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(54) **AMYLOID BETA EXPRESSION CONSTRUCTS AND USES THEREFOR**

AMYLOID-BETA-EXPRESSION-KONSTRUKTE UND VERWENDUNGEN DAVON

CONSTRUCTIONS D'EXPRESSION DE LA PROTÉINE BÊTA-AMYLOÏDE ET LEURS UTILISATIONS

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Description

Technical Field

[0001] The invention relates to protein chemistry and cellular and molecular biology.

Background

[0002] Alzheimer's disease is a neurodegenerative disorder characterized by neurofibrillary tangles and plaques containing an amyloid beta peptide. Patients with Alzheimer's disease exhibit progressive dementia and personality dysfunction. Proteolytic cleavage of the amyloid precursor protein (APP) results in the generation of an amyloid beta peptide having a length ranging from 38 to 49 amino acids (e.g., 38-43 amino acids). The amyloid beta 1-42 peptide is particularly prone to self-aggregation and is strongly linked to development of Alzheimer's disease.

Summary

[0003] The invention is based, at least in part, on the discovery that a yeast expression construct encoding a polypeptide containing a signal sequence, a Golgi-directing pro sequence, and a human amyloid beta protein can result in significant toxicity when expressed in yeast cells. The invention is also based, at least in part, on the discovery that a mammalian expression construct encoding a polypeptide containing a selected signal sequence and a human amyloid beta protein can result in significant toxicity when expressed in mammalian cells. This discovery permits the carrying out of screening assays using amyloid beta-expressing cells to identify compounds or genetic factors that modulate amyloid beta-induced toxicity. Compounds identified by such screens can be used for the treatment or prevention of neurodegenerative diseases such as Alzheimer's disease.

[0004] Described herein is an expression construct comprising a promoter operably linked to a nucleic acid encoding a polypeptide comprising a signal sequence, a Golgi-directing pro sequence, and a human amyloid beta protein.

[0005] Also provided herein is an expression construct comprising a promoter operably linked to a nucleic acid encoding a polypeptide comprising a signal sequence, a Golgi-directing pro sequence, and a polypeptide having the formula $[X\text{-}Y]_n$, wherein each X is, independently, absent or is a linker; each Y is, independently, a human amyloid beta peptide; and n is an integer between two and eight, inclusive, wherein expression of the nucleic acid and production of the polypeptide in a yeast cell results in a decrease in growth or viability of the cell.

[0006] A signal sequence causes the polypeptide containing it to be targeted to the endoplasmic reticulum within a cell. In some embodiments, the signal sequence is located at the amino terminus of the polypeptide encoded by the expression construct. The signal sequence can be identical to a naturally occurring signal sequence or can be an artificial (non-naturally occurring) signal sequence. In some embodiments, the signal sequence is identical to the signal sequence of a naturally occurring yeast protein. In some embodiments, the signal sequence is the yeast mating factor alpha signal sequence. In some embodiments, the signal sequence is the signal sequence of yeast killer toxin K1, secreted acid phosphatase, invertase or sucrase, or protoplast secreted protein 1 (Pst1).

[0007] A Golgi-directing pro sequence causes the transport of the polypeptide containing it to the Golgi. The Golgi-directing pro sequence can be identical to a naturally occurring Golgi-directing pro sequence or can be an artificial (non-naturally occurring) Golgi-directing pro sequence. In some embodiments, the Golgi-directing pro sequence is identical to the Golgi-directing pro sequence of a naturally occurring yeast protein. In some embodiments, the Golgi-directing pro sequence is the yeast mating factor alpha pro sequence. In some embodiments, the Golgi-directing pro sequence is the pro sequence of yeast KEX2, carboxypeptidase Y, Pep4, or Prb1.

[0008] The terms "human amyloid beta protein" and "human amyloid beta peptide," as used interchangeably herein, include proteins whose amino acid sequences are identical to naturally occurring wild type amyloid beta peptides as well as naturally occurring mutant amyloid beta peptides. Wild type amyloid beta peptides include amyloid beta 1-38 (Aβ38), amyloid beta 1-39 (Aβ39), amyloid beta 1-40 (Aβ40), amyloid beta 1-41 (Aβ41), amyloid beta 1-42 (Aβ42), amyloid beta 1-43 (Aβ43), amyloid beta 1-48 (Aβ48), and amyloid beta 1-49 (Aβ49). Amyloid beta mutations include A2T, A2V, H6R, D7N, E11K, F20E, A21G, E22G, E22Q, E22K, E22 deletion, D23N, I31E, E22G/I31E, A42T, and A42V. These mutations may optionally be present in any of the amyloid beta peptides 1-38, 1-39, 1-40, 1-41, 1-42, 1-43, 1-48, and 1-49.

[0009] In alternate embodiments, a variant of a human amyloid beta protein can be used. A "variant human amyloid beta protein" differs (via substitution, deletion, and/or insertion) from a naturally occurring amyloid beta peptide at up to 10 amino acids (e.g., differs at no more than 5 amino acids, differs at no more than 4 amino acids, differs at no more than 3 amino acids, differs at no more than 2 amino acids, or differs at 1 amino acid) and retains the ability to cause a decrease in growth or viability of a cell when expressed in a fusion polypeptide described herein.

[0010] An expression construct described herein can optionally be integrated in the genome of the yeast cell. For

example, the expression construct can be an integrative plasmid such as pAG303, pAG304, pAG305, pAG306, pRS303, pRS304, pRS305, pRS306, or a derivative thereof.

[0011] An expression construct described herein can optionally be an episomal plasmid.

[0012] The promoter can be an inducible promoter such as GAL 1-10, GAL1, GALL, GALS, GPD, ADH, TEF, CYC1, MRP7, MET25, TET, VP16, or VP16-ER. Alternatively, the promoter can be a constitutively active promoter.

[0013] In some embodiments, the polypeptide encoded by an expression construct described herein can include a plurality of human amyloid beta peptides. The polypeptide encoded by an expression construct described herein can optionally contain two, three, four, five, six, seven, eight, or more human amyloid beta peptides. In some embodiments, the polypeptide includes at least two human amyloid beta peptides. In some embodiments, the polypeptide includes at least four human amyloid beta peptides. In some embodiments, each of the human amyloid beta peptides has the same amino acid sequence. In other embodiments, the polypeptide includes at least two human amyloid beta peptides having different amino acid sequences.

[0014] The polypeptide encoded by an expression construct described herein can optionally comprise or consist of the amino acid sequence of SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, or SEQ ID NO:101.

[0015] Also disclosed is a yeast cell comprising an expression construct described herein, wherein expression of the nucleic acid and production of the polypeptide in the cell results in a decrease in growth or viability of the cell. In some embodiments, expression of the nucleic acid and production of the polypeptide renders the cell non-viable.

[0016] A yeast cell can have one or more (e.g., at least two, at least three, or at least four) copies (e.g., integrated or episomal copies) of an expression construct.

[0017] In some embodiments, the yeast is Saccharomyces cerevisiae, Saccharomyces uvae, Saccharomyces kluyveri, Schizosaccharomyces pombe, Kluyveromyces lactis, Hansenula polymorpha, Pichia pastoris, Pichia methanolica, Pichia kluyveri, Yarrowia lipolytica, Candida sp., Candida utilis, Candida cacaoi, Geotrichum sp., or Geotrichum fermentans.

[0018] In some embodiments, at least one gene that encodes a protein involved in drug efflux or cell permeability is disrupted in the yeast cell. For example, one or more of the genes PDR1, PDR3, PDR5, SNQ2, or ERG6 can be disrupted in the yeast cell.

[0019] Also disclosed is an expression construct comprising a promoter operably linked to a nucleic acid encoding a polypeptide comprising a signal sequence and a human amyloid beta protein, wherein the signal sequence is an insulin signal sequence (e.g., the human insulin signal sequence) or a trypsin signal sequence (e.g., the human trypsin signal sequence). In some embodiments, the human amyloid beta protein is fused directly to the signal sequence.

[0020] The invention further provides an expression construct comprising a promoter operably linked to a nucleic acid encoding a polypeptide comprising a signal sequence and a polypeptide having the formula $[X\text{-}Y]_n$, wherein each X is, independently, absent or is a linker; each Y is, independently, a human amyloid beta peptide; and n is an integer between two and eight, inclusive, wherein the signal sequence is an insulin signal sequence (e.g., the human insulin signal sequence) or a trypsin signal sequence (e.g., the human trypsin signal sequence), wherein expression of the nucleic acid and production of the polypeptide in a yeast cell results in a decrease in growth or viability of the cell.

[0021] The polypeptide encoded by an expression construct described herein can optionally comprise or consist of the amino acid sequence of SEQ ID NO:93, SEQ ID NO:95, or SEQ ID NO:103.

[0022] In some embodiments, the expression construct is a plasmid or viral vector.

[0023] Also disclosed is a mammalian cell comprising an expression construct comprising a promoter operably linked to a nucleic acid encoding a polypeptide comprising a signal sequence and a human amyloid beta protein, wherein the signal sequence is an insulin signal sequence (e.g., the human insulin signal sequence) or a trypsin signal sequence (e.g., the human trypsin signal sequence), wherein expression of the nucleic acid and production of the polypeptide in the cell results in a decrease in growth or viability of the cell. Expression of the nucleic acid and production of the polypeptide may render the cell non-viable. The cell may be a neuronal cell. The cell may also be a 293T cell.

[0024] Also disclosed is a method of inducing toxicity in a cell by: providing a yeast or mammalian cell described herein; and allowing a level of inductive or constitutive expression of the nucleic acid in the cell that is toxic to the cell.

[0025] Also disclosed is a method of identifying a compound that prevents or suppresses amyloid beta-induced toxicity by: culturing a yeast or mammalian cell described herein in the presence of a candidate agent and under conditions that allow for expression of the nucleic acid at a level that, in the absence of the candidate agent, is sufficient to induce toxicity in the cell; measuring cell growth or viability in the presence of the candidate agent; and comparing cell growth or viability measured in the presence of the candidate agent to cell growth or viability in the absence of the candidate agent, wherein if cell growth or viability is increased in the presence of the candidate agent as compared to in the absence of the candidate agent, then the candidate agent is identified as a compound that prevents or suppresses amyloid beta-induced toxicity.

[0026] Also disclosed is a method of identifying a genetic suppressor or enhancer of amyloid beta-induced toxicity by: providing a yeast or mammalian cell described herein, wherein the cell has been genetically modified to overexpress a

gene; culturing the cell under conditions that allow for expression of the protein at a level that, in the absence of overexpression of the gene, is sufficient to induce toxicity in the cell; measuring cell growth or viability in the presence of overexpression of the gene; and comparing cell growth or viability measured in the presence of overexpression of the gene to cell growth or viability in the absence of overexpression of the gene, wherein (i) if cell growth or viability is increased in the presence of overexpression of the gene as compared to in the absence of overexpression of the gene, then the gene is identified as a genetic suppressor of amyloid beta-induced toxicity, and (ii) if cell growth or viability is decreased in the presence of overexpression of the gene as compared to in the absence of overexpression of the gene, then the gene is identified as a genetic enhancer of amyloid beta-induced toxicity.

[0027] Also disclosed is a method of identifying a genetic suppressor or enhancer of amyloid beta-induced toxicity by: providing a yeast or mammalian cell described herein, wherein an endogenous gene of the cell has been disrupted; culturing the cell under conditions that allow for expression of the protein at a level that, in the absence of disruption of the endogenous gene, is sufficient to induce toxicity in the cell; measuring cell growth or viability in the presence of disruption of the endogenous gene; and comparing cell growth or viability measured in the presence of disruption of the endogenous gene to cell growth or viability in the absence of disruption of the endogenous gene, wherein (i) if cell growth or viability is increased in the presence of disruption of the endogenous gene as compared to in the absence of disruption of the endogenous gene, then the gene is identified as a genetic enhancer of amyloid beta-induced toxicity, and (ii) if cell growth or viability is decreased in the presence of disruption of the endogenous gene as compared to in the absence of disruption of the endogenous gene, then the gene is identified as a genetic suppressor of amyloid beta-induced toxicity.

[0028] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application, including definitions, will control.

[0029] Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

[0030]

Fig. 1A depicts the yeast Aβ42 expression construct "Matα-Aβ42_4X", which contains four copies of Aβ42 in a single construct.

Fig. 1B depicts yeast expression constructs containing varying numbers of copies of Aβ42 or Aβ40.

Fig. 2 depicts the map of the "pAG425Gal-Matβ_4X" expression vector.

Fig. 3 is a series of photographs depicting yeast toxicity resulting from expression of constructs containing varying numbers of copies of Aβ42 or Aβ40.

Fig. 4 is a series of photographs depicting yeast toxicity resulting from expression of constructs containing four copies of various Aβ42 mutants.

Fig. 5 depicts mammalian Aβ42 expression constructs containing the signal sequences of human insulin (Ins), human trypsin (Tryp), human amyloid precursor protein (APP), human placental secreted alkaline phosphatase (SEAP), or Metridia luciferase (mLuc) of the marine copepod *Metridia longa* fused to Aβ42.

Fig. 6 depicts the map of the "pCMV-Ins-Abeta" expression vector.

Fig. 7A-7B are graphs depicting the amount of human Aβ42 present in the cell lysate and supernatant of 293T cells 48 hours (Fig. 7A) and 72 hours (Fig. 7B) after transfection with mammalian expression constructs containing the signal sequences of human insulin (Ins), human trypsin (Tryp), human amyloid precursor protein (APP), human placental secreted alkaline phosphatase (SEAP), or Metridia luciferase (mLuc) of the marine copepod *Metridia longa* fused to Aβ42.

Fig. 8 is a graph depicting the presence of adenylate kinase (an indicator of cell damage/death) in the supernatant of transfected 293T cells 48 hours following transfection with constructs expressing Aβ42 or Aβ40 fused to the insulin signal peptide or a vector control without an inserted transgene.

Fig. 9 depicts the map of the "pLV-hSyn-Abeta_spIns" expression vector.

Fig. 10A is a graph depicting the amount of human Aβ42 or Aβ40 present in the cell lysate and supernatant of rat embryonic cortical neurons after infection with lentiviral constructs expressing human Aβ42 or Aβ40.

Fig. 10B is a graph depicting the cytotoxicity of rat embryonic cortical neurons, as determined by measuring ATP levels, after infection with lentiviral constructs expressing human Aβ42 or Aβ40.

Fig. 11A is a series of images of live rat cortical neurons infected with a GFP expression construct and the indicated lentiviral Aβ construct. Cells infected with only the GFP expression construct served as a control ("GFP only"). The top panel depicts a schematic of the expression constructs and the experimental protocol.

Fig. 11B is a series of images depicting Map2-stained rat cortical neurons infected with the indicated lentiviral Aβ construct or with GFP ("GFP only"). The top panel depicts a schematic of the expression constructs and the exper-

imental protocol.

Fig. 12 depicts the map of the poly-Aβ42 "pLIX402-Ins-Aβ42-4X" expression vector, which contains four copies of Aβ42 linked by 2 amino acid linkers. The poly-Aβ is linked at the N-terminus to the insulin signal peptide. Expression of the transgene is driven by a tetracycline (doxycycline)-inducible promoter. Similar expression vectors were generated for Aβ40-4X, Aβ40-6x, Aβ40-8X, Aβ42_Arctic-4X (also referred to as "4X_Arc"), Aβ42-6X, and Aβ42-8X.

Fig. 13A-13D show that poly-Aβ42 expression causes cytotoxicity in mammalian cells. Fig. 13A depicts a doxycycline-inducible poly-Aβ42 expression construct containing the signal sequence of human insulin (Ins). Fig. 13B depicts mammalian poly-Aβ expression constructs. Fig. 13C is a graph depicting the cytotoxic effect of the indicated poly-Aβ expression construct upon doxycycline-induced expression in 293T cells. Fig. 13D is a graph depicting the amount of Aβ42 present in the cell supernatant of 293T cells 96 hours post-induction.

Fig. 14 shows that poly-Aβ expression is toxic in rat cortical neurons. The top panel shows a schematic of the experimental protocol. The bottom panel is a graph depicting the cytotoxicity of rat embryonic cortical neurons, as determined by measuring ATP levels, following infection with lentiviral constructs expressing the indicated poly-Aβ42 or poly-Aβ40 expression construct.

Fig. 15 is a graph depicting the effect of clioquinol on Aβ-mediated cytotoxicity as determined by measuring adenylate kinase released from the damaged cells.

Fig. 16A depicts yeast poly-Aβ-4X (4 tandem Aβ peptides) expression constructs.

Fig. 16B is a series of photographs depicting yeast toxicity resulting from expression of the indicated poly-Aβ-4X expression construct and a graph depicting the quantification of the growth. Glc, glucose; Gal, galactose. *** indicates P-value $\leq$ 0.001.

Fig. 17A-17C depicts a comparison of Aβ43-mediated toxicity to toxicity caused by other naturally-occurring Aβ peptide fragments in mammalian cells (293T). Fig. 17A shows a CMV promoter driven expression system for Aβ peptide in mammalian models and a cytotoxic end-point measurement. Fig. 17B depicts mammalian expression constructs for different Aβ peptides. Fig. 17C is a graph depicting cytotoxicity in 293T cells caused by expression of the indicated Aβ peptides.

Detailed Description

[0031] The expression constructs and amyloid beta-expressing cells described herein can be used to identify compounds or genetic factors that modulate amyloid beta-induced toxicity. Compounds identified by such screens can be used for the treatment or prevention of neurodegenerative diseases such as Alzheimer's disease.

Proteins and Nucleic Acids

[0032] Described herein are compositions and methods for identifying candidate compounds that prevent or suppress amyloid beta-induced toxicity and genetic suppressors or enhancers of amyloid beta-induced toxicity. A yeast expression construct used in the compositions and methods described herein contains a signal sequence, a Golgi-directing pro sequence, and a human amyloid beta protein (i.e., a human amyloid beta peptide). A mammalian expression construct used in the compositions and methods described herein contains a selected signal sequence and a human amyloid beta protein.

[0033] Described herein is a nucleic acid encoding a polypeptide comprising a signal sequence, a Golgi-directing pro sequence, and a polypeptide having the formula $[X\text{-}Y]_n$, wherein each X is, independently, absent or is a linker; each Y is, independently, a human amyloid beta peptide; and n is an integer between two and twenty, inclusive.

[0034] Further described herein is a nucleic acid encoding a polypeptide comprising a signal sequence and a polypeptide having the formula $[X\text{-}Y]_n$, wherein each X is, independently, absent or is a linker; each Y is, independently, a human amyloid beta peptide; and n is an integer between two and twenty, inclusive, wherein the signal sequence is an insulin signal sequence (e.g., the human insulin signal sequence) or a trypsin signal sequence (e.g., the human trypsin signal sequence).

[0035] "Human amyloid beta" refers to an amino acid sequence identical to human amyloid beta peptide that is derived through proteolytic processing of the human amyloid precursor protein (APP) and is associated with amyloid pathologies. The term amyloid beta (Aβ) includes naturally occurring wild type amyloid beta peptides as well as naturally occurring mutant amyloid beta peptides. The term "human amyloid beta protein" as used herein encompasses proteins produced synthetically by recombinant methods. Wild type amyloid beta peptides include Aβ38, Aβ39, Aβ40, Aβ41, Aβ42, Aβ43, Aβ48, and Aβ49, wherein the number next to Aβ denotes the length of the peptide defined by the number of amino acids after proteolytic processing from the amyloid precursor protein. Amyloid beta mutations include A2T, A2V, H6R (English), D7N (Tottori), E1 1K (Leuven), F20E, A21G (Flemish), E22G (Arctic), E22Q (Dutch), E22K (Italian), E22 deletion, D23N (Iowa), I31E, E22G/I3IE, A42T, and A42V. In these mutations the first letter represents the naturally occurring amino acid, the middle number represents the position of the amino acid in the context of the Aβ peptide (1-42) and the last

letter following the numbered position represents the amino acid to which the wild type peptide is mutated. The mutation "E22 deletion" represents an Aβ peptide where amino acid glutamic acid (E) at position 22 is deleted and E22G/I31E represents Aβ peptide in which both amino acids glutamic acid (E) and isoleucine (I) are replaced by glycine (G) and glutamic acid (E), respectively. These mutations may be present in any of the naturally occurring amyloid beta peptides Aβ38, Aβ39, Aβ40, Aβ41, Aβ42, Aβ43, Aβ48, and Aβ49, or their synthetic derivatives.

[0036] Amino acids 1-43 of human amyloid beta, which amino acids are used as the backbone of the amyloid beta peptides described herein, are as follows: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAT (SEQ ID NO:1). In other embodiments, amino acids 1-48 of human amyloid beta are used as the backbone of amyloid beta peptides described herein. Amino acids 1-48 of human amyloid beta are as follows: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVIT (SEQ ID NO:104). In yet other embodiments, amino acids 1-49 of human amyloid beta are used as the backbone of amyloid beta peptides described herein. Amino acids 1-49 of human amyloid beta are as follows: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITL (SEQ ID NO:105).

[0037] As used herein, the term "signal sequence" refers to a peptide sequence that is present within a polypeptide and causes the polypeptide to be targeted to the endoplasmic reticulum within a cell. Exemplary signal sequences described in the working examples are the yeast mating factor alpha signal sequence (for a yeast expression construct) and the human insulin and human trypsin signal sequences (for mammalian expression constructs). Examples of additional signal sequences that can be used in a yeast expression construct include the signal sequences of killer toxin K1, secreted acid phosphatase, invertase (sucrase), and Pst1. Examples of additional signal sequences that can be used in a mammalian expression construct include the signal sequences of human serum albumin, orexin, human preproparathyroid hormone, somatostatin, shadoo, brain-derived neurotrophic factor, neuropeptide Y, vasoactive intestinal peptide, enkephalin, cholecystokinin, neurotensin, pro-thyrotropin-releasing hormone, neuropeptide W, somatoliberin, and somatotropin. Signal sequences are reviewed in e.g., Wilkinson et al. (1997) J Membr Biol. 155(3):189-97, Haguenauer-Tsapis (1992) Mol Microbiol. 6(5):573-9, and Pool (2005) Mol Membr Biol. 22(1-2):3-15.

[0038] A polypeptide containing a signal sequence and a human amyloid beta protein may optionally contain one or more heterologous sequences. The heterologous sequence of the fusion protein can optionally be a linker, an immunoglobulin element, a dimerizing domain, a targeting domain, a stabilizing domain, or a purification domain. Alternatively, an amyloid beta protein can be fused with a heterologous sequence such as a detection protein. Exemplary detection proteins include: a fluorescent protein such as green fluorescent protein (GFP), cyan fluorescent protein (CFP) or yellow fluorescent protein (YFP); an enzyme such as β-galactosidase or alkaline phosphatase (AP); and an epitope such as glutathione-S-transferase (GST) or hemagglutinin (HA). To illustrate, an amyloid beta protein can be fused to GFP at the N- or C-terminus or other parts of the amyloid beta protein. These fusion proteins provide methods for rapid and easy detection and identification of the amyloid beta protein in the recombinant yeast cell.

[0039] The term "linker," as used herein, refers to a sequence of amino acids linking two polypeptide moieties. Exemplary, non-limiting functions of a linker can include introduction of a flexible component or space-creating region between two protein domains, introduction of proteolytic processing sites (e.g., proteolytic cleavage sites), or creation of an affinity tag. A linker may be any suitable length, for example, a linker may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, or more amino acids. In some embodiments, a linker is a spacer peptide as described herein. In some embodiments, the spacer peptide is from the yeast mating factor alpha 1 gene. For example, in some embodiments, a linker may include an amino acid sequence selected from SEQ ID NOs:44-49 or a fragment thereof.

[0040] In some embodiments, a polypeptide containing a signal sequence and a human amyloid beta peptide may include a plurality of human amyloid beta peptides. In some embodiments, a polypeptide containing a signal sequence, a Golgi-directing pro sequence, and a human amyloid beta peptide may include a plurality of human amyloid beta peptides. The polypeptide encoded by an expression construct described herein can optionally contain two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, or more human amyloid beta peptides. In some embodiments, the polypeptide includes at least two human amyloid beta peptides. In some embodiments, the polypeptide includes at least four human amyloid beta peptides. In some embodiments, each of the human amyloid beta peptides includes the same amino acid sequence. For instance, in some embodiments, each of the human amyloid beta peptides is Aβ42. In other embodiments, the polypeptide includes at least two human amyloid beta peptides having different amino acid sequences. For instance, in some embodiments, the polypeptide includes Aβ42 and Aβ43.

[0041] Also described herein are methods of preparing and transferring nucleic acids encoding an amyloid beta protein into a cell so that the cell expresses the amyloid beta protein. The term "amyloid beta nucleic acid" encompasses a nucleic acid containing a sequence encoding any of the amyloid beta proteins described herein. Exemplary amyloid beta nucleic acids include those encoding Aβ42 or Aβ43.

[0042] The term "nucleic acid" generally refers to at least one molecule or strand of DNA, RNA or a derivative or mimic thereof, containing at least one nucleobase, for example, a naturally occurring purine or pyrimidine base found in DNA or RNA. Generally, the term "nucleic acid" refers to at least one single-stranded molecule, but in specific embodiments will also encompass at least one additional strand that is partially, substantially or fully complementary to the at least one single-stranded molecule. Thus, a nucleic acid may encompass at least one double-stranded molecule or at least

one triple-stranded molecule that comprises one or more complementary strand(s) or "complement(s)" of a particular sequence comprising a strand of the molecule.

[0043] The working examples describe the use of several exemplary yeast expression constructs. The following are several additional exemplary yeast expression constructs.

Matα-Aβ342_4X_NC (Not Cleaved, Prepro and 4 copies of Aβ are linked) Nucleotide Sequence (SEQ ID NO:2)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTTTCTTTGGACGGTTCTGCTGGTTCTGGTG
ATGCAGAATTCAGACATGATTCTGGTTACGAAGTTCACCACCAAAAGTTGGTT
TTTTTCGCTGAAGATGTCGGTTCTAACAAGGGTGCTATTATTGGTTTGATGGTT
GGTGGTGTAGTTATTGCTGGTTCAGCAGGTTCAGCTGGTAGTGACGCCGAATT

CAGACACGATAGTGGTTATGAAGTCCATCATCAAAAATTAGTTTTCTTTGCCGA
AGATGTTGGTAGTAACAAAGGTGCAATCATCGGTTTAATGGTCGGTGGTGTCG
TAATAGCAGGTAGTGCAGGTTCCGCCGGTTCTGATGCCGAATTCAGACACGAC
TCCGGTTATGAAGTACATCACCAAAAGTTGGTATTCTTCGCAGAAGATGTAGG
TTCAAACAAAGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCGCAG
GTTCTGCCGGTAGTGCTGGTTCAGACGCAGAATTCAGACATGACAGTGGTTAC
GAAGTACACCATCAAAAATTAGTCTTTTTCGCAGAAGATGTTGGTAGTAACAA
GGGTGCTATAATAGGTTTGATGGTCGGTGGTGTAGTCATAGCTTAAACCCAGC
TTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_NC (Not Cleaved, Prepro and 4 copies of Aβ are linked) Amino Acid Sequence (SEQ ID NO:3)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDGSAGSGDAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_Clinked (prepro cleaved, Aβ linked) Nucleotide Sequence (SEQ ID NO:4)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTGGTTCTGCAGGTTCAGCTGGTTCAGATGCCGAATT

CAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCG
AAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTC
GTAATAGCAGGTAGTGCCGGTTCTGCTGGTAGTGATGCAGAATTCAGACACGA
CTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAAGATGTCG
GTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCGCC
GGTAGTGCTGGTAGTGCAGGTTCCGACGCCGAATTCAGACATGACTCAGGTTA
CGAAGTACACCATCAAAAGTTGGTATTTTTCGCAGAAGATGTAGGTTCCAACA
AAGGTGCAATCATAGGTTTGATGGTTGGTGGTGTCGTAATTGCCTAAACCCAG
CTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Clinked (prepro cleaved, Aβ linked) Amino Acid Sequence (SEQ ID NO:5)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_Clinked2 Nucleotide Sequence (SEQ ID NO:6)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTGGTTCTGCAGGTTCAGCTGGTTCAGATGCCGAATT
CAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCG

AAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTC
GTAATCGCTAAAAGAGAAGCCGACGCTGAAGCTGATGCCGAATTCAGACATG
ACTCAGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAAGATGTC
GGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATAGC
AGGTAGTGCTGGTTCCGCTGGTAGTGATGCAGAATTCAGACATGACAGTGGTT
ATGAAGTCCACCATCAAAAATTGGTCTTTTTCGCAGAAGATGTAGGTTCCAAC
AAAGGTGCAATCATAGGTTTGATGGTTGGTGGTGTCGTAATTGCCTAAACCCA
GCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Clinked2 Amino Acid Sequence (SEQ ID NO:7)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAGSAGSAGSDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ40_1X Nucleotide Sequence (SEQ ID NO:8)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATT
AGCTGCTCCAGTCAACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTG
AAGCTGTCATCGGTTACTTAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGC
CATTTTCCAACAGCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCA
GCATTGCTGCTAAAGAAGAAGGGGTATCTTTGGATAAAAGAGAGGCTGAAGC
TGATGCTGAATTTAGACATGATTCTGGTTATGAAGTTCATCATCAAAAATTGGT
TTTTTTTGCTGAAGATGTTGGTTCTAATAAAGGTGCTATTATTGGTTTGATGGTT
GGTGGTGTTGTCTAAACCCAGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCG
AG

Matα-Aβ40_1X Amino Acid Sequence (SEQ ID NO:9)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVV

Matα-Aβ40_2X Nucleotide Sequence (SEQ ID NO: 10)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTAAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAATTCAGA
CACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCGAAGA
TGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTCGTTTG
AACCCAGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ40_2X Amino Acid Sequence (SEQ ID NO:11)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVKREAEAEADAEFRHDSGYEVHHQKLVFFAEDVGS
NKGAIIGLMVGGVV

Matα-Aβ42_2X- Aβ40_2X Nucleotide Sequence (SEQ ID NO: 12)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC

CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCG
AAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTC
GTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACG
ACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAAGATGTC
GGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTAAGAG
AGAAGCTGACGCTGAAGCAGACGCCGAATTCAGACACGACTCAGGTTATGAA
GTACACCATCAAAAATTGGTATTTTTCGCAGAAGATGTTGGTTCCAACAAAGG
TGCCATTATTGGTTTGATGGTTGGTGGTGTCGTTTAAACCCAGCTTTCTTGTAC
AAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_2X- Aβ40_2X Amino Acid Sequence (SEQ ID NO: 13)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL
MVGGVV

Matα-Aβ42_2X- Aβ40scr_2X, Nucleotide Sequence (SEQ ID NO:14)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA

GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCG
AAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTC
GTAATAGCCAAGAGAGAAGCAGACGCCGAAGCTGCTATTGCTGAAGGTGATT
CACATGTTTTGAAAGAAGGTGCCTACATGGAAATCTTCGATGTTCAAGGTCAT
GTTTTCGGTGGTAAGATCTTCAGAGTTGTTGATTTGGGTTCCCACAACAAAAGA
GAAGCTGACGCAGAAGCCGCAATAGCCGAAGGTGACTCTCACGTCTTAAAAG
AAGGTGCTTATATGGAAATTTTTGACGTCCAAGGTCACGTCTTTGGTGGTAAG
ATTTTTAGAGTAGTCGACTTGGGTAGTCATAACTAAACCCAGCTTTCTTGTACA
AAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_2X- Aβ40scr_2X, Amino Acid Sequence (SEQ ID NO:15)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAKREADAEAAIAEGDSHVLKEGAYMEIFDVQGHVFGG
KIFRVVDLGSHNKREADAEAAIAEGDSHVLKEGAYMEIFDVQGHVFGGKIFRVVD
LGSHN

[0044] The working examples describe the use of several exemplary mammalian expression constructs. The following are several additional exemplary mammalian expression constructs.

Ins-Aβ42_Arc_1X Nucleotide Sequence (SEQ ID NO:16)

CACCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCC
CTCTGGGGACCTGACCCAGCCGCAGCCGATGCGGAATTTCGCCATGATTCTGG
CTATGAAGTGCATCATCAGAAACTGGTGTTTTTTGCGGGAGATGTGGGCTCTA
ACAAAGGCGCGATTATTGGCCTGATGGTGGGCGGCGTGGTGATTGCGTAA

Ins-Aβ42_Arc_1X Amino Acid Sequence (SEQ ID NO:17)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAGDV
GSNKGAIIGLMVGGVVIA

Ins-Aβ42_F20E_1X Nucleotide Sequence (SEQ ID NO:18)

CACCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCC
CTCTGGGGACCTGACCCAGCCGCAGCCGATGCGGAATTTCGCCATGATTCTGG
CTATGAAGTGCATCATCAGAAACTGGTGTTTGAGGCGGAAGATGTGGGCTCTA
ACAAAGGCGCGATTATTGGCCTGATGGTGGGCGGCGTGGTGATTGCGTAA

Ins-Aβ42_F20E_1X Amino Acid Sequence (SEQ ID NO:19)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFEAEDV
GSNKGAIIGLMVGGVVIA

Ins-Aβ42_I31E_1X Nucleotide Sequence (SEQ ID NO:20)

CACCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCC
CTCTGGGGACCTGACCCAGCCGCAGCCGATGCGGAATTTCGCCATGATTCTGG
CTATGAAGTGCATCATCAGAAACTGGTGTTTTTTGCGGAAGATGTGGGCTCTA
ACAAAGGCGCGGAGATTGGCCTGATGGTGGGCGGCGTGGTGATTGCGTAA

Ins-Aβ42_I31E_1X Amino Acid Sequence (SEQ ID NO:21)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAEIGLMVGGVVIA

Ins-Aβ42_Arc_I31E_1X Nucleotide Sequence (SEQ ID NO:22)

CACCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCC
CTCTGGGGACCTGACCCAGCCGCAGCCGATGCGGAATTTCGCCATGATTCTGG
CTATGAAGTGCATCATCAGAAACTGGTGTTTTTTGCGGGAGATGTGGGCTCTA
ACAAAGGCGCGGAGATTGGCCTGATGGTGGGCGGCGTGGTGATTGCGTAA

Ins-Aβ42_Arc_I31E_1X Amino Acid Sequence (SEQ ID NO:23)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAGDV
GSNKGAEIGLMVGGVVIA

Tryp-Aβ40_1X Nucleotide Sequence (SEQ ID NO:24)

CACCATGTCTGCACTTCTGATCCTAGCTCTTGTTGGAGCTGCAGTTGCTG
ATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAAACTGGTG
TTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCCTGATGGT
GGGCGGCGTGGTGTAA

Tryp-Aβ40_1X Amino Acid Sequence (SEQ ID NO:25)

MSALLILALVGAAVADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLM
VGGVV

SEAP-Aβ40_1X Nucleotide Sequence (SEQ ID NO:26)

CACCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCC
CTGGGCGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAA
ACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCC
TGATGGTGGGCGGCGTGGTGTAA

SEAP-Aβ40_1X Amino Acid Sequence (SEQ ID NO:27)

MLLLLLLLGLRLQLSLGDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL
MVGGVV

mLuc-Aβ40_1X Amino Acid Sequence (SEQ ID NO:28)

CACCATGGACATCAAGGTGGTGTTCACCCTGGTGTTCAGCGCCCTGGTG
CAGGCCGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAA
ACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCC
TGATGGTGGGCGGCGTGGTGTAA

mLuc-Aβ40_1X Amino Acid Sequence (SEQ ID NO:29)

MDIKVVFTLVFSALVQADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL
MVGGVV

APP-Aβ40_1X Nucleotide Sequence (SEQ ID NO:30)

CACCATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCT
CGGGCGGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAA
ACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCC
TGATGGTGGGCGGCGTGGTGTAA

APP-Aβ40_1X Amino Acid Sequence (SEQ ID NO:31)

MLPGLALLLLAAWTARADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVV

NSP-Aβ40_1X Nucleotide Sequence (SEQ ID NO:32)

CACCATGGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCAT
CAGAAACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTAT
TGGCCTGATGGTGGGCGGCGTGGTGTAA

NSP-Aβ40_1X Amino Acid Sequence (SEQ ID NO:33)

MDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV

Ins-Aβ42_4X Nucleotide Sequence (SEQ ID NO:34)

CACCATGGCCCTGTGGATGAGACTGCTGCCCCTGCTGGCTCTGCTGGCA
CTGTGGGGACCTGATCCTGCCGCCGCTGATGCCGAGTTCAGACACGATAGCGG
CTACGAGGTGCACCACCAGAAACTGGTGTTCTTCGCCGAGGACGTGGGCAGCA
ACAAGGGCGCCATCATCGGCCTGATGGTGGGAGGCGTCGTGATCGCCAGAAG
GGACGCCGAGTTTAGGCACGACTCTGGATATGAAGTGCATCATCAGAAACTGG
TGTTTTTTGCTGAAGATGTGGGGTCCAACAAAGGGGCCATTATTGGACTGATG
GTGGGCGGAGTCGTGATTGCTAAGCGCGACGCCGAATTCCGGCACGATTCCGG
CTACGAAGTGCACCATCAGAAACTGGTGTTTTTCGCAGAGGATGTGGGCTCTA
ACAAGGGGGCTATCATCGGACTGATGGTGGGCGGGGTCGTGATCGCTCGGAG
AGATGCCGAGTTCCGGCATGACAGCGGATATGAAGTGCACCACCAGAAACTG
GTGTTTTTTGCCGAGGATGTGGGAAGTAACAAAGGGGCAATCATTGGCCTGAT
GGTGGGAGGGGTCGTGATTGCCTGA

Ins-Aβ42_4X Amino Acid Sequence (SEQ ID NO:35)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAIIGLMVGGVVIARRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMV
GGVVIAKRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIARRDA
EFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Ins-Aβ42_Arc 4X Nucleotide Sequence (SEQ ID NO:36)

CACCATGGCCCTGTGGATGAGACTGCTGCCCCTGCTGGCTCTGCTGGCA
CTGTGGGGACCTGATCCTGCCGCCGCTGATGCCGAGTTCAGACACGATAGCGG
CTACGAGGTGCACCACCAGAAACTGGTGTTCTTTGCCGGCGACGTGGGCAGCA
ACAAGGGCGCCATCATCGGCCTGATGGTGGGAGGCGTCGTGATCGCCAGAAG
GGACGCCGAGTTTAGGCACGACTCTGGATATGAAGTGCATCATCAGAAACTGG
TGTTTTTCGCTGGGGATGTGGGGTCCAACAAAGGGGCCATTATTGGACTGATG
GTGGGCGGAGTCGTGATTGCTAAGCGCGACGCCGAATTCCGGCACGATTCCGG
CTACGAAGTGCACCATCAGAAACTGGTGTTCTTCGCCGGGGACGTGGGATCTA
ACAAGGGGGCTATCATTGGGCTGATGGTGGGAGGGGTCGTGATTGCTCGGCGG

GATGCTGAGTTCCGGCATGACAGCGGATATGAGGTGCACCATCAGAAACTGGT
GTTTTTTGCCGGGGACGTGGGCTCAAACAAAGGCGCAATTATCGGGCTGATGG
TGGGCGGGGTCGTGATCGCTTAA

Ins-Aβ42_Arc_4X Amino Acid Sequence (SEQ ID NO:37)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAGDV
GSNKGAIIGLMVGGVVIARRDAEFRHDSGYEVHHQKLVFFAGDVGSNKGAIIGLM
VGGVVIAKRDAEFRHDSGYEVHHQKLVFFAGDVGSNKGAIIGLMVGGVVIARRD
AEFRHDSGYEVHHQKLVFFAGDVGSNKGAIIGLMVGGVVIA

Ins-Aβ42_6X Nucleotide Sequence (SEQ ID NO:38)

CACCATGGCCCTGTGGATGAGACTGCTGCCCCTGCTGGCTCTGCTGGCA
CTGTGGGGACCTGATCCTGCCGCCGCTGATGCCGAGTTCAGACACGATAGCGG
CTACGAGGTGCACCACCAGAAACTGGTGTTCTTCGCCGAGGACGTGGGCAGCA
ACAAGGGCGCCATCATCGGCCTGATGGTGGGAGGCGTCGTGATCGCCAGAAG
GGACGCCGAGTTTAGGCACGACTCTGGATATGAAGTGCATCATCAGAAACTGG
TGTTTTTTGCTGAAGATGTGGGGTCCAACAAAGGGGCCATTATTGGACTGATG
GTGGGCGGAGTCGTGATTGCTAAGCGCGACGCCGAATTCCGGCACGATTCCGG
CTACGAAGTGCACCATCAGAAACTGGTGTTTTTCGCAGAGGATGTGGGCTCTA
ACAAGGGGGCTATCATCGGACTGATGGTGGGCGGGGTCGTGATCGCTCGGAG
AGATGCCGAGTTCCGGCATGACAGCGGATATGAAGTGCACCACCAGAAACTG
GTGTTTTTTGCCGAGGATGTGGGAAGTAACAAAGGGGCAATCATTGGCCTGAT
GGTGGGAGGGGTCGTGATTGCTAAACGGGATGCTGAGTTCCGCCACGACTCAG
GCTATGAGGTGCACCATCAGAAACTGGTGTTCTTTGCCGAAGATGTGGGATCT
AACAAGGGCGCAATTATTGGGCTGATGGTGGGCGGCGTCGTGATCGCAAGAC
GGGATGCAGAATTCAGACATGACTCCGGATACGAGGTGCACCATCAGAAACT
GGTGTTTTTTGCTGAGGACGTGGGGAGCAACAAAGGGGCTATTATCGGGCTGA
TGGTGGGAGGCGTCGTGATTGCCTGA

Ins-Aβ42_6X Amino Acid Sequence (SEQ ID NO:39)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAIIGLMVGGVVIARRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMV
GGVVIAKRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIARRDA
EFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAKRDAEFRHDSGYEV
HHQKLVFFAEDVGSNKGAIIGLMVGGVVIARRDAEFRHDSGYEVHHQKLVFFAE
DVGSNKGAIIGLMVGGVVIA

Ins-Aβ40_4X Nucleotide Sequence (SEQ ID NO:40)

CACCATGGCCCTGTGGATGAGACTGCTGCCCCTGCTGGCTCTGCTGGCA
CTGTGGGGACCTGATCCTGCCGCCGCTGATGCCGAGTTCAGACACGATAGCGG
CTACGAGGTGCACCACCAGAAACTGGTGTTCTTCGCCGAGGACGTGGGCAGCA
ACAAGGGCGCCATCATCGGCCTGATGGTGGGAGGCGTCGTGCGGAGAGATGC
CGAATTCCGGCACGACTCCGGATATGAAGTGCATCATCAGAAACTGGTGTTTT
TTGCTGAAGATGTGGGGTCCAACAAAGGGGCCATTATTGGACTGATGGTGGGC
GGAGTCGTGAAGCGGGACGCCGAGTTTAGGCATGACTCTGGCTACGAAGTGCA
CCATCAGAAACTGGTGTTTTTCGCAGAGGATGTGGGCTCTAACAAGGGGGCTA
TCATCGGACTGATGGTGGGCGGGGTCGTGCGCAGAGATGCTGAGTTTAGACAC
GATTCTGGATATGAAGTGCACCACCAGAAACTGGTGTTTTTTGCCGAGGATGT
GGGAAGTAACAAAGGGGCAATCATTGGCCTGATGGTGGGAGGGGTGGTGTAA

Ins-Aβ40_4X Amino Acid Sequence (SEQ ID NO:41)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAIIGLMVGGVVRRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG
GVVKRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVRRDAEFRHD
SGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV

Ins-Aβ40_6X Nucleotide Sequence (SEQ ID NO:42)

CACCATGGCCCTGTGGATGAGACTGCTGCCCCTGCTGGCTCTGCTGGCA
CTGTGGGGACCTGATCCTGCCGCCGCTGATGCCGAGTTCAGACACGATAGCGG

CTACGAGGTGCACCACCAGAAACTGGTGTTCTTCGCCGAGGACGTGGGCAGCA
ACAAGGGCGCCATCATCGGCCTGATGGTGGGAGGCGTCGTGCGGAGAGATGC
CGAATTCCGGCACGACTCCGGATATGAAGTGCATCATCAGAAACTGGTGTTTT
TTGCTGAAGATGTGGGGTCCAACAAAGGGGCCATTATTGGACTGATGGTGGGC
GGAGTCGTGAAGCGGGACGCCGAGTTTAGGCATGACTCTGGCTACGAAGTGCA
CCATCAGAAACTGGTGTTTTTCGCAGAGGATGTGGGCTCTAACAAGGGGGCTA
TCATCGGACTGATGGTGGGCGGGGTCGTGCGCAGAGATGCTGAGTTTAGACAC
GATTCTGGATATGAAGTGCACCACCAGAAACTGGTGTTTTTTGCCGAGGATGT
GGGAAGTAACAAAGGGGCAATCATTGGCCTGATGGTGGGAGGGGTCGTGAAA
AGGGATGCAGAGTTTCGGCACGACAGTGGCTATGAAGTGCATCACCAGAAACT
GGTGTTCTTCGCAGAAGATGTGGGGAGTAACAAGGGCGCTATTATCGGGCTGA
TGGTGGGCGGAGTCGTGCGGAGGGACGCTGAGTTCCGCCATGACAGCGGATAT
GAGGTGCACCATCAGAAACTGGTGTTCTTTGCCGAAGATGTGGGATCAAACAA
GGGCGCAATCATTGGGCTGATGGTGGGCGGCGTGGTGTAA

Ins-Aβ40_6X Amino Acid Sequence (SEQ ID NO:43)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAIIGLMVGGVVRRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG
GVVKRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVRRDAEFRHD
SGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVKRDAEFRHDSGYEVHHQKLVF
FAEDVGSNKGAIIGLMVGGVVRRDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAII
GLMVGGVV

Yeast Expression Constructs, Cells, and Screening Assays

[0045] Yeast strains that can be used in the compositions and methods described herein include, but are not limited to, Saccharomyces cerevisiae, Saccharomyces uvae, Saccharomyces kluyveri, Schizosaccharomyces pombe, Kluyveromyces lactis, Hansenula polymorpha, Pichia pastoris, Pichia methanolica, Pichia kluyveri, Yarrowia lipolytica, Candida sp., Candida utilis, Candida cacaoi, Geotrichum sp., and Geotrichum fermentans. Although much of the discussion herein relates to Saccharomyces cerevisiae which ectopically expresses an abnormally processed protein, this is merely for illustrative purposes. Other yeast strains can be substituted for S. cerevisiae.

[0046] Certain aspects of the disclosure relate to screening methods for identifying candidate therapeutic agents (e.g., pharmaceutical, chemical, or genetic agents). The methods described herein can optionally be carried out in yeast strains bearing mutations in the ERG6 gene, the PDR1 gene, the PDR3 gene, the PDR5 gene, the SNQ2 gene, and/or any other gene which affects membrane efflux pumps and/or increases permeability for drugs.

[0047] A nucleic acid encoding a polypeptide described herein may be transfected into a yeast cell using nucleic acid vectors that include, but are not limited to, plasmids, linear nucleic acid molecules, artificial chromosomes, and episomal vectors.

[0048] Three well known systems used for recombinant plasmid expression and replication in yeast cells include integrative plasmids, low-copy-number ARS-CEN plasmids, and high-copy-number 2μ plasmids. See Sikorski, "Extrachromosomal cloning vectors of Saccharomyces cerevisiae," in Plasmid, A Practical Approach, Ed. K. G. Hardy, IRL

Press, 1993; and Yeast Cloning Vectors and Genes, Current Protocols in Molecular Biology, Section II, Unit 13.4, Eds., Ausubel et al., 1994.

[0049] An example of the integrative plasmids is YIp, which is maintained at one copy per haploid genome, and is inherited in Mendelian fashion. Such a plasmid, containing a gene of interest, a bacterial origin of replication and a selectable gene (typically an antibiotic-resistance marker), is produced in bacteria. The purified vector is linearized within the selectable gene and used to transform competent yeast cells.

[0050] An example of the low-copy-number ARS-CEN plasmids is YCp, which contains the autonomous replicating sequence (ARS1) and a centromeric sequence (CEN4). These plasmids are usually present at 1-2 copies per cell. Removal of the CEN sequence yields a YRp plasmid, which is typically present in 100-200 copies per cell. However, this plasmid is both mitotically and meiotically unstable.

[0051] An example of the high-copy-number 2μ plasmids is YEp, which contains a sequence approximately 1 kb in length (named the 2μ sequence). The 2μ sequence acts as a yeast replicon giving rise to higher plasmid copy number. However, these plasmids are unstable and require selection for maintenance. Copy number is increased by having on the plasmid a selection gene operatively linked to a crippled promoter.

[0052] A wide variety of plasmids can be used in the compositions and methods described herein. In one embodiment, the plasmid is an integrative plasmid (e.g., pAG303, pAG304, pAG305, pAG306, pRS303, pRS304, pRS305, pRS306, or a derivative thereof). See, e.g., Alberti et al. (2007) "A suite of Gateway cloning vectors for high-throughput genetic analysis in Saccharomyces cerevisiae" Yeast 24(10):913-19. In further embodiments, the plasmid is an episomal plasmid (e.g., p426GPD, p416GPD, p426TEF, p423GPD, p425GPD, p424GPD or p426GAL).

[0053] Regardless of the type of plasmid used, yeast cells are typically transformed by chemical methods (e.g., as described by Rose et al., 1990, Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The cells are typically treated with lithium acetate to achieve transformation efficiencies of approximately 10^4 colony-forming units (transformed cells)/μg of DNA. Yeast perform homologous recombination such that the cut, selectable marker recombines with the mutated (usually a point mutation or a small deletion) host gene to restore function. Transformed cells are then isolated on selective media. Of course, any suitable means of introducing nucleic acids into yeast cells can be used.

[0054] The yeast vectors (plasmids) described herein typically contain a yeast origin of replication, an antibiotic resistance gene, a bacterial origin of replication (for propagation in bacterial cells), multiple cloning sites, and a yeast nutritional gene for maintenance in yeast cells. The nutritional gene (or "auxotrophic marker") is most often one of the following: 1) TRP1 (Phosphoribosylanthranilate isomerase); 2) URA3 (Orotidine-5'-phosphate decarboxylase); 3) LEU2 (3-Isopropylmalate dehydrogenase); 4) HIS3 (Imidazoleglycerolphosphate dehydratase or IGP dehydratase); or 5) LYS2 (α-aminoadipate-semialdehyde dehydrogenase).

[0055] The yeast vectors (plasmids) described herein also contain promoter sequences. A "promoter" is a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind, such as RNA polymerase and other transcription factors, to initiate the specific transcription a nucleic acid sequence. The phrases "operatively linked" and "operatively positioned" mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence.

[0056] A promoter may be one naturally associated with a nucleic acid sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Alternatively, a promoter may be a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid sequence in its natural environment. Such promoters may include promoters of other genes and promoters not "naturally occurring." The promoters employed may be either constitutive or inducible.

[0057] For example, various yeast-specific promoters (elements) may be employed to regulate the expression of a RNA in yeast cells. Examples of inducible yeast promoters include GAL1-10, GAL1, GALL, GALS, TET, VP16 and VP16-ER. Examples of repressible yeast promoters include Met25. Examples of constitutive yeast promoters include glyceraldehyde 3-phosphate dehydrogenase promoter (GPD), alcohol dehydrogenase promoter (ADH), translation-elongation factor-1-alpha promoter (TEF), cytochrome c-oxidase promoter (CYC1), and MRP7. Autonomously replicating expression vectors of yeast containing promoters inducible by glucocorticoid hormones have also been described (Picard et al., 1990), including the glucocorticoid responsive element (GRE). These and other examples are described in Mumber et al., 1995; Ronicke et al., 1997; Gao, 2000. Yet other yeast vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel et al. and Grant et al., 1987.

[0058] In some embodiments, a yeast strain is used that allows for expression, e.g., inducible expression, from GAL promoters on carbon sources other than galactose. In some embodiments, the strain carries an integrated or episomal (e.g., plasmid-borne) gene encoding a fusion protein, wherein the Gal4 DNA binding domain is fused to a transcriptional activation domain and a regulatory domain. The fusion protein is characterized in that its ability to activate transcription is regulated by binding of a small molecule to the regulatory domain. For example, in some embodiments, the fusion

protein does not activate transcription in the absence of the small molecule, whereas in the presence of the small molecule, the fusion protein activates transcription. Exemplary small molecules include, e.g., steroid hormones, wherein the corresponding regulatory domain comprises at least a portion of a receptor for the small molecule. For example, the small molecule may be an estrogen (e.g., estradiol), or analog thereof (e.g., tamoxifen), and the corresponding regulatory domain comprises at least a portion of the estrogen receptor (ER). Exemplary activation domains include, e.g., viral protein activation domains such as the herpes simplex virus protein VP16 activation domain. In some embodiments, the strain carries an integrated or episomal (e.g., plasmid-borne) gene encoding a Gal4-ER-VP16 fusion protein. The presence of an estrogen receptor ligand, e.g., estradiol, in the medium, allows for expression from GAL promoters on carbon sources other than galactose. Numerous ways exist to render expression of a molecule of interest, e.g., an amyloid beta peptide, conditional, e.g., on culture media containing galactose or other carbon sources.

[0059] Certain aspects of the present disclosure provide methods of screening for a candidate drug (agent or compound) or a genetic factor that modulates amyloid beta-induced toxicity. Various types of candidate drugs may be screened by the methods described herein, including nucleic acids, polypeptides, small molecule compounds, and peptidomimetics. In some cases, genetic agents can be screened by contacting the yeast cell with a nucleic acid construct coding for a gene. For example, one may screen cDNA libraries expressing a variety of genes, to identify genes that modulate amyloid beta-induced toxicity.

[0060] For example, the identified drugs may modulate amyloid beta-induced toxicity. Accordingly, irrespective of the exact mechanism of action, drugs identified by the screening methods described herein are expected to provide therapeutic benefit to Alzheimer's disease.

[0061] Certain aspects of the present disclosure provide methods of identifying compounds or genes that modulate amyloid beta-induced toxicity. One of the strongest aspects of yeast is the possibility of performing high throughput screens that may identify genes, peptides and other compounds with the potential to ameliorate toxicity. A large number of compounds can be screened under a variety of growth conditions and in a variety of genetic backgrounds. The toxicity screen has the advantage of not only selecting for compounds that interact with amyloid beta, but also upstream or downstream targets that are not themselves cytotoxic and that are not yet identified.

[0062] In certain embodiments, candidate drugs can be screened from large libraries of synthetic or natural compounds. One example is an FDA approved library of compounds that can be used by humans. In addition, compound libraries are commercially available from a number of companies including but not limited to Maybridge Chemical Co. (Trevillet, Cornwall, UK), Comgenex (Princeton, NJ), Microsource (New Milford, CT), Aldrich (Milwaukee, WI), AKos Consulting and Solutions GmbH (Basel, Switzerland), Ambinter (Paris, France), Asinex (Moscow, Russia), Aurora (Graz, Austria), BioFocus DPI, Switzerland, Bionet (Camelford, UK), ChemBridge, (San Diego, CA), ChemDiv, (San Diego, CA), Chemical Block Lt, (Moscow, Russia), ChemStar (Moscow, Russia), Exclusive Chemistry, Ltd (Obninsk, Russia), Enamine (Kiev, Ukraine), Evotec (Hamburg, Germany), Indofine (Hillsborough, NJ), Interbioscreen (Moscow, Russia), Interchim (Montlucon, France), Life Chemicals, Inc. (Orange, CT), Microchemistry Ltd. (Moscow, Russia), Otava, (Toronto, ON), PharmEx Ltd.(Moscow, Russia), Princeton Biomolecular (Monmouth Junction, NJ), Scientific Exchange (Center Ossipee, NH), Specs (Delft, Netherlands), TimTec (Newark, DE), Toronto Research Corp. (North York ON), UkrOrgSynthesis (Kiev, Ukraine), Vitas-M, (Moscow, Russia), Zelinsky Institute, (Moscow, Russia), and Bicoll (Shanghai, China). Combinatorial libraries are available and can be prepared. Libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are commercially available or can be readily prepared by methods well known in the art. It is proposed that compounds isolated from natural sources, such as animals, bacteria, fungi, plant sources, including leaves and bark, and marine samples may be assayed as candidates for the presence of potentially useful pharmaceutical agents. It will be understood that the pharmaceutical agents to be screened could also be derived or synthesized from chemical compositions or man-made compounds. Several commercial libraries can be used in the screens.

[0063] Another embodiment relates to genetic screens. For example, genomic libraries and disruption libraries can be screened to find extragenic suppressors or enhancers of amyloid beta-induced toxicity. Because the yeast genome is small, 10,000 transformants of each type should be sufficient for good coverage.

[0064] Another embodiment contemplates screening assays using fluorescent resonance energy transfer (FRET). FRET occurs when a donor fluorophore is in close proximity (10-60 A) to an acceptor fluorophore, and when the emission wavelength of the first overlaps the excitation wavelength of the second (Kenworthy AK et al., 2001. Methods. 24:289-96). FRET should occur when cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) fusion proteins are actually part of the same complex.

[0065] For example, an amyloid beta protein can be fused to CFP and to YFP respectively, and integrated in the yeast genome under the regulation of a GAL1-10 promoter. Cells are grown in galactose to induce expression. Upon induction, cells produce the fusion proteins, which aggregate and bring the CFP and YFP close together. Because proteins in the aggregates are tightly packed, the distance between the CFP and YFP is less than the critical value of 100 A that is necessary for FRET to occur. In this case, the energy released by the emission of CFP will excite the YFP, which in turn will emit at its characteristic wavelength. FRET based screening can be used to identify candidate compounds including, drugs, genes or other factors that can disrupt the interaction of CFP and YFP by maintaining the proteins in a state that

does not allow aggregation to occur.

[0066] One embodiment contemplates screening assays using fluorescence activated cell sorting (FACS) analysis. FACS provides the means of scanning individual cells for the presence of fluorescently labeled/tagged moiety. The method is unique in its ability to provide a rapid, reliable, quantitative, and multiparameter analysis on either living or fixed cells. For example, an amyloid beta protein can be suitably labeled, and provide a useful tool for the analysis and quantitation of protein aggregation as a result of other genetic or growth conditions of individual yeast cells as described above.

[0067] Screens (e.g., for compounds and/or for genetic suppressors or enhancers) can be carried out under a variety of different conditions. For example, a variety of different culture media can be used. Culture media can contain different carbon sources, e.g., different sugars such as glucose, glycerol, galactose, raffinose, etc. In some embodiments, multiple screens are performed using two, three, or more different culture conditions (e.g., culture media containing different carbon sources), and compounds or genes identified as "hits" under at least two different culture conditions are identified. In some embodiments, screens are performed under two or more different culture conditions (e.g., using culture media containing different carbon sources), wherein the different culture conditions (e.g., different carbon sources) result in different levels of mitochondrial respiration. For example, growth using culture media containing glucose, glycerol, or galactose result in different levels of mitochondrial respiration. In glucose, yeast cells ferment and respiration remains low until all glucose is converted to ethanol. In galactose, respiration is moderately active. In glycerol, yeast cells are completely dependent on respiration for growth. In some embodiments, a screen is performed in parallel using media containing glucose, galactose, or glycerol as a carbon source.

[0068] Certain embodiments provide methods of further testing those potential drugs that have been identified in the yeast system, in other model systems. The model systems include, but are not limited to, worms, flies, mammalian cells, and *in vivo* animal models.

Mammalian Expression Constructs, Cells, and Screening Assays

[0069] An amyloid beta nucleic acid can be transfected into a mammalian cell using nucleic acid vectors that include, but are not limited to, plasmids, linear nucleic acid molecules, artificial chromosomes, and viral vectors. The vectors can contain as a transgene any of the amyloid beta nucleic acids described herein.

[0070] Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) can be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. Where the vector is a viral vector (e.g., a lentiviral vector), the vector can be delivered to the cell by direct infection. Preferably the vector is stably integrated into the host genome. Methods of producing a cell line containing a stably integrated nucleic acid (e.g., a vector) are well known in the art (see, for example, Sambrook et al. in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989)). Briefly, a cell transfected with a nucleic acid can be selected for using a host of antibiotics including, for example, G418, neomycin, or hygromycin B. Generally, the transfected nucleic acid contains a suitable antibiotic resistance gene or is co-transfected with a vector containing a suitable antibiotic resistance gene. Thus, only cells and their progeny which contain a stably integrated nucleic acid encoding the antibiotic resistance gene will survive when grown in antibiotic.

[0071] Amyloid beta can be expressed under the control of an inducible promoter. Methods for assessing the induction of amyloid beta following administration of an inducer include western blotting using an antibody specific for amyloid beta or RT-PCR or northern blotting techniques to detect mRNA expression of amyloid beta. Such methods are well known to those in the art and are described in detail in Sambrook et al. (in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol. 1, 2, 3 (1989)). Expression of amyloid beta can be detected, e.g., at about one hour (e.g., about 30 minutes, about 90 minutes, about two hours, about three hours, about 4 hours, about 6 hours, about 8 hours, about 12 hours, about 12 hours, about 16 hours, about 20 hours, about 24 hours, about 36 hours, about 48 hours or more) post-induction. One can assess (evaluate) a change in expression over time post-induction. For example, the amount of expression of amyloid beta before induction (i.e., before the inducer is added) can be compared to the expression of amyloid beta by the cells at various time points (e.g., 1, 4, 8, and 12 hours; 4, 8, 12 and 16 hours; 8, 12, 16, and 20 hours; 12, 24, 36, and 48 hours) post-induction.

[0072] A suitable starting concentration of an inducing agent is about 0.001 μM (e.g., about 0.01 μM, about 0.1 μM, about 1.0 μM, about 10.0 μM, about 100 μM). It is understood that the concentration of the inducing agent can be optimized for the particular experiment and can depend on, for example, the cell line, the transgene, or the culture conditions the cells are grown in (e.g., low serum conditions).

[0073] A mammalian cell (e.g., a mammalian neuronal cell) containing a nucleic acid vector encoding amyloid beta under control of an inducible promoter may be a non-human mammal (e.g., mouse, rat, or guinea pig). The vector can be introduced to the mammalian cell (e.g., a neuronal cell) *ex vivo,* i.e., the cell can be transfected *in vitro* and then implanted or otherwise delivered to the mammal (e.g., surgically implanted). Alternatively, a non-human transgenic

animal can be established wherein the nucleic acid vector using any of a variety of techniques known in the art (see, for example, Manson et al. (2001) Exp. Rev. Mol. Med. 11; and Hofker et al. Transgenic Mouse: Methods and Protocols (Methods in Molecular Biology) Humana Press, Clifton, N.J., Vol. 29 (2002)).

[0074] Where an inducible promoter is used, induction of expression of amyloid beta in an animal can be accomplished by administering to the animal an appropriate amount of the inducer. The inducer can be delivered to the mammal as part of food or water (i.e., in the food or water) or can be administered intravenously or parenterally (e.g., subcutaneous injection). Suitable dosages of inducing agent and methods for detecting induction of a transgene in an animal model are described in, for example, Teng et al. (2002) Physiol. Genomics 11:99-107; Kim et al. (2003) Am. J. Pathol. 162(5):1693-1707; and Zabala et al. (2004) Cancer Res. 64:2799-2804.

[0075] It is understood that any of the *in vitro* or *in vivo* aspects of the disclosure of the mammalian cell described above can be used in the following screening methods.

[0076] Certain aspects of the present disclosure provide methods of screening for and identifying a "candidate agent" (e.g., a compound or a drug) that prevents or suppresses cytotoxicity resulting from overexpression of amyloid beta in a mammalian neuronal cell. Thus, a "candidate agent," as referred to herein, is any substance with a potential to reduce, interfere with or curtail (i.e., prevent or suppress) cytotoxicity resulting from overexpression of amyloid beta in a mammalian neuronal cell.

[0077] Various types of candidate agents can be screened by the methods described herein, including, but not limited to, nucleic acids, polypeptides, small molecule compounds, large molecule compounds, peptidomimetics or any other compounds described herein (e.g., see "Compounds" below). In some instances, the candidate agents are genetic agents that reduce, interfere with, or curtail cytotoxicity resulting from overexpression of amyloid beta in a mammalian neuronal cell. For example, a cDNA library containing coding sequences for a variety of genes can be screened to identify potential therapeutic genes for the diseases described herein. Alternatively, a screen can be performed to identify genetic elements that contribute to cytotoxicity resulting from amyloid beta expression in a mammalian cell. For example, a library of siRNAs or antisense oligonucleotides can be screened such that the level or amount of cytotoxicity in the absence of one or more genes could be determined. In another example, a mammalian neuronal cell could be mutagenized to inactivate one or more genes prior to performing the screening assay. In these examples, a reduced level of amyloid beta-induced cytotoxicity in a cell in the absence of a gene (through mutational inactivation or silencing) indicates that the gene contributes to amyloid beta-induced cytotoxicity. Accordingly, siRNAs or antisense oligonucleotides that target that gene, for example, can be useful in treating Alzheimer's disease.

[0078] Screening methods to identify an agent capable of preventing or suppressing cytotoxicity resulting from overexpression of amyloid beta can involve the steps of: (i) culturing the cell in the presence of a candidate agent and under conditions that allow for expression of the nucleic acid encoding amyloid beta at a level that, in the absence of the candidate agent, is sufficient to induce toxicity in the cell; (ii) measuring cell viability in the presence of the candidate agent; and (iii) comparing cell viability measured in the presence of the candidate agent to cell viability in the absence of the candidate agent, where if cell viability is greater in the presence of the candidate agent as compared to in the absence of the candidate agent, then the candidate agent is identified as a compound that prevents or suppresses amyloid beta-induced toxicity.

[0079] The screening assays can involve a mammalian cell containing a stably integrated nucleic acid encoding amyloid beta (optionally under the control of an inducible promoter). Although the cell can be any mammalian cell, preferably the cell is a neuronal cell (e.g., primary neuronal cells or a neural cell line such as PC12, H4, SK-N-SH, SH-SY5Y, Neuro-2a, SVG p12, CCF-STTG1, SW 1088, SW 1783, LN-18, A172, U-138 MG, T98G, U-87 MG, U-118 MG, Hs 683, M059K, M059J, H4, LN-229, Daoy, or PFSK-1). Additional cell lines are available at the American Type Culture Collection (ATCC), Manassas, VA.

[0080] The cells can be treated with two or more concentrations of a compound, where, for example, a concentration-dependence or EC50 is to be determined. Suitable concentrations of a candidate compound for the assay include, for example, about 0.01 μM to 1 mM of the agent (e.g., about 0.01 μM to 0.1 μM, about 0.1 μM to 1 μM, about 1 μM to 10 μM, about 10 to 1 mM, or about 100 μM to 1 mM).

[0081] Methods of assessing the efficacy of an agent to prevent or suppress amyloid beta-induced cytotoxicity can be quantitative, semi-quantitative, or qualitative. Thus, for example, the activity of an agent can be determined as a discrete value. An example of a quantitative determination of an agent's is a 50% Effective Concentration, or EC50 value, which is the molar concentration of an agent (e.g., a compound) that gives one-half the maximal response of that agent. Alternatively, the efficacy of an agent can be assessed using a variety of semi-quantitative/qualitative systems known in the art. Thus, the efficacy of an agent to prevent or suppress amyloid beta-induced cytotoxicity in a mammalian cell can be expressed as, for example, (a) one or more of "excellent", "good", "satisfactory", "unsatisfactory", and/or "poor"; (b) one or more of "very high", "high", "average", "low", and /or "very low"; or (c) one or more of "+++++", "++++", "+++", "++", "+", "+/-", and/or "-".

[0082] Methods include determining the efficacy of agents in preventing or suppressing amyloid beta-induced cytotoxicity in a mammalian cell (e.g., a compound such as any of those described herein). Cells are generally plated on

solid support matrix (e.g., a plastic tissue culture plate, or a multi-well (96 or 386-well) tissue culture plate) and grown in appropriate medium. Cells are then contacted with serial dilutions of a candidate agent generally ranging, for example, from 10 μM to 0.1 μM concentration. Often, a control compound (e.g., a known inhibitor of known concentration) is also added to a set of cells as an internal standard. Often, a set of cells are grown in the presence of a carrier, buffer, or solvent, in which the compound is delivered. Cells are grown in the presence or absence of test compounds for varying times, for example, from 1 to three days (1 day, 2 days, 3 days, 4 days, 1 week, 2 weeks), followed by a test for the number of cells remaining on the plate or the viability of the cells remaining on the plate. Methods of detecting (e.g., determining or measuring) the extent of amyloid beta-induced cytotoxicity in the presence or absence of an agent are myriad and well known to those of ordinary skill in the art. These methods can include, for example, measuring ATP concentration in a cell. The amount of ATP present in a cell or population of cells is proportional to the number of viable cells in that population. In one example, ATP concentration can be determined enzymatically, for example, by using luciferase/luciferin. These enzymes produce a light signal in a reaction requiring ATP hydrolysis. Thus, the more ATP present in a sample, the more light produced. In this method, cells are first harvested and lysed. Cell lysates are then incubated with luciferase/luciferin and the amount of ATP-dependent light produced from the sample can be detected and/or quantitated using a luminometer (e.g., Turner BioSystems TD-20/20 Luminometer, Turner Biosystems, Sunnyvale, CA). In this case, to determine the efficacy of a given agent in preventing or suppressing amyloid beta-induced cytotoxicity, the amount of light signal produced from induced cells in the presence of the compound can be compared to the light signal produced from induced cells in the absence of the agent. Where more light signal is produced from lysates of cells cultured in the presence of the agent as compared to cells cultured in the absence of the agent, this indicates that the compound prevents or suppresses cytotoxicity. Further examples of this method are set forth in the Examples.

[0083] Other suitable methods for determining the efficacy of agents in preventing or suppressing amyloid beta-induced cytotoxicity include, for example, counting the number of cells remaining after the period of induction in the absence or presence of the agent. In this method, cells can be trypsinized from the plate, washed, stained with a dye (e.g., trypan blue), and counted using a microscope or mechanical cell counter (Beckman-Coulter Z1™ Series COULTER COUNTER® Cell and Particle Counter). Since dyes like trypan blue are only taken up by dead or dying cells, this method allows for discrimination (i.e., blue or white cell) between viable and non-viable cells in a population. Another method for determining prevention or suppression of amyloid beta-induced cytotoxicity by an agent (e.g., any one of the compositions described herein) following treatment is a metabolic assay, for example, an MTT-metabolic assay (Invitrogen, USA). MTT Diphenyltetrazolium Bromide, is a tetrazolium salt (yellowish) that is cleaved to formazan crystals by the succinate dehydrogenase system which belongs to the mitochondrial respiratory chain, and is only active in viable cells. The mitochondrial succinate dehydrogenase reduces the MTT crystals into purple formazan in the presence of an electron coupling reagent. Following the treatment of the cells with a compound, the cells are exposed to the MTT reagent and the more viable cells are present in a well, the more formazan dye is produced. The amount of formazan dye can be measured, for example, using a spectrophotometer.

[0084] Other commonly used methods of testing for prevention or suppression of cytotoxicity in a cell (e.g., cytotoxicity resulting from overexpression of amyloid beta in a mammalian cell) by an agent (e.g., a compound or a composition described herein) include the monitoring of DNA synthesis in the cell. For example, induced cells grown in the presence or absence of an agent are also treated with a nucleotide analog that can incorporate into the DNA of the cell upon cell division. Examples of such nucleotide analogs include, for example, BrdU or ^{3}H-Thymidine. In each case, the amount of label incorporated into the induced cells (grown in the presence and absence of a given agent) is quantitated, and the amount of label incorporation is directly proportional to the amount of cell growth in the population of cells. The amount of label incorporated in the induced cells in the presence and absence of an agent can be normalized to the amount of label incorporated into uninduced cells. More signal (i.e., more DNA synthesis) in an induced cell set treated with the agent as compared to induced cells not treated with the agent indicates that the agent prevents or suppresses amyloid beta-induced cytotoxicity.

[0085] Other suitable methods for assessing suppression or prevention of amyloid beta-induced cytotoxicity by an agent include the detection of apoptosis in a cell. Such methods of detecting or measuring apoptosis include, for example, monitoring DNA fragmentation, caspase activation, or annexin V expression.

[0086] It should be understood that the screening methods described herein can also be used as secondary, or cell-based screens to identify compounds useful in treating a Alzheimer's disease. For example, the screening methods can be used following a primary screen where, for example, a compound is first selected based on an ability to inhibit amyloid beta-induced toxicity in another system (e.g., yeast).

[0087] Screening assays can be performed in any format that allows for rapid preparation, processing, and analysis of multiple reactions. This can be, for example, in multi-well assay plates (e.g., 96 wells or 386 wells). Stock solutions for various agents can be made manually or robotically, and all subsequent pipetting, diluting, mixing, distribution, washing, incubating, sample readout, data collection and analysis can be done robotically using commercially available analysis software, robotics, and detection instrumentation capable of detecting the signal generated from the assay. Examples of such detectors include, but are not limited to, spectrophotometers, luminometers, fluorimeters, and devices

that measure radioisotope decay.

Compounds

[0088] Compounds to be screened or identified using any of the methods described herein can include various chemical classes, though typically small organic molecules having a molecular weight in the range of 50 to 2,500 daltons. These compounds can comprise functional groups necessary for structural interaction with proteins (e.g., hydrogen bonding), and typically include at least an amine, carbonyl, hydroxyl, or carboxyl group, and preferably at least two of the functional chemical groups. These compounds often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures (e.g., purine core) substituted with one or more of the above functional groups.

[0089] In alternative embodiments, compounds can also include biomolecules including, but not limited to, peptides, polypeptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives or structural analogues thereof, polynucleotides, nucleic acid aptamers, and polynucleotide analogs.

[0090] Compounds can be identified from a number of potential sources, including: chemical libraries, natural product libraries, and combinatorial libraries comprised of random peptides, oligonucleotides, or organic molecules. Chemical libraries consist of diverse chemical structures, some of which are analogs of known compounds or analogs or compounds that have been identified as "hits" or "leads" in other drug discovery screens, while others are derived from natural products, and still others arise from non-directed synthetic organic chemistry. Natural product libraries are collections of microorganisms, animals, plants, or marine organisms which are used to create mixtures for screening by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms, or (2) extraction of plants or marine organisms. Natural product libraries include polypeptides, non-ribosomal peptides, and variants (non-naturally occurring) thereof. For a review, see Science 282:63-68 (1998). Combinatorial libraries are composed or large numbers of peptides, oligonucleotides, or organic compounds as a mixture. These libraries are relatively easy to prepare by traditional automated synthesis methods, PCR, cloning, or proprietary synthetic methods. Of particular interest are non-peptide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, Curr. Opin. Biotechnol. 8:701-707 (1997).

[0091] Identification of test compounds through the use of the various libraries herein permits subsequent modification of the test compound "hit" or "lead" to optimize the capacity of the "hit" or "lead" to prevent or suppress amyloid beta-induced toxicity and/or amyloid beta-induced aggregation.

[0092] The compounds identified above can be synthesized by any chemical or biological method. The compounds identified above can also be pure, or may be in a composition (e.g., a pharmaceutical composition) that contains one or more additional component(s), and can be prepared in an assay-, physiologic-, or pharmaceutically- acceptable diluent or carrier (see below).

Pharmaceutical Compositions and Methods of Treatment

[0093] A compound that is found to prevent or suppress amyloid beta-induced toxicity or the formation of amyloid beta aggregates in a cell can be formulated as a pharmaceutical composition, e.g., for administration to a subject to treat a neurodegenerative disease such as Alzheimer's disease.

[0094] A pharmaceutical composition typically includes a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The composition can include a pharmaceutically acceptable salt, *e.g.,* an acid addition salt or a base addition salt (see *e.g.,* Berge et al., J. Pharm. Sci. 66:1-19, 1977).

[0095] The compound can be formulated according to standard methods. Pharmaceutical formulation is a well-established art, and is further described, *e.g.,* in Gennaro (ed.), Remington: The Science and Practice of Pharmacy, 20th ed., Lippincott, Williams & Wilkins (2000) (ISBN: 0683306472); Ansel et al., Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott Williams & Wilkins Publishers (1999) (ISBN: 0683305727); and Kibbe (ed.), Handbook of Pharmaceutical Excipients American Pharmaceutical Association, 3rd ed. (2000) (ISBN: 091733096X).

[0096] A compound that prevents or suppresses amyloid beta-induced toxicity and/or amyloid beta aggregate formation in a cell can be formulated with excipient materials, such as sodium chloride, sodium dibasic phosphate heptahydrate, sodium monobasic phosphate, and a stabilizer. It can be provided, for example, in a buffered solution at a suitable concentration and can be stored at 2-8°C.

[0097] The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.,* injectable and infusible solutions), dispersions or suspensions, tablets, capsules, pills, powders, liposomes and suppositories. The preferred form can depend on the intended mode

of administration and therapeutic application. Compositions for the agents described herein may be in the form of injectable or infusible solutions.

[0098] Such compositions can be administered by a parenteral mode (*e.g.,* intravenous, subcutaneous, intraperitoneal, or intramuscular injection). The phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intracerebral, intracranial, intracarotid and intrasternal injection and infusion.

[0099] The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions can be prepared by incorporating an agent described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating a compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of a compound plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0100] In certain embodiments, the compound can be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0101] A compound identified as one that prevents or suppresses amyloid beta-induced toxicity and/or amyloid beta aggregate formation in a cell can be modified, *e.g.,* with a moiety that improves its stabilization and/or retention in circulation, *e.g.,* in blood, serum, or other tissues, *e.g.,* by at least 1.5, 2, 5, 10, or 50 fold. The modified compound can be evaluated to assess whether it can reach treatment sites of interest (e.g., locations of aggregate amyloid beta) such as can occur in a cell in a subject with a neurodegenerative disease such as Alzheimer's disease (*e.g.,* by using a labeled form of the compound).

[0102] For example, the compound can be associated with a polymer, *e.g.,* a substantially non-antigenic polymer, such as a polyalkylene oxide or a polyethylene oxide. Suitable polymers will vary substantially by weight. Polymers having molecular number average weights ranging from about 200 to about 35,000 Daltons (or about 1,000 to about 15,000, and 2,000 to about 12,500) can be used. For example, a compound can be conjugated to a water soluble polymer, *e.g.,* a hydrophilic polyvinyl polymer, *e.g.,* polyvinylalcohol or polyvinylpyrrolidone. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided that the water solubility of the block copolymers is maintained. Additional useful polymers include polyoxyalkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene (Pluronics); polymethacrylates; carbomers; and branched or unbranched polysaccharides.

[0103] When the compound is used in combination with a second agent (*e.g.,* any additional therapies for Alzheimer's disease), the two agents can be formulated separately or together. For example, the respective pharmaceutical compositions can be mixed, *e.g.,* just prior to administration, and administered together or can be administered separately, *e.g.,* at the same or different times.

[0104] Compounds identified as described herein can be used to treat a subject (e.g., a human subject) that is at risk for or has a disorder associated with amyloid beta mediated toxicity and/or the formation, deposition, accumulation, or persistence of amyloid beta aggregates, e.g., amyloid beta oligomers and/or dimers. In certain embodiments, the disorder is Alzheimer's disease, Down Syndrome, Fragile X syndrome, or systemic amyloidosis. Methods of identifying an individual at risk for or having such disorders are known in the art. For example, Alzheimer's disease can be diagnosed based on, e.g., patient history (e.g., memory loss), clinical observations, the presence of characteristic neurological and neuropsychological features, and the absence of other conditions that might be responsible for the foregoing. Imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), or positron emission tomography (PET) can be of use. Diagnosis can be confirmed by post-mortem examination of brain material. Exemplary criteria for diagnosis of Alzheimer's disease are found in the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV (text revision, 2000) or DSM-V and the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS)-Alzheimer's Disease and Related Disorders Association (ADRDA) criteria (McKhann G, et al. (1984) Neurology 34 (7): 939-44), e.g., as updated (Dubois B, et al. (2007) Lancet

Neurol 6 (8): 734-46). Analysis of cerebrospinal fluid (CSF) for various biomarkers, e.g., amyloid beta or tau proteins (e.g., total tau protein and phosphorylated tau) and/or imaging (e.g., PET imaging) with labeled compounds that bind to amyloid beta deposits (e.g., 11C-labeled Pittsburgh Compound-B (11C-PIB) or18F-AV-45 (flobetapir F18)) can be used to predict the onset of AD, e.g., to identify individuals who have a significant likelihood of progressing to Alzheimer's disease in the future (e.g., within the next two years). Such imaging methods may also be of use in the instant invention to assess the in vivo effect of compounds identified herein. In some embodiments, a subject has a mutation in a gene encoding amyloid precursor protein (APP), presenilin 1, or presenilin 2. In some embodiments, the mutation increases the production of Aβ42 or alters the ratio of Aβ42 to Aβ40. In some embodiments the subject has at least one copy of the ε4 allele of the apolipoprotein E (APOE) gene.

[0105] Down Syndrome can be diagnosed based on presence of trisomy 21.

[0106] Fragile X Syndrome is caused by expansion of a trinucleotide gene sequence (CGG) on the X chromosome that results in a failure to express the protein coded by the FMR1 gene, which encodes FMRP. It may be suspected based on the presence of characteristic signs and symptoms, with diagnostic confirmation from genetic testing.

[0107] Thus, methods and compositions for treating a subject at risk of (or susceptible to) an amyloid beta mediated disease are described herein. For example, an individual who is at risk of developing Alzheimer's disease and/or has signs suggesting that he or she will develop Alzheimer's disease can be treated with the compounds and methods described herein.

[0108] As used herein, the term "treatment" is defined as the application or administration of a therapeutic compound to a patient, or application or administration of a therapeutic compound to a subject (e.g., a human subject, who may be referred to as a "patient") who has a disease (or other medically recognized disorder or syndrome), a symptom of disease or a predisposition toward a disease (e.g., one or more risk factors associated with the disease), with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect (in a manner beneficial to the subject) the disease, the symptoms of disease or the predisposition toward disease. In some embodiments, treatment is prophylactic, i.e., it is administered to a subject who has not developed the disease (and who may or may not have a predisposition to develop the disease) with an intent to delay, prevent, or reduce the likelihood that the subject will develop the disease or reduce the severity should the subject develop the disease. Compounds may also or alternately be administered to a subject for purposes of testing, research, or diagnosis and/or may be contacted with an isolated tissue, cells, or cell line from a patient, e.g., for purposes of testing, research, diagnosis, or with an intent to subsequently administer the isolated tissue, cells, or cell line to the subject for treatment.

[0109] The following are examples of the practice of the invention.

EXAMPLES

Example 1: Amyloid Beta Expression Constructs for Yeast Cells

[0110] The mating factor alpha 1 gene in yeast encodes a precursor protein of 165 amino acids, called the prepro-alpha-factor. This prepro-alpha-factor consists of a 19-amino acid signal peptide, a 64-amino acid pro region and 4 tandem repeats of alpha-factor sequence, each preceded by a spacer peptide with signature proteolytic cleavage sites. The precursor protein undergoes a series of sequential and defined posttranslational enzymatic modification and proteolytic processing steps in the secretory pathway of the cell to generate the mature 13-amino acid long peptide pheromone, alpha-factor. The nascent prepro-alpha polypeptide is translocated into the lumen of ER, where the signal sequence is cleaved by a signal peptidase to produce the pro-alpha-factor. In the ER lumen, N-linked carbohydrates are added to three specific glycosylation sites within the pro region, a step that is necessary for the transport of the pro-alpha-factor from the ER to Golgi. Further carbohydrate modification takes place in the Golgi before the final proteolytic processing steps. The first of these three proteolytic processing steps is by the KEX2 endopeptidase that cleaves on the carboxyl side of Lys-Arg (KR) residues at the N terminus of each of the spacer repeats (KREAEA (SEQ ID NO:44) or KREAEAEA (SEQ ID NO:45) or KREADAEA (SEQ ID NO:46)). The second proteolytic processing occurs when the dibasic KR residues are removed from the C terminus of alpha-factor by KEX1 carboxypeptidase B (CoxyPdaseB). Finally, the remaining amino acids of the spacer (EAEA (SEQ ID NO:47) or EAEAEA (SEQ ID NO:48) or EADAEA (SEQ ID NO:49)) are cleaved from the N terminus of alpha-factor by STEB3 encoded dipeptidyl aminopeptidase (DAPdaseA). Mature alpha-factor is then secreted into the extracellular medium.

[0111] The human Aβ42 coding sequence was inserted into the mating factor alpha 1 prepro sequence, wherein the 13-amino acid long alpha-factor peptide sequence was replaced by the Aβ42 sequence, leaving all the other sequence components of the prepro-alpha factor intact (Fig. 1A). Constructs were prepared based upon the mating factor alpha 1 prepro sequence backbone and containing (i) one (1X), two (2X), four (4X), six (6X), or eight (8X) copies of Aβ42, (ii) four (4X) or six (6X) copies of Aβ40, or four (4X) copies of scrambled Aβ42 (Fig. 1B). The nucleotide and amino acid sequences of the fusion polypeptides encoded by the expression constructs are as follows.

Matα-Aβ42_1X Nucleotide Sequence (SEQ ID NO:50)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATT
AGCTGCTCCAGTCAACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTG
AAGCTGTCATCGGTTACTTAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGC
CATTTTCCAACAGCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCA
GCATTGCTGCTAAAGAAGAAGGGGTATCTTTGGATAAAAGAGAGGCTGAAGC
TGATGCTGAATTTAGACATGATTCTGGTTATGAAGTTCATCATCAAAAATTGGT
TTTTTTTGCTGAAGATGTTGGTTCTAATAAAGGTGCTATTATTGGTTTGATGGTT
GGTGGTGTTGTCATTGCTAAGAGAGAAGCCGAAGCTGAAGCTGATGCTGAATT
TAGACATGATTCTGGTTATGAAGTTCATCATCAAAAATTGGTTTTTTTTGCTGA
AGATGTTGGTTCTAATAAAGGTGCTATTATTGGTTTGATGGTTGGTGGTGTTGT
CATTGCTTAAACCCAGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_1X Amino Acid Sequence (SEQ ID NO:51)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIA

Matα-Aβ42_2X Nucleotide Sequence (SEQ ID NO:52)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCG
AAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTC
GTAATCGCTTAAACCCAGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_2X Amino Acid Sequence (SEQ ID NO:53)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIA

Matα-Aβ42_4X Nucleotide Sequence (SEQ ID NO:54)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTTCCTTCAATTTTTACTGCAGTTTTATTCGCAGCATCCTCCGCATT
AGCTGCTCCAGTCAACACTACAACAGAAGATGAAACGGCACAAATTCCGGCTG
AAGCTGTCATCGGTTACTTAGATTTAGAAGGGGATTTCGATGTTGCTGTTTTGC
CATTTTCCAACAGCACAAATAACGGGTTATTGTTTATAAATACTACTATTGCCA
GCATTGCTGCTAAAGAAGAAGGGGTATCTTTGGATAAAAGAGAGGCTGAAGC
TGATGCTGAATTTAGACATGATTCTGGTTATGAAGTTCATCATCAAAAATTGGT
TTTTTTTGCTGAAGATGTTGGTTCTAATAAAGGTGCTATTATTGGTTTGATGGTT
GGTGGTGTTGTCATTGCTAAGAGAGAAGCCGAAGCTGAAGCTGATGCTGAATT
TAGACATGATTCTGGTTATGAAGTTCATCATCAAAAATTGGTTTTTTTTGCTGA
AGATGTTGGTTCTAATAAAGGTGCTATTATTGGTTTGATGGTTGGTGGTGTTGT
CATTGCTAAAAGAGAAGCCGACGCTGAAGCTGATGCTGAATTTAGACATGATT
CTGGTTATGAAGTTCATCATCAAAAATTGGTTTTTTTTGCTGAAGATGTTGGTT
CTAATAAAGGTGCTATTATTGGTTTGATGGTTGGTGGTGTTGTCATTGCTAAAA
GAGAAGCCGACGCTGAAGCTGATGCTGAATTTAGACATGATTCTGGTTATGAA
GTTCATCATCAAAAATTGGTTTTTTTTGCTGAAGATGTTGGTTCTAATAAAGGT
GCTATTATTGGTTTGATGGTTGGTGGTGTTGTCATTGCTTAAACCCAGCTTTCTT
GTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X Amino Acid Sequence (SEQ ID NO:55)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_6X Nucleotide Sequence (SEQ ID NO:56)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCG
AAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTC
GTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACG
ACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAAGATGTC
GGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCGC
AAAAAGAGAAGCTGACGCAGAAGCAGACGCCGAATTCAGACACGATTCAGGT
TACGAAGTTCACCATCAAAAATTGGTATTTTTCGCAGAAGATGTTGGTTCCAAC
AAAGGTGCCATTATTGGTTTGATGGTTGGTGGTGTCGTCATTGCCAAGAGAGA
AGCTGAAGCTGAAGCCGACGCAGAATTCAGACACGACAGTGGTTATGAAGTC
CACCATCAAAAGTTGGTCTTTTTTGCTGAAGATGTTGGTTCTAACAAAGGTGCA
ATCATAGGTTTGATGGTTGGTGGTGTAGTCATAGCAAAAAGAGAAGCAGACGC
TGAAGCAGATGCCGAATTCAGACATGACAGTGGTTATGAAGTTCATCACCAAA
AATTAGTATTCTTCGCTGAAGATGTAGGTAGTAACAAAGGTGCCATAATCGGT
TTGATGGTCGGTGGTGTCGTTATAGCTTAAACCCAGCTTTCTTGTACAAAGTGG
TGCGGCCGCACTCGAG

Matα-Aβ42_6X Amino Acid Sequence (SEQ ID NO:57)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDV

GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG
GVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVI
A

Matα-Aβ42_8X Nucleotide Sequence (SEQ ID NO:58)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTACGGTACCTA
CAAAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCT
TTGGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCT
GAAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTG
CCATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCT
TCTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGC
AGACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGG
TTTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGG
TTGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAA
TTCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCC
GAAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGT
CGTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACAC
GACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAAGATGT
CGGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCG
CAAAAAGAGAAGCTGACGCAGAAGCAGACGCCGAATTCAGACACGATTCAGG
TTACGAAGTTCACCATCAAAAATTGGTATTTTTCGCAGAAGATGTTGGTTCCAA
CAAAGGTGCCATTATTGGTTTGATGGTTGGTGGTGTCGTCATTGCCAAGAGAG
AAGCTGAAGCTGAAGCCGACGCAGAATTCAGACACGACAGTGGTTATGAAGT
CCACCATCAAAAGTTGGTCTTTTTTGCTGAAGATGTTGGTTCTAACAAAGGTGC
AATCATAGGTTTGATGGTTGGTGGTGTAGTCATAGCAAAAAGAGAAGCAGACG
CTGAAGCAGATGCCGAATTCAGACATGACAGTGGTTATGAAGTTCATCACCAA
AAATTAGTATTCTTCGCTGAAGATGTAGGTAGTAACAAAGGTGCCATAATCGG
TTTGATGGTCGGTGGTGTCGTTATCGCTAAGAGAGAAGCAGACGCTGAAGCTG

ACGCAGAATTCAGACATGACTCAGGTTACGAAGTACACCATCAAAAGTTAGTA
TTCTTCGCCGAAGATGTAGGTTCAAACAAAGGTGCTATCATCGGTTTAATGGTT
GGTGGTGTCGTAATTGCTAAAAGAGAAGCTGAAGCCGAAGCAGATGCAGAAT
TCAGACATGATTCAGGTTACGAAGTCCATCACCAAAAATTGGTCTTTTTCGCTG
AAGATGTCGGTTCAAACAAGGGTGCAATTATTGGTTTGATGGTCGGTGGTGTA
GTAATTGCCTAAACCCAGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_8X Amino Acid Sequence (SEQ ID NO:59)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG
GVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVI
AKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIAKRE
AEAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Matα-Aβ40_4X Nucleotide Sequence (SEQ ID NO:60)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTAAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAATTCAGA
CACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCGAAGA
TGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTCGTTA

AGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACGACTCCGGTTA
CGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAAGATGTCGGTTCAAACA
AGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTCAAAAGAGAAGCTGAC
GCTGAAGCAGACGCCGAATTCAGACACGACTCAGGTTATGAAGTACACCATCA
AAAATTGGTATTTTTCGCAGAAGATGTTGGTTCCAACAAAGGTGCCATTATTG
GTTTGATGGTTGGTGGTGTCGTTTAAACCCAGCTTTCTTGTACAAAGTGGTGCG
GCCGCACTCGAG

Matα-Aβ40_4X Amino Acid Sequence (SEQ ID NO:61)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVKREAEAEADAEFRHDSGYEVHHQKLVFFAEDVGS
NKGAIIGLMVGGVVKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAII
GLMVGGVVKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG
GVV

Matα-Aβ40_6X Nucleotide Sequence (SEQ ID NO:62)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTAAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAATTCAGA
CACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGCCGAAGA
TGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTCGTTA
AGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACGACTCCGGTTA
CGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAAGATGTCGGTTCAAACA

AGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTCAAAAGAGAAGCTGAC
GCTGAAGCAGACGCCGAATTCAGACACGACTCAGGTTATGAAGTACACCATCA
AAAATTGGTATTTTTCGCAGAAGATGTTGGTTCCAACAAAGGTGCCATTATTG
GTTTGATGGTTGGTGGTGTCGTCAAGAGAGAAGCCGAAGCCGAAGCTGACGCA
GAATTCAGACATGACAGTGGTTACGAAGTCCACCATCAAAAGTTGGTCTTTTTT
GCTGAAGATGTTGGTTCTAACAAAGGTGCAATCATAGGTTTGATGGTTGGTGG
TGTAGTTAAGAGAGAAGCTGACGCTGAAGCTGATGCAGAATTCAGACATGATT
CAGGTTACGAAGTCCATCATCAAAAATTGGTTTTCTTCGCCGAAGATGTAGGTT
CAAACAAAGGTGCTATCATCGGTTTAATGGTTGGTGGTGTCGTTTGAACCCAG
CTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ40_6X Amino Acid Sequence (SEQ ID NO:63)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVKREAEAEADAEFRHDSGYEVHHQKLVFFAEDVGS
NKGAIIGLMVGGVVKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAII
GLMVGGVVKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVG
GVVKREAEAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVKRE
ADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV

[0112] The following yeast expression constructs containing four copies (4X) of various Aβ42 mutants were also prepared.

Matα-Aβ42_4X_English Nucleotide Sequence (SEQ ID NO:64)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA

GACGCAGAATTTAGAAGAGATTCTGGTTACGAAGTTCACCACCAAAAGTTGGT
TTTTTTCGCTGAAGATGTCGGTTCTAACAAGGGTGCTATTATTGGTTTGATGGT
TGGTGGTGTCGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGAAGAGACTCAGGTTACGAAGTACATCATCAAAAATTAGTATTCTTTGCC
GAAGATGTTGGTAGTAACAAAGGTGCAATCATCGGTTTAATGGTCGGTGGTGT
AGTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGACGCAGAATTCAGAAGA
GATTCAGGTTACGAAGTCCATCACCAAAAGTTAGTTTTCTTCGCAGAAGATGT
CGGTTCAAACAAAGGTGCCATAATAGGTTTAATGGTTGGTGGTGTAGTTATCG
CTAAGAGAGAAGCTGACGCTGAAGCAGATGCAGAATTCAGAAGAGACTCCGG
TTACGAAGTTCACCATCAAAAATTAGTCTTTTTCGCAGAAGATGTTGGTAGTAA
CAAGGGTGCTATAATAGGTTTGATGGTCGGTGGTGTCGTCATAGCTTAAACCC
AGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_English Amino Acid Sequence (SEQ ID NO:65)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRRDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRRDSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRRDSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRRDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_F20E Nucleotide Sequence (SEQ ID NO:66)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTTGAAGCCGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT

TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTATTCGAAGCT
GAAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGT
CGTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACAC
GACTCCGGTTACGAAGTCCATCACCAAAAGTTAGTCTTTGAAGCTGAAGATGT
CGGTTCAAACAAAGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCG
CAAAAAGAGAAGCTGACGCAGAAGCAGACGCCGAATTCAGACACGATTCAGG
TTACGAAGTTCACCATCAAAAATTGGTCTTTGAAGCTGAAGATGTTGGTAGTA
ACAAGGGTGCCATAATAGGTTTGATGGTCGGTGGTGTAGTCATAGCTTAAACC
CAGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_F20E Amino Acid Sequence (SEQ ID NO:67)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFE
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFEAEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFEAEDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFEAEDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_Flemish Nucleotide Sequence (SEQ ID NO:68)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGGTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTGGTG

AAGATGTCGGTAGTAACAAAGGTGCCATAATTGGTTTAATGGTCGGTGGTGTC
GTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACG
ACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTTTTTTTTGGTGAAGATGTCG
GTTCCAACAAGGGTGCAATCATAGGTTTAATGGTTGGTGGTGTCGTTATCGCA
AAAAGAGAAGCTGACGCAGAAGCAGACGCCGAATTCAGACACGATTCAGGTT
ACGAAGTTCACCATCAAAAATTGGTATTCTTTGGTGAAGATGTAGGTTCAAAC
AAAGGTGCCATCATTGGTTTGATGGTTGGTGGTGTCGTAATTGCCTAAACCCA
GCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Flemish Amino Acid Sequence (SEQ ID NO:69)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
GEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFGEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFGEDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFGEDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_Dutch Nucleotide Sequence (SEQ ID NO:70)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTCAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTCGCCC
AAGACGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGTC
GTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACG

ACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTTTTTGCCCAAGACGTA
GGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCGC
AAAAAGAGAAGCTGACGCAGAAGCAGACGCCGAATTCAGACACGATTCAGGT
TACGAAGTTCACCATCAAAAATTGGTATTCTTTGCCCAAGATGTTGGTTCCAAC
AAAGGTGCCATTATTGGTTTGATGGTTGGTGGTGTCGTCATAGCTTAAACCCAG
CTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Dutch Amino Acid Sequence (SEQ ID NO:71)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AQDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAQDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAQDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAQDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_Italian Nucleotide Sequence (SEQ ID NO:72)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTAAAGATGTCGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTCGCC
AAGGACGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGT
CGTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACAC
GACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTTTTTGCCAAGGACGTT
GGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCGC

AAAAAGAGAAGCTGACGCAGAAGCAGACGCCGAATTCAGACACGATTCAGGT
TACGAAGTTCACCATCAAAAATTGGTATTCTTTGCCAAAGATGTAGGTAGTAA
CAAGGGTGCCATAATTGGTTTGATGGTCGGTGGTGTAGTCATAGCTTAAACCC
AGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Italian Amino Acid Sequence (SEQ ID NO:73)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AKDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAKDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAKDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAKDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_Arctic Nucleotide Sequence (SEQ ID NO:74)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTTTTCGCTGGTGATGTTGGTTCTAACAAGGGTGCTATTATTGGTTTGATGGTT
GGTGGTGTCGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAATT
CAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTTTTCTTTGCCG
GTGACGTCGGTAGTAACAAAGGTGCAATCATAGGTTTAATGGTCGGTGGTGTA
GTCATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACG
ACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGGTGACGTA
GGTTCAAACAAGGGTGCCATAATCGGTTTAATGGTTGGTGGTGTAGTAATCGC
AAAAAGAGAAGCTGACGCTGAAGCAGACGCCGAATTCAGACACGACTCAGGT
TATGAAGTACACCATCAAAAATTGGTCTTTTTCGCCGGTGATGTAGGTAGTAA

CAAGGGTGCAATTATCGGTTTGATGGTCGGTGGTGTAGTTATCGCTTAAACCC
AGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Arctic Amino Acid Sequence (SEQ ID NO:75)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AGDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAGDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAGDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAGDVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_E22G (Arctic)/I31E Nucleotide Sequence (SEQ ID NO:76)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTTTTCGCTGGTGATGTTGGTTCTAACAAGGGTGCTGAAATTGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTTTTCTTTGCCG
GTGACGTCGGTAGTAACAAAGGTGCAGAAATAGGTTTAATGGTCGGTGGTGTC
GTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACG
ACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGGTGACGTA
GGTTCAAACAAGGGTGCAGAAATCGGTTTAATGGTTGGTGGTGTAGTCATAGC
AAAGAGAGAAGCTGACGCTGAAGCAGACGCCGAATTCAGACACGACTCAGGT
TATGAAGTACACCATCAAAAATTGGTCTTTTTCGCCGGTGATGTAGGTAGTAA
CAAGGGTGCCGAAATCGGTTTGATGGTCGGTGGTGTCGTTATCGCTTAAACCC
AGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_E22G/I31E Amino Acid Sequence (SEQ ID NO:77)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AGDVGSNKGAEIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAGDV
GSNKGAEIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAGDVGSNK
GAEIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAGDVGSNKGAEI
GLMVGGVVIA

Matα-Aβ42_4X_E22del Nucleotide Sequence (SEQ ID NO:78)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGATGTTGGTTCTAACAAGGGTGCTATTATTGGTTTGATGGTTGG
TGGTGTCGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAATTCA
GACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTCGCCGAT
GTCGGTAGTAACAAAGGTGCAATCATAGGTTTAATGGTCGGTGGTGTAGTCAT
AGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACGACTCC
GGTTACGAAGTCCATCACCAAAAGTTGGTATTTTTTGCCGACGTAGGTTCAAA
CAAAGGTGCCATAATCGGTTTAATGGTTGGTGGTGTAGTAATCGCAAAAAGAG
AAGCTGACGCTGAAGCAGACGCCGAATTCAGACACGACTCAGGTTATGAAGT
ACACCATCAAAAATTGGTATTCTTTGCTGACGTTGGTAGTAACAAGGGTGCCA
TAATAGGTTTGATGGTCGGTGGTGTCGTAATCGCTTAAACCCAGCTTTCTTGTA
CAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_E22del Amino Acid Sequence (SEQ ID NO:79)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
ADVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFADVGS
NKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFADVGSNKGAII
GLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFADVGSNKGAIIGLMVG
GVVIA

Matα-Aβ42_4X_Iowa Nucleotide Sequence (SEQ ID NO:80)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAAATGTCGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTCGCC
GAAAACGTTGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGTGT
CGTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACAC
GACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTTTTTGCCGAAAACGTT
GGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGTTATCGC
AAAAAGAGAAGCTGACGCAGAAGCAGACGCCGAATTCAGACACGATTCAGGT
TACGAAGTTCACCATCAAAAATTGGTATTCTTCGCAGAAAACGTTGGTTCCAA
CAAAGGTGCTATTATTGGTTTAATGGTTGGTGGTGTCGTCATTGCTTAAACCCA
GCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Iowa Amino Acid Sequence (SEQ ID NO:81)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AENVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHDSGYEVHHQKLVFFAENV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAENVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHDSGYEVHHQKLVFFAENVGSNKGAIIG
LMVGGVVIA

Matα-Aβ42_4X_Tottori Nucleotide Sequence (SEQ ID NO:82)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATAATTCTGGTTACGAAGTTCACCACCAAAAGTTGGT
TTTTTTCGCTGAAGATGTCGGTTCTAACAAGGGTGCTATTATTGGTTTGATGGT
TGGTGGTGTCGTTATTGCTAAAAGAGAAGCCGAAGCTGAAGCTGATGCCGAAT
TCAGACACAATAGTGGTTATGAAGTCCATCATCAAAAATTAGTTTTCTTTGCCG
AAGATGTTGGTAGTAACAAAGGTGCAATCATCGGTTTAATGGTCGGTGGTGTA
GTAATAGCCAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCAGACACA
ACTCCGGTTATGAAGTACATCACCAAAAGTTAGTCTTTTTCGCCGAAGATGTTG
GTTCAAACAAAGGTGCCATTATAGGTTTAATGGTTGGTGGTGTAGTTATCGCTA
AGAGAGAAGCTGACGCTGAAGCAGACGCCGAATTCAGACACAACTCAGGTTA
CGAAGTCCACCATCAAAAATTGGTATTCTTCGCAGAAGATGTAGGTAGTAACA
AGGGTGCCATAATAGGTTTGATGGTCGGTGGTGTCGTCATAGCTTAAACCCAG
CTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ42_4X_Tottori Amino Acid Sequence (SEQ ID NO:83)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHNSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIAKREAEAEADAEFRHNSGYEVHHQKLVFFAEDV
GSNKGAIIGLMVGGVVIAKREADAEADAEFRHNSGYEVHHQKLVFFAEDVGSNK
GAIIGLMVGGVVIAKREADAEADAEFRHNSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIA

[0113] The Aβ sequence was codon optimized for expression in yeast. The Aβ expression construct consists of attB Gateway flanking regions at the 5' and 3' ends (nucleotides ACAAGTTTGTACAAAAAAGCAGGCT (SEQ ID NO:84) and ACCCAGCTTTCTTGTACAAAGTGGT (SEQ ID NO:85) , respectively) for Gateway cloning of MataPrepro-Aβ42 sequence. The entire construct was synthesized into pUC57-Kan plasmid and cloned into the Gateway entry vector pDONR221. These constructs were then transferred into the pAG425Gal expression vector by Gateway LR cloning (Alberti et al., "A suite of Gateway cloning vectors for high throughput genetic analysis in Saccharomyces cerevisiae," Yeast 24:913 (2007)). The GAL1 promoter enables expression of Aβ fusion transgenes under galactose-inducible conditions. The vector has an ampicillin resistant gene for amplification of the vector in E. coli and leucine auxotrophic marker (LEU2) for selection of transformed yeast cells on media lacking leucine (Fig. 2).

[0114] Yeast cells transformed with either an expression vector containing galactose-inducible green fluorescent protein (GFP) or a galactose-inducible expression plasmid encoding a yeast mating factor alpha signal sequence/human amyloid beta 1-42 (Aβ42) or 1-40 (Aβ40) or scrambled Aβ42 fusion polypeptide were grown on glucose or galactose containing synthetic media and growth was assessed. Transformed cells were selected in medium lacking leucine. Individual transformants were grown overnight in complete synthetic medium (CSM) lacking leucine with 2% raffinose as the carbon source. Cell concentrations (OD600) were adjusted in a 96-well plate to OD 0.5 or 1. Cells were then 5-fold serially diluted and spotted on SD media containing glucose (uninduced) and galactose (induced). Plates were incubated at 30° C for 2 days (glucose) or 3 to 4 days (galactose).

[0115] The experimental and control transformants grew equally well on glucose containing media (uninduced), whereas amyloid beta expression in galactose containing media (induced) profoundly inhibited cell growth in a dose number-dependent manner (Fig. 3). Expression of a single copy Aβ42 (1X) on a 2μ high-copy expression plasmid was not toxic, whereas increased Aβ42 copy number (2, 4, 6 and 8-copies) on the same expression plasmid yielded toxicity in transformed cells in a dose-dependent manner (Fig. 3). Expression of Aβ40 (4 and 6 copies) did not produce significant toxicity as compared to the similar copy number of Aβ42 (Fig. 3). The control expression construct of scrambled Aβ42 (4 copies), in which the amino acid sequence of Aβ42 was randomly scrambled, did not produce significant toxicity (Fig. 3).

[0116] Yeast cells were transformed with galactose-inducible expression vector (pAG425Gal) harboring different mutations in Aβ (4 copies). Among these were nine disease-related mutations: *English (H6R), Tottori (D7N), Flemish (A21G), Dutch (E22Q), Italian (E22K), Arctic (E22G), E22_deletion, Iowa (D23N),* and "wild-type" and two detoxifying mutations: *F20E* and *Arctic_I31E.* Growth of transformed yeast cells was tested under induced (galactose) and uninduced (glucose) conditions. Different Aβ mutations caused varying degrees of toxicity upon expression under galactose induction (Fig. 4). For example, the Arctic, E22_deletion, and Dutch mutations caused profound toxicity whereas Arctic_I31E and F20E mutations caused significantly lower amount of toxicity (Fig. 4).

Example 2: Amyloid Beta Expression Constructs for Mammalian Cells

[0117] A human Aβ sequence was fused at the C-terminus of the signal sequence of several eukaryotic secretory proteins. The signal sequences were human insulin (Ins), human trypsin (Tryp), human amyloid precursor protein (APP), human placental secreted alkaline phosphatase (SEAP) and Metridia luciferase (mLuc) of the marine copepod *Metridia longa.* The Aβ peptide was fused to these signal peptides without any linker in between the sequences (Fig. 5). For cytoplasmic expression, human Aβ peptide without any secretory signal was also constructed in similar expression vector (labeled "no signal sequence" in Fig. 5).

[0118] The nucleotide and amino acid sequences of the fusion polypeptides encoded by the expression constructs are as follows.

SEAP-Aβ42_1X Nucleotide Sequence (SEQ ID NO:86)

CACCATGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCC
CTGGGCGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAA
ACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCC
TGATGGTGGGCGGCGTGGTGATTGCGTAA

SEAP-Aβ42_1X Amino Acid Sequence (SEQ ID NO:87)

MLLLLLLLGLRLQLSLGDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL
MVGGVVIA

mLuc-Aβ42_1X Amino Acid Sequence (SEQ ID NO:88)

CACCATGGACATCAAGGTGGTGTTCACCCTGGTGTTCAGCGCCCTGGTG
CAGGCCGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAA
ACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCC
TGATGGTGGGCGGCGTGGTGATTGCGTAA

mLuc-Aβ42_1X Amino Acid Sequence (SEQ ID NO:89)

MDIKVVFTLVFSALVQADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL
MVGGVVIA

APP-Aβ42_1X Nucleotide Sequence (SEQ ID NO:90)

CACCATGCTGCCCGGTTTGGCACTGCTCCTGCTGGCCGCCTGGACGGCT
CGGGCGGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAA
ACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCC
TGATGGTGGGCGGCGTGGTGATTGCGTAA

APP-Aβ42_1X Amino Acid Sequence (SEQ ID NO:91)

MLPGLALLLLAAWTARADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIG
LMVGGVVIA

Ins-Aβ42_1X Nucleotide Sequence (SEQ ID NO:92)

CACCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCC
CTCTGGGGACCTGACCCAGCCGCAGCCGATGCGGAATTTCGCCATGATTCTGG
CTATGAAGTGCATCATCAGAAACTGGTGTTTTTTGCGGAAGATGTGGGCTCTA
ACAAAGGCGCGATTATTGGCCTGATGGTGGGCGGCGTGGTGATTGCGTAA

Ins-Aβ42_1X Amino Acid Sequence (SEQ ID NO:93)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAIIGLMVGGVVIA

Tryp-Aβ42_1X Nucleotide Sequence (SEQ ID NO:94)

CACCATGTCTGCACTTCTGATCCTAGCTCTTGTTGGAGCTGCAGTTGCTG
ATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCATCAGAAACTGGTG
TTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTATTGGCCTGATGGT
GGGCGGCGTGGTGATTGCGTAA

Tryp-Aβ42_1X Amino Acid Sequence (SEQ ID NO:95)

MSALLILALVGAAVADAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLM
VGGVVIA

NSP-Aβ42_1X Nucleotide Sequence (SEQ ID NO:96)

CACCATGGATGCGGAATTTCGCCATGATTCTGGCTATGAAGTGCATCAT
CAGAAACTGGTGTTTTTTGCGGAAGATGTGGGCTCTAACAAAGGCGCGATTAT
TGGCCTGATGGTGGGCGGCGTGGTGATTGCGTAA

NSP-Aβ42_1X Amino Acid Sequence (SEQ ID NO:97)
MDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

[0119] The following mammalian expression construct encoding Aβ40 was also prepared.

Ins-Aβ40_1X Nucleotide Sequence (SEQ ID NO:98)

CACCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCC
CTCTGGGGACCTGACCCAGCCGCAGCCGATGCGGAATTTCGCCATGATTCTGG
CTATGAAGTGCATCATCAGAAACTGGTGTTTTTTGCGGAAGATGTGGGCTCTA
ACAAAGGCGCGATTATTGGCCTGATGGTGGGCGGCGTGGTGTAA

Ins-Aβ40_1X Amino Acid Sequence (SEQ ID NO:99)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAIIGLMVGGVV

[0120] The human Aβ sequence fused to different signal peptide sequence was PCR amplified and cloned in the p3XFLAG-CMV-13 vector. The p3XFLAG-CMV-13 expression vector is a 6.3 kb plasmid for transient expression of fusion proteins in mammalian cells. The prepro-trypsin leader signal sequence, which is an integral part of the vector, was deleted by quick change mutagenesis. Deletion of this prepro-trypsin sequence from the 5' of the multiple cloning

site enabled use of this vector for cloning of Aβ fused to various signal sequences at the N-terminal of the peptide. The vector encodes three adjacent FLAG epitopes downstream of the multiple cloning region. Aβ sequence with a stop codon at the C-terminal end was inserted using the EcoR1 and BglII restriction sites so that the Flag tags are not fused to Aβ. The human cytomegalovirus (CMV) promoter-regulatory region upstream of the transgene drives transcription of the Aβ construct (Fig. 6). The vector also enabled selection of stable transfectants by virtue of the aminoglycoside phosphotransferase II gene (Neo), which confers resistance to aminoglycosides such as G418. It is a shuttle vector for mammalian cells and *E. coli,* in which the plasmid can be maintained and amplified because of the ampicillin resistant gene (AmpR) in the vector backbone.

[0121] The constructs were transiently transfected in 293T cells using FUGENE® 6 transfection reagent following the manufacture's instruction. 293T cells were routinely propagated in adhesion culture in DMEM medium (Invitrogen) supplemented with 10 % FBS and incubated at 37° C with 5% CO_2. For transfection of the expression vector, cells were plated to approximately 90% confluency (approximately 1.2×10^5 cells/ml) in each well of a 6-well tissue culture plate. Before transfection, the spent medium was replaced by 1 ml of serum free or low serum medium (DMEM without FBS or DMEM + 1% FBS). For each transfection of cells in a single well of a 6-well plate, 1 μg of the expression construct was used. Upon transfection cells are incubated for up to 96 hours at 37°C with 5% CO_2.

[0122] Supernatants were harvested 48 and 72 hours post transfection and cells were lysed in RIPA buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 0.25% Deoxycholate, 10% Glycerol, 25 mM NaF, 10 mM $MgCl_2$, 1 mM EDTA, 1% TRITON™ X-100, 0.5 mM PMSF, protease inhibitor cocktail). A solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect and quantify recombinant Aβ from the supernatant and soluble fractions of the cell lysates using ELISA Kit specific for the detection of human Aβ42 (Life Technologies, Catalogue no. KHB3441). Two of the signal peptides (Ins and Tryp) were efficient in secreting pathobiologically relevant Aβ concentration in the supernatant of 293T cells (Figs. 7A-7B).

[0123] Cytotoxicity of the transfected cells was measured by TOXILIGHT™ BioAssay Kit (Lonza) following the manufacturer's instruction. TOXILIGHT™ assay is a bioluminescent, non-destructive cytotoxicity assay designed to measure the release of adenylate kinase from damaged or dying cells. Adenylate kinase is ubiquitous in all eukaryotic cells. It is released into the culture medium when cells are damaged or when cells die. The enzyme actively phosphorylates ADP to form ATP and the resultant ATP is measured using the bioluminescent firefly luciferase reaction. The amount of adenylate kinase in the supernatant is quantitatively proportional to cytolysis and is measured by the intensity of the emitted light by the TOXILIGHT™ reagent. Constructs harboring either Aβ42 or Aβ40 fused to the insulin signal peptide or a vector control without any inserted transgene were used for transfecting the cells. Supernatant from the transfected cells was harvested 48, 72, or 96 hours post-transfection to measure Aβ-mediated cytotoxicity. The construct encoding the insulin signal peptide fused to Aβ42 elicited particularly strong cytotoxicity (Fig. 8).

Example 3: Amyloid Beta Expression in Rat Embryonic Cortical Neurons

[0124] Rat embryonic cortical neurons were used for expressing Aβ. For generating lentiviral constructs, an expression vector was used with the human synapsin 1 promoter upstream of the inserted insulin signal peptide-Ap fusion polypeptide transgene (Fig. 9). The encoded fusion polypeptide is processed in the secretory pathway of the neuron, releasing Aβ in the extracellular milieu.

[0125] The pLV-Aβ constructs along with the psPAX2 packaging vector and pMD2.G envelope vector were transfected in 293T cells using LIPOFECTAMINE® 2000 (Invitrogen) transfection reagent following the manufacture's instruction to generate lentivirus containing the Aβ transgene. The virus was purified using LENTI-X™ Maxi Purification Kit (Clontech) from the supernatant of the transfected 293T cells according to the protocol provided by the manufacturer. Viral titer were determined by using Lentivirus-Associated p24 ELISA Kit (Cell BioLabs).

[0126] Rat embryonic cortices were dissected out of embryonic (E18) rat brains, and neurons were enriched in adherent monolayer culture. These cortical neurons were infected with lentiviral preparation harboring an Aβ transgene or a control gene (RFP in the same vector backbone). Infected cultures were harvested to quantify the amount of Aβ in the supernatant and cell lysate by Aβ-specific ELISA 21 days after infection (Fig. 10A). Corresponding cytotoxicity of the infected neurons was measured by quantifying ATP levels using VIALIGHT™ assay for cell viability (Lonza). Expression of human Aβ in the secretory pathway of rat embryonic cortical neurons caused profound cytotoxicity (Fig. 10B).

[0127] When rat embryonic cortical neurons were infected with lentiviral constructs (as depicted in Fig. 9) expressing wild-type human Aβ42 or various mutations (i.e., F20E, Arctic (E22G), I31E, and E22G/I31E) in the secretory pathway of these cells, differential toxicity was observed, reminiscent of the differential cytotoxic effects caused by these mutations in yeast (see Example 1). The Arctic mutation (E22G) of human Aβ42 had a more toxic effect compared to the wild-type human Aβ42. Three mutations -- F20E, I31E, and E22G/I31E -- exerted less cytotoxic effects compared to either the wild-type human Aβ42 or the Arctic variation of human Aβ42.

Example 4: Amyloid Beta-Induced Cytotoxicity in Neuronal Cultures

[0128] Rat cortical neurons were infected with a doxycycline-inducible lentiviral GFP construct. Two days post-infection, the cells were infected again with neuron-specific (synapsin promoter) lentiviral Aβ constructs (i.e., Aβ40, Aβ42, and various mutations). The doubly-infected cells were treated with doxycycline 7 days post-infection to express GFP. After 21 days of the initial infection, live neurons were visualized using fluorescence microscopy to observe the effect of different Aβ variants on neuronal survival and morphology by visualizing neurons expressing GFP in presence of Aβ expressed from the transgenes. Wild-type human Aβ42 and the Aβ42_Arctic (E22G) mutation caused neuronal death and adversely affected neurite outgrowth and structures, which were detected by microscopic observation of GFP-stained live neurons (Fig. 11A). Both the Aβ42_I31E and the Aβ42_Arc_/I31E mutations demonstrated reduced cytotoxicity and no significant morphological distortions of the neurons and neurites in rat cortical neurons infected at multiplicity of infection (MOI) identical to the wild-type Aβ42 and Aβ42_Arctic (E22G) viral constructs.

[0129] Similar observations were also made by immunohistochemical analysis of rat cortical neurons infected with neuron-specific (synapsin promoter) lentiviral Aβ constructs. Twenty-one days post-infection, the neurons were stained with a neuron-specific microtubule-associated protein 2 (Map2) primary antibody (rabbit polyclonal, Millipore) and ALEXA FLUOR® (red fluorophore conjugated) secondary antibody (goat anti-rabbit polyclonal, Life Technologies). Neuronal cells and morphology were visualized to observe the effect of different Aβ mutations using fluorescence microscopy. Similar to the results obtained from live neurons described above, wild-type Aβ42 and the Aβ42_Arctic (E22G) mutation caused profound cytotoxicity, resulting in massive neuronal loss and distorted neurite structures and outgrowth (Fig. 11B). Also similar to the observations with live neuronal imaging, the Aβ42_I31E and Aβ42_Arc/I31E mutations demonstrated reduced cytotoxicity and had no significant effects on the neuronal morphology (Figure 11B).

Example 5: Poly-Aβ Expression in Mammalian Cells

[0130] Similar to the poly-Aβ (Aβ40 or Aβ42_1X/4X/6X/8X) expression constructs in yeast (see Example 1), a poly-Aβ toxicity model was developed in mammalian cells. Stable cell lines with chromosomally-integrated tetracycline (doxycycline)-inducible poly-Aβ transgenes (Aβ42-4X, Aβ42-6X, Aβ40-4X, Aβ40-6X and Aβ42-Arctic-4X) linked to an N-terminal insulin signal peptide were generated (Figs. 13A-13B). These expression constructs were generated using the pLix402 lentiviral plasmid (Fig. 12). The poly-Aβ constructs were individually transfected into 293T cells along with the lentiviral packaging plasmid psPAX and the viral envelope-expressing plasmid mMD2.G using LIPOFECTAMINE® 2000 (Invitrogen) transfection reagent to generate lentivirus containing the poly-Aβ transgene. The virus was harvested from the supernatant of transfected 293T cells 2 and 3 days post-transfection and subsequently purified and concentrated using the LENTI-X™ Maxi Purification kit (Clontech) according to the manufacturer's instructions.

[0131] To generate stable cells harboring the expression transgene for the poly-Aβ constructs, 293T cells were infected using POLYBRENE® infection reagent (Sigma Aldrich), which is used to introduce retroviral vectors into mammalian cells. Infected cells were then selected using puromycin (2 -10 μg/ml). Infected cells harboring the transgene survive this selection pressure whereas the control cells without the transgene die in 2-3 days. Aliquots of the selected cells were frozen in liquid nitrogen or maintained and propagated for further applications.

[0132] Expression of Aβ peptide from these stable cell lines was measured in the culture supernatant 72-96 hours following doxycycline induction (Fig. 13D). Toxicity was measured 72-96 hours following doxycycline induction using a TOXILIGHT™ cytotoxicity assay (Lonza) as described in Example 2. These stable lines demonstrated an Aβ peptide-mediated cytotoxic effect (Aβ42-6X ≥ Aβ42-Arctic-4X > Aβ42-4X > AB40-6X ≥ Aβ40-4X), as determined by adenylate kinase release in the cell culture supernatant from damaged cells upon doxycycline-induction of the transgenes (Fig. 13C).

Example 6: Poly-Aβ Expression in Neuronal Cells

[0133] Rat embryonic cortical neurons were infected with lentiviral poly-Aβ expression constructs to model poly-Aβ toxicity. Tetracycline (doxycycline)-inducible poly-Aβ (Aβ42-4X, Aβ42-6X, Aβ40-4X, Aβ40-6X, and Aβ42-Arctic-4X) viral constructs described in Example 5 were used to express poly-Aβ constructs in rat cortical neurons. In each expression construct, the poly-Aβ coding sequence is linked to an N-terminal insulin signal peptide. The poly-Aβ expression plasmids were packaged in lentiviral particles, which were generated by 293T cells as described in Example 3.

[0134] Rat cortical neurons were enriched from cortices dissected out of embryonic (E18) rat brains and enriched in adherent monolayer culture as described in Example 3. Purified lentiviral preparations at different MOI were used to infect these cortical neurons to express poly-Aβ transgenes. Three days post-infection, expression of poly-Aβ was induced by adding doxycycline (2 μg/ml) to the infected cultures. The viability of the infected neurons was measured three weeks post doxycycline-induction by quantifying ATP levels using VIALIGHT™ assay for cell viability (Lonza) to determine the cytotoxicity of the poly-Aβ expression constructs. Expression of human Aβ42-4X-WT or Aβ42-4X-Arctic in the secretory pathway of rat embryonic cortical neurons caused profound cytotoxicity compared to Aβ40-4X (Fig. 14).

Example 7: Rescue of Aβ-Toxicity by Small Molecule Compounds in Mammalian Cells

[0135] The cytotoxic model in 293T cells was used to test the effect of small molecule compounds to alleviate Aβ-mediated toxicity. The Aβ42 or Aβ40 expression construct was transiently transfected and small molecules were added in different dosages 12 hours post-transfection. Cytotoxicity was measured at different compound dosages by adenylate kinase released from the damaged cells by TOXILIGHT™ BioAssay Kit (Lonza) 96 hours post-transfection. One example of a compound-mediated effect of toxicity alleviation was by clioquinol (CQ), which rescued Aβ-mediated toxicity between concentrations of 2 and 5 μM (Fig. 15). Similar assays are also performed using virally-infected stable 293T cells harboring tetracycline (doxycycline)-inducible poly-Aβ expression constructs to identify compounds that rescue cytotoxicity caused by poly-Aβ expression constructs.

Example 8: An Aβ43 Expression Construct is Toxic to Yeast Cells

[0136] Aβ43 was expressed in the yeast *S. cerevisiae* as a tandem polypeptide (four tandem Aβ peptides linked by 6-8 amino acid linkers) using a poly-Aβ expression construct under the control of the galactose-inducible GAL1 promoter. The Aβ polypeptide coding sequence is linked at the N-terminus to the mating factor α pre-pro signal sequence for directed expression of the transgene in the secretory pathway (Fig. 16A).

[0137] The yeast expression construct containing four copies (4X) of Aβ43 had the following nucleotide and amino acid sequences.

Matα-Aβ43_4X Nucleotide Sequence (SEQ ID NO:100)

GTCGACTGGATCCACAAGTTTGTACAAAAAAGCAGGCTGGTACCAAAA
GAATGAGATTCCCATCTATTTTCACCGCTGTTTTGTTTGCTGCTTCTTCTGCTTT
GGCTGCTCCAGTTAACACTACTACTGAAGATGAAACTGCTCAAATTCCAGCTG
AAGCTGTTATTGGTTACTTGGATTTGGAAGGTGATTTCGATGTTGCTGTTTTGC
CATTCTCTAACTCTACCAACAATGGTTTGTTGTTCATCAACACCACCATTGCTT
CTATTGCTGCTAAAGAAGAAGGTGTCTCTTTGGATAAGAGAGAAGCTGAAGCA
GACGCAGAATTCAGACATGATTCTGGTTATGAAGTTCACCACCAAAAGTTGGT
TTTCTTCGCTGAAGATGTTGGTTCTAACAAGGGTGCTATTATCGGTTTGATGGT
TGGTGGTGTAGTTATTGCTACAAAAAGAGAAGCCGAAGCTGAAGCTGATGCCG
AATTCAGACACGATAGTGGTTACGAAGTACATCATCAAAAATTAGTCTTTTTTG
CCGAAGATGTCGGTAGTAACAAAGGTGCAATCATTGGTTTAATGGTCGGTGGT
GTCGTAATAGCAACTAAGAGAGAAGCAGACGCCGAAGCCGATGCAGAATTCA

GACACGACTCCGGTTACGAAGTCCATCACCAAAAGTTGGTATTCTTTGCCGAA
GATGTCGGTTCAAACAAGGGTGCCATAATAGGTTTAATGGTTGGTGGTGTCGT
TATCGCTACCAAGAGAGAAGCTGACGCTGAAGCAGACGCCGAATTCAGACAC
GACTCAGGTTATGAAGTACACCATCAAAAATTGGTATTTTTCGCAGAAGATGT
TGGTTCCAACAAAGGTGCCATTATTGGTTTGATGGTTGGTGGTGTCGTCATAGC
TACTTAAACCCAGCTTTCTTGTACAAAGTGGTGCGGCCGCACTCGAG

Matα-Aβ43_4X Amino Acid Sequence (SEQ ID NO:101)

MRFPSIFTAVLFAASSALAAPVNTTTEDETAQIPAEAVIGYLDLEGDFDVAV
LPFSNSTNNGLLFINTTIASIAAKEEGVSLDKREAEADAEFRHDSGYEVHHQKLVFF
AEDVGSNKGAIIGLMVGGVVIATKREAEAEADAEFRHDSGYEVHHQKLVFFAED
VGSNKGAIIGLMVGGVVIATKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGS
NKGAIIGLMVGGVVIATKREADAEADAEFRHDSGYEVHHQKLVFFAEDVGSNKG
AIIGLMVGGVVIAT

[0138] The toxicity of Aβ43 to yeast cells was compared to that of similar poly-Aβ expression constructs expressing Aβ42, Aβ40, Aβ48, or Aβ49. The Aβ_scrambled construct, in which the amino acid sequence of Aβ42 was randomly scrambled, served as a control tandem peptide, and a GFP construct served as a vector control. The transgenes were expressed from an episomal expression plasmid (2-micron) under the selection of leucine auxotrophy. The growth of the transformed yeast was measured on synthetic agar media lacking leucine and containing galactose for induction of the transgene. The transformed strains were also grown on similar media with glucose as a measure of growth without the induction of transgene expression. An ImageJ spot assay quantification macro was used to quantify yeast growth on solid media. Aβ43 was significantly more cytotoxic than other Aβ peptides when expressed in yeast (Fig. 16B). The relative toxicity was Aβ43 > Aβ49 ≥ Aβ48 ≥ Aβ42 > Aβ40.

Example 9: An Aβ43 Expression Construct is Toxic to Mammalian Cells

[0139] To express Aβ43 peptide in mammalian cells, an expression construct was generated by fusing the insulin signal peptide to the N-terminus of the Aβ43 peptide coding sequence (Figs. 17A and 17B).

[0140] The mammalian expression construct encoding Aβ43 had the following nucleotide and amino acid sequences.

Ins-Aβ43_1X Nucleotide Sequence (SEQ ID NO:102)

CACCATGGCCCTGTGGATGCGCCTCCTGCCCCTGCTGGCGCTGCTGGCC
CTCTGGGGACCTGACCCAGCCGCAGCCGATGCGGAATTTCGCCATGATTCTGG
CTATGAAGTGCATCATCAGAAACTGGTGTTTTTTGCGGAAGATGTGGGCTCTA
ACAAAGGCGCGATTATTGGCCTGATGGTGGGCGGCGTGGTGATTGCGACATAA

Ins-Aβ43_1X Amino Acid Sequence (SEQ ID NO:103)

MALWMRLLPLLALLALWGPDPAAADAEFRHDSGYEVHHQKLVFFAEDVG
SNKGAIIGLMVGGVVIAT

[0141] The expression construct is expressed under the CMV promoter using pCMV vector as described above (see Example 2 and Fig. 6). The cytotoxic effect of Aβ43 in mammalian cells was tested in 293T cells by transiently transfecting the expression construct and measuring adenylate kinase released from the damaged cells 96 hours post-transfection. Cytotoxicity was measured using the TOXILIGHT™ BioAssay Kit (Lonza) following the manufacturer's instructions (see Example 2). Similar to the results from yeast cells described in Example 8, Aβ43 was significantly more cytotoxic compared to Aβ42, Aβ48, Aβ49, or Aβ40 (Fig. 17C).

Other Embodiments

[0142] It is to be understood that claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a

given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all embodiments in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise and such embodiments do not constitute added matter or extend beyond the content of the application as filed. Where elements are presented as lists, it is to be understood that each subgroup of the elements is also disclosed, and any one or more element(s) can be removed from the group, and such subgroup or resulting list is explicitly disclosed herein and does not constitute added matter or extend beyond the content of the application as filed. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. It should also be understood that any embodiment of the invention, can be explicitly excluded from the claims without constituting added matter or extending beyond the content of the application as filed.

Claims

1. An expression construct comprising a promoter operably linked to a nucleic acid encoding a polypeptide comprising a signal sequence, a Golgi-directing pro sequence, and a polypeptide having the formula $[X\text{-}Y]_n$, wherein each X is, independently, absent or is a linker; each Y is, independently, a human amyloid beta peptide; and n is an integer between two and eight, inclusive, wherein expression of the nucleic acid and production of the polypeptide in a yeast cell results in a decrease in growth or viability of the cell.

2. The expression construct of claim 1, wherein:

   (i) the signal sequence is identical to the signal sequence of a naturally occurring yeast protein, optionally wherein the signal sequence is the yeast mating factor alpha signal sequence or the signal sequence of yeast killer toxin K1, secreted acid phosphatase, invertase (sucrase), or Pst1;
   (ii) the Golgi-directing pro sequence is the yeast mating factor alpha pro sequence or the pro sequence of yeast KEX2, carboxypeptidase Y, Pep4, or Prb1;
   (iii) the promoter is an inducible promoter, optionally wherein the inducible promoter is GAL1-10, GAL1, GALL, GALS, GPD, ADH, TEF, CYC1, MRP7, MET25, TET, VP16, or VP16-ER; and/or
   (iv)

      a) each human amyloid beta protein is independently selected from the group consisting of wild type Aβ38, wild type Aβ39, wild type Aβ40, wild type Aβ41, wild type Aβ42, wild type Aβ43, wild type Aβ48, and wild type Aβ49;
      b) each human amyloid beta protein is independently selected from the group consisting of Aβ38, Aβ39, Aβ40, Aβ41, Aβ42, Aβ43, Aβ48, and Aβ49 and comprises a mutation selected from the group consisting of A2T, A2V, H6R, D7N, E11K, F20E, A21G, E22G, E22Q, E22K, E22 deletion, D23N, I31E, E22G/I31E, A42T, and A42V, wherein the mutation is relative to the amino acid sequence of SEQ ID NO:105; or
      c) the human amyloid beta protein is wild type Aβ42.

3. The expression construct of claim 1 or 2, wherein the polypeptide comprises:

   (i) at least four human amyloid beta peptides, and/or
   (ii)

      a) each human amyloid beta peptide comprises the same amino acid sequence; or
      b) the polypeptide comprises at least two human amyloid beta peptides having different amino acid sequences.

4. The expression construct of any one of claims 1 to 3, wherein:

   (i) the polypeptide comprises the amino acid sequence of SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, or SEQ ID

NO:101; and/or
(ii) the expression construct is an episomal plasmid or an integrative plasmid, optionally wherein the integrative plasmid is pAG303, pAG304, pAG305, pAG306, pRS303, pRS304, pRS305, pRS306, or a derivative thereof.

5. A yeast cell comprising the expression construct of any of the preceding claims.

6. The yeast cell of claim 6, wherein:

(i) expression of the nucleic acid and production of the polypeptide renders the cell non-viable;
(ii) the expression construct is episomal or is integrated in the genome of the yeast cell;
(iii) the yeast is Saccharomyces cerevisiae, Saccharomyces uvae, Saccharomyces kluyveri, Schizosaccharomyces pombe, Kluyveromyces lactis, Hansenula polymorpha, Pichia pastoris, Pichia methanolica, Pichia kluyveri, Yarrowia lipolytica, Candida sp., Candida utilis, Candida cacaoi, Geotrichum sp., or Geotrichum fermentans;
(iv) at least one gene that encodes a protein involved in drug efflux or cell permeability is disrupted, optionally wherein the at least one gene is PDR1, PDR3, PDR5, SNQ2, or ERG6; and/or
(v) the yeast cell allows for expression from GAL promoters on carbon sources other than galactose; optionally wherein the yeast cell comprises a gene encoding a fusion protein comprising the Gal4 DNA binding domain fused to a transcriptional activation domain and a regulatory domain; optionally wherein the fusion protein activates transcription in the presence of estrogen or an analog thereof; preferably wherein the yeast cell comprises a gene encoding a Gal4-ER-VP16 fusion protein.

7. A method of inducing toxicity in a cell, the method comprising:
providing the cell of claim 5 or 6; and
inducing a level of expression of the nucleic acid in the cell that is toxic to the cell.

8. A method of identifying a compound that prevents or suppresses amyloid beta-induced toxicity, the method comprising:

culturing the cell of claim 5 or 6, in the presence of a candidate agent and under conditions that allow for expression of the nucleic acid at a level that, in the absence of the candidate agent, is sufficient to induce toxicity in the cell;
measuring cell growth or viability in the presence of the candidate agent; and
comparing cell growth or viability measured in the presence of the candidate agent to cell growth or viability in the absence of the candidate agent,
wherein if cell growth or viability is increased in the presence of the candidate agent as compared to in the absence of the candidate agent, then the candidate agent is identified as a compound that prevents or suppresses amyloid beta-induced toxicity.

9. A method of identifying a genetic suppressor or enhancer of amyloid beta-induced toxicity, the method comprising:

providing the cell of claim 5 or 6, wherein the cell has been genetically modified to overexpress a gene;
culturing the cell under conditions that allow for expression of the protein at a level that, in the absence of overexpression of the gene, is sufficient to induce toxicity in the cell;
measuring cell growth or viability in the presence of overexpression of the gene; and
comparing cell growth or viability measured in the presence of overexpression of the gene to cell growth or viability in the absence of overexpression of the gene,
wherein (i) if cell growth or viability is increased in the presence of overexpression of the gene as compared to in the absence of overexpression of the gene, then the gene is identified as a genetic suppressor of amyloid beta-induced toxicity, and (ii) if cell growth or viability is decreased in the presence of overexpression of the gene as compared to in the absence of overexpression of the gene, then the gene is identified as a genetic enhancer of amyloid beta-induced toxicity.

10. A method of identifying a genetic suppressor or enhancer of amyloid beta-induced toxicity, the method comprising:

providing the cell of claim 5 or 6, wherein an endogenous gene of the cell has been disrupted;
culturing the cell under conditions that allow for expression of the protein at a level that, in the absence of disruption of the endogenous gene, is sufficient to induce toxicity in the cell;
measuring cell growth or viability in the presence of disruption of the endogenous gene; and

comparing cell growth or viability measured in the presence of disruption of the endogenous gene to cell growth or viability in the absence of disruption of the endogenous gene,
wherein (i) if cell growth or viability is increased in the presence of disruption of the endogenous gene as compared to in the absence of disruption of the endogenous gene, then the gene is identified as a genetic enhancer of amyloid beta-induced toxicity, and (ii) if cell growth or viability is decreased in the presence of disruption of the endogenous gene as compared to in the absence of disruption of the endogenous gene, then the gene is identified as a genetic suppressor of amyloid beta-induced toxicity.

Patentansprüche

1. Expressionskonstrukt, umfassend einen Promotor, der operativ mit einer Nukleinsäure verknüpft ist, die ein Polypeptid, umfassend eine Signalsequenz, eine Golgi-leitende Pro-Sequenz und ein Polypeptid mit der Formel $[X-Y]_n$, kodiert, wobei jedes X, unabhängig, fehlt oder ein Linker ist; jedes Y, unabhängig, ein humanes Amyloid-beta-Peptid ist; und n eine ganze Zahl zwischen zwei und einschließlich acht ist, wobei die Expression der Nukleinsäure und die Produktion des Polypeptids in einer Hefezelle in einer Abnahme des Wachstums oder der Lebensfähigkeit der Zelle resultiert.

2. Expressionskonstrukt nach Anspruch 1, wobei:

   (i) die Signalsequenz identisch mit der Signalsequenz eines natürlich vorkommenden Hefeproteins ist, wobei wahlweise die Signalsequenz die Signalsequenz des Hefe-Paarungsfaktors Alpha oder die Signalsequenz des Hefegift-Toxins K1, sekretierter saurer Phosphatase, Invertase (Sucrase) oder Pst1 ist;
   (ii) die Golgi-leitende Pro-Sequenz die Signalsequenz des Hefe-Paarungsfaktors Alpha oder die Pro-Sequenz von Hefe KEX2, einer Carboxypeptidase Y, Pep4 oder Prb1 ist;
   (iii) der Promoter ein induzierbarer Promotor ist, wobei wahlweise der induzierbare Promotor GAL1-10, GAL1, GALL, GALS, GPD, ADH, TEF, CYC1, MRP7, MET25, TET, VP16 oder VP16-ER ist; und/oder
   (iv)

      a) jedes humane Amyloid-beta-Protein unabhängig ausgewählt ist aus der Gruppe, bestehend aus Wildtyp-Aß38, Wildtyp-Aß39, Wildtyp-Aß40, Wildtyp-Aß41, Wildtyp-Aß42, Wildtyp-Aß43, Wildtyp-Aß48 und Wildtyp-Aß49;
      b) jedes humane Amyloid-beta-Protein unabhängig ausgewählt ist aus der Gruppe, bestehend aus Aβ38, Aβ39, Aβ40, Aβ41, Aβ42, Aβ43, Aβ48 und Aβ49 und eine Mutation umfasst, ausgewählt aus der Gruppe, bestehend aus A2T, A2V, H6R, D7N, E11K, F20E, A21G, E22G, E22Q, E22K, E22-Deletion, D23N, I31E, E22G/I31 E, A42T und A42V, wobei die Mutation in Bezug auf die Aminosäuresequenz von SEQ ID NO:105 ist; oder
      c) das humane Amyloid-beta-Protein Wildtyp-Aß42 ist.

3. Expressionskonstrukt nach Anspruch 1 oder 2, wobei das Polypeptid umfasst:

   (i) mindestens vier humane Amyloid-beta-Peptide, und/oder
   (ii)

      a) jedes humane Amyloid-beta-Peptid die gleiche Aminosäuresequenz umfasst; oder
      b) das Polypeptid mindestens zwei humane Amyloid-beta-Peptide mit unterschiedlichen Aminosäuresequenzen umfasst.

4. Expressionskonstrukt nach einem der Ansprüche 1 bis 3, wobei:

   (i) das Polypeptid die Aminosäuresequenz von SEQ ID NO:53, SEQ ID NO NO:55, SEQ ID NO NO:57, SEQ ID NO NO:59, SEQ ID NO NO:61, SEQ ID NO NO:63, SEQ ID NO NO:65, SEQ ID NO NO:67, SEQ ID NO NO:69, SEQ ID NO NO:71, SEQ ID NO NO:73, SEQ ID NO NO:75, SEQ ID NO NO:77, SEQ ID NO NO:79, SEQ ID NO NO:81, SEQ ID NO NO:83 oder SEQ ID NO:101 umfasst; und/oder
   (ii) das Expressionskonstrukt ein episomales Plasmid oder ein integratives Plasmid ist, wobei wahlweise das integrative Plasmid pAG303, pAG304, pAG305, pAG306, pRS303, pRS304, pRS305, pRS306 oder ein Derivat davon ist.

5. Hefezelle, umfassend das Expressionskonstrukt nach einem der vorstehenden Ansprüche.

6. Hefezelle nach Anspruch 6, wobei:

(i) die Expression der Nukleinsäure und die Produktion des Polypeptids die Zelle nicht lebensfähig macht;
(ii) das Expressionskonstrukt episomal ist oder in das Genom der Hefezelle integriert ist;
(iii) die Hefe Saccharomyces cerevisiae, Saccharomyces uvae, Saccharomyces kluyveri, Schizosaccharomyces pombe, Kluyveromyces lactis, Hansenula polymorpha, Pichia pastoris, Pichia methanolica, Pichia kluyveri, Yarrowia lipolytica, Candida sp., Candida utilis, Candida cacaoi, Geotrichum sp. oder Geotrichum fermentans ist;
(iv) mindestens ein Gen, das ein Protein kodiert, das an Arzneimittel-Efflux oder Zellpermeabilität beteiligt ist, unterbrochen wird, wobei wahlweise das mindestens eine Gen PDR1, PDR3, PDR5, SNQ2 oder ERG6 ist; und/oder
(v) die Hefezelle die Expression von GAL-Promotoren auf anderen Kohlenstoffquellen als Galaktose ermöglicht; wobei wahlweise die Hefezelle ein Gen umfasst, das ein Fusionsprotein kodiert, umfassend die Gal4-DNA-Bindungsdomäne, das an eine Transkriptionsaktivierungsdomäne und eine regulatorische Domäne fusioniert ist; wobei wahlweise das Fusionsprotein die Transkription in Gegenwart von Östrogen oder einem Analogon davon aktiviert; vorzugsweise wobei die Hefezelle ein Gen umfasst, das ein Gal4-ER-VP16-Fusionsprotein kodiert.

7. Verfahren zum Induzieren von Toxizität in einer Zelle, das Verfahren umfassend:
Bereitstellen der Zelle nach Anspruch 5 oder 6; und
Induzieren eines Expressionsniveaus der Nukleinsäure in der Zelle, das für die Zelle toxisch ist.

8. Verfahren zum Identifizieren einer Verbindung, die Amyloid-beta-induzierte Toxizität verhindert oder unterdrückt, das Verfahren umfassend:

Kultivieren der Zelle nach Anspruch 5 oder 6 in Gegenwart eines Kandidatenmittels und unter Bedingungen, die die Expression der Nukleinsäure auf einem Niveau ermöglichen, das, in Abwesenheit des Kandidatenmittels, ausreichend ist, um Toxizität in der Zelle zu induzieren;
Messen des Zellwachstums oder der Lebensfähigkeit in Gegenwart des Kandidatenmittels; und
Vergleichen des Zellwachstums oder der Lebensfähigkeit, gemessen in Gegenwart des Kandidatenmittels, mit dem Zellwachstum oder der Lebensfähigkeit in Abwesenheit des Kandidatenmittels,
wobei, wenn das Zellwachstum oder die Lebensfähigkeit in Gegenwart des Kandidatenmittels im Vergleich zu der Abwesenheit des Kandidatenmittels erhöht wird, dann das Kandidatenmittel als eine Verbindung identifiziert wird, die Amyloid-beta-induzierte Toxizität verhindert oder unterdrückt.

9. Verfahren zum Identifizieren eines genetischen Suppressors oder Enhancers von Amyloid-beta-induzierter Toxizität, das Verfahren umfassend:

Bereitstellen der Zelle nach Anspruch 5 oder 6, wobei die Zelle genetisch modifiziert wurde, um ein Gen zu überexprimieren;
Kultivieren der Zelle unter Bedingungen, die eine Expression des Proteins auf einem Niveau ermöglichen, das in Abwesenheit einer Überexpression des Gens ausreichend ist, um Toxizität in der Zelle zu induzieren;
Messen des Zellwachstums oder der Lebensfähigkeit in Gegenwart der Überexpression des Gens; und
Vergleichen des Zellwachstums oder der Lebensfähigkeit, gemessen in Gegenwart der Überexpression des Gens, mit dem Zellwachstum oder der Lebensfähigkeit in Abwesenheit der Überexpression des Gens,
wobei (i), wenn das Zellwachstum oder die Lebensfähigkeit in Gegenwart einer Überexpression des Gens im Vergleich zur Abwesenheit einer Überexpression des Gens erhöht wird, dann das Gen als ein genetischer Suppressor der Amyloid-beta-induzierten Toxizität identifiziert wird, und (ii), wenn das Zellwachstum oder die Lebensfähigkeit in Gegenwart einer Überexpression des Gens verringert wird, im Vergleich zur Abwesenheit der Überexpression des Gens, dann das Gen als ein genetischer Enhancer der Amyloid-beta-induzierten Toxizität identifiziert wird.

10. Verfahren zum Identifizieren eines genetischen Suppressors oder Enhancers von Amyloid-beta-induzierter Toxizität, das Verfahren umfassend:

Bereitstellen der Zelle nach Anspruch 5 oder 6, wobei ein endogenes Gen der Zelle unterbrochen wurde;
Kultivieren der Zelle unter Bedingungen, die eine Expression des Proteins auf einem Niveau ermöglichen, das

in Abwesenheit einer Unterbrechung des endogenen Gens ausreichend ist, um Toxizität in der Zelle zu induzieren;
Messen des Zellwachstums oder der Lebensfähigkeit in Gegenwart einer Unterbrechung des endogenen Gens; und
Vergleichen des Zellwachstums oder der Lebensfähigkeit, gemessen in Gegenwart einer Unterbrechung des endogenen Gens, mit dem Zellwachstum oder der Lebensfähigkeit in Abwesenheit der Unterbrechung des endogenen Gens,
wobei (i), wenn das Zellwachstum oder die Lebensfähigkeit in Gegenwart einer Unterbrechung des endogenen Gens im Vergleich zur Abwesenheit einer Unterbrechung des endogenen Gens erhöht wird, dann das Gen als ein genetischer Enhancer der Amyloid-beta-induzierten Toxizität identifiziert wird, und (ii), wenn das Zellwachstum oder die Lebensfähigkeit in Gegenwart einer Unterbrechung des endogenen Gens verringert wird, dann das Gen als ein genetischer Suppressor der Amyloid-beta-induzierten Toxizität identifiziert wird.

Revendications

1. Construction d'expression comprenant un promoteur lié de manière fonctionnelle à un acide nucléique codant pour un polypeptide comprenant une séquence de signal, une séquence pro d'orientation de Golgi, et un polypeptide ayant la formule $[X\text{-}Y]_n$, dans laquelle chaque X est, indépendamment, absent ou est un lieur ; chaque Y est, indépendamment, un peptide bêta-amyloïde humain ; et n est un nombre entier entre deux et huit, inclus, dans laquelle une expression de l'acide nucléique et une production du polypeptide dans une cellule de levure résultent en une diminution de croissance ou de viabilité de la cellule.

2. Construction d'expression selon la revendication 1, dans laquelle :

(i) la séquence de signal est identique à la séquence de signal d'une protéine de levure présente naturellement, facultativement dans laquelle la séquence de signal est la séquence de signal alpha de facteur de conjugaison de levure ou la séquence de signal de toxine tueuse de levure K1, de phosphatase acide sécrétée, d'invertase (sucrase), ou de Pst1 ;
(ii) la séquence pro d'orientation de Golgi est la séquence pro alpha de facteur de conjugaison de levure ou la séquence pro de KEX2 de levure, de carboxypeptidase Y, de Pep4, ou de Prb1 ;
(iii) le promoteur est un promoteur inductible, facultativement dans laquelle le promoteur inductible est GAL1-10, GAL1, GALL, GALS, GPD, ADH, TEF, CYC1, MRP7, MET25, TET, VP16, ou VP16-ER ; et/ou
(iv)

a) chaque protéine bêta-amyloïde humaine est indépendamment choisie dans le groupe constitué d'Aβ38, de type sauvage, Aβ39 de type sauvage, Aβ40de type sauvage, Aβ41 de type sauvage, Aβ42de type sauvage, Aβ43de type sauvage, Aβ48 de type sauvage, et Aβ49 de type sauvage ;
b) chaque protéine bêta-amyloïde humaine est indépendamment choisie dans le groupe constitué d'Aβ38, Aβ39, Aβ40, Aβ41, Aβ42, Aβ43, Aβ48 et Aβ49 et comprend une mutation choisie dans le groupe constitué d'A2T, A2V, H6R, D7N, E11K, F20E, A21G, E22G, E22Q, E22K, délétion de E22, D23N, I31E, E22G/I31E, A42T et A42V, dans laquelle la mutation est par rapport à la séquence d'acides aminés de SEQ ID NO:105 ; ou
c) la protéine bêta-amyloïde humaine est Aβ42 de type sauvage.

3. Construction d'expression selon la revendication 1 ou 2, dans laquelle le polypeptide comprend :

(i) au moins quatre peptides bêta-amyloïdes humains, et/ou
(ii)

a) chaque peptide bêta-amyloïde humain comprend la même séquence d'acides aminés ; ou
b) le polypeptide comprend au moins deux peptides bêta-amyloïdes humains ayant des séquences d'acides aminés différentes.

4. Construction d'expression selon l'une quelconque des revendications 1 à 3, dans laquelle :

(i) le polypeptide comprend la séquence d'acides aminés de SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71,

SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, ou SEQ ID NO:101 ; et/ou
(ii) la construction d'expression est un plasmide épisomique ou un plasmide intégratif, facultativement dans laquelle le plasmide intégratif est pAG303, pAG304, pAG305, pAG306, pRS303, pRS304, pRS305, pRS306, ou un dérivé de ceux-ci.

5. Cellule de levure comprenant la construction d'expression selon l'une quelconque des revendications précédentes.

6. Cellule de levure selon la revendication 6, dans laquelle :

(i) une expression de l'acide nucléique et une production du polypeptide rendent la cellule non viable ;
(ii) la construction d'expression est épisomique ou est intégrée dans le génome de la cellule de levure ;
(iii) la levure est Saccharomyces cerevisiae, Saccharomyces uvae, Saccharomyces kluyveri, Schizosaccharomyces pombe, Kluyveromyces lactis, Hansenula polymorpha, Pichia pastoris, Pichia methanolica, Pichia kluyveri, Yarrowia lipolytica, Candida sp., Candida utilis, Candida cacaoi, Geotrichum sp., ou Geotrichum fermentans ;
(iv) au moins un gène qui code pour une protéine impliquée dans un efflux de médicament ou une perméabilité de cellule est perturbé, facultativement dans laquelle l'au moins un gène est PDR1, PDR3, PDR5, SNQ2, ou ERG6 ; et/ou
(v) la cellule de levure permet l'expression à partir de promoteurs GAL sur des sources de carbone autres que galactose ; facultativement dans laquelle la cellule de levure comprend un gène codant pour une protéine hybride comprenant le domaine de liaison d'ADN Gal4 fusionné à un domaine d'activation transcriptionnelle et un domaine régulateur ; facultativement dans laquelle la protéine hybride active une transcription en présence d'oestrogène ou d'un analogue de celui-ci ; de préférence dans laquelle la cellule de levure comprend un gène codant pour une protéine de fusion Gal4-ER-VP16.

7. Procédé d'induction de toxicité dans une cellule, le procédé comprenant :
la fourniture de la cellule selon la revendication 5 ou 6 ; et
l'induction d'un niveau d'expression de l'acide nucléique dans la cellule qui est toxique pour la cellule.

8. Procédé d'identification d'un composé qui empêche ou supprime une toxicité induite par bêta-amyloïdes, le procédé comprenant :

la culture de la cellule selon la revendication 5 ou 6, en présence d'un agent candidat et dans des conditions qui permettent l'expression de l'acide nucléique à un niveau qui, en l'absence de l'agent candidat, est suffisant pour induire une toxicité dans la cellule ;
la mesure de croissance ou viabilité cellulaire en présence de l'agent candidat ; et
la comparaison de la croissance ou viabilité cellulaire mesurée en présence de l'agent candidat à une croissance ou viabilité cellulaire en l'absence de l'agent candidat,
dans lequel si la croissance ou viabilité cellulaire est accrue en présence de l'agent candidat par rapport à en l'absence de l'agent candidat, alors l'agent candidat est identifié en tant que composé qui empêche ou supprime une toxicité induite par bêta-amyloïdes.

9. Procédé d'identification d'un suppresseur ou amplificateur génétique de toxicité induite par bêta-amyloïdes, le procédé comprenant :

la fourniture de la cellule selon la revendication 5 ou 6, dans lequel la cellule a été génétiquement modifiée pour surexprimer un gène ;
la culture de la cellule dans des conditions qui permettent l'expression de la protéine à un niveau qui, en l'absence de surexpression du gène, est suffisant pour induire une toxicité dans la cellule ;
la mesure de la croissance ou viabilité cellulaire en présence de surexpression du gène ; et
la comparaison de la croissance ou viabilité cellulaire mesurée en présence de surexpression du gène à la croissance ou viabilité cellulaire en l'absence de surexpression du gène,
dans lequel (i) si la croissance ou viabilité cellulaire est accrue en présence d'une surexpression du gène par rapport à en l'absence de surexpression du gène, alors le gène est identifié en tant que suppresseur génétique de toxicité induite par bêta-amyloïdes, et (ii) si la croissance ou viabilité cellulaire est diminuée en présence de surexpression du gène par rapport à en l'absence de surexpression du gène, alors le gène est identifié en tant qu'amplificateur génétique de toxicité induite par bêta-amyloïdes.

10. Procédé d'identification d'un suppresseur ou amplificateur génétique de toxicité induite par bêta-amyloïdes, le procédé comprenant :

la fourniture de la cellule selon la revendication 5 ou 6, dans lequel un gène endogène de la cellule a été perturbé ;
la culture de la cellule dans des conditions qui permettent l'expression de la protéine à un niveau qui, en l'absence de perturbation du gène endogène, est suffisant pour induire une toxicité dans la cellule ;
la mesure de la croissance ou viabilité cellulaire en présence de perturbation du gène endogène ; et
la comparaison de la croissance ou viabilité cellulaire mesurée en présence de perturbation du gène endogène à la croissance ou viabilité cellulaire en l'absence de perturbation du gène endogène,
dans lequel (i) si la croissance ou viabilité cellulaire est accrue en présence de perturbation du gène endogène par rapport à en l'absence de perturbation du gène endogène, alors le gène est identifié en tant qu'amplificateur génétique de toxicité induite par bêta-amyloïdes, et (ii) si la croissance ou viabilité cellulaire est diminuée en présence de perturbation du gène endogène par rapport à en l'absence de perturbation du gène endogène, alors le gène est identifié en tant que suppresseur génétique de toxicité induite par bêta-amyloïdes.

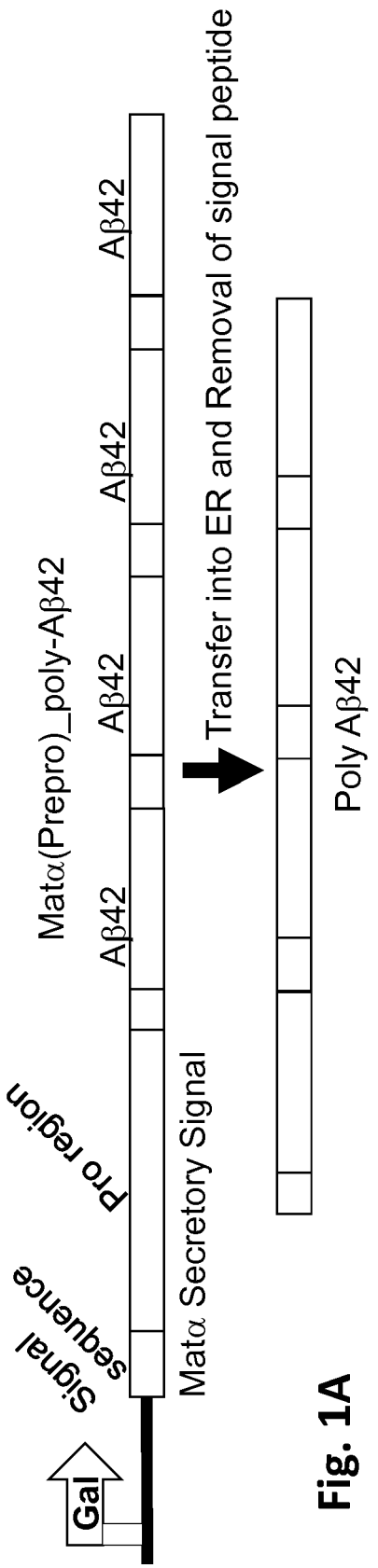

Gal
Signal sequence
Pro region
Matα Secretory Signal
Aβ42
Aβ42
Aβ42
Aβ42
Matα(Prepro)_poly-Aβ42
Transfer into ER and Removal of signal peptide
Poly Aβ42

Fig. 1A

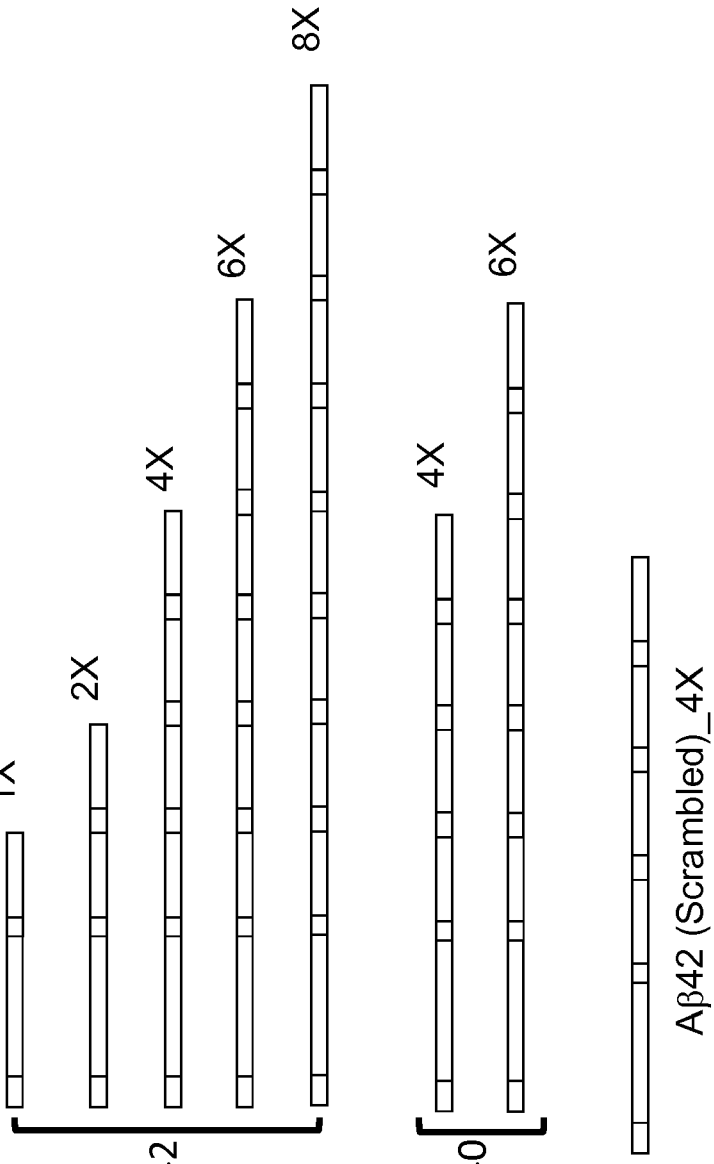

1X
2X
4X
6X
8X
Aβ42
4X
6X
Aβ40
Aβ42 (Scrambled)_4X

Fig. 1B

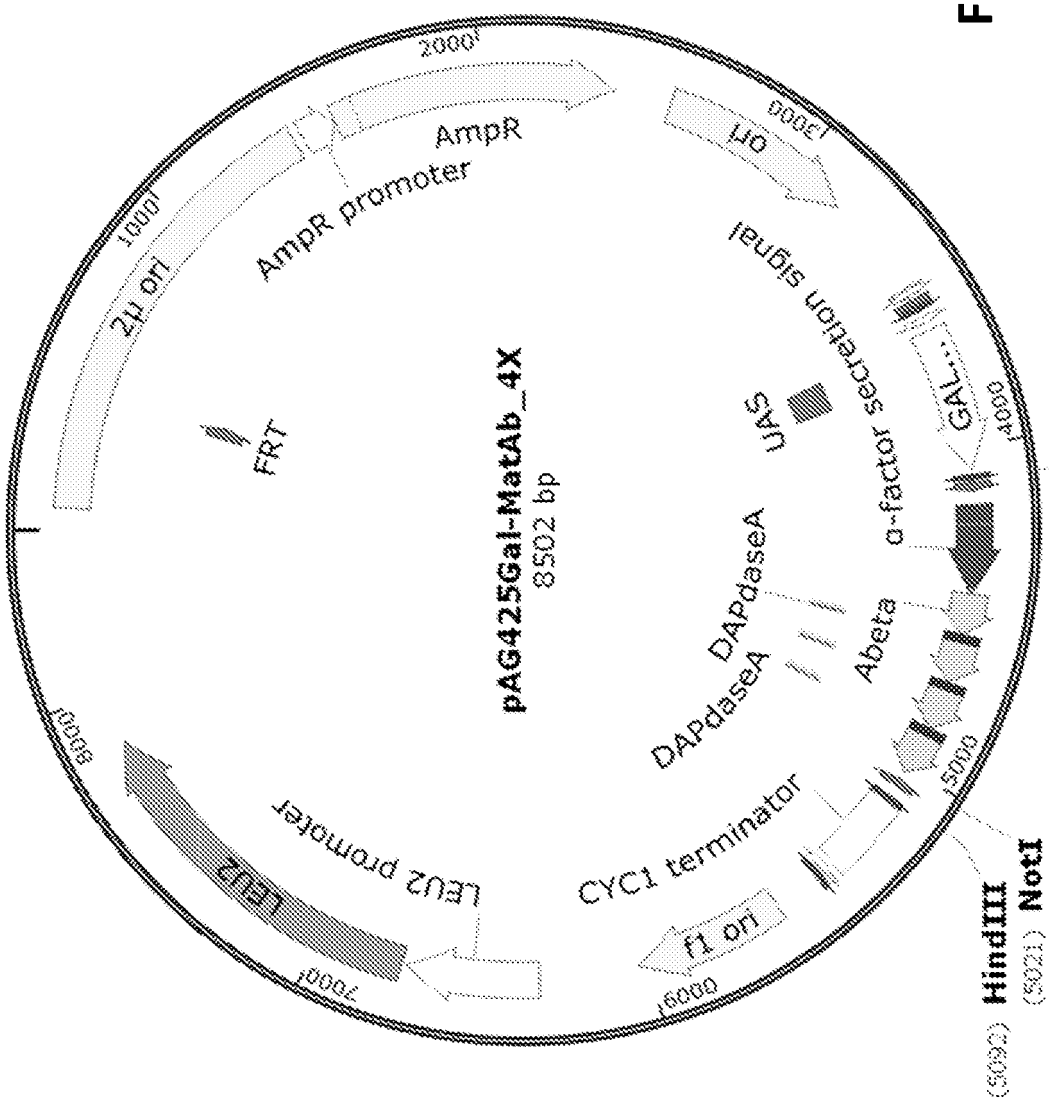

pAG425Gal-MatAb_4X
8502 bp
AmpR
AmpR promoter
2µ ori
ori
Secretion signal
GAL...
SpeI (4002)
α-factor secretion signal
Abeta
DAPdaseA
DAPdaseA
UAS
FRT
CYC1 terminator
f1 ori
LEU2 promoter
LEU2
HindIII (5090)
NotI (5021)
1000
2000
3000
4000
5000
6000
7000
8000

Fig. 2

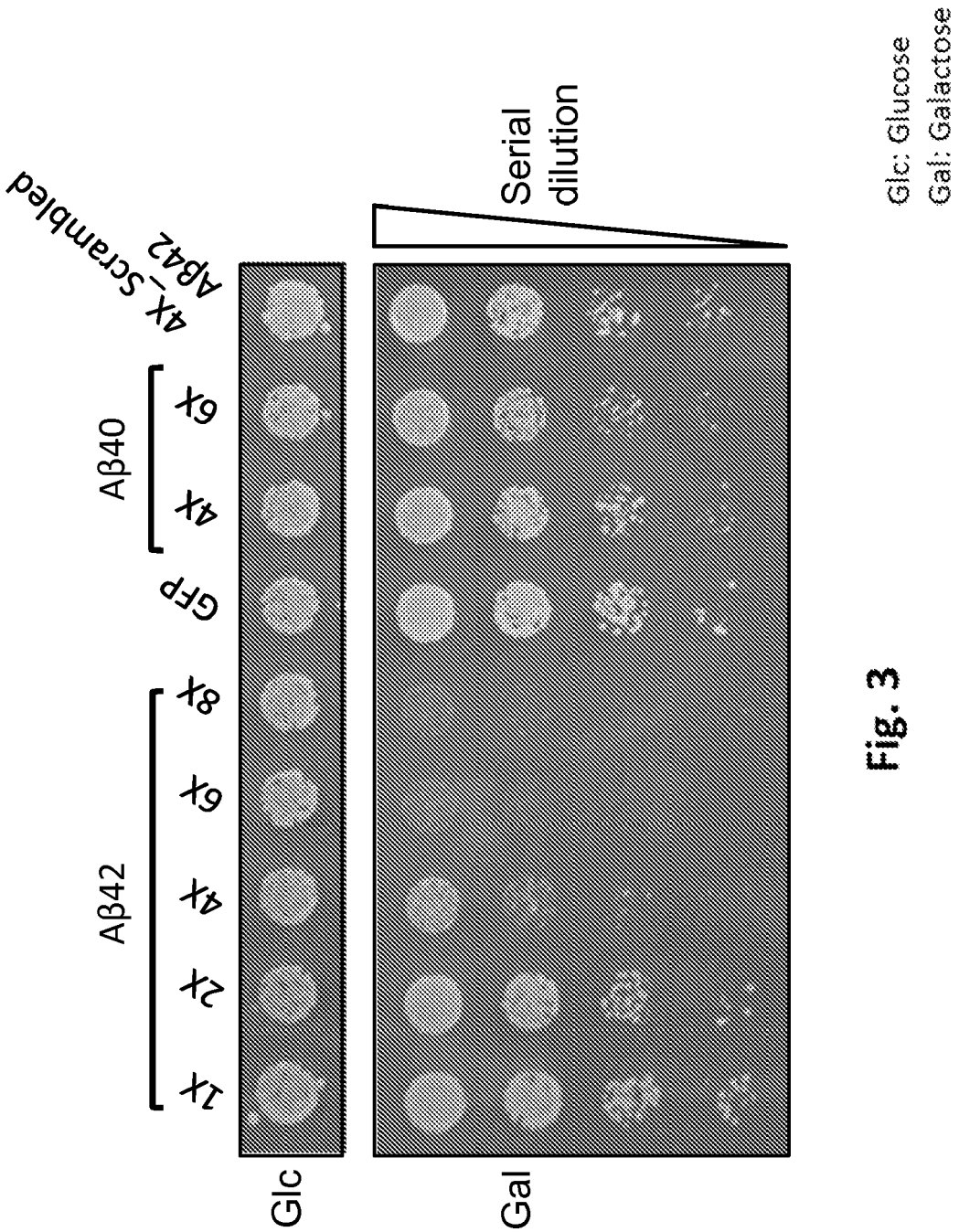

Glc: Glucose
Gal: Galactose
Serial dilution
Glc
Gal
Aβ42
1x
2x
4x
6x
8x
GFP
Aβ40
4x
6x
4x_Scrambled Aβ42

Fig. 3

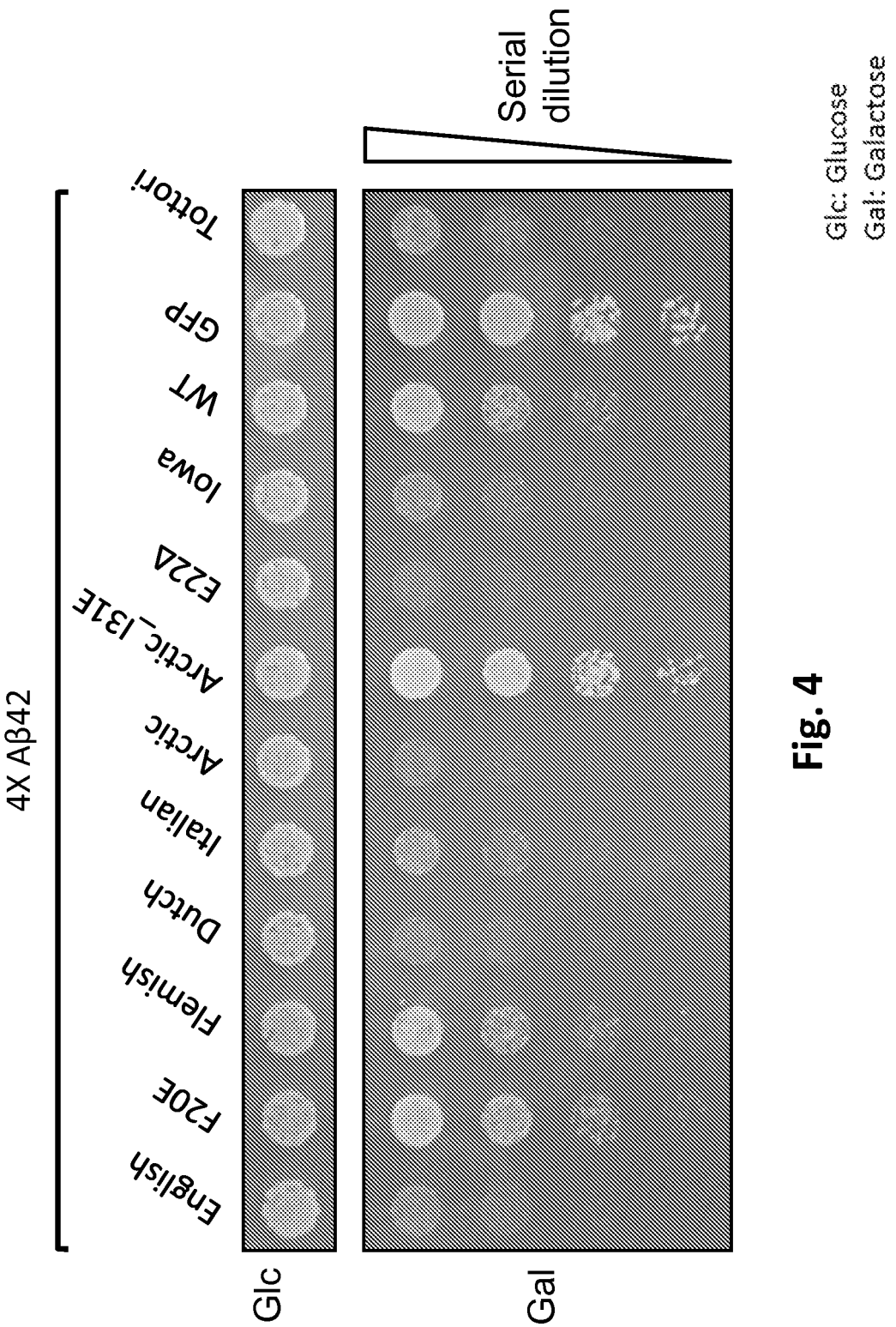

Serial dilution
Glc: Glucose
Gal: Galactose
4X Aβ42
English
F20E
Flemish
Dutch
Italian
Arctic
Arctic_I31E
E22A
Iowa
WT
GFP
Tottori
Glc
Gal

Fig. 4

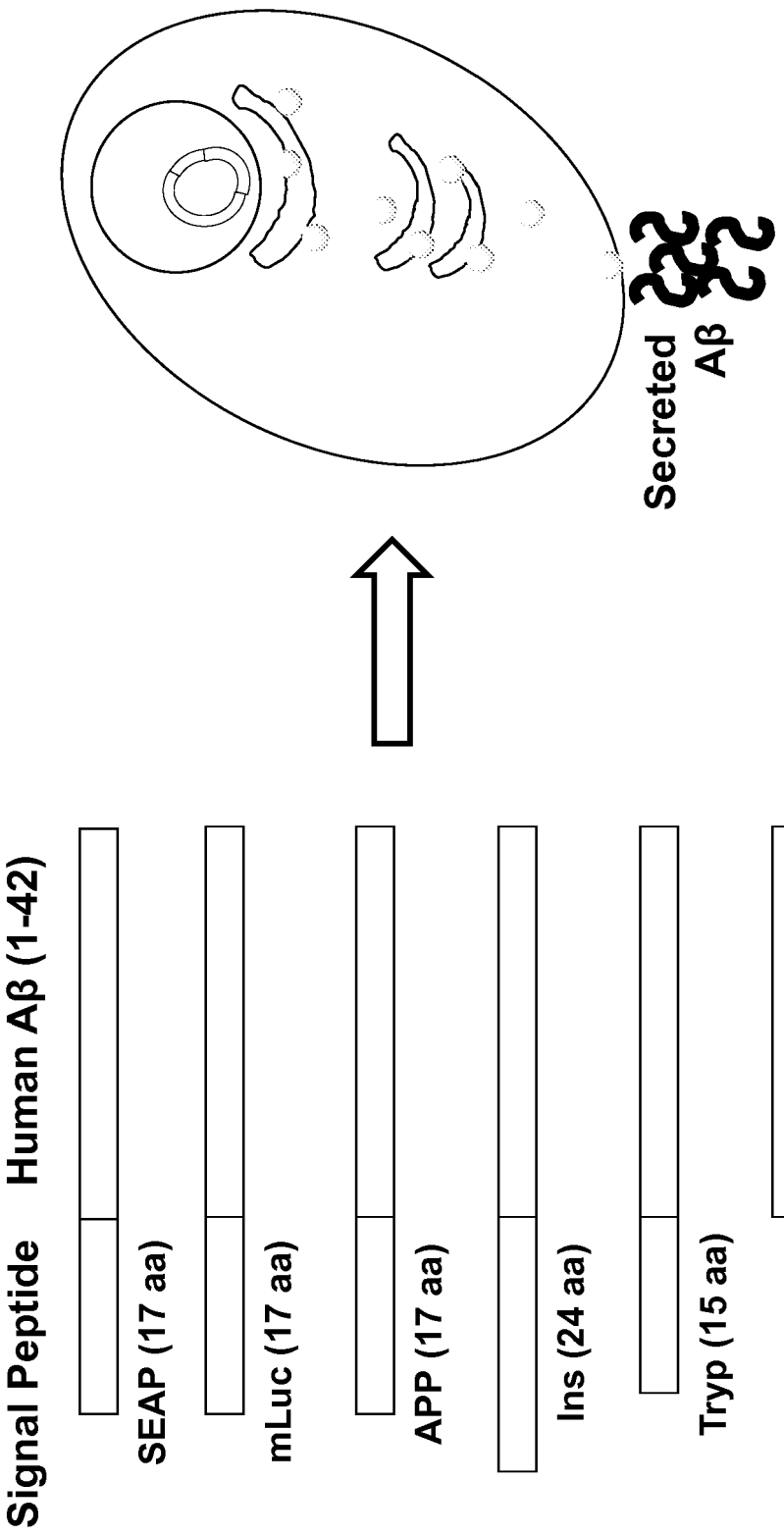

Signal Peptide
Human Aβ (1-42)
SEAP (17 aa)
mLuc (17 aa)
APP (17 aa)
Ins (24 aa)
Tryp (15 aa)
No signal sequence
Secreted Aβ

Fig. 5

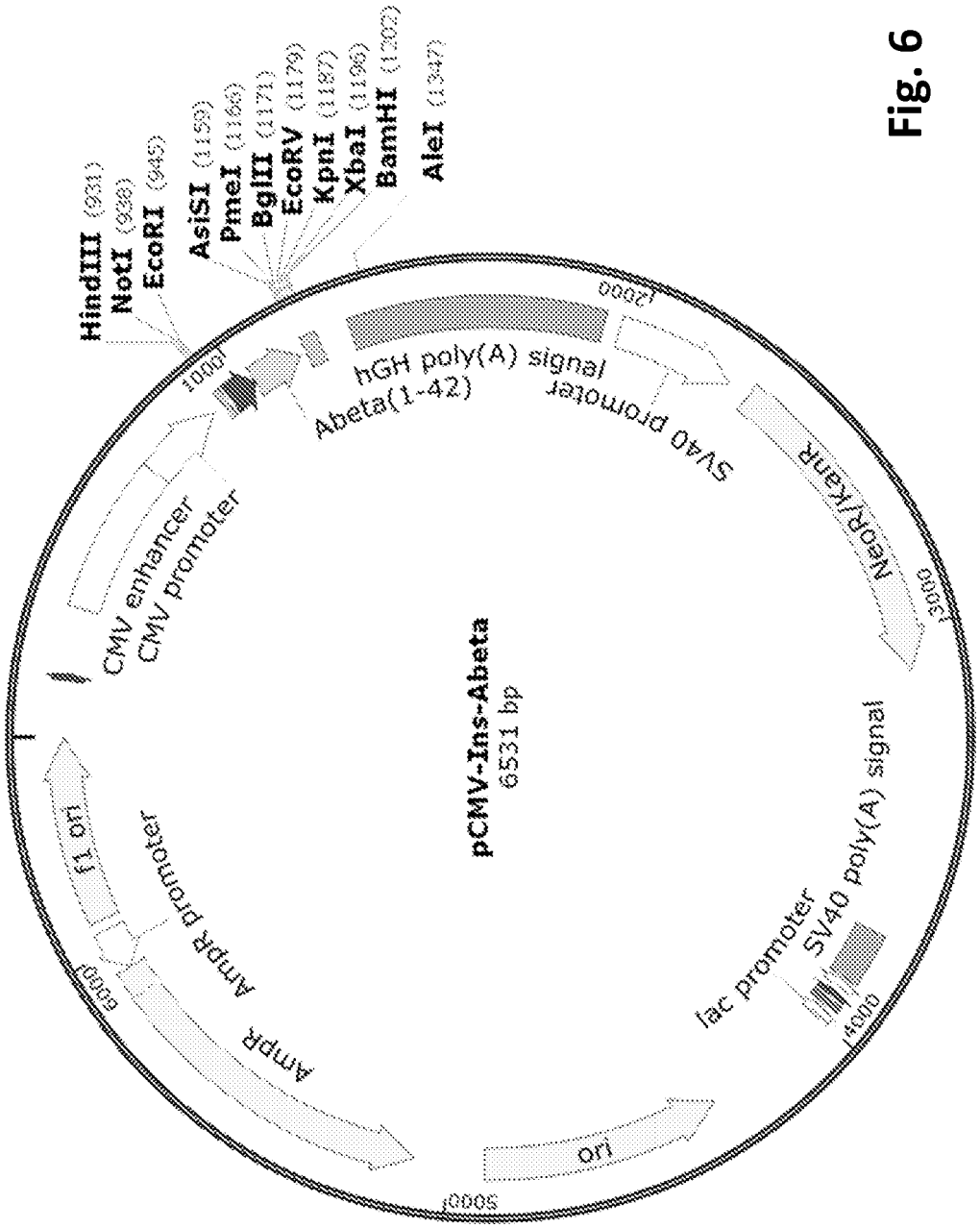

pCMV-Ins-Abeta
6531 bp
HindIII (931)
NotI (938)
EcoRI (945)
AsiSI (1159)
PmeI (1164)
BglII (1171)
EcoRV (1179)
KpnI (1187)
XbaI (1196)
BamHI (1202)
AleI (1347)
hGH poly(A) signal
Abeta(1-42)
SV40 promoter
Neo R/Kan R
CMV enhancer
CMV promoter
f1 ori
AmpR promoter
AmpR
ori
lac promoter
SV40 poly(A) signal
1000
2000
3000
4000
5000
6000

Fig. 6

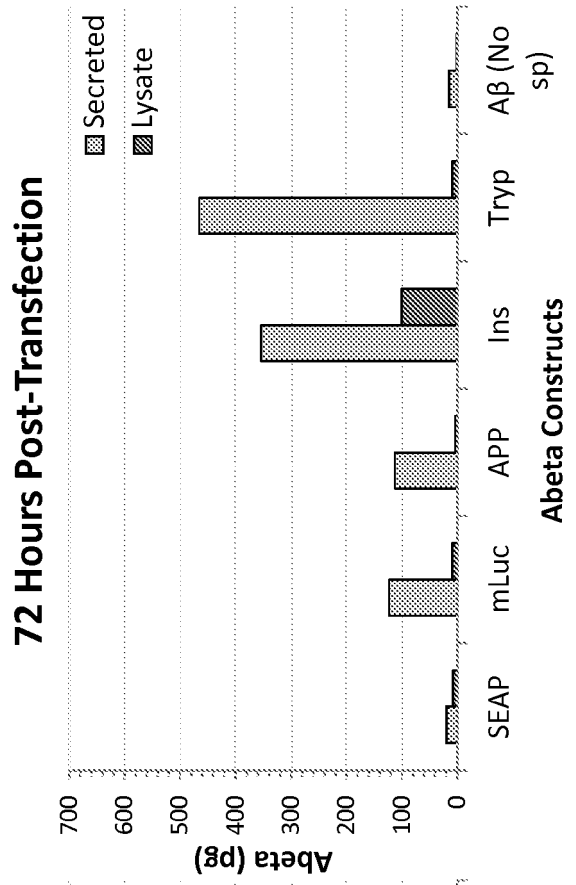

48 Hours Post-Transfection
Secreted
Lysate
Abeta (pg)
700
600
500
400
300
200
100
0
SEAP
mLuc
APP
Ins
Tryp
Aβ (No sp)
Abeta Constructs

Fig. 7A

72 Hours Post-Transfection
Secreted
Lysate
Abeta (pg)
700
600
500
400
300
200
100
0
SEAP
mLuc
APP
Ins
Tryp
Aβ (No sp)
Abeta Constructs

Fig. 7B

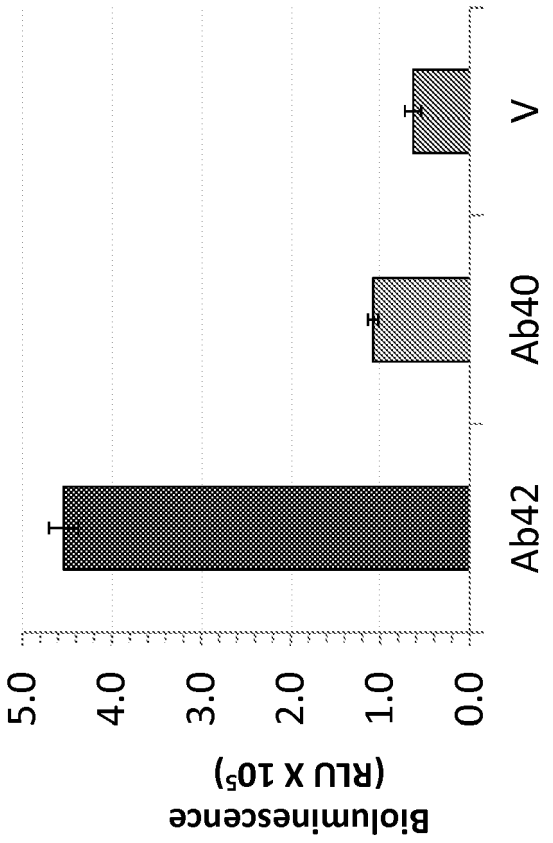


Fig. 8

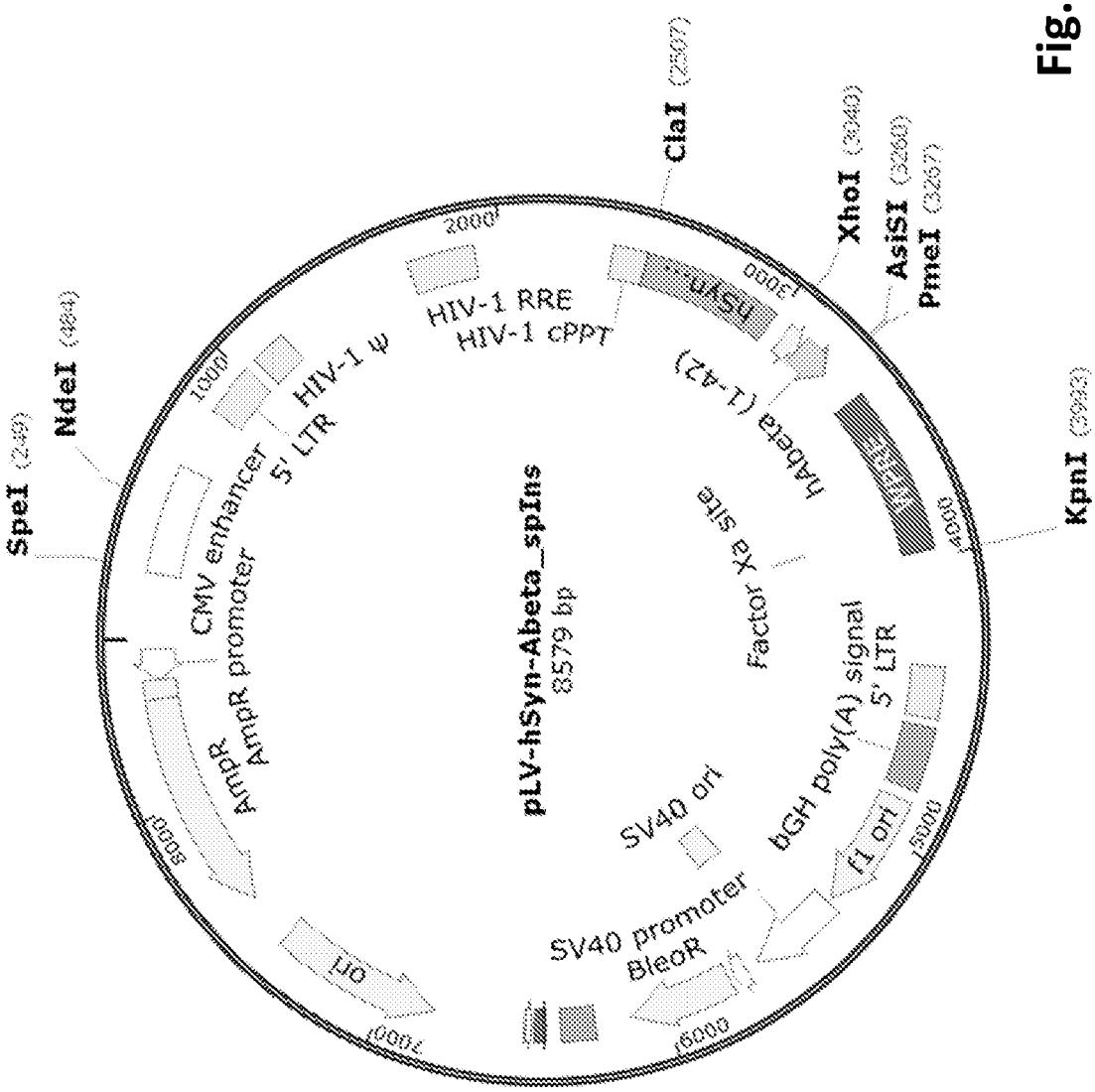

pLV-hSyn-Abeta_spIns
8579 bp
SpeI (249)
NdeI (464)
ClaI (2507)
XhoI (2940)
AsiSI (3260)
PmeI (3297)
KpnI (3943)
1000
2000
3000
4000
5000
6000
7000
8000
CMV enhancer
AmpR promoter
AmpR
HIV-1 Ψ
5' LTR
HIV-1 RRE
HIV-1 cPPT
WPRE
hAbeta (1-42)
Factor Xa site
bGH poly(A) signal
5' LTR
f1 ori
SV40 ori
SV40 promoter
BleoR
ori

Fig. 9

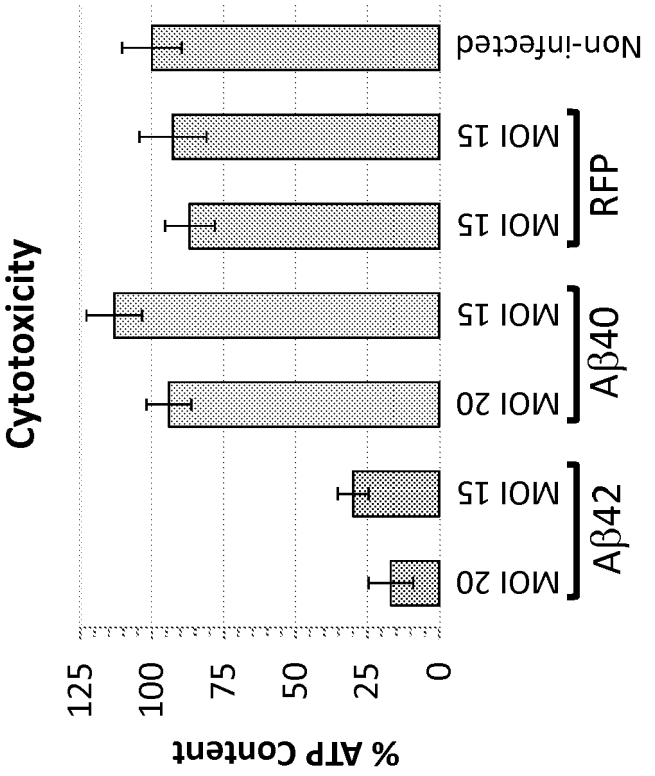


Fig. 10B

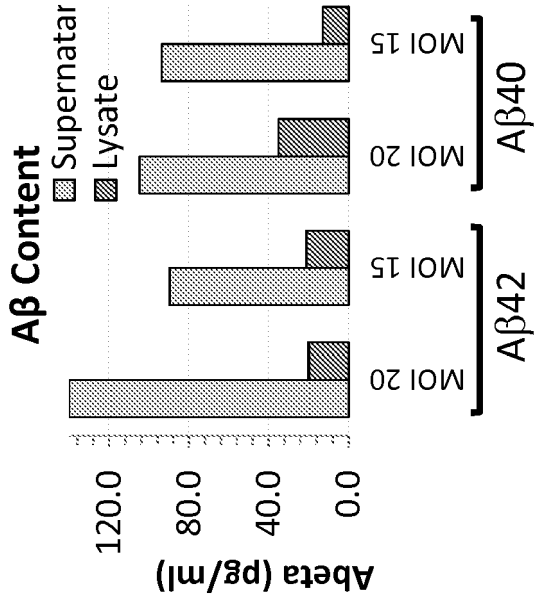


Fig. 10A

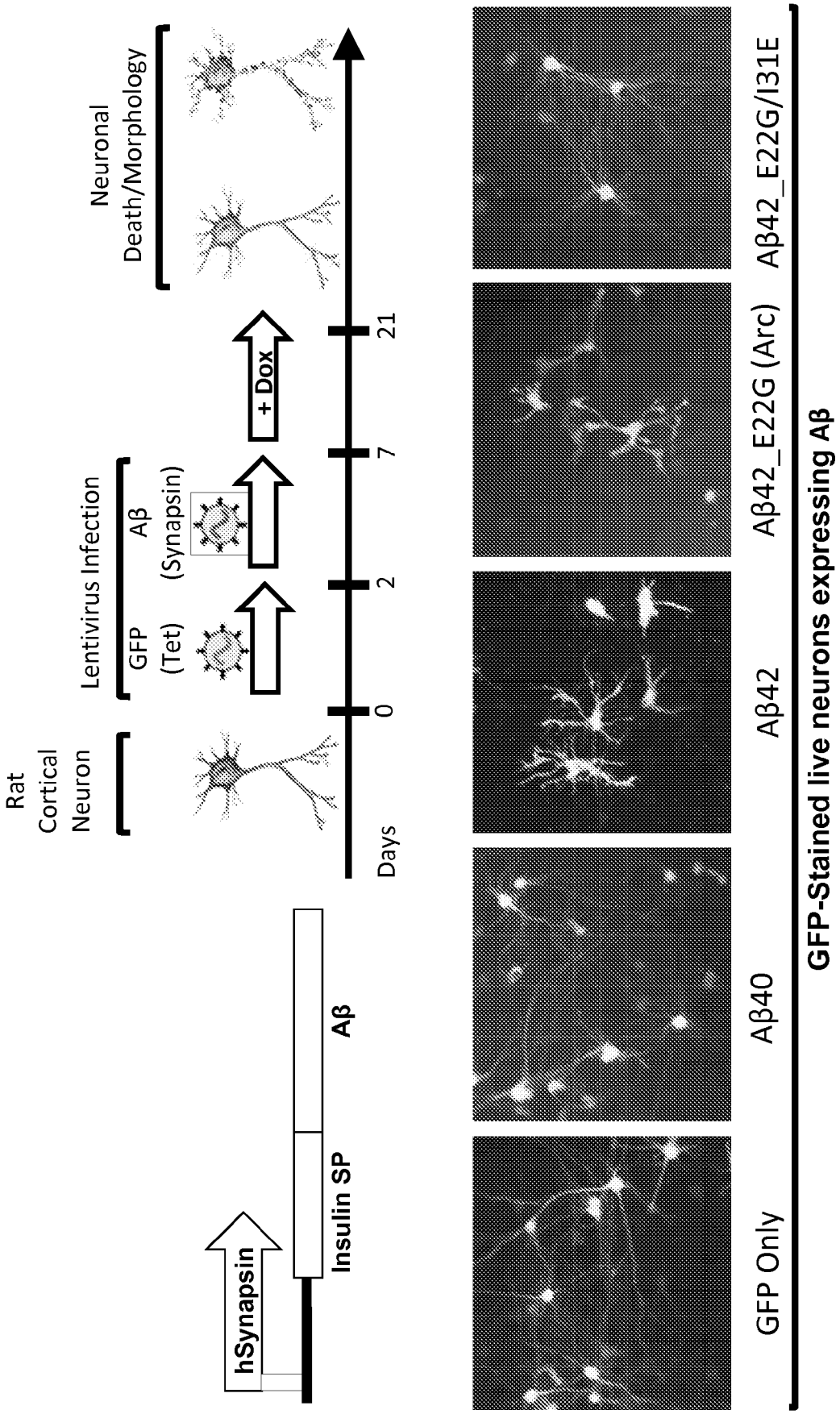

Rat Cortical Neuron
Lentivirus Infection
GFP (Tet)
Aβ (Synapsin)
+ Dox
Neuronal Death/Morphology
Days
0
2
7
21
hSynapsin
Insulin SP
Aβ
GFP Only
Aβ40
Aβ42
Aβ42_E22G (Arc)
Aβ42_E22G/I31E
GFP-Stained live neurons expressing Aβ

Fig. 11A

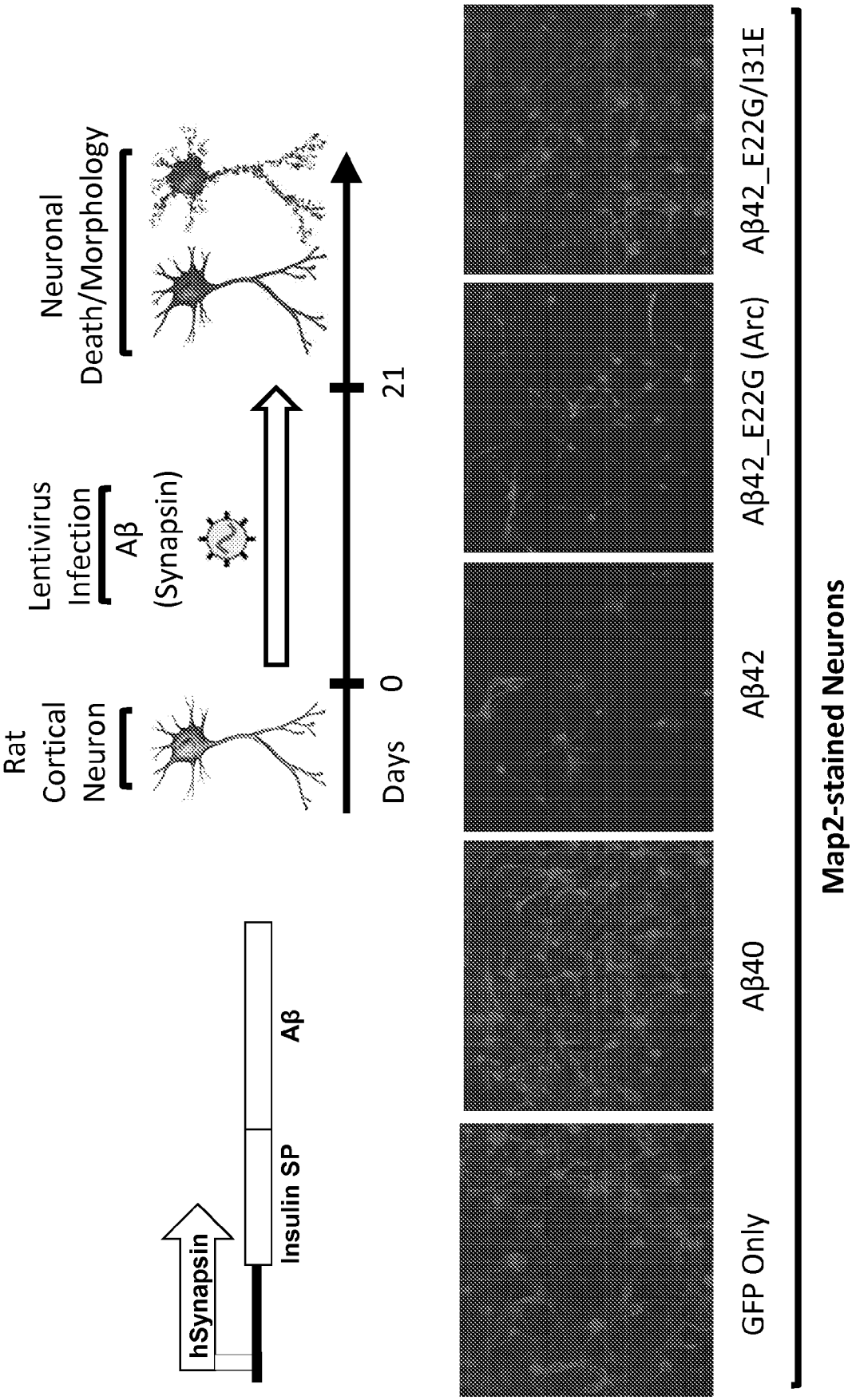

Rat Cortical Neuron
Lentivirus Infection
Aβ (Synapsin)
Neuronal Death/Morphology
Days 0
21
hSynapsin
Insulin SP
Aβ
GFP Only
Aβ40
Aβ42
Aβ42_E22G (Arc)
Aβ42_E22G/I31E
Map2-stained Neurons

Fig. 11B

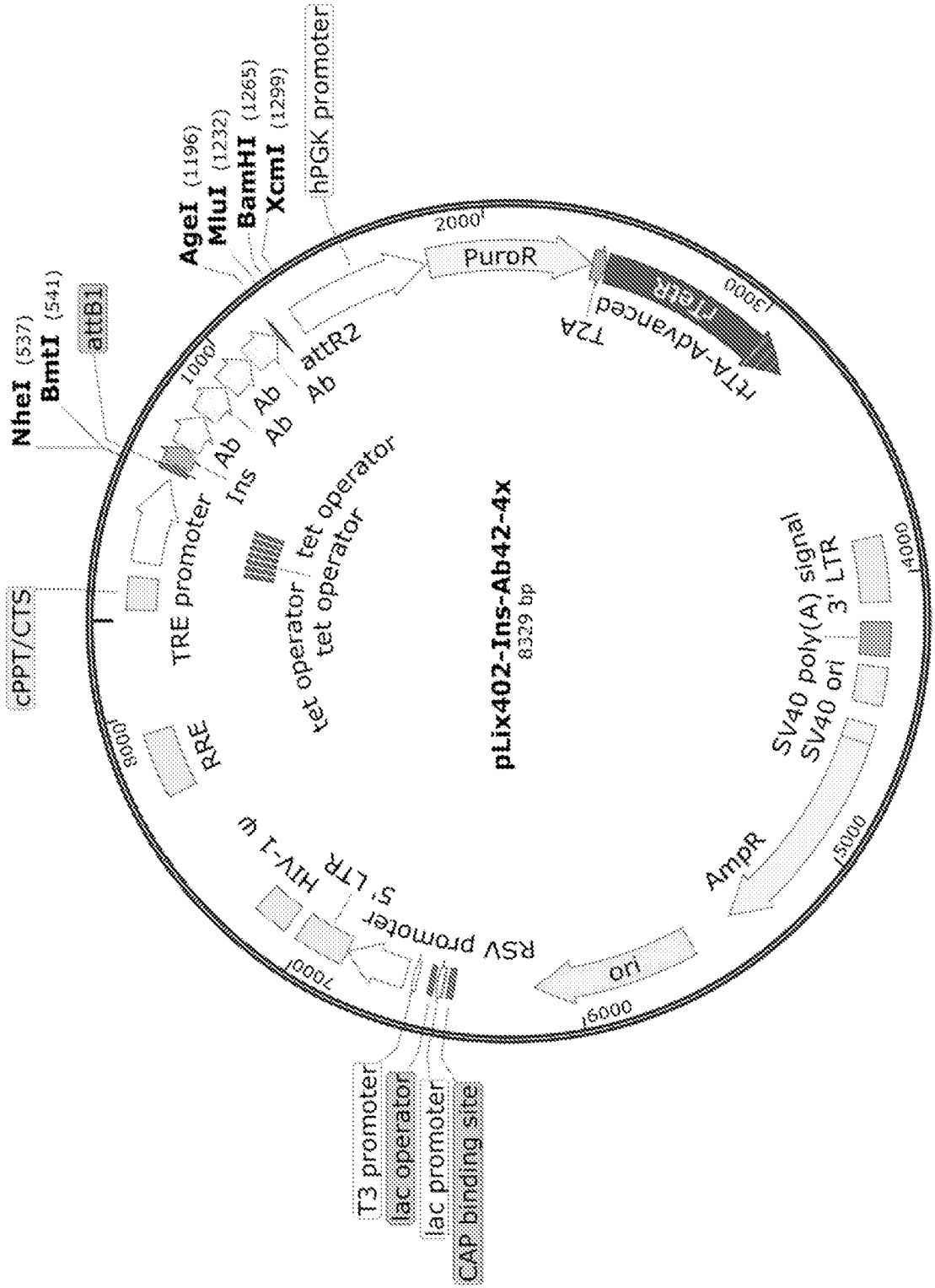

pLix402-Ins-Ab42-4x
8329 bp
cPPT/CTS
NheI (537)
BmtI (541)
attB1
AgeI (1196)
MluI (1232)
BamHI (1265)
XcmI (1299)
hPGK promoter
PuroR
T2A
rtTA-Advanced
TRE promoter
Ins
Ab
Ab
Ab
Ab
attR2
tet operator
tet operator
tet operator
RRE
HIV-1 ψ
5' LTR
RSV promoter
T3 promoter
lac operator
lac promoter
CAP binding site
ori
AmpR
SV40 ori
SV40 poly(A) signal
3' LTR
1000
2000
3000
4000
5000
6000
7000
8000

Fig. 12

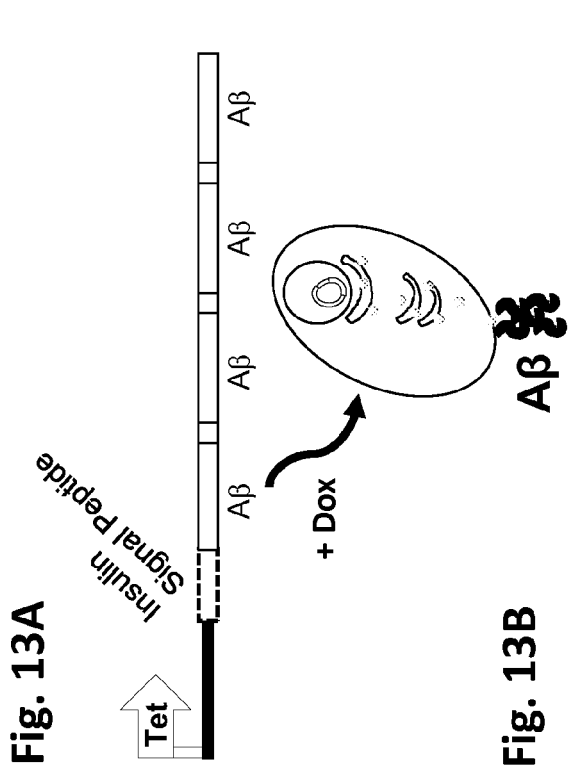


Fig. 13A
Tet
Insulin Signal Peptide
Aβ
Aβ
Aβ
Aβ
+ Dox
Fig. 13B
Aβ
Aβ42
1X
4X
Arc_4X
6X
Aβ40
1X
4X
6X

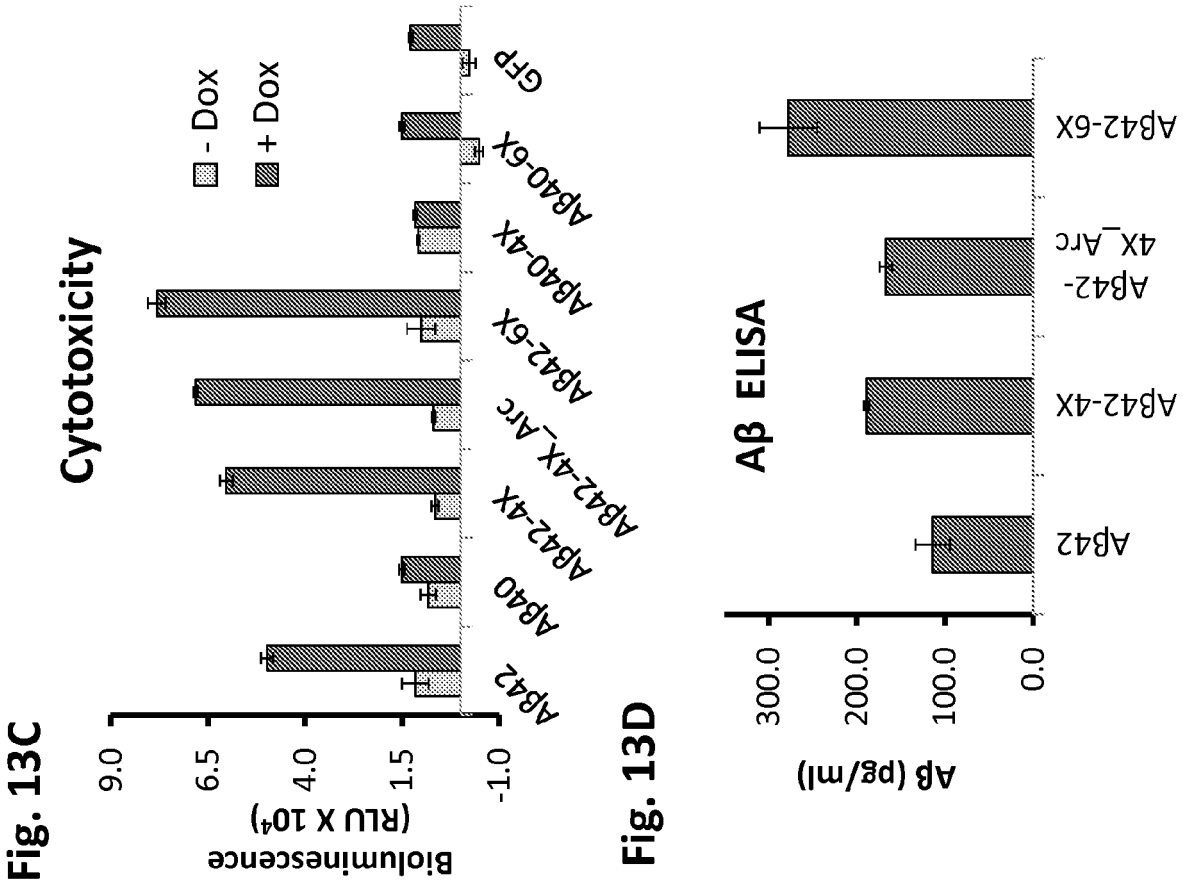


Fig. 13C
Cytotoxicity
- Dox
+ Dox
Bioluminescence (RLU X 10⁴)
9.0
6.5
4.0
1.5
-1.0
Aβ42
Aβ40
Aβ42-4x
Aβ42-4x_Arc
Aβ42-6x
Aβ40-4x
Aβ40-6x
GFP
Fig. 13D
Aβ ELISA
Aβ (pg/ml)
300.0
200.0
100.0
0.0
Aβ42
Aβ42-4X
Aβ42-4x_Arc
Aβ42-6X

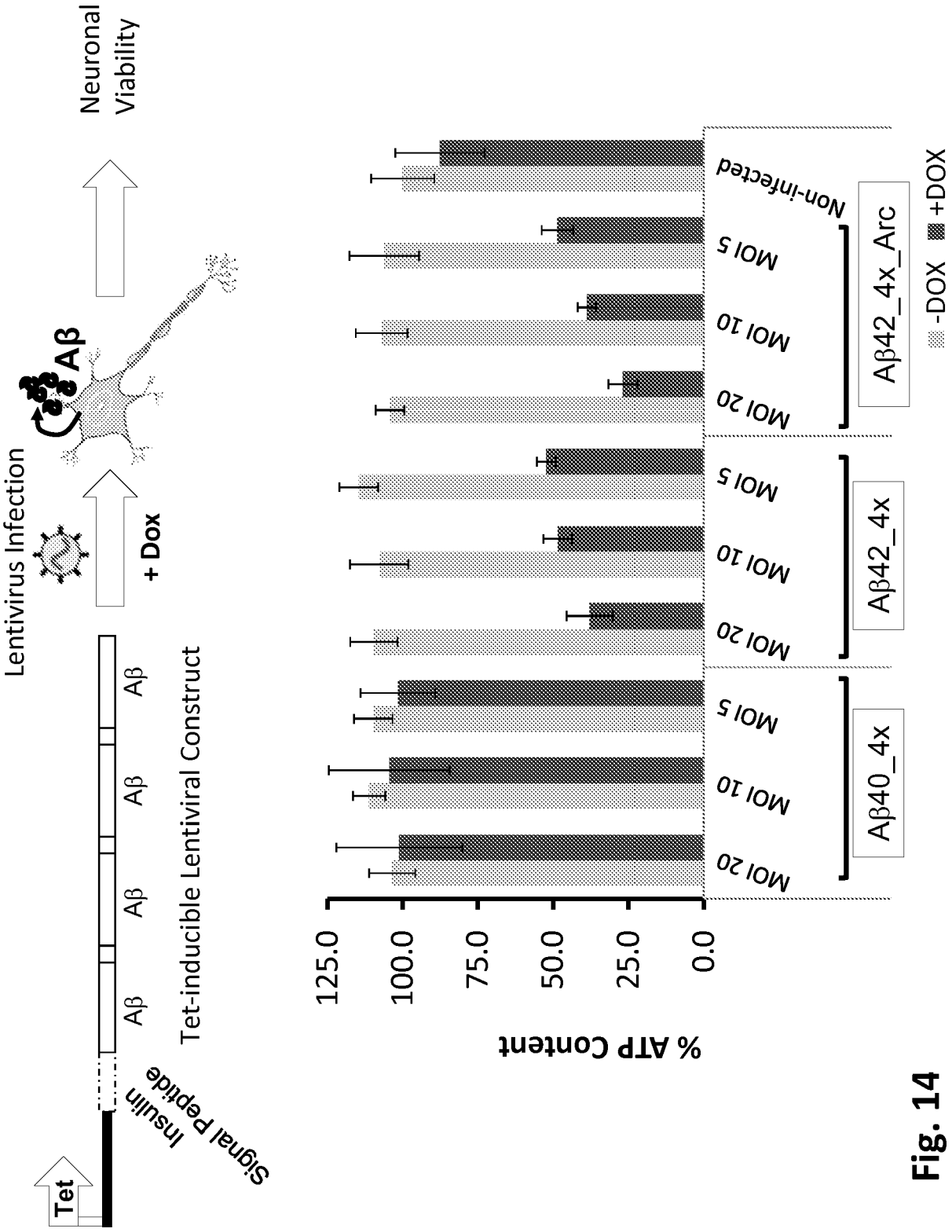
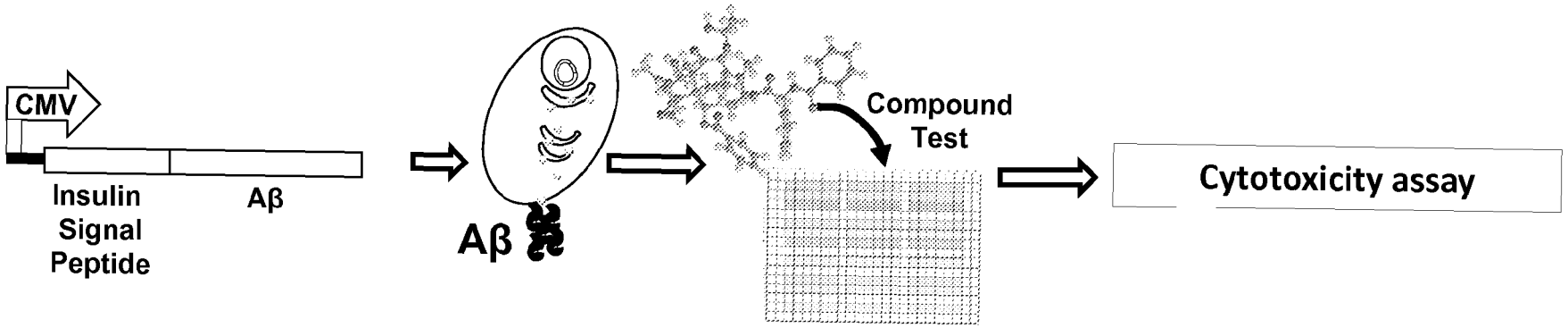

Neuronal Viability
Aβ
Lentivirus Infection
+ Dox
Tet
Insulin Signal Peptide
Aβ
Aβ
Aβ
Aβ
Tet-inducible Lentiviral Construct
% ATP Content
125.0
100.0
75.0
50.0
25.0
0.0
MOI 20
MOI 10
MOI 5
Aβ40_4x
MOI 20
MOI 10
MOI 5
Aβ42_4x
MOI 20
MOI 10
MOI 5
Non-infected
Aβ42_4x_Arc
-DOX
+DOX

Fig. 14


CMV
Insulin
Signal
Peptide
Aβ
Aβ
Compound
Test
Cytotoxicity assay

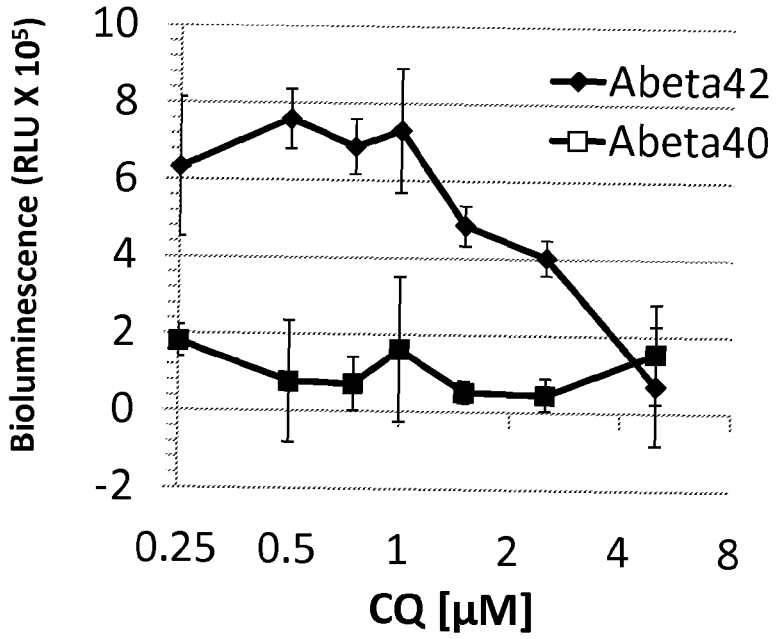

Bioluminescence (RLU X 10^5)
10
8
6
4
2
0
-2
Abeta42
Abeta40
0.25
0.5
1
2
4
8
CQ [μM]

Fig. 15

Fig. 16A

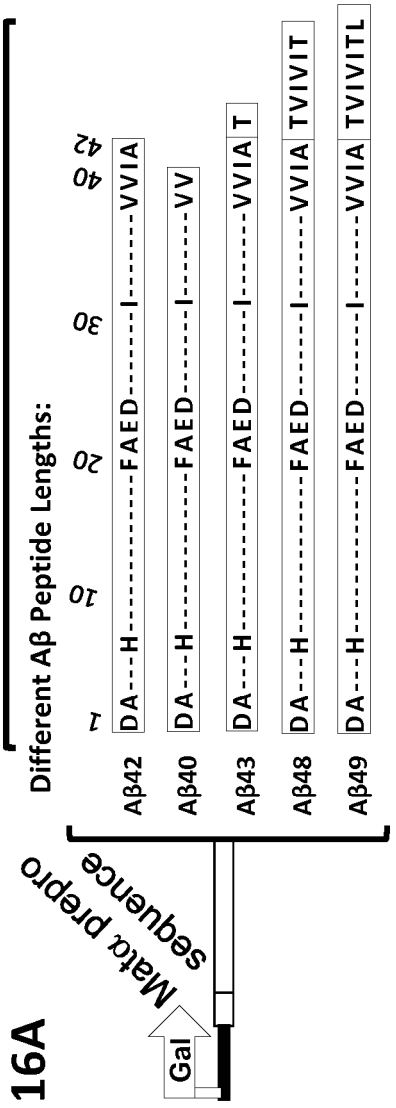


Fig. 16B

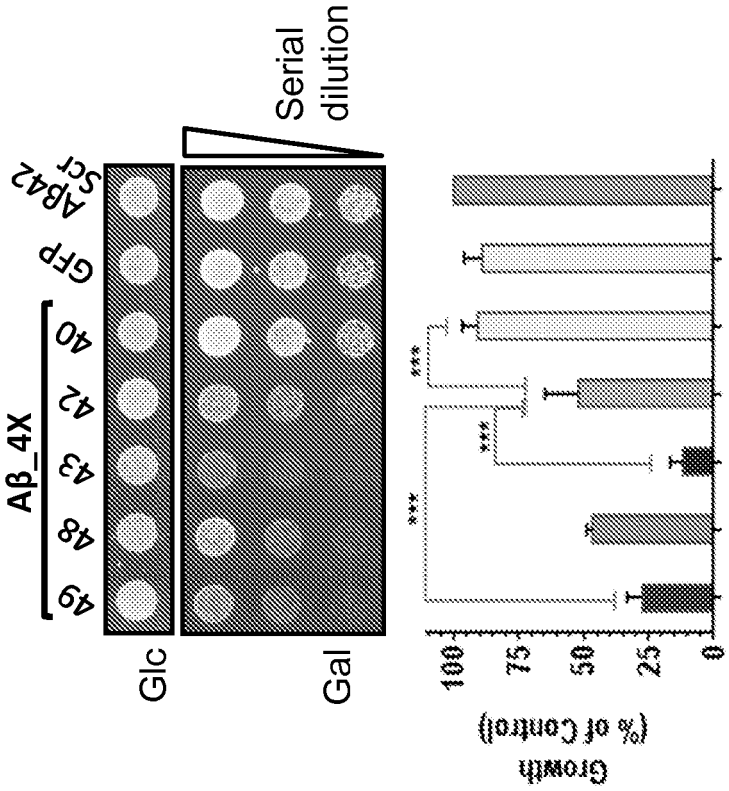


Fig. 17A

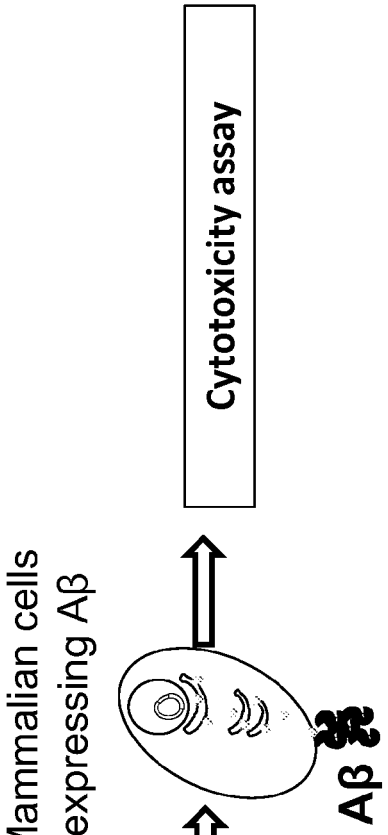


Fig. 17B

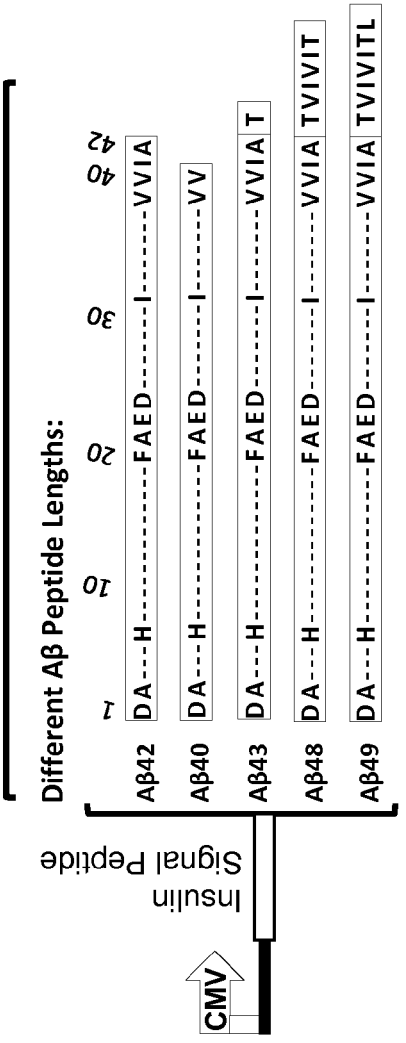
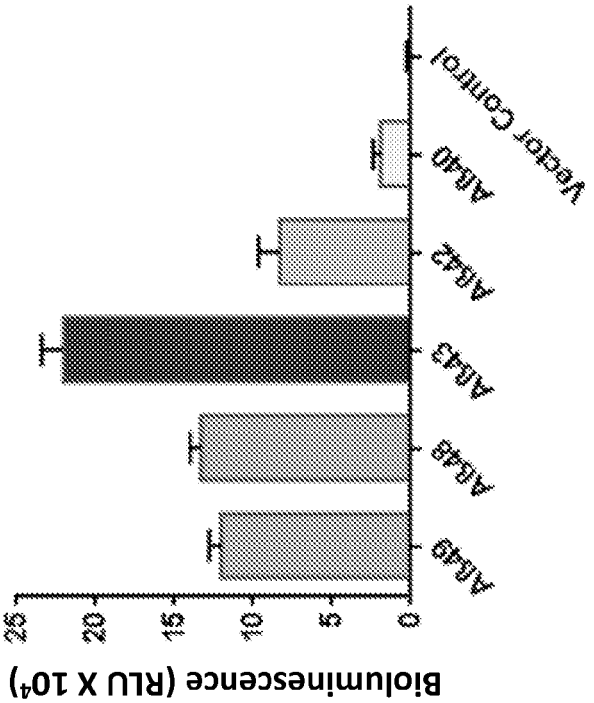


Fig. 17C



REFERENCES CITED IN THE DESCRIPTION

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