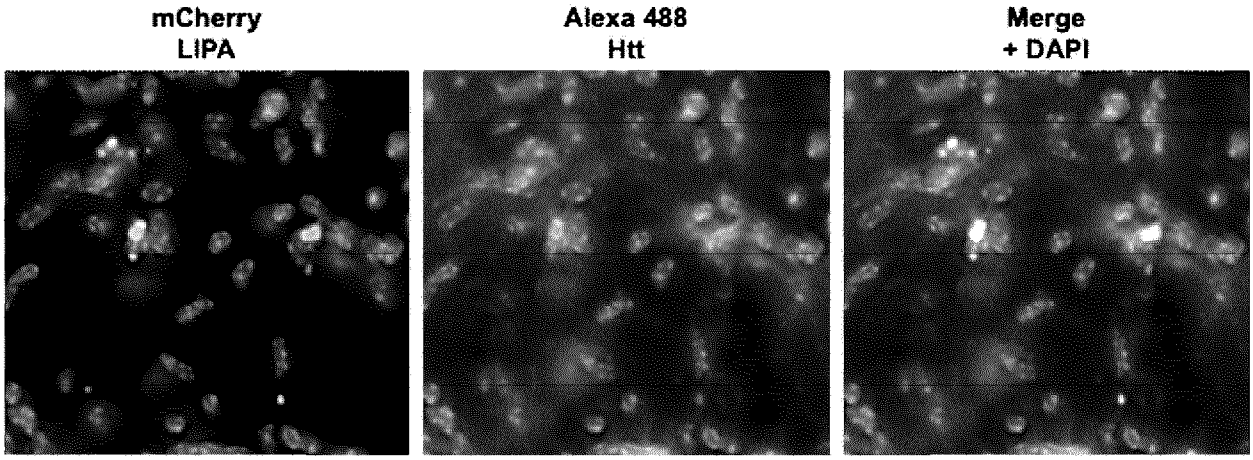


Fig. 54F



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/050914

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12N 15/63*** (2006.01) , ***A61K 38/16*** (2006.01) , ***A61K 45/00*** (2006.01) , ***C07K 1/00*** (2006.01) , ***C07K 14/415*** (2006.01) , ***C12N 15/29*** (2006.01) (more IPCs on the last page)

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)
Keywords searched over entire IPC

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, PubMed, Questel Orbit, Google
Keywords: optogenetics, synuclein, CRY, photoactive polypeptide, fusion, chimeric, neurodegenerative

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US2012/0237966 A1 (DOLMETSCH and YAZAWA) 20 September 2012 (20-09-2012) See abstract, [0052], [0053], [0056], [0062]-[0064], [0070]	1-11, 13-25 and 34-43
P,X	WO 2018/165293A1 (DONNELLY and MANN) 13 September 2018 (13-09-2018) See whole document	1-11 and 13-48
A	BENEDETTI, L. et al, "*Light-activated protein interaction with high spatial subcellular confinement*". PNAS, 20 February 2018 (20-02-2018), Vol. 115(10), pp. E2238-E2245,1091-6490	
A	TASLIMI, A. et al., "*An optimized optogenetic clustering tool for probing protein interaction and function*". Nature Communications, 18 March 2015 (18-03-2015), Vol. 5, pp. 4925, 2041-1723	

☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D" document cited by the applicant in the international application	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 07 August 2019 (07-08-2019)	Date of mailing of the international search report 10 September 2019 (10-09-2019)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Tera Mosher (819) 639-6843

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2019/050914

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Group A - Claims 1-11, 13-25, 28-45 and 48 (partially) and claims 26, 27, 46 and 47 (wholly)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Patent Document Cited in Search Report	Publication Date	Publication Family Member(s)	Publication Date
US2012/0237966 A1	20 September 2012 (20-09-2012)	WO2011041327A1	07 April 2011(07-04-2011)
WO2018/165293A1	13 September 2018 (13-09-2018)		

Continuation of Box III

Group A - Claims 1-11, 13-25, 28-45 and 48 (partially) and claims 26, 27, 46 and 47 (wholly) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing an alpha-synuclein polypeptide linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group B - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing Aβ precursor protein or Aβ peptide linked to a photoactive polypeptide;

Group C - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing a Tau protein linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group D - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing a prion polypeptide linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group E - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing an Ig light chain polypeptide linked to a photoactive polypeptide. Methods of using said system expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group F - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing serum amyloid A linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group G - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing transthyretin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group H - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing cystatin C linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group I - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing beta-2-microglobulin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group J - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing apolipoprotein AI or AII linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group K - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing gelsolin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group L - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing amylin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group M - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing calcitonin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group N - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing atrial natriuretic factor linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group O - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing lysozyme linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Continued on extra sheet II

C12N 15/85 (2006.01) , ***C12N 5/10*** (2006.01) , ***C12P 21/00*** (2006.01) , ***C12Q 1/00*** (2006.01) , ***C12Q 1/02*** (2006.01) , ***C12Q 1/6897*** (2018.01) , ***G01N 33/48*** (2006.01)

Continuation of Box III from extra sheet I

Group P - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing insulin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group Q - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing fibrinogen α-A chain linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group R - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing superoxide dismutase 1 linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group S - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing huntingtin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group T - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing an androgen receptor linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group U - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing an ataxin linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group V- Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing a TATA box-binding protein linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed;

Group W - Claims 1-25, 28-45 and 48 (partially) are directed to a light-inducible intracellular protein aggregation system, the system comprising a cell expressing TDP-43 linked to a photoactive polypeptide. Methods of using said system, expression cassettes, vectors, cells and animal models comprising said system are also claimed; and

Group X - Claims 49-54 (wholly) are directed to a variant alpha-synuclein polypeptide for use in preventing aggregation of a proteopathic polypeptide other than alpha-synuclein. An expression cassette encoding said variant alpha-synuclein polypeptide is also claimed.

The proteins defined in Groups A-X are not linked by a special technical feature. The claims must be limited to one inventive concept as set out in PCT Rule 13.