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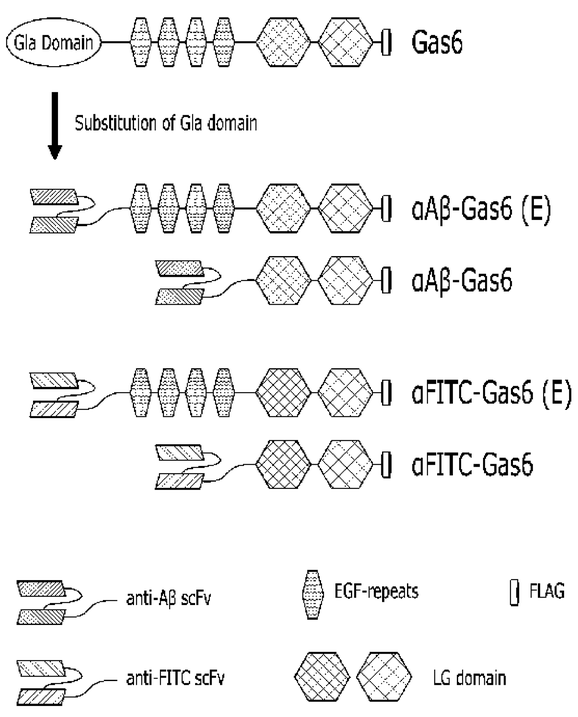
(54) **Title:** FUSION MOLECULE HAVING NON-INFLAMMATORY PHAGOCYTOSIS INDUCING ACTIVITY

(54) **발명의 명칭:** 비염증성 식세포작용 유도 활성을 갖는 융합분자



(57) **Abstract:** The present invention relates to a fusion molecule having phagocytosis inducing activity, which can address the issue of tissue damage caused by activation of an inflammatory reaction of the prior art, and thus accumulated abnormal proteins, such as beta-amyloid, tau, alpha-synuclein, huntingtin, or prion, or the like, are effectively removed, so that the present invention can be used for prevention or treatment of proteinosis caused by the abnormal accumulation of substances.

(57) 요약서: 본 발명은 식세포작용 유도 활성을 갖는 융합분자에 관련된 것으로서, 종래기술이 갖는 염증반응 활성화에 따른 조직 손상 문제를 해결할 수 있으며, 이에 따라 축적된 이상 단백질, 예컨대 베타-아밀로이드, 타우, 알파-시누클린, 헌팅틴 또는 프라이온 등을 효과적으로 제거하여, 이러한 물질의 이상 축적에 의해 일어나는 단백질증 등의 예방 또는 치료용도로 활용이 가능하다.

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DESCRIPTION

Invention Title

FUSION MOLECULE HAVING NON-INFLAMMATORY PHAGOCYTOSIS INDUCING ACTIVITY

Technical Field

[0001] The present invention relates to fusion molecules having non-inflammatory phagocytosis-inducing activity and suggests the possibility of using the fusion molecules for prevention or treatment of diseases that are caused by abnormal accumulation of substances, such as proteopathy.

[0002]

Background Art

[0003] Numerous degenerative diseases are characterized by aberrant folding, polymerization and accumulation of proteins. These proteopathies include various types of amyloidosis.

[0004] Amyloidosis is a disease in which abnormal proteins called amyloid accumulate in tissues. Amyloid is a protein aggregate that has a diameter of 7-13 nm and a beta-sheet structure and exhibits a fibrous morphology when viewed under a microscope, and it is characterized by being stained with Thioflavin T (ThioT) and Congo red. Amyloid is not normally found in the body, and to date, 36 proteins have been identified as being amyloidogenic (Picken, *Acta*

Haematol.(2020), 143:322-334). Representative examples of amyloidosis diseases include neurological diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease and prion disease. In addition, there are a number of amyloid diseases having various aspects depending on amyloid-causing proteins and affected organs.

[0005] Alzheimer's disease is the biggest cause of dementia and is a fatal disease accompanied by learning and memory impairment. 130 million people are expected to suffer from Alzheimer's disease by 2050 worldwide, and 1 in 9 people among the population above 65 years old have already been diagnosed with Alzheimer's disease.

[0006] A hallmark of Alzheimer's disease is that beta-amyloid (Aβ) protein caused by abnormal degradation of amyloid precursor protein (APP), deposits and accumulates around the brain cell membrane. Another hallmark is abnormal hyperphosphorylation of microtubule-associated tau protein.

[0007] It has recently been reported that beta-amyloid oligomers and fibrils cause synaptic dysfunction and cytotoxicity through various pathways, and create a vicious cycle that adversely affects nerve cells through functional changes in astrocytes and microglia, which are responsible for immunity in the brain.

[0008] Therapeutic drugs for Alzheimer's disease approved by the FDA to date are drugs that inhibit acetylcholine

degradation or inhibit the activity of NMDA receptors, and these drugs aim only for temporary relief of symptoms, not fundamental treatment of the disease. Therefore, there is currently no method capable of fundamentally treating Alzheimer's disease, and accordingly, Alzheimer's disease is known to be the most expensive disease for patient treatment and care in the aging population.

[0009] For a fundamental treatment of Alzheimer's disease, drug development has been conducted for decades with a focus on inhibiting formation of and eliminating beta-amyloid. Unfortunately, however, most of the therapeutic drugs for Alzheimer's disease developed to inhibit formation of and eliminate beta-amyloid failed because they turned out to be ineffective in the clinical stage. For example, in the case of BACE inhibitors for reducing beta-amyloid, strategies that prevent additional beta-amyloid production are largely ineffective, because in Alzheimer's patients with cognitive decline, beta-amyloid plaques have already accumulated and neuronal cell death is taking place.

[0010] Since the recent studies reporting that monoclonal antibodies which specifically bind to beta-amyloid oligomers and fibrils induced beta-amyloid clearance and restored cognitive function in Alzheimer's disease patients, a strategy to treat Alzheimer's disease through anti-beta-amyloid antibodies has emerged as a new hope.

[0011] The mechanisms of action of beta-amyloid monoclonal antibodies proposed to date include inhibition of aggregation of beta-amyloid oligomers and fibrils by binding thereto, or the induction of microglial phagocytosis of beta-amyloid through Fc receptors that recognize the monoclonal antibodies.

[0012] However, despite the advances in the development of therapeutic drugs for Alzheimer's disease, current immunotherapy using anti-beta-amyloid monoclonal antibodies shows amyloid-related imaging abnormalities (ARIAs) accompanied by severe edema in 55% of patients treated with the antibodies, and for this reason, about 35% of the ARIA patients were dropped from clinical trials. The ARIA phenomenon is known to be due to synaptotoxicity and cytotoxicity caused by inflammatory responses that are inevitably activated when anti-beta-amyloid monoclonal antibodies stimulate Fc receptors of microglia cells.

[0013] Since synapses and neurons in the brain respond sensitively to inflammatory cytokines, treatment using anti-beta-amyloid monoclonal antibodies has an inherent problem in that it inevitably causes damages to neurons and synapses, even if it clears beta-amyloid to some extent. In addition to monoclonal antibodies, companies such as Alector and Denali presented strategies to improve the microglia's ability to clear beta-amyloid by activating targets such as TREM2 that regulate the immunological mechanism of microglia,

and these strategies have received a lot of attention. However, even in these strategies, when microglia are excessively activated, synaptic damage due to an increase in overall phagocytotic capacity is expected.

[0014] Therefore, an important task in the treatment of Alzheimer's disease is to develop a method to selectively clear only beta-amyloid oligomers and fibrils without causing inflammatory responses and synaptic damage, and these drugs are expected to make a significant contribution to the treatment of Alzheimer's disease.

[0015] Furthermore, if a method to selectively clear only an abnormally accumulated substance as a target, for example, abnormally accumulated proteins causing proteopathy, without causing inflammatory responses and consequent additional tissue damage as described above, is extensively applied, it will be possible to develop a method for selectively clearing abnormally accumulated proteins such as tau, α-synuclein, and huntingtin proteins. These drugs are expected to make a significant contribution to the treatment of not only neurological diseases such as Huntington's disease, but also overall diseases related to abnormal accumulation of certain substances.

[0016]

DISCLOSURE

Technical Problem

[0017] The present invention relates to fusion molecules having phagocytosis-inducing activity, and an object of the present invention is to suggest the possibility of using the fusion molecules for preventing or treating diseases caused by abnormal accumulation of target substances.

[0018] Objects to be achieved by the present invention are not limited to the above-mentioned object, and other objects not mentioned herein will be clearly understood by those skilled in the art from the following description.

[0019]

Technical Solution

[0020] One aspect of the present invention provides a fusion molecule having phagocytosis-inducing activity, the fusion molecule comprising: a first region that is able to bind to TAM receptor; and a second region that specifically binds to a target substance.

[0021] Here, the TAM receptor may specifically be at least one selected from the group consisting of Tyro3, Axl, and MerTK, which are able to induce phagocytosis by binding to a laminin G-like domain (or LG domain).

[0022] The first region may comprise Gas6, ProS1, Tubby, Tulp1, Gal3, or active fragments thereof. The type or range of the first region is not particularly limited as long as the first region has a conserved ability to induce phagocytosis by interaction with a TAM receptor. The first region may

preferably be selected from Gas6, ProS1, or active fragments thereof.

[0023] More specifically, the first region may comprise a laminin G-like domain of Gas6 or ProS1, or an active fragment thereof, which contains a laminin G-like domain as a phagocytosis-related bridging molecule which is abundantly expressed in various tissue, and thus is able to induce phagocytosis through a TAM receptor.

[0024] Specifically, the laminin G-like domain may comprise an LG1 domain, an LG2 domain, or a combination thereof, and may preferably include both an LG1 domain and an LG2 domain, which are able to induce phagocytosis by binding to the TAM receptor.

[0025] The first region may be a peptide comprising the sequence of at least one of SEQ ID NO: 1 and SEQ ID NO: 2, or the sequence of at least one of SEQ ID NO: 3 and SEQ ID NO: 4. Preferably, the first region may be a peptide comprising the sequences of both SEQ ID NO: 1 and SEQ ID NO: 2, the sequences of both SEQ ID NO: 3 and SEQ ID NO: 4, and more preferably may be a peptide comprising the sequence of SEQ ID NO: 5 or SEQ ID NO: 6. The peptide comprising the sequence of any one of SEQ ID Nos above includes not only the amino acid sequence of the peptide but also an amino acid sequence variant thereof. The term "sequence variant" refers to a protein having a sequence in which one or more amino

acid residues differ from the amino acid sequence. As long as the activity of the fusion molecule is maintained, any truncation, deletion, insertion, substitution, or a combination thereof in the final structure of the protein is possible. One example of the sequence variant is a form in which amino acid residues at sites not essential for activity are truncated or deleted, or amino acid residues at sites important for autoinhibition are substituted. In some cases, it may also be modified by phosphorylation, glycosylation, methylation, farnesylation, or the like. These sequence variations and modifications are more preferable when the function and/or stability (thermal stability, pH stability, structural stability, etc.) and/or solubility of the protein are increased by mutation in the amino acid sequence.

[0026] The method for mutagenesis of the amino acid sequence is based on a method of producing a nucleic acid molecule comprising a nucleotide sequence corresponding to the amino acid sequence to be mutated by mutating a nucleotide sequence encoding the protein, and a method for obtaining the gene encoding the protein may be performed *in vivo* or *in vitro* using any mutagenesis technique well known in the art, for example, site-directed mutagenesis (Hutchinson et al., *J. Biol. Chem.*, 253:6551, 1978; Zoller and Smith, *DNA*, 3:479-488, 1984; Oliphant et al., *Gene*, 44:177, 1986; Hutchinson et al ., *Proc. Natl. Acad. Sci. U.S.A.*, 83:710, 1986), TAB

linker (Pharmacia), PCR technique (Higuchi, 1989, "Using PCR to Engineer DNA" in *PCR Technology: Principles and Applications for DNA Amplification*, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70), or the like.

[0027] In addition, when the first region comprises a laminin G-like domain of Gas6 or ProS1, or an active fragment thereof, the first region may not comprise a Gla domain. In this case, the first region may not recognize phosphatidylserine (PS), while the second region is able to induce phagocytosis by recognizing a target substance.

[0028] In addition, when the first region comprises a laminin G-like domain of Gas6 or ProS1, or an active fragment thereof, the first region does not comprise both the Gla domain and the EGF domain. In this case, it is possible not only to obtain technical effects by not comprising the above-mentioned Gla domain, but also to increase the yield by suppressing aggregation in the process of purifying the fusion molecule.

[0029]

[0030] The target substance may be a substance that accumulates in living tissue, causing a disease. For example, it may be a substance accumulated in an affected (i.e., diseased) tissue of a patient. The substance accumulated in a disease may be protein. That is, the disease may be proteopathy, without being limited thereto. For example, the

target substance may be amyloid. That is, the proteopathy may be amyloidosis. The target substance may be selected from abnormally accumulated substances listed in Table 1 below, and in this case, the disease may be a disease in which each abnormally accumulated substance is detected. For example, the proteopathy may be selected from Alzheimer's disease, Parkinson's disease, Huntington's disease, and prion disease, and in this case, target substances may be abnormally accumulated proteins that cause the diseases. That is, the target substances may be β-amyloid, tau, α-synuclein, huntingtin, and prion proteins, respectively.

[0031] [Table 1]

Abnormally accumulated substances	Abbreviation	Diseases
Amyloid precursor protein-derived β-amyloid	Aβ	Alzheimer's disease, Hereditary cerebral haemorrhage with amyloidosis, etc.
α-Synuclein	AαSyn	Parkinson's disease, Parkinson's dementia, dementia with Lewy bodies, multiple system atrophy, etc.
PrP^{Sc}	APrP	Transmissible spongiform encephalopathy (fatal familial insomnia, Gerstmann-Straussler-

		Scheinker disease, Creutzfeldt-Jacob disease, new variant Creutzfeldt-Jacob disease, etc.), etc.
Microtubule-associated protein tau	ATau	Tauopathies (Pick's disease, progressive supranuclear palsy, corticobasal degeneration, frontotemporal dementia with parkinsonism linked to chromosome 17, argyrophilic grain disease, etc.), Alzheimer's disease, Parkinson's disease, etc.
Huntingtin exon 1	(no abbreviation)	Huntington's disease, etc.
TAR DNA-binding protein 43 (TDP43)	(no abbreviation)	Frontotemporal dementia, amyotrophic lateral sclerosis (ALS), etc.
Superoxide dismutase 1 (SOD1)	(no abbreviation)	Amyotrophic lateral sclerosis (ALS), etc.
ABri peptide	ABri	Familial British dementia
ADan peptide	ADan	Familial Danish dementia
Immunoglobulin light-chain fragment	AL	Light-chain amyloidosis

Immunoglobulin heavy-chain fragment	AH	Heavy-chain amyloidosis
N-terminal fragment of serum amyloid A protein	AA	AA amyloidosis
Transthyretin	ATTR	Senile systemic amyloidosis, familial amyloid polyneuropathy, familial amyloid cardiomyopathy, leptomeningeal amyloidosis
β-2 microglobulin	Aβ2M	Dialysis-related amyloidosis, hereditary visceral amyloidosis
N-terminal fragment of apolipoprotein AI	AApoAI	ApoAI amyloidosis
C-terminally extended apolipoprotein AII	AApoAII	ApoAII amyloidosis
N-terminal fragment of apolipoprotein AIV	AApoAIV	ApoAIV amyloidosis
apolipoprotein C-II	AApoCII	ApoCII amyloidosis
apolipoprotein C-III	AApoCIII	ApoCIII amyloidosis
Gelsoliin fragment	AGel	Familial amyloidosis, Finnish type
Lysozyme	ALys	Hereditary non-neuropathic systemic amyloidosis
Fibrinogen alpha chain fragment	AFib	Fibrinogen amyloidosis

N-terminally truncated cystatin C	ACys	Hereditary cerebral hemorrhage with amyloidosis, Icelandic type
Amylin, IAPP	AIAPP	Diabetes mellitus type 2, insulinoma
Calcitonin	ACal	Medullary carcinoma of the thyroid
Atrial natriuretic factor	AANF	Cardiac arrhythmias, isolated atrial amyloidosis
Prolactin	APro	Pituitary prolactinoma
Insulin	AIns	Injection-localized amyloidosis
Lactadherin or medin	AMed	Aortic medial amyloidosis
Lactotransferrin or lactoferrin	ALac	Gelatinous drop-like corneal dystrophy
ODAM (odontogenic ameloblast-associated protein)	AOAAP	Calcifying epithelial odontogenic tumors
SP-C (pulmonary surfactant-associated protein C)	ASPC	Pulmonary alveolar proteinosis
LECT-2(Leukocyte cell-derived chemotaxin-2)	ALECT2	Renal LECT2 amyloidosis
Galectin-7	Agal7	Lichen amyloidosis, macular amyloidosis
Corneodesmosin	ACor	Hypotrichosis simplex of the scalp

C-terminal fragment of TGFBI (or keratoepithelin)	AKer	Lattice corneal dystrophy; type I, 3A or Avellino
SGI (Semenogelin-1)	ASem1	Seminal vesicle amyloidosis
S100 protein (A8 or A9)	(no abbreviation)	Prostate cancer
Enfuvirtide	AEnf	Injection-localized amyloidosis

[0032]

[0033] The second region that specifically binds to the target substance may be selected from among an antibody, an active fragment thereof, an antibody-like protein, a peptide, an aptamer, and a soluble receptor, and is not particularly limited as long as it specifically binds to the target substance.

[0034] Here, the antibody or active fragment thereof may be selected from among, for example, i) immunoglobulins such as IgG1, IgG2, IgG3 and IgG4; ii) native antibody fragments such as Fv, Fab, Fab', F(ab')2, VHH, VNAR, etc.; and iii) engineered antibodies such as scFv, dsFv, ds-scFv, (scFv)2, diabody, triabody, tetrabody, pentabody, etc. The antibody or active fragment thereof may be, for example, a Mab, Fab, or single-chain variable fragment (scFv) based on an antibody that specifically binds to a corresponding target substance, or six complementarity-determining regions (CDRs) derived from the antibody. That is, the protein or active fragment

thereof that specifically binds to the target substance comprises a portion necessary for an activity that specifically binds to the target substance, and the type or range thereof is not particularly limited as long as the protein or active fragment thereof is linked to the first region and does not cause an inflammatory response and synaptic damage. For example, the target substance may be beta-amyloid, and in this case, the protein or active fragment thereof that specifically binds to the target substance may comprise aducanumab or a single-chain variable fragment thereof. The second region comprise a Mab, Fab, or single-chain variable fragment based on six complementarity determining regions (CDRs) derived from any one selected from the group consisting of aducanumab, semorinemab, and cinpanemab.

[0035] The antibody or active fragment thereof may not comprise an Fc region, and preferably may comprise an Fc region variant that does not bind to an Fc receptor (particularly an Fcγ receptor). This Fc region variant may serve to improve properties such as purification.

[0036] The antibody-like protein refers to a protein scaffold capable of specifically binding to a target substance, like an antibody. Antibody-like proteins may be designed to have a size of about 2 to 20 kDa, which is smaller than antibodies (about 150 kDa on average), and thus target a binding site

that antibodies cannot reach. It is known that antibody-like proteins are more stable at high temperatures than antibodies and are much easier to synthesize using non-mammalian cells such as viruses and yeast or synthesize chemically, compared to antibodies.

[0037] As used herein, the term "aptamer" refers to a single-stranded DNA (ssDNA) or RNA having high specificity and affinity for a specific substance. Aptamers have a very high affinity for specific substances, are stable, may be synthesized in a relatively simple way, may be modified in various ways to increase the binding affinity thereof, and can target cells, proteins, and even small organic substances. Thus, the aptamers are characterized by having very high specificity and stability compared to antibodies that have already been developed. In addition, the aptamer may be produced through a known SELEX (Systematic Evolution of Ligands by Exponential enrichment) method. As this aptamer, an aptamer that specifically binds to, for example, beta-amyloid, tau, or alpha-synuclein, may be produced through a known SELEX (Systematic Evolution of Ligands by Exponential enrichment) method and then linked to the first region, thereby producing the fusion molecule according to the present invention.

[0038] The aptamer of the present invention is not limited as long as it is able to specifically bind to beta-amyloid, tau,

or alpha-synuclein, and bases that are used for the aptamer may be selected from among A, G, C, U, and deoxy forms thereof, unless otherwise specified.

[0039] In addition, the aptamer may be modified by linkage of at least one, selected from the group consisting of polyethylene glycol (PEG), inverted deoxythymidine (idT), locked nucleic acid (LNA), 2'-methoxy nucleoside, 2'-amino nucleoside, 2'F-nucleoside, amine linker, thiol linker, and cholesterol, at the 5'-end region, intermediate region, 3'-end region, or both ends thereof in order to increase the stability thereof. Inverted deoxythymidine (idT) is a molecule that is generally used to prevent nuclease degradation of an aptamer having weak nuclease resistance. In the case of a nucleic acid unit, the 3'-OH of the previous nucleotide is attached to the 5'-OH of the next nucleotide to form a chain, but in the case of idT, the 3'-OH of the previous nucleotide is attached to the 3'-OH of the next unit so that 5′-OH, not 3′-OH, is exposed. Thus, idT is a molecule that has the effect of inhibiting degradation by 3' exonuclease, a type of nuclease.

[0040] The soluble receptor of the present invention comprises a domain having an activity capable of binding to a target substance, that is, an endogenous ligand, wherein the domain may be one derived from an endogenous membrane receptor or an intracellular receptor, or a derivative

thereof. In this case, the soluble receptor comprised in the second region of the fusion molecule of the present invention may preferably be one in which regions having activities other than binding to a target substance have been removed from the endogenous receptor.

[0041] In the present invention, the peptide, which may be the second region, means an entity other than the antibody or an active fragment thereof, antibody-like protein or soluble receptor among polypeptides having amino acids as monomers capable of binding specifically to a target substance.

[0042]

[0043] Since the fusion molecule according to the present invention induces phagocytosis through interaction with the TAM receptor(s), the phagocytosis may be induced in cells expressing the TAM receptor(s). Phagocytosis generally means ingestion of cells or particles of 0.5 μm or more in size, and includes a process of tethering, engulfing, and degrading the cells or particles. In this case, phagocytosis forms a phagosome that surrounds the internalized cell or particle, and includes degradation within the phagolysosome by fusion of the phagosome and the lysosome. In phagocytosis, the process of cell death by apoptosis or necrosis is also referred to as efferocytosis.

[0044] The cells expressing the TAM receptor(s) may be at

least one type of professional phagocytes, at least one type of non-professional phagocytes, or a combination thereof. Here, the professional phagocytes refer to cells whose main role is to remove dead cells and accumulated debris through phagocytosis, and examples thereof include macrophages, neutrophils, dendritic cells, and mast cells. Macrophages usually stay in each tissue that can become a path of infection, and in many cases, they are called different names for tissues, including, for example, adipose tissue macrophages, bone marrow or blood monocytes, hepatic Kupffer cells, lymph node sinus histiocytes, alveolar macrophages, connective tissue histiocytes or giant cells, microglia of the central nervous system, placental Hofbauer cells, renal intraglomerular mesangial cells, bone osteoclasts, epithelioid cells of granulomas, red pulp macrophages of the spleen, peritoneal macrophage of the peritoneal cavity, LysoMac of Peyer's patch, and the like. On the other hand, the non-professional phagocytes refer to cells that mainly perform functions specific to the tissue in which the phagocytes reside, but can perform phagocytosis when necessary, and examples thereof epithelial cells, endothelial cells, fibroblasts, mesenchymal cells, some tissue-specific cells, for example, astrocytes or oligodendrocyte of the central nervous system, retinal Muller glia, hepatocytes, muscular satellite cells, testicular

Sertoli cells, etc., and some lymphocytes such as natural killer cells, large granular lymphocytes, eosinophils, basophils, B cells, etc. The fusion molecule according to the present invention is able to induce phagocytosis in phagocytes specific to a tissue in which a target substance to be cleared accumulates. For example, when abnormal proteins accumulated in the brain are to be cleared, the phagocytosis may be induced in astrocytes, microglia, oligodendrocytes, or combinations thereof. It may be induced, for example, by topically administering the fusion molecule according to the present invention to this tissue or by manipulating cells in the tissue to express and secrete the fusion molecule.

[0045] The induction of phagocytosis may not involve an inflammatory response. This enables clearance of the target substance without inducing an inflammatory response and tissue damage caused by an inflammatory response to be suppressed so that tissue dysfunction caused by accumulation of the target substance can be treated more safely than conventional techniques.

[0046]

[0047] The fusion molecule may further comprise a tag. When such a label is added to the fusion molecule, it may be used to check the purification, expression, action or mechanism of action of the fusion molecule.

[0048] Examples of the tag include, but are not limited to, His-tag, T7-tag, S-tag, FLAG-tag, Strep-tag, thioredoxin (Trx)-tag, His-patch thioredoxin-tag, *lacZ* (L-galactosidase)-tag, chloramphenicol acetyltransferase-tag, trpE-tag, avidin/streptavidin/Strep-tag, T7gene10-tag, staphylococcal protein A-tag, streptococcal protein G-tag, glutathione-*S*-transferase (GST)-tag, dihydrofolate reductase (DHFR)-tag, cellulose binding domains (CBDs)-tag, maltose binding protein (MBP)-tag, galactose-binding protein-tag, calmodulin binding protein (CBP)-tag, hemagglutinin influenza virus (HAI)-tag, HSV-tag, B-(VP7 protein region of bluetongue virus)-tag, polycysteine-tag, polyphenyalanine-tag, $(Ala\text{-}Trp\text{-}Trp\text{-}Pro)_n$-tag, polyaspartic acid-tag, c-myc-tag, *lac* repressor-tag, and the like. The tag may be located at the N-terminus, C-terminus or internally of the target protein.

[0049] The fusion molecule may further comprise a signal peptide or leader sequence at the N-terminus. It is known that a signal peptide is a short peptide present at the N-terminus at the initial stage of protein synthesis toward the secretory pathway, and directs the intracellular localization of the corresponding protein, membrane topology (in the case of a membrane protein), and the like. The signal peptide may be cleaved during expression and extracellular secretion of the fusion molecule.

[0050] The above-mentioned first region, second region, tag, signal peptide, or regions having minimal functionality (e.g., LG1 and LG2 regions or scFv heavy chain variable region and light chain variable region) included in the fusion molecule may be linked together directly or by a linker comprising a short oligopeptide or polypeptide. In general, the linker may comprise 2 to 500 amino acid residues. The length or type of the linker is not particularly limited as long as the linker can link the above-described regions together so as to have the intended activity, thereby forming the fusion molecule. An example of the linker may be the commonly used oligopeptide linker (GGGGS)n, that is, a linker in which one or more Gly-Gly-Gly-Gly-Ser units are repeated. Other examples of the linker include, but are not limited to, (GSSGGS)n, KESGSVSSEQLAQFRSLD, EGKSSGSGSESKST, GSAGSAAGSGEF, (EAAAK)n, CRRRRRREAEAC, A(EAAAK)4ALEA(EAAAK)4A, GGGGGGGG, GGGGGG, AEAAAKEAAAAKA, PAPAP, (Ala-Pro)n, VSQTSKLTRAETVFPDV, PLGLWA, TRHRQPRGWE, AGNRVRRSVG, RRRRRRRR, GFLG, and GSSGGSGSSGGSGGGDEADGSRGSQKAGVDE.

[0051]

[0052] Another aspect of the present invention provides a nucleic acid molecule encoding the fusion molecule, and an expression vector containing the same.

[0053] As described above, the nucleic acid molecule sequence encoding the fusion molecule may be mutated by substitution,

deletion, insertion, or a combination thereof, of one or more nucleotide residues, as long as it encodes a protein having an activity equivalent thereto.

[0054] The nucleic acid molecule sequence encoding the fusion molecule may be isolated from nature or artificially produced through synthesis or genetic recombination. The nucleic acid molecule sequence encoding the fusion molecule is operatively linked to an expression vector capable of expressing the same.

[0055] The term "expression vector" is a vector capable of expressing a protein or RNA of interest by introducing a nucleic acid sequence encoding a gene of interest into a suitable host cell, and refers to a gene construct containing essential regulatory elements operably linked to express the gene insert. Such expression vectors include all vectors such as plasmid vectors, cosmid vectors, bacteriophage vectors, and viral vectors.

[0056] A suitable expression vector has expression control elements such as a promoter, a start codon, a stop codon, a polyadenylation signal and an enhancer. The start codon and the stop codon are generally considered to be part of a nucleic acid sequence encoding a protein, and the sequence encoding the protein is designed to be in frame so as to be operable in the vector. The promoter may be constitutive or inducible. In addition, a conventional expression vector contains a selectable marker. Operational linkage with the

expression vector can be performed using genetic recombination techniques well known in the art, and site-specific DNA cleavage and ligation can be performed using enzymes generally known in the art.

[0057] The expression vector may preferably be configured to express the fusion molecule in a host cell for isolation and purification of the fusion molecule or such that the vector may be introduced into a cell *in vivo* and the corresponding cell may express and secrete the fusion molecule. For the purpose of introducing into cells *in vivo*, the vector may preferably be a non-integrating vector, that is, a vector that is not integrated into the genome of a host cell.

[0058]

[0059] Still another aspect of the present invention provides a cell expressing the fusion molecule.

[0060] The cells may be transformed to contain the nucleic acid molecule or an expression vector containing the same, and the "transformation" may be performed using suitable standard techniques selected depending on the host cell as known in the art, including any method of introducing the nucleic acid molecule into an organism, cell, tissue, or organ. These methods include, but are not limited to, electroporation, protoplast fusion, calcium phosphate ($CaPO_4$) precipitation, calcium chloride ($CaCl_2$) precipitation, agitation using silicon carbide fibers, agrobacterium-

mediated transformation, PEG-, dextran sulfate-, lipofectamine-, and desiccation/inhibition-mediated transformation methods.

[0061] Examples of the host cells include, but are not limited to, prokaryotic host cells such as *Escherichia coli*, *Bacillus subtilis*, *Streptomyces*, *Pseudomonas* (e.g., *Pseudomonas putida*), *Proteus mirabilis*, or *Staphylococcus* (e.g., *Staphylocus carnosus*). Other examples of the host cell include fungal cells such as *Aspergillus*, yeast cells, including *Pichia pastoris*, *Saccharomyces cerevisiae*, *Schizosaccharomyces*, and *Neurospora crassa*, lower eukaryotic cells, or cells derived from higher eukaryotes including insect cells, plant cells, or mammalian cells.

[0062] After the fusion molecule is expressed in the cells, it may be isolated and purified using conventional biochemical isolation techniques, such as treatment with a protein precipitating agent (salting out method), centrifugation, sonication, ultrafiltration, dialysis, or various chromatography such as molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, and affinity chromatography, which are generally used in combination in order to isolate proteins with high purity (Sambrook et al., Molecular Cloning: A laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press(1989); Deuscher, M., Guide to Protein Purification

Methods Enzymology, Vol. 182. Academic Press. Inc., San Diego, CA (1990)).

[0063]

[0064] Yet another aspect of the present invention provides a pharmaceutical composition for preventing or treating a disease caused by accumulation of the target substance in living tissue, the pharmaceutical composition containing the fusion molecule or the expression vector. Here, the composition may be administered topically to a site where the substance that causes the disease, that is, the target substance, accumulates.

[0065] Still yet another aspect of the present invention provides a method for preventing or treating proteopathy, the method comprising a step of administering to a subject a pharmaceutically effective amount of the fusion molecule.

[0066] A further aspect of the present invention provides the use of the fusion molecule for manufacture of a medicament for preventing or treating proteopathy.

[0067] The fusion molecule, which is an active ingredient in the pharmaceutical composition, is contained in a "pharmaceutically effective amount". The term "pharmaceutically effective amount" means an amount sufficient to achieve the above-mentioned efficacy or activity of the fusion molecule.

[0068] The pharmaceutical composition may be administered

orally or parenterally, preferably parenterally. More preferably, it may be administered topically to a tissue in which the target substance to be cleared accumulates.

[0069] As used herein, the term "parenteral administration" includes subcutaneous injection, intravenous, intramuscular, intrasternal injection or infusion techniques.

[0070] When the pharmaceutical composition is prepared as an injectable formulation, it may be prepared as the injectable formulation a conventional method known in the art. The injectable formulation may be in a form dispersed in a sterile medium so that it may be administered directly to a patient or may be in a form that may be administered after being dispersed in distilled water for injection at an appropriate concentration.

[0071] When the pharmaceutical composition is formulated for oral administration, it may contain one or more carriers selected from among diluents, lubricants, binders, disintegrants, sweeteners, stabilizers, and preservatives, and may contain one or more additives selected from among flavorings, vitamins, and antioxidants.

[0072] Techniques necessary for formulation of the pharmaceutical composition, and pharmaceutically acceptable carriers, additives, etc. are widely known to those skilled in the art (see, for example, the Handbook of Pharmaceutical Excipients, 4^{th} edition, Rowe et al., Eds., American

Pharmaceuticals Association (2003); Remington: the Science and Practice of Pharmacy, 20th edition, Gennaro, Ed., Lippincott Williams & Wilkins (2000); Remington's Pharmaceutical Sciences (19th ed., 1995)).

[0073] The appropriate dosage of the pharmaceutical composition may vary depending on factors such as formulation method, administration mode, patient's age, weight, sex, medical condition, diet, administration time, administration route, excretion rate, and response sensitivity. The dosage of the pharmaceutical composition of the present invention is 0.0001 to 1,000 μg/kg body weight for an adult.

[0074]

Advantageous Effects

[0075] The present invention relates to a fusion molecule having phagocytosis-inducing activity, which can solve the problem of tissue damage caused by activation of an inflammatory response, which occurs in the prior art. Accordingly, the fusion molecule is able to effectively clear abnormally accumulated substances such as beta-amyloid, tau, alpha-synuclein, huntingtin or prion protein, and thus may be used to prevent or treat diseases caused by these abnormally accumulated substances, for example, Alzheimer's disease, Parkinson's disease, Huntington's disease, or prion disease. The fusion molecule may be administered to a patient in the form of a purified fusion molecule or a gene therapy

vector capable of expressing and secreting the fusion molecule when introduced into a cell.

[0076] However, it should be understood that effects of the present invention are not limited to the above effects, and include all effects that may be inferred from the configuration of the invention described in the detailed description or claims.

[0077]

Brief Description of Drawings

[0078] FIG. 1 schematically shows beta-amyloid- and FITC-specific chimeric phagocytosis inducer based on Gas6.

[0079] FIG. 2 shows the results of Western blot analysis of chimeric phagocytosis inducer comprising a FLAG Tag, produced according to Preparation Example 1.

[0080] FIG. 3 schematically shows the action of chimeric phagocytosis inducer, produced according to Preparation Example 1, on TAM receptor.

[0081] FIG. 4 shows the evaluation results for selective beta-amyloid clearing ability of αAβ-Gas6.

[0082] FIG. 5 shows the evaluation results for beta-amyloid clearing ability of αAβ-Gas6 in the HMC3 cell line by beta-amyloid engulfment assay *in vitro*.

[0083] FIG. 6 shows the evaluation results for beta-amyloid clearing ability of αAβ-Gas6 in the HMC3 cell line by beta-amyloid engulfment assay *in vitro*.

[0084] FIG. 7 shows results indicating that the beta-amyloid clearing ability of αAβ-Gas6 is dependent on Axl among TAM receptors.

[0085] FIG. 8 shows results indicating that the beta-amyloid clearing ability of αAβ-Gas6 is dependent on Axl among TAM receptors.

[0086] FIG. 9 shows results indicating that the beta-amyloid clearing ability of αAβ-Gas6 is dependent on Axl among TAM receptors.

[0087] FIG. 10 shows the results of comparative analysis of the activation of inflammatory response signaling by αAβ-Gas6 and aducanumab using THP-Axl cells.

[0088] FIG. 11 shows the results of comparative analysis of the levels of pro-inflammatory cytokine secretion by αAβ-Gas6 and aducanumab using THP-Axl cells.

[0089] FIG. 12 shows the evaluation results for anti-inflammatory activity of αAβ-Gas6.

[0090] FIG. 13 shows results indicating that the beta-amyloid clearing ability of microglia was significantly increased by αAβ-Gas6.

[0091] FIG. 14 shows results indicating that the beta-amyloid clearing ability of astrocytes was significantly increased by αAβ-Gas6.

[0092] FIG. 15 shows results indicating that the transcriptional levels of pro-inflammatory cytokines in

astrocytes were changed by αAβ-Gas6 and aducanumab.

[0093] FIG. 16 shows results indicating that the transcriptional levels of pro-inflammatory cytokines in BV2 were changed by αAβ-Gas6 and aducanumab.

[0094] FIG. 17 shows the evaluation results for beta-amyloid plaque clearing ability of αAβ-Gas6 through administration of αAβ-Gas6 protein in 5XFAD Alzheimer's disease model mice.

[0095] FIG. 18 shows the evaluation results for beta-amyloid plaque clearing ability of αAβ-Gas6 through administration of αAβ-Gas6 virus in 5XFAD Alzheimer's disease model mice.

[0096] FIG. 19 shows results indicating that beta-amyloid contained in lysosomes were increased by microglia-mediated clearance in 5XFAD Alzheimer's disease model mice upon administration of αAβ-Gas6 protein.

[0097] FIG. 20 shows results indicating that beta-amyloid contained in lysosomes were increased by astrocyte-mediated clearance in 5XFAD Alzheimer's disease model mice upon administration of αAβ-Gas6 protein.

[0098] FIG. 21 shows results indicating that beta-amyloid contained in lysosomes were increased by microglia-mediated clearance in 5XFAD Alzheimer's disease model mice upon administration of αAβ-Gas6 virus.

[0099] FIG. 22 shows results indicating that beta-amyloid contained in lysosomes were increased by astrocyte-mediated clearance in 5XFAD Alzheimer's disease model mice upon

administration of αAβ-Gas6 virus.

[00100] FIG. 23 shows results indicating that microglia-mediated synapse engulfment that abnormally increased in 5XFAD Alzheimer's disease model mice due to the side effect of aducanumab was significantly restored upon administration of αAβ-Gas6 virus.

[00101] FIG. 24 shows results indicating that microglia-mediated synapse engulfment that abnormally increased in 5XFAD Alzheimer's disease model mice due to the side effect of aducanumab was significantly restored upon administration of αAβ-Gas6 virus.

[00102] FIG. 25 shows an experimental protocol for evaluating cognitive and memory abilities in 5XFAD Alzheimer's disease model mice upon administration of αAβ-Gas6 virus.

[00103] FIG. 26 shows results indicating that cognitive and memory abilities in 5XFAD Alzheimer's disease model mice were more restored upon administration of αAβ-Gas6 virus than administration of aducanumab.

[00104] FIG. 27 shows the evaluation results for tau clearing ability of αTau-Gas6 in the HMC3 cell line by *in vitro* tau engulfment assay.

[00105] FIG. 28 shows the evaluation results for alpha-synuclein clearing ability of αTau-Gas6 in the HMC3 cell line by *in vitro* tau engulfment assay.

[00106] FIG. 29 shows the evaluation results for beta-amyloid

clearing ability of αAβ-ProS1 in primary-cultured astrocytes by *in vitro* tau engulfment assay.

[00107] FIG. 30 shows the evaluation results for beta-amyloid clearing ability of αAβ(Fab)-Gas6 in the HMC3 cell line by *in vitro* tau engulfment assay.

[00108] FIG. 31 shows the evaluation results for beta-amyloid clearing ability of αAβ(Fab)-Gas6 in the HMC3 cell line by *in vitro* tau engulfment assay.

[00109]

Best Mode

[00110] Hereinafter, the present invention will be described in more detail with reference to examples and experimental examples. However, the following examples and experimental examples are illustrative only, and the scope of the invention is not limited thereto.

[00111]

[00112] Preparation Example 1. Preparation of Gas6-based fusion molecule having beta-amyloid clearance activity (I): beta-amyloid binding region in the form of scFv

[00113] To prepare a beta-amyloid (Aβ)-specific chimeric phagocytosis inducer based on Gas6 protein, the Gla domain, which recognizes PS (phosphatidylserine) in apoptotic cells, was first removed, and a single-chain variable fragment (scFv) of aducanumab, an amyloid-specific antibody, was introduced at that position [αAβ-Gas6(E)].

[00114] In addition, for the efficiency of protein production, the EGF repeat domain present in the internal residues of the Gas6 protein was also removed and an scFv of aducanumab was introduced at that position, thereby preparing αAβ-Gas6 (FIG. 1).

[00115] In addition, as controls for verifying beta-amyloid-specific binding of the scFv of aducanumab, αFITC-Gas6(E) and αFITC-Gas6, each introduced with an E2 scFv that selectively recognizes FITC, instead of the scFv of aducanumab, were prepared.

[00116] Table 2 below shows amino acid sequences related to the preparation of the fusion molecules, and Table 3 below shows nucleotide sequences related to the preparation of the fusion molecules (the underlined sequences are flag tags).

[00117] [Table 2]

1. αAβ-Gas6(E) **(FLAG tag, Gla delete, G-/-)**
MAPSLSPGPAALRRAPQLLLLLLAAECALADIQMTQSPSSLSASVGDRVTITCRASQSI
SSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
QQSYSTPLTFGGGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGVVQPGRSLRLSCAA
SGFAFSSYGMHWVRQAPGKGLEWVAVIWFDGTKKYYTDSVKGRFTISRDNSKNTLYLQM
NTLRAEDTAVYYCARDRGIGARRGPYYMDVWGKGTTVTVSSGGGGSGGGGSCINKYGSP
YTKNSGFATCVQNLPDQCTPNPCDRKGTQACQDLMGNFFCLCKAGWGGRLCDKDVNECS
QENGGCLQICHNKPGSFHCSCHSGFELSSDGRTCQDIDECADSEACGEARCKNLPGSYS
C

LCDEGFAYSSQEKACRDVDECLQGRCEQVCVNSPGSYTCHCDGRGGLKLSQDMDTCEDI
LPCVPFSVAKSVKSLYLGRMFSGTPVIRLRFKRLQPTRLVAEFDFRTFDPEGILLFAGG
HQDSTWIVLALRAGRLELQLRYNGVGRVTSSGPVINHGMWQTISVEELARNLVIKVNRD
AVMKIAVAGDLFQPERGLYHLNLTVGGIPFHEKDLVQPINPRLDGCMRSWNWLNGEDTT
IQETVKVNTRMQCFSVTERGSFYPGSGFAFYSLDYMRTPLDVGTESTWEVEVVAHIRPA
ADTGVLFALWAPDLRAVPLSVALVDYHSTKKLKKQLVVLAVEHTALALMEIKVCDGQEH
VVTVSLRDGEATLEVDGTRGQSEVSAAQLQERLAVLERHLRSPVLTFAGGLPDVPVTSA
PVTAFYRGCMTLEVNRRLLDLDEAAYKHSDITAHSCPPVEPAAAQGSRADYKDHDGDYK
DHDIDYKDDDDK*

2. αFITC-Gas6(E) **(FLAG tag, Gla delete, G-/-)**

MAPSLSPGPAALRRAPQLLLLLLAAECALAQVQLVESGGNLVQPGGSLRLSCAASGFTF
GSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVKGRFTISRDNAKNSVYLQMNSLRV
EDTAVYYCARRSYDSSGYWGHFYSYMDVWGQGTLVTVSGGGGSGGGGSGGGGSSVLTQP
SSVSAAPGQKVTISCSGSTSNIGNNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGS
KSGNSASLDISGLQSEDEADYYCAAWDDSLSEFLFGTGTKLTVLGGGGGSGGGGSCINK
YGSPYTKNSGFATCVQNLPDQCTPNPCDRKGTQACQDLMGNFFCLCKAGW
GGRLCDKDVNECSQENGGCLQICHNKPGSFHCSCHSGFELSSDGRTCQDIDECADSEAC
GEARCKNLPGSYSCLCDEGFAYSSQEKACRDVDECLQGRCEQVCVNSPGSYTCHCDGRG
GLKLSQDMDTCEDILPCVPFSVAKSVKSLYLGRMFSGTPVIRLRFKRLQPTRLVAEFDF
RTFDPEGILLFAGGHQDSTWIVLALRAGRLELQLRYNGVGRVTSSGPVINHGMWQTISV
EELARNLVIKVNRDAVMKIAVAGDLFQPERGLYHLNLTVGGIPFHEKDLVQPINPRLDG
CMRSWNWLNGEDTTIQETVKVNTRMQCFSVTERGSFYPGSGFAFYSLDYMRTPLDVGTE
STWEVEVVAHIRPAADTGVLFALWAPDLRAVPLSVALVDYHSTKKLKKQLVVLAVEHTA
LALMEIKVCDGQEHVVTVSLRDGEATLEVDGTRGQSEVSAAQLQERLAVLERHLRSPVL

TFAGGLPDVPVTSAPVTAFYRGCMTLEVNRRLLDLDEAAYKHSDITAHSCPPVEPAAAQ
GSRADYKDHDGDYKDHDIDYKDDDDK*

3. αAβ-Gas6 **(FLAG tag, Gla EGF delete, GE-/-)**

MAPSLSPGPAALRRAPQLLLLLLAAECALADIQMTQSPSSLSASVGDRVTITCRASQSI
SSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
QQSYSTPLTFGGGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGVVQPGRSLRLSCAA
SGFAFSSYGMHWVRQAPGKGLEWVAVIWFDGTKKYYTDSVKGRFTISRDNSKNTLYLQM
NTLRAEDTAVYYCARDRGIGARRGPYYMDVWGKGTTVTVSSGGGGSGGGGSDILPCVPF
SVAKSVKSLYLGRMFSGTPVIRLRFKRLQPTRLVAEFDFRTFDPEGILLF
AGGHQDSTWIVLALRAGRLELQLRYNGVGRVTSSGPVINHGMWQTISVEELARNLVIKV
NRDAVMKIAVAGDLFQPERGLYHLNLTVGGIPFHEKDLVQPINPRLDGCMRSWNWLNGE
DTTIQETVKVNTRMQCFSVTERGSFYPGSGFAFYSLDYMRTPLDVGTESTWEVEVVAHI
RPAADTGVLFALWAPDLRAVPLSVALVDYHSTKKLKKQLVVLAVEHTALALMEIKVCDG
QEHVVTVSLRDGEATLEVDGTRGQSEVSAAQLQERLAVLERHLRSPVLTFAGGLPDVPV
TSAPVTAFYRGCMTLEVNRRLLDLDEAAYKHSDITAHSCPPVEPAAAQGSRADYKDHDG
DYKDHDIDYKDDDDK*

4. αFITC-Gas6 **(FLAG tag, Gla EGF delete, GE-/-)**

MAPSLSPGPAALRRAPQLLLLLLAAECALAQVQLVESGGNLVQPGGSLRLSCAASGFTF
GSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVKGRFTISRDNAKNSVYLQMNSLRV
EDTAVYYCARRSYDSSGYWGHFYSYMDVWGQGTLVTVSGGGGSGGGGSGGGGSSVLTQP
SSVSAAPGQKVTISCSGSTSNIGNNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGS
KSGNSASLDISGLQSEDEADYYCAAWDDSLSEFLFGTGTKLTVLGGGGGSGGGGSCINK
YGSPYTKNSGFATCVQNKDILPCVPFSVAKSVKSLYLGRMFSGTPVIRLRFKRLQPTRL
VAEFDFRTFDPEGILLFAGGHQDSTWIVLALRAGRLELQLRYNGVGRVTSSGPVINHGM
W

QTISVEELARNLVIKVNRDAVMKIAVAGDLFQPERGLYHLNLTVGGIPFHEKDLVQPIN
PRLDGCMRSWNWLNGEDTTIQETVKVNTRMQCFSVTERGSFYPGSGFAFYSLDYMRTPL
DVGTESTWEVEVVAHIRPAADTGVLFALWAPDLRAVPLSVALVDYHSTKKLKKQLVVLA
VEHTALALMEIKVCDGQEHVVTVSLRDGEATLEVDGTRGQSEVSAAQLQERLAVLERHL
RSPVLTFAGGLPDVPVTSAPVTAFYRGCMTLEVNRRLLDLDEAAYKHSDITAHSCPPVE
PAAAQGSRADYKDHDGDYKDHDIDYKDDDDK*

5. αAβ-Gas6 **(HA tag, Gla EGF delete, GE-/-)**

MAPSLSPGPAALRRAPQLLLLLLAAECALADIQMTQSPSSLSASVGDRVTITCRASQSI
SSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
QQSYSTPLTFGGGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGVVQPGRSLRLSCAA
SGFAFSSYGMHWVRQAPGKGLEWVAVIWFDGTKKYYTDSVKGRFTISRDNSKNTLYLQM
NTLRAEDTAVYYCARDRGIGARRGPYYMDVWGKGTTVTVSSGGGGSGGGGSDILPCVPF
SVAKSVKSLYLGRMFSGTPVIRLRFKRLQPTRLVAEFDFRTFDPEGILLF
AGGHQDSTWIVLALRAGRLELQLRYNGVGRVTSSGPVINHGMWQTISVEELARNLVIKV
NRDAVMKIAVAGDLFQPERGLYHLNLTVGGIPFHEKDLVQPINPRLDGCMRSWNWLNGE
DTTIQETVKVNTRMQCFSVTERGSFYPGSGFAFYSLDYMRTPLDVGTESTWEVEVVAHI
RPAADTGVLFALWAPDLRAVPLSVALVDYHSTKKLKKQLVVLAVEHTALALMEIKVCDG
QEHVVTVSLRDGEATLEVDGTRGQSEVSAAQLQERLAVLERHLRSPVLTFAGGLPDVPV
TSAPVTAFYRGCMTLEVNRRLLDLDEAAYKHSDITAHSCPPVEPAAAGSGSGSGSGSGS
YPYDVPDYA*

6. Lentiviral Aducanumab IgG_IRES Zsgreen

MGWSCIILFLVATATGDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKA
PKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGT
KVEIKRKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECRRKR

GSGEGRGSLLTCGDVEENPGPMGWSCIILFLVATATGEVQLVESGGGVVQPGRSLRLSC AASGFAFSSYGMHWVRQAPGKGLEWVAVIWFDGTKKYYTDSVKGRFTISR DNSKNTLYLQMNTLRAEDTAVYYCARDRGIGARRGPYYMDVWGKGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSSDKTHTSPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK*
7. Endogenous full sequence human Gas6 protein MAPSLSPGPAALRRAPQLLLLLLAAECALAALLPAREATQFLRPRQRRAFQVFEEAKQG HLERECVEELCSREEAREVFENDPETDYFYPRYLDCINKYGSPYTKNSGFATCVQNLPD QCTPNPCDRKGTQACQDLMGNFFCLCKAGWGGRLCDKDVNECSQENGGCLQICHNKPGS FHCSCHSGFELSSDGRTCQDIDECADSEACGEARCKNLPGSYSCLCDEGFAYSSQEKAC RDVDECLQGRCEQVCVNSPGSYTCHCDGRGGLKLSQDMDTCEDILPCVPFSVAKSVKSL YLGRMFSGTPVIRLRFKRLQPTRLVAEFDFRTFDPEGILLFAGGHQDSTW IVLALRAGRLELQLRYNGVGRVTSSGPVINHGMWQTISVEELARNLVIKVNRDAVMKIA VAGDLFQPERGLYHLNLTVGGIPFHEKDLVQPINPRLDGCMRSWNWLNGEDTTIQETVK VNTRMQCFSVTERGSFYPGSGFAFYSLDYMRTPLDVGTESTWEVEVVAHIRPAADTGVL FALWAPDLRAVPLSVALVDYHSTKKLKKQLVVLAVEHTALALMEIKVCDGQEHVVTVSL RDGEATLEVDGTRGQSEVSAAQLQERLAVLERHLRSPVLTFAGGLPDVPVTSAPVTAFY RGCMTLEVNRRLLDLDEAAYKHSDITAHSCPPVEPAAAQGSRADYKDHDGDYKDHDIDY KDDDDK*

[00118]

[00119] [Table 3]

1. αAβ-Gas6(E) **(FLAG tag, Gla delete, G-/-)**

ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT

GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCGACATTCAGATGACTCAATCTCCTAGCT

CTCTGAGCGCCTCCGTTGGAGATAGAGTCACTATTACCTGCAGAGCCAGCCAATCCATC

AGCTCTTATCTAAATTGGTACCAACAGAAGCCCGGCAAAGCGCCAAAGCTGCTCATCTA

CGCTGCAAGCTCCTTACAGAGCGGAGTACCCAGCAGATTCTCAGGCAGTGGCAGTGGGA

CTGACTTCACATTGACGATTAGCTCTCTGCAGCCTGAAGACTTTGCCACATACTATTGT

CAGCAGAGCTATAGCACCCCGCTGACGTTTGGAGGCGGAACTAAGGTGGAAATCAAGAG

AGGAGGCGGGGGCTCCGGCGGGGGTGGCTCGGGGGGAGGAGGCTCAGAGGTTCAGCTTG

TCGAGTCTGGGGGGGGAG

TCGTTCAGCCAGGTAGAAGCCTCAGACTGAGCTGTGCCGCAAGTGGGTTTGCTTTTTCA

TCTTACGGTATGCACTGGGTGAGACAGGCTCCTGGCAAAGGACTCGAGTGGGTCGCTGT

AATATGGTTCGATGGTACAAAGAAATACTATACCGATAGTGTGAAAGGAAGATTCACCA

TTTCACGAGACAACAGTAAAAATACCTTGTACCTTCAGATGAACACCCTGAGAGCAGAA

GACACAGCCGTGTACTACTGCGCCAGAGATAGAGGTATCGGAGCAAGGCGTGGTCCCTA

TTATATGGATGTGTGGGGGAAGGGAACAACAGTGACTGTGAGCTCTGGCGGGGGCGGCA

GCGGCGGCGGTGGCAGCTGCATCAACAAGTATGGGTCTCCGTACACCAAAAACTCAGGC

TTCGCCA

CCTGCGTGCAAAACCTGCCTGACCAGTGCACGCCCAACCCCTGCGATAGGAAGGGGACC

CAAGCCTGCCAGGACCTCATGGGCAACTTCTTCTGCCTGTGTAAAGCTGGCTGGGGGGG

CCGGCTCTGCGACAAAGATGTCAACGAATGCAGCCAGGAGAACGGGGGCTGCCTCCAGA

TCTGCCACAACAAGCCGGGTAGCTTCCACTGTTCCTGCCACAGCGGCTTCGAGCTCTCC

TCTGATGGCAGGACCTGCCAAGACATAGACGAGTGCGCAGACTCGGAGGCCTGCGGGGA

GGCGCGCTGCAAGAACCTGCCCGGCTCCTACTCCTGCCTCTGTGACGAGGGCTTT

GCGTACAGCTCCCAGGAGAAGGCTTGCCGAGATGTGGACGAGTGTCTGCAGGGCCGCTG
TGAGCAGGTCTGCGTGAACTCCCCAGGGAGCTACACCTGCCACTGTGACGGGCGTGGGG
GCCTCAAGCTGTCCCAGGACATGGACACCTGTGAGGACATCTTGCCGTGCGTGCCCTTC
AGCGTGGCCAAGAGTGTGAAGTCCTTGTACCTGGGCCGGATGTTCAGTGGGACCCCCGT
GATCCGACTGCGCTTCAAGAGGCTGCAGCCCACCAGGCTGGTAGCTGAGTTTGACTTCC
GGACCTTTGACCCCGAGGGCATCCTCCTCTTTGCCGGAGGCCACCAGGACAGCACCTGG
ATCGTGCTGGCCCTGAGAGCCGGCCGGCTGGAGCTGCAGCTGCGCTACAACGGTGTCGG
CCGTGTC

ACCAGCAGCGGCCCGGTCATCAACCATGGCATGTGGCAGACAATCTCTGTTGAGGAGCT
GGCGCGGAATCTGGTCATCAAGGTCAACAGGGATGCTGTCATGAAAATCGCGGTGGCCG
GGGACTTGTTCCAACCGGAGCGAGGACTGTATCATCTGAACCTGACCGTGGGAGGTATT
CCCTTCCATGAGAAGGACCTCGTGCAGCCTATAAACCCTCGTCTGGATGGCTGCATGAG
GAGCTGGAACTGGCTGAACGGAGAAGACACCACCATCCAGGAAACGGTGAAAGTGAACA
CGAGGATGCAGTGCTTCTCGGTGACGGAGAGAGGCTCTTTCTACCCCGGGAGCGGCTTC
GCCTTCTACAGCCTGGACTACATGCGGACCCCTCTGGACGTCGGGACTGAATCAACCTG
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TCTGGGCCCCCGACCTCC

GTGCCGTGCCTCTCTCTGTGGCACTGGTAGACTATCACTCCACGAAGAAACTCAAGAAG
CAGCTGGTGGTCCTGGCCGTGGAGCATACGGCCTTGGCCCTAATGGAGATCAAGGTCTG
CGACGGCCAAGAGCACGTGGTCACCGTCTCGCTGAGGGACGGTGAGGCCACCCTGGAGG
TGGACGGCACCAGGGGCCAGAGCGAGGTGAGCGCCGCGCAGCTGCAGGAGAGGCTGGCC
GTGCTCGAGAGGCACCTGCGGAGCCCCGTGCTCACCTTTGCTGGCGGCCTGCCAGATGT
GCCGGTGACTTCAGCGCCAGTCACCGCGTTCTACCGCGGCTGCATGACACTGGAGGTCA
ACCGGAGGCTGCTGGACCTGGACGAGGCGGCGTACAAGCACAGCGACATCACGGCCCAC

TCCTGCCCCCCCGTGGAGCCCGCCGCAGCCcaagGATCCCGGGCTGACTACAAAGACCA

TGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGtga

2. αFITC-Gas6(E) **(FLAG tag, Gla delete, G-/-)**

ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT

GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCCAGGTTCAGCTGGTTGAGAGCGGAGGCA

ATCTGGTTCAGCCCGGTGGTAGTCTGCGTCTGTCTTGTGCGGCGTCAGGGTTCACTTTC

GGTAGTTTTTCAATGAGCTGGGTCCGTCAGGCACCAGGCGGTGGGCTGGAATGGGTGGC

AGGTCTGTCTGCACGTAGCTCCCTGACCCACTATGCAGATAGTGTTAAAGGGCGGTTCA

CAATTTCACGCGACAACGCTAAGAATAGCGTCTACCTGCAAATGAACTCCCTGCGGGTC

GAGGATACCGCAGTGTATTACTGCGCTCGCCGTTCTTATGACTCTAGTGGATACTGGGG

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GCCTGCCAGGACCTCATGGGCAACTTCTTCTGCCTGTGTAAAGCTGGCTGGGGGGGCCG

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GCGCTGCAAGAACCTGCCCGGCTCCTACTCCTGCCTCTGTGACGAGGGCTTTGCGTACA

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CCGTGCCTCTCTCTGTGGCACTGGTAGACTATCACTCCACGAAGAAACTCAAGAAGCAG

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CGGCCAAGAGCACGTGGTCACCGTCTCGCTGAGGGACGGTGAGGCCACCCTGGAGGTGG

ACGGCACCAGGGGCCAGAGCGAGGTGAGCGCCGCGCAGCTGCAGGAGAGGCTGGCCGTG

CTCGAGAGGCACCTGCGGAGCCCCGTGCT

CACCTTTGCTGGCGGCCTGCCAGATGTGCCGGTGACTTCAGCGCCAGTCACCGCGTTCT

ACCGCGGCTGCATGACACTGGAGGTCAACCGGAGGCTGCTGGACCTGGACGAGGCGGCG

TACAAGCACAGCGACATCACGGCCCACTCCTGCCCCCCCGTGGAGCCCGCCGCAGCCca

agGATCCCGGGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACT
ACAAGGATGACGATGACAAGtga

3. αAβ-Gas6 **(FLAG tag, Gla EGF delete, GE-/-)**

ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT
GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCGACATTCAGATGACTCAATCTCCTAGCT
CTCTGAGCGCCTCCGTTGGAGATAGAGTCACTATTACCTGCAGAGCCAGCCAATCCATC
AGCTCTTATCTAAATTGGTACCAACAGAAGCCCGGCAAAGCGCCAAAGCTGCTCATCTA
CGCTGCAAGCTCCTTACAGAGCGGAGTACCCAGCAGATTCTCAGGCAGTGGCAGTGGGA
CTGACTTCACATTGACGATTAGCTCTCTGCAGCCTGAAGACTTTGCCACATACTATTGT
CAGCAGAGCTATAGCACCCCGCTGACGTTTGGAGGCGGAACTAAGGTGGAAATCAAGAG
AGGAGGCGGGGGCTCCGGCGGGGGTGGCTCGGGGGGAGGAGGCTCAGAGGTTCAGCTTG
TCGAGTCTGGGGGGGGAG
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TCTTACGGTATGCACTGGGTGAGACAGGCTCCTGGCAAAGGACTCGAGTGGGTCGCTGT
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TTTCACGAGACAACAGTAAAAATACCTTGTACCTTCAGATGAACACCCTGAGAGCAGAA
GACACAGCCGTGTACTACTGCGCCAGAGATAGAGGTATCGGAGCAAGGCGTGGTCCCTA
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GCTGGTAGCTGAGTTTGACTTCCGGACCTTTGACCCCGAGGGCATCCTCCTCTTTGCCG
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TATAAACCCTCGTCTGGATGGCTGCATGAGGAGCTGGAACTGGCTGAACGGAGAAGACA
CCACCATCCAGGAAACGGTGAAAGTGAACACGAGGATGCAGTGCTTCTCGGTGACGGAG
AGAGGCTCTTTCTACCCC
GGGAGCGGCTTCGCCTTCTACAGCCTGGACTACATGCGGACCCCTCTGGACGTCGGGAC
TGAATCAACCTGGGAAGTAGAAGTCGTGGCTCACATCCGCCCAGCCGCAGACACAGGCG
TGCTGTTTGCGCTCTGGGCCCCCGACCTCCGTGCCGTGCCTCTCTCTGTGGCACTGGTA
GACTATCACTCCACGAAGAAACTCAAGAAGCAGCTGGTGGTCCTGGCCGTGGAGCATAC
GGCCTTGGCCCTAATGGAGATCAAGGTCTGCGACGGCCAAGAGCACGTGGTCACCGTCT
CGCTGAGGGACGGTGAGGCCACCCTGGAGGTGGACGGCACCAGGGGCCAGAGCGAGGTG
AGCGCCGCGCAGCTGCAGGAGAGGCTGGCCGTGCTCGAGAGGCACCTGCGGAGCCCCGT
GCTCACC
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CGGCTGCATGACACTGGAGGTCAACCGGAGGCTGCTGGACCTGGACGAGGCGGCGTACA
AGCACAGCGACATCACGGCCCACTCCTGCCCCCCCGTGGAGCCCGCCGCAGCCcaaGGA
TCCCGGGCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAA
GGATGACGATGACAAGtga

4. αFITC-Gas6 **(FLAG tag, Gla EGF delete, GE-/-)**

ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT
GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCCAGGTTCAGCTGGTTGAGAGCGGAGGCA
ATCTGGTTCAGCCCGGTGGTAGTCTGCGTCTGTCTTGTGCGGCGTCAGGGTTCACTTTC
GGTAGTTTTTCAATGAGCTGGGTCCGTCAGGCACCAGGCGGTGGGCTGGAATGGGTGGC
AGGTCTGTCTGCACGTAGCTCCCTGACCCACTATGCAGATAGTGTTAAAGGGCGGTTCA
CAATTTCACGCGACAACGCTAAGAATAGCGTCTACCTGCAAATGAACTCCCTGCGGGTC

GAGGATACCGCAGTGTATTACTGCGCTCGCCGTTCTTATGACTCTAGTGGATACTGGGG

CCATTTTTATAGCTACATGGATGTGTGGGGACAGGGCACTCTGGTGACCGTTTCCGGAG

GCGGTGGGTCTGGAGGCGGTGGGAGTGGAGGCGGTGGGTCAAGCGTTCTGACCCAGCCG

TCCTCTGTCAGCGCCGCGCCAGGCCAGAAAGTGACAATTTCCTGTTCTGGAAGTACTTC

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CCGGGCAAAGCGCCCAAGCTGATGATTTATGATGTGTCTAAACGTCCAAGTGGTGTTCC

TGACCGGTTCAGCGGTTCCAAGTCTGGGAATAGTGCCTCACTGGACATCTCAGGCCTGC

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GGCCGGCTGGAGCTGCAGCTGCGCTACAA

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GGCGTGCTGTTTGCGCTC

TGGGCCCCCGACCTCCGTGCCGTGCCTCTCTCTGTGGCACTGGTAGACTATCACTCCAC
GAAGAAACTCAAGAAGCAGCTGGTGGTCCTGGCCGTGGAGCATACGGCCTTGGCCCTAA
TGGAGATCAAGGTCTGCGACGGCCAAGAGCACGTGGTCACCGTCTCGCTGAGGGACGGT
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GCAGGAGAGGCTGGCCGTGCTCGAGAGGCACCTGCGGAGCCCCGTGCTCACCTTTGCCG
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CTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGAC
GATGACAAGtga

5. αAβ-Gas6 **HA tag (Gla EGF delete, GE-/-)**

ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT
GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCGACATTCAGATGACTCAATCTCCTAGCT
CTCTGAGCGCCTCCGTTGGAGATAGAGTCACTATTACCTGCAGAGCCAGCCAATCCATC
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TCGAGTCTGGGGGGGGAG
TCGTTCAGCCAGGTAGAAGCCTCAGACTGAGCTGTGCCGCAAGTGGGTTTGCTTTTTCA
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GAGGCCACCAGGACAGCACCTGGATCGTGCTGGCCCTGAGAGCCGGCCGGCTGGAGCTG
CAGCTGCGCTACAACGGTGTCGGCCGTGTCACCAGCAGCGGCCCGGTCATCAACCATGG
CATGTGGCAGACAATCTCTGTTGAGGAGCTGGCGCGGAATCTGGTCATCAAGGTCAACA
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CCACCAT
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CTTTCTACCCCGGGAGCGGCTTCGCCTTCTACAGCCTGGACTACATGCGGACCCCTCTG
GACGTCGGGACTGAATCAACCTGGGAAGTAGAAGTCGTGGCTCACATCCGCCCAGCCGC
AGACACAGGCGTGCTGTTTGCGCTCTGGGCCCCCGACCTCCGTGCCGTGCCTCTCTCTG
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GGTCACCGTCTCGCTGAGGGACGGTGAGGCCACCCTGGAGGTGGACGGCACCAGGGGCC
AGAGCGAGGTGAGCGCCGCGCAGCTGCAGGAGAGGCTGGCCGTGCTCGAGAGGCACCTG
CGGAGCCCCGTGCTCACCTTTGCTGGCGGCCTGCCAGATGTGCCGGTGACTTCAGCGCC
AGTCACCGCGTTCTACCGCGGCTGCATGACACTGGAGGTCAACCGGAGGCTGCTGGACC
TGGACGAGGCGGCGTACAAGCACAGCGACATCACGGCCCACTCCTGCCCCCCCGTGGAG
CCCGCCGCAGCCggcagcggcagcggcagcggcagcggcagctacccatacga
tgttccagattacgctTGA

6. Lentiviral Aducanumab IgG_IRES Zsgreen

GGATCCATGGGCTGGTCCTGCATCATCCTGTTCCTGGTGGCCACCGCCACCGGCGACAT

TCAGATGACTCAATCTCCTAGCTCTCTGAGCGCCTCCGTTGGAGATAGAGTCACTATTA

CCTGCAGAGCCAGCCAATCCATCAGCTCTTATCTAAATTGGTACCAACAGAAGCCCGGC

AAAGCGCCAAAGCTGCTCATCTACGCTGCAAGCTCCTTACAGAGCGGAGTACCCAGCAG

ATTCTCAGGCAGTGGCAGTGGGACTGACTTCACATTGACGATTAGCTCTCTGCAGCCTG

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CCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTCGTGTGCCTGCTGAATA

ACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGT

AACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAG

CACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAA

GTCTACGCCTGCGAAGTCACCCATCAGGGCCTGTCCTCGCCCGTCACAAAGAGCTTCAA

CAGGGGAGAGTGTCGCAGAAAACGCGGAAGCGGAgagggcagaggaagtcttctaacat

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TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACC

AGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAG
CGT
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AGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTAGCGACAAAACTCAC
ACAAGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCC
CCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGG
TGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGT
CAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGG
TCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG
CCCCGAGAACCACAGGTG
TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCT
GGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG
AGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTAC
AGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGT
GATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCCCCGGGTA
AAtga

7. Endogenous full sequence human Gas6 protein

ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT
GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCGCGCTGTTGCCGGCGCGCGAGGCCACGC
AGTTCCTGCGGCCCAGGCAGCGCCGCGCCTTTCAGGTCTTCGAGGAGGCCAAGCAGGGC
CACCTGGAGAGGGAGTGCGTGGAGGAGCTGTGCAGCCGCGAGGAGGCGCGGGAGGTGTT
CGAGAACGACCCCGAGACGGATTATTTTTACCCAAGATACTTAGACTGCATCAACAAGT
ATGGGTCTCCGTACACCAAAAACTCAGGCTTCGCCACCTGCGTGCAAAACCTGCCTGAC

CAGTGCACGCCCAACCCCTGCGATAGGAAGGGGACCCAAGCCTGCCAGGACCTCATGGG
CAACTTC
TTCTGCCTGTGTAAAGCTGGCTGGGGGGGCCGGCTCTGCGACAAAGATGTCAACGAATG
CAGCCAGGAGAACGGGGGCTGCCTCCAGATCTGCCACAACAAGCCGGGTAGCTTCCACT
GTTCCTGCCACAGCGGCTTCGAGCTCTCCTCTGATGGCAGGACCTGCCAAGACATAGAC
GAGTGCGCAGACTCGGAGGCCTGCGGGGAGGCGCGCTGCAAGAACCTGCCCGGCTCCTA
CTCCTGCCTCTGTGACGAGGGCTTTGCGTACAGCTCCCAGGAGAAGGCTTGCCGAGATG
TGGACGAGTGTCTGCAGGGCCGCTGTGAGCAGGTCTGCGTGAACTCCCCAGGGAGCTAC
ACCTGCCACTGTGACGGGCGTGGGGGCCTCAAGCTGTCCCAGGACATGGACACCTGTGA
GGACATCTTGCCGTGCGTGCCCTTCAGCGTGGCCAAGAGTGTGAAGTCCTTGTACCTGG
GCCGGATGTTCAGTGGGACCCCCGTGATCCGACTGCGCTTCAAGAGGCTGCAGCCCACC
AGGCTGGTAGCTGAGTTTGACTTCCGGACCTTTGACCCCGAGGGCATCCTCCTCTTTGC
CGGAGGCCACCAGGACAGCACCTGGATCGTGCTGGCCCTGAGAGCCGGCCGGCTGGAGC
TGCAGCTGCGCTACAACGGTGTCGGCCGTGTCACCAGCAGCGGCCCGGTCATCAACCAT
GGCATGTGGCAGACAATCTCTGTTGAGGAGCTGGCGCGGAATCTGGTCATCAAGGTCAA
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ATCATCTGAACCTGACCGTGGGAGGTATTCCCTTCCATGAGAAGGACCTCGTGCAGCCT
ATAAACCCTCGTCTGGATGGCTGCATGAGGAGCTGGAACTGGCTGAACGGAGAAGACAC
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CCTCTGGACGTCGGGACTGAATCAACCTGGGAAGTAGAAGTCGTGGCTCACATCC
GCCCAGCCGCAGACACAGGCGTGCTGTTTGCGCTCTGGGCCCCCGACCTCCGTGCCGTG
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GGTCCTGGCCGTGGAGCATACGGCCTTGGCCCTAATGGAGATCAAGGTCTGCGACGGCC

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GAGGCACCTGCGGAGCCCCGTGCTCACCTTTGCTGGCGGCCTGCCAGATGTGCCGGTGA
CTTCAGCGCCAGTCACCGCGTTCTACCGCGGCTGCATGACACTGGAGGTCAACCGGAGG
CTGCTGGACCTGGACGAGGCGGCGTACAAGCACAGCGACATCACGGCCCACTCCTGCCC
CCCCGTGGAGCCCGCCGCAGCCcaaGGATCCCGGGCTGACTACAAAGACCATGACGGTG
ATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGtga

[00120]

[00121] **Preparation Example 2. Gas6-based fusion molecule targeting tau**

[00122] To prepare a tau-specific chimeric phagocytosis inducer based on Gas6 protein, the Gla domain and the EGF repeat domain were first removed, and a single-chain variable fragment (scFv) of semorinemab, a tau-specific antibody fragment; scFv), was introduced at that position (αTau-Gas6). Table 4 below shows the amino acid sequence and nucleotide sequence of the chimeric phagocytosis inducer.

[00123] [Table 4]

1. αTau-Gas6 **(Tau-VL-G4Sx3-VH-LG-HA-T2A-EGFP, amino acid sequence)**

MAPSLSPGPAALRRAPQLLLLLLAAECALADDVLTQTPLSLPVTPGQPASISCRSSQSI
VHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDV
GVYYCFQGSLVPWTFGQGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRL
SCAASGLIFRSYGMSWVRQAPGKGLEWVATINSGGTYTYYPDSVKGRFTISRDNSKNTL
YLQMNSLRAEDTAVYYCANSYSGAMDYWGQGTLVTVSSGGGGSGGGGSDILPCVPFSVA

KSVKSLYLGRMFSGTPVIRLRFKRLQPTRLVAEFDFRTFDPEGILLFAGGHQDSTWIVL
ALRAGRLELQLRYNGVGRVTSSGPVINHGMWQTISVEELARNLVIKVNRDAVMKIAVAG
DLFQPERGLYHLNLTVGGIPFHEKDLVQPINPRLDGCMRSWNWLNGEDTTIQETVKVNT
RMQCFSVTERGSFYPGSGFAFYSLDYMRTPLDVGTESTWEVEVVAHIRPAADTGVLFAL
WAPDLRAVPLSVALVDYHSTKKLKKQLVVLAVEHTALALMEIKVCDGQEHVVTVSLRDG
EATLEVDGTRGQSEVSAAQLQERLAVLERHLRSPVLTFAGGLPDVPVTSAPVTAFYRGC
MTLEVNRRLLDLDEAAYKHSDITAHSCPPVEPAAAGSGSGSGSGSGSYPYDVPDYAEGR
GSLLTCGDVEENPGPVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTL
KFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDD
GNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIK
VNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLL
EFVTAAGITLGMDELYK*

2. αTau-Gas6 **(Tau-VL-G4Sx3-VH-LG-HA-T2A-EGFP, nucleotide sequence)**

ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT
GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCGACGATGTATTAACACAAACTCCCCTAT
CATTGCCGGTGACCCCGGGCCAACCAGCTTCGATCAGCTGCCGTAGCTCTCAGAGCATC
GTGCACAGCAACGGTAATACCTACCTGGAATGGTATTTGCAAAAACCGGGTCAATCCCC
GCAGTTGCTGATTTATAAAGTTTCGAATCGTTTCAGCGGTGTTCCGGATCGTTTCAGCG
GCTCTGGCTCCGGCACCGATTTTACGCTGAAGATCAGTCGCGTGGAAGCGGAGGACGTG
GGTGTCTACTACTGCTTTCAGGGTAGTTTGGTGCCGTGGACCTTTGGTCAGGGTACTAA
GGTGGAAATTAAGGGTGGTGGGGGATCAGGTGGCGGCGGCAGCGGCGGTGGCGGGAGCG
AGGTACAACTAGTTGAATCAGGTGGAGGGTTGGTTCAGCCAGGTGGTTCGCTGCGTCTG
AGTTGTGCGGCAAGCGGTTTGATCTTTCGCAGCTATGGTATGAGCTGGGTTCGTCAGGC
GCCGGGCAAGGGTCTGGAGTGGGTGGCGACCATTAACTCT

GGCGGCACGTACACCTACTATCCCGACTCCGTGAAAGGCCGTTTCACCATCTCCCGCGA

CAATAGCAAAAACACCCTGTATTTGCAGATGAACTCGCTCCGCGCAGAGGACACCGCTG

TGTACTACTGCGCCAATTCCTACAGCGGTGCTATGGATTATTGGGGTCAGGGCACATTG

GTGACTGTAAGCAGCGGCGGGGGCGGCAGCGGCGGCGGTGGCAGCGACATCTTGCCGTG

CGTGCCCTTCAGCGTGGCCAAGAGTGTGAAGTCCTTGTACCTGGGCCGGATGTTCAGTG

GGACCCCCGTGATCCGACTGCGCTTCAAGAGGCTGCAGCCCACCAGGCTGGTAGCTGAG

TTTGACTTCCGGACCTTTGACCCCGAGGGCATCCTCCTCTTTGCCGGAGGCCACCAGGA

CAGCACCTGGATCGTGCTGGCCCTGAGAGCCGGCCGGCTGGAGCTGCAGCTGCGCTACA

ACGGTGTCGGCCGTGTCACCAGCAGCGGCCCGGTCATCAACCATGGCATGTGGCAGACA

ATCTCTGTTGAGGAGCTGGCGCGGAATCTGGTCATCAAGGTCAACAGGGATGCTGTCAT

GAAAATCGCGGTGGCCGGGGACTTGTTCCAACCGGAGCGA

GGACTGTATCATCTGAACCTGACCGTGGGAGGTATTCCCTTCCATGAGAAGGACCTCGT

GCAGCCTATAAACCCTCGTCTGGATGGCTGCATGAGGAGCTGGAACTGGCTGAACGGAG

AAGACACCACCATCCAGGAAACGGTGAAAGTGAACACGAGGATGCAGTGCTTCTCGGTG

ACGGAGAGAGGCTCTTTCTACCCCGGGAGCGGCTTCGCCTTCTACAGCCTGGACTACAT

GCGGACCCCTCTGGACGTCGGGACTGAATCAACCTGGGAAGTAGAAGTCGTGGCTCACA

TCCGCCCAGCCGCAGACACAGGCGTGCTGTTTGCGCTCTGGGCCCCCGACCTCCGTGCC

GTGCCTCTCTCTGTGGCACTGGTAGACTATCACTCCACGAAGAAACTCAAGAAGCAGCT

GGTGGTCCTGGCCGTGGAGCATACGGCCTTGGCCCTAATGGAGATCAAGGTCTGCGACG

GCCAAGAGCACGTGGTCACCGTCTCGCTGAGGGACGGTGAGGCCACCCTGGAGGTGGAC

GGCACCAGGGGCCAGAGCGAGGTGAGCGC

CGCGCAGCTGCAGGAGAGGCTGGCCGTGCTCGAGAGGCACCTGCGGAGCCCCGTGCTCA

CCTTTGCTGGCGGCCTGCCAGATGTGCCGGTGACTTCAGCGCCAGTCACCGCGTTCTAC

CGCGGCTGCATGACACTGGAGGTCAACCGGAGGCTGCTGGACCTGGACGAGGCGGCGTA

CAAGCACAGCGACATCACGGCCCACTCCTGCCCCCCCGTGGAGCCCGCCGCAGCCGGCA

GCGGCAGCGGCAGCGGCAGCGGCAGCGGCAGCtacccatacgatgttccagattacgct
GAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCCAGTGAG
CAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACG
TAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAG
CTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGT
GACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGC
ACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCA
GGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGT
TCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGAC
GGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCAT
GGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGG
ACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCC
GTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAA
CGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCG
GCATGGACGAGCTGTACAAGtaa

[00124]

[00125] **Preparation Example 3. Gas6-based fusion molecule targeting alpha-synuclein**

[00126] To prepare an alpha-synuclein-specific chimera phagocytosis inducer based on Gas6 protein, the Gla domain and the EGF repeat domain were first removed, and a single-chain variable region (scFv) of cinpanemab, an alpha-synuclein-specific antibody, was introduced at that position (ααSyn-Gas6). Table 5 below shows the amino acid sequence and nucleotide sequence of the chimeric phagocytosis inducer.

[00127] [Table 5]

1. ααSyn-Gas6 **(Cinpanemab (aSyn)_VL-G4Sx3-VH-LG-HA-T2A-EGFP, amino acid sequence)**
MAPSLSPGPAALRRAPQLLLLLLAAECALASYELTQPPSVSVSPGQTARITCSGEALPM
QFAHWYQQRPGKAPVIVVYKDSERPSGVPERFSGSSSGTTATLTITGVQAEDEADYYCQ
SPDSTNTYEVFGGGTKLTVLGGGGSGGGGSGGGGSEVQLVESGGGLVEPGGSLRLSCAV
SGFDFEKAWMSWVRQAPGQGLQWVARIKSTADGGTTSYAAPVEGRFIISRDDSRNMLYL
QMNSLKTEDTAVYYCTSAHWGQGTLVTVSSGGGGSGGGGSDILPCVPFSVAKSVKSLYL
GRMFSGTPVIRLRFKRLQPTRLVAEFDFRTFDPEGILLFAGGHQDSTWIVLALRAGRLE
LQLRYNGVGRVTSSGPVINHGMWQTISVEELARNLVIKVNRDAVMKIAVAGDLFQPERG
LYHLNLTVGGIPFHEKDLVQPINPRLDGCMRSWNWLNGEDTTIQETVKVNTRMQCFSVT
ERGSFYPGSGFAFYSLDYMRTPLDVGTESTWEVEVVAHIRPAADTGVLFALWAPDLRAV
PLSVALVDYHSTKKLKKQLVVLAVEHTALALMEIKVCDGQEHVVTVSLRDGEATLEVDG
TRGQSEVSAAQLQERLAVLERHLRSPVLTFAGGLPDVPVTSAPVTAFYRGCMTLEVNRR
LLDLDEAAYKHSDITAHSCPPVEPAAAGSGSGSGSGSGSYPYDVPDYAEGRGSLLTCGD
VEENPGPVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGK
LPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAE
VKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHN
IEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGI
TLGMDELYK*
2. ααSyn-Gas6 **(Cinpanemab (aSyn)_VL-G4Sx3-VH-LG-HA-T2A-EGFP, nucleotide sequence)**
ATGGCCCCTTCGCTCTCGCCCGGGCCCGCCGCCCTGCGCCGCGCGCCGCAGCTGCTGCT
GCTGCTGCTGGCCGCGGAGTGCGCGCTTGCCTCCTATGAGCTGACTCAGCCACCCTCGG
TGTCAGTGTCCCCAGGACAGACGGCCAGGATCACCTGCTCTGGAGAAGCATTGCCAATG

CAATTTGCTCATTGGTACCAACAGAGGCCAGGCAAGGCCCCAGTGATAGTGGTGTACAA

AGACAGTGAGAGACCGTCAGGTGTCCCTGAGCGATTCTCTGGCTCCAGCTCAGGGACAA

CAGCCACGTTGACCATCACTGGAGTCCAGGCAGAAGATGAGGCTGACTATTACTGCCAG

TCGCCAGACAGCACTAACACTTATGAAGTCTTCGGCGGAGGGACCAAGCTGACCGTCCT

AGGTGGTGGGGGATCAGGTGGCGGCGGCAGCGGCGGTGGCGGGAGCGAGGTGCAGCTGG

TGGAGTCTGGGGGAGGTCTGGTCGAGCCGGGGGGGTCCCTAAGACTCTCCTGTGCAGTC

TCCGGATTCGATTTCGAAAAAGCCTGGAT

GAGTTGGGTCCGCCAGGCTCCAGGGCAGGGGCTACAGTGGGTTGCCCGTATCAAGAGCA

CAGCTGATGGTGGGACAACAAGCTACGCCGCCCCCGTGGAAGGCAGGTTCATCATCTCA

AGAGATGATTCGAGAAACATGCTTTATCTGCAAATGAACAGTCTGAAAACTGAAGACAC

AGCCGTCTATTATTGTACATCAGCCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCT

CGGGCGGGGGCGGCAGCGGCGGCGGTGGCAGCGACATCTTGCCGTGCGTGCCCTTCAGC

GTGGCCAAGAGTGTGAAGTCCTTGTACCTGGGCCGGATGTTCAGTGGGACCCCCGTGAT

CCGACTGCGCTTCAAGAGGCTGCAGCCCACCAGGCTGGTAGCTGAGTTTGACTTCCGGA

CCTTTGACCCCGAGGGCATCCTCCTCTTTGCCGGAGGCCACCAGGACAGCACCTGGATC

GTGCTGGCCCTGAGAGCCGGCCGGCTGGAGCTGCAGCTGCGCTACAACGGTGTCGGCCG

TGTCACCAGCAGCGGCCCGGTCATCAACC

ATGGCATGTGGCAGACAATCTCTGTTGAGGAGCTGGCGCGGAATCTGGTCATCAAGGTC

AACAGGGATGCTGTCATGAAAATCGCGGTGGCCGGGGACTTGTTCCAACCGGAGCGAGG

ACTGTATCATCTGAACCTGACCGTGGGAGGTATTCCCTTCCATGAGAAGGACCTCGTGC

AGCCTATAAACCCTCGTCTGGATGGCTGCATGAGGAGCTGGAACTGGCTGAACGGAGAA

GACACCACCATCCAGGAAACGGTGAAAGTGAACACGAGGATGCAGTGCTTCTCGGTGAC

GGAGAGAGGCTCTTTCTACCCCGGGAGCGGCTTCGCCTTCTACAGCCTGGACTACATGC

GGACCCCTCTGGACGTCGGGACTGAATCAACCTGGGAAGTAGAAGTCGTGGCTCACATC

CGCCCAGCCGCAGACACAGGCGTGCTGTTTGCGCTCTGGGCCCCCGACCTCCGTGCCGT

GCCTCTCTCTGTGGCACTGGTAGACTATCACTCCACGAAGAAACTCAAGAAGCAGCTGG
TGGTCCTGGCCGTGGAGCATACGGCCTTGGCCCTAATGGAGATCAAGGTCTGCGACGGC
CAAGAGCACGTGGTCACCGTCTCGCTGAGGGACGGTGAGGCCACCCTGGAGGTGGACGG
CACCAGGGGCCAGAGCGAGGTGAGCGCCGCGCAGCTGCAGGAGAGGCTGGCCGTGCTCG
AGAGGCACCTGCGGAGCCCCGTGCTCACCTTTGCTGGCGGCCTGCCAGATGTGCCGGTG
ACTTCAGCGCCAGTCACCGCGTTCTACCGCGGCTGCATGACACTGGAGGTCAACCGGAG
GCTGCTGGACCTGGACGAGGCGGCGTACAAGCACAGCGACATCACGGCCCACTCCTGCC
CCCCCGTGGAGCCCGCCGCAGCCGG
CAGCGGCAGCGGCAGCGGCAGCGGCAGCGGCAGCtacccatacgatgttccagattacg
ctGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGCCCAGTG
AGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGA
CGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCA
AGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTC
GTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGA
AGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATC
TTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACAC
CCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGG
GGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG
AAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCA
GCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCG
ACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGAT
CACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCT
GTACAAGtaa

[00128]

[00129] **Preparation Example 4. ProS1-based fusion molecule**

targeting beta-amyloid

[00130] To prepare a beta-amyloid (Aβ)-specific chimeric phagocytosis inducer based on ProS1 protein, the Gla domain and the EGF repeat domain were first removed, and a single-chain variable fragment (scFv) of aducanumab, a beta-amyloid-specific antibody, was introduced at that position (αAβ-ProS1). Table 6 below shows the amino acid sequence and nucleotide sequence of the chimeric phagocytosis inducer.

[00131] [Table 6]

1. αAβ-ProS1 **(**αAβ-ProS1**(GE-)-FLAG-IRES-ZsGreen, amino acid sequence)**

MRVLGGRCGALLACLLLVLPVSEADIQMTQSPSSLSASVGDRVTITCRASQSISSYLNW
YQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYST
PLTFGGGTKVEIKRGGGGSGGGGSGGGGSEVQLVESGGGVVQPGRSLRLSCAASGFAFS
SYGMHWVRQAPGKGLEWVAVIWFDGTKKYYTDSVKGRFTISRDNSKNTLYLQMNTLRAE
DTAVYYCARDRGIGARRGPYYMDVWGKGTTVTVSSGGGGSGGGGSVVSVCLPLNLDTKY
ELLYLAEQFAGVVLYLKFRLPEISRFSAEFDFRTYDSEGVILYAESIDHSAWLLIALRG
GKIEVQLKNEHTSKITTGGDVINNGLWNMVSVEELEHSISIKIAKEAVMDINKPGPLFK
PENGLLETKVYFAGFPRKVESELIKPINPRLDGCIRSWNLMKQGASGIKEIIQEKQNKH
CLVTVEKGSYYPGSGIAF
HIDYNNVSSAEGWHVNVTLNIRPSTGTGVMLALVSGNNTVPFAVSLVDSTSEKSQDILL
SVENTVIYRIQALSLCSDQQSHLEFRVNRNNLELSTPLKIETISHEDLQRQLAVLDKAM
KAKVATYLGGLPDVPFSATPVNAFYNGCMEVNINGVQLDLDEAISKHNDIRAHSCPSVW
KKTKNSQGSRADYKDHDGDYKDHDIDYKDDDDK*ASAPLPPPPLTLLAEAAWNKAGVRL

SICYFPPYCRLLAM*GPGNLALSS*RAFLGVFPLSPKECKVC*MS*RKQFLWKLLEDKQ
RL*RPFAGSGTPHLATGASAAKSHVYKIHLQRRHNPSATL*VG*LWKESNGSPQA
YSTRG*RMPRRYPIVWDLIWGLGTHALHVFSRG*KNV*APRTTGTWFSFEKHDDNMATT
MAQSKHGLTKEMTMKYRMEGCVDGHKFVITGEGIGYPFKGKQAINLCVVEGGPLPFAED
ILSAAFMYGNRVFTEYPQDIVDYFKNSCPAGYTWDRSFLFEDGAVCICNADITVSVEEN
CMYHESKFYGVNFPADGPVMKKMTDNWEPSCEKIIPVPKQGILKGDVSMYLLLKDGGRL
RCQFDTVYKAKSVPRKMPDWHFIQHKLTREDRSDAKNQKWHLTEHAIASGSALP*

2. αAβ-ProS1 **(**αAβ-ProS1**(GE-)-FLAG-IRES-ZsGreen, nucleotide sequence)**

ATGAGGGTCCTGGGTGGGCGCTGCGGGGCGCTGCTGGCGTGTCTCCTCCTAGTGCTTCC
CGTCTCAGAGGCAGACATTCAGATGACTCAATCTCCTAGCTCTCTGAGCGCCTCCGTTG
GAGATAGAGTCACTATTACCTGCAGAGCCAGCCAATCCATCAGCTCTTATCTAAATTGG
TACCAACAGAAGCCCGGCAAAGCGCCAAAGCTGCTCATCTACGCTGCAAGCTCCTTACA
GAGCGGAGTACCCAGCAGATTCTCAGGCAGTGGCAGTGGGACTGACTTCACATTGACGA
TTAGCTCTCTGCAGCCTGAAGACTTTGCCACATACTATTGTCAGCAGAGCTATAGCACC
CCGCTGACGTTTGGAGGCGGAACTAAGGTGGAAATCAAGAGAGGAGGCGGGGGCTCCGG
CGGGGGT
GGCTCGGGGGGAGGAGGCTCAGAGGTTCAGCTTGTCGAGTCTGGGGGGGGAGTCGTTCA
GCCAGGTAGAAGCCTCAGACTGAGCTGTGCCGCAAGTGGGTTTGCTTTTTCATCTTACG
GTATGCACTGGGTGAGACAGGCTCCTGGCAAAGGACTCGAGTGGGTCGCTGTAATATGG
TTCGATGGTACAAAGAAATACTATACCGATAGTGTGAAAGGAAGATTCACCATTTCACG
AGACAACAGTAAAAATACCTTGTACCTTCAGATGAACACCCTGAGAGCAGAAGACACAG
CCGTGTACTACTGCGCCAGAGATAGAGGTATCGGAGCAAGGCGTGGTCCCTATTATATG
GATGTGTGGGGGAAGGGAACAACAGTGACTGTGAGCTCTGGCGGGGGCGGCAGCGGCGG
CGGTGGCAGCGTTGTTTCAGTGTGCCTTCCCTTGAACCTTGACACAAAGTATGAATTAC

TTTACTTGGCGGAGCAGTTTGCAGGGGTTGTTTTATATTTAAAATTTCGTTTGCCAGAA
ATCAGCAGATTTTCAGCAGAATTTGATTTCCGGACATATGATTCAGAAGGCGTGATACT
GTACGCAGAATCTATCGATCACTCAGCGTGGCTCCTGATTGCACTTCGTGGTGGAAAGA
TTGAAGTTCAGCTTAAGAATGAACATACATCCAAAATCACAACTGGAGGTGATGTTATT
AATAATGGTCTATGGAATATGGTGTCTGTGGAAGAATTAGAACATAGTATTAGCATTAA
AATAGCTAAAGAAGCTGTGATGGATATAAATAAACCTGGACCCCTTTTTAAGCCGGAAA
ATGGATTGCTGGAA

ACCAAAGTATACTTTGCAGGATTCCCTCGGAAAGTGGAAAGTGAACTCATTAAACCGAT
TAACCCTCGTCTAGATGGATGTATACGAAGCTGGAATTTGATGAAGCAAGGAGCTTCTG
GAATAAAGGAAATTATTCAAGAAAAACAAAATAAGCATTGCCTGGTTACTGTGGAGAAG
GGCTCCTACTATCCTGGTTCTGGAATTGCTCAATTTCACATAGATTATAATAATGTATC
CAGTGCTGAGGGTTGGCATGTAAATGTGACCTTGAATATTCGTCCATCCACGGGCACTG
GTGTTATGCTTGCCTTGGTTTCTGGTAACAACACAGTGCCCTTTGCTGTGTCCTTGGTG
GACTCCACCTCTGAAAAATCACAGGATATTCTGTTATCTGTTGAAAATACTGTAATATA
TCGGATA

CAGGCCCTAAGTCTATGTTCCGATCAACAATCTCATCTGGAATTTAGAGTCAACAGAAA
CAATCTGGAGTTGTCGACACCACTTAAAATAGAAACCATCTCCCATGAAGACCTTCAAA
GACAACTTGCCGTCTTGGACAAAGCAATGAAAGCAAAAGTGGCCACATACCTGGGTGGC
CTTCCAGATGTTCCATTCAGTGCCACACCAGTGAATGCCTTTTATAATGGCTGCATGGA
AGTGAATATTAATGGTGTACAGTTGGATCTGGATGAAGCCATTTCTAAACATAATGATA
TTAGAGCTCACTCATGTCCATCAGTTTGGAAAAAGACAAAGAATTCTCAAGGATCCCGG
GCTGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGA
CGATGACAAGtgaGCTAGCGCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAG
CCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGT

CTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGG GGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGA ACGCCAAGAACCAGAAGTGGCACCTGACCGAGCACGCCATCGCCTCCGGCTCCGCCTTG CCCtga

[00132]

[00133] **Preparation Example 5. Gas6-based fusion molecules targeting beta-amyloid (II): beta-amyloid-binding regions in the form of Fab or Mab**

[00134] To prepare gas6 protein-based beta-amyloid (Aβ)-specific chimera phagocytosis inducer, the Gla domain, which recognizes PS (phosphatidylserine) in apoptotic cells, was first removed, and an antigen-binding fragment (Fab) or monoclonal antibody (Mab) of the beta-amyloid-specific antibody aducanumab was introduces at that position (αAβ[Fab]-Gas6, and αAβ[Mab]-Gas6). Tables 7 and 8 below show the amino acid sequences and nucleotide sequences of the two chimeric phagocytosis inducers.

[00135] [Table 7]

1. αAβ[Fab]-Gas6 (Aducanumab (Fab)-Gas6-FLAG, amino acid sequence)
METDTLLLWVLLLWVPGSTGDEVQLVESGGGVVQPGRSLRLSCAASGFAFSSYGMHWVR QAPGKGLEWVAVIWFDGTKKYYTDSVKGRFTISRDNSKNTLYLQMNTLRAEDTAVYYCA RDRGIGARRGPYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHGGGGSGGGGSDILPCVPFSVAKSVKSLYLGRMFSGTPVIRLRF

KRLQPTRLVAEFDFRTFDPEGILLFAGGHQDSTWIVLALRAGRLELQLRYNGVGRVTSS
GPVINHGMWQTISVEELARNLVIKVNRDAVMKIAVAGDLFQPERGLYHLNLTVGGIPFH
EKDLVQPINPRLDGCMRSWNWLNGEDTTIQETVKVNTRMQCFSVTERGSFYPGSGFAFY
SLDYMRTPLDVGTESTWEVEVVAHIRPAADTGVLFALWAPDLRAVPLSVALVDYHSTKK
LKKQLVVLAVEHTALALMEIKVCDGQEHVVTVSLRDGEATLEVDGTRGQSEVSAAQLQE
RLAVLERHLRSPVLTFAGGLPDVPVTSAPVTAFYRGCMTLEVNRRLLDLDEAAYKHSDI
TAHSCPPVEPAAADYKDHDGDYKDHDIDYKDDDDK*

2 αAβ[Fab]-Gas6 (Aducanumab (Fab)-Gas6-FLAG, nucleotide sequence)

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGG
TGACGAGGTTCAGCTTGTCGAGTCTGGGGGGGGAGTCGTTCAGCCAGGTAGAAGCCTCA
GACTGAGCTGTGCCGCAAGTGGGTTTGCTTTTTCATCTTACGGTATGCACTGGGTGAGA
CAGGCTCCTGGCAAAGGACTCGAGTGGGTCGCTGTAATATGGTTCGATGGTACAAAGAA
ATACTATACCGATAGTGTGAAAGGAAGATTCACCATTTCACGAGACAACAGTAAAAATA
CCTTGTACCTTCAGATGAACACCCTGAGAGCAGAAGACACAGCCGTGTACTACTGCGCC
AGAGATAGAGGTATCGGAGCAAGGCGTGGTCCCTATTATATGGATGTGTGGGGGAAGGG
AACAACAGTGACTGTGAGCTCTGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCAC
CCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTAC
TTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACAC
CTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGC
CCTCTAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGC
CCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACGGC
GGAGGTGGAAGCGGAGGCGGTGGAAGCGACATCTTGCCGTGCGTGCCCTTCAGCGTGGC
CAAGAGTGTGAAGTCCTTGTACCTGGGCCGGATGTTCAGTGGGACCCCCGTGATCCGAC
TGCGCTTCAAGAGGCTGCAGCCCACCAGGCTGGTAGCTGAGTTTGACTTCCGGACCTTT

GACCCCGAGGGCATCCTCCTCTTTGCCGGAGGCCACCAGGACAGCACCTGGATCGTGCT
GGCCCTGAGAGCCGGCCGGCTGGAGCTGCAGCTGCGCTACAACGGTGTCGGCCGT
GTCACCAGCAGCGGCCCGGTCATCAACCATGGCATGTGGCAGACAATCTCTGTTGAGGA
GCTGGCGCGGAATCTGGTCATCAAGGTCAACAGGGATGCTGTCATGAAAATCGCGGTGG
CCGGGGACTTGTTCCAACCGGAGCGAGGACTGTATCATCTGAACCTCACCGTGGGAGGT
ATTCCCTTCCATGAGAAGGACCTCGTGCAGCCTATAAACCCTCGTCTGGATGGCTGTAT
GAGGAGCTGGAACTGGCTGAACGGAGAAGACACCACCATCCAGGAAACGGTGAAAGTGA
ACACGAGGATGCAGTGCTTCTCGGTGACGGAGAGAGGCTCTTTCTACCCCGGGAG
CGGCTTCGCCTTCTACAGCCTGGACTACATGCGGACCCCTCTGGACGTCGGGACTGAAT
CAACCTGGGAAGTAGAAGTCGTGGCTCACATCCGCCCAGCCGCAGACACAGGCGTGCTG
TTTGCGCTCTGGGCCCCCGACCTCCGTGCCGTGCCTCTCTCTGTGGCACTGGTAGACTA
TCACTCCACGAAGAAACTCAAGAAGCAGCTGGTGGTCCTGGCCGTGGAGCATACGGCCT
TGGCCCTAATGGAGATCAAGGTCTGCGACGGCCAAGAGCACGTGGTCACCGTCTCGCTG
AGGGACGGTGAGGCCACCCTGGAGGTGGACGGCACCAGGGGCCAGAGCGAGGTGAGCGC
CGCGCAGCTGCAGGAGAGGCTGGCCGTGCTCGAGAGGCACCTGCGGAGCCCCGTGCTCA
CCTTTGC
CGGCGGCCTGCCAGATGTGCCGGTGACTTCAGCGCCAGTCACCGCGTTCTACCGCGGCT
GCATGACACTGGAGGTCAACCGGAGGCTGCTGGACCTGGACGAGGCGGCGTACAAGCAC
AGCGACATCACGGCCCACTCCTGCCCCCCCGTGGAGCCCGCCGCAGCCGACTACAAAGA
CCATGACGGTGATTATAAAGATCATGACATCGACTACAAGGATGACGATGACAAGtga

[00136]

[00137] [Table 8]

1. αAβ[Mab]-Gas6 (Aducanumab (Mab)-Gas6-FLAG, amino acid sequence)

METDTLLLWVLLLWVPGSTGDEVQLVESGGGVVQPGRSLRLSCAASGFAFSSYGMHWVR
QAPGKGLEWVAVIWFDGTKKYYTDSVKGRFTISRDNSKNTLYLQMNTLRAEDTAVYYCA
RDRGIGARRGPYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY
FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG
QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGKGGGGSGGGGSDILPC
VPFSVAKSVKSLYLGRMFSGTPVIRLRFKRLQPTRLVAEFDFRTFDPEGILLFAGGHQD
STWIVLALRAGRLELQLRYNGVGRVTSSGPVINHGMWQTISVEELARNLVIKVNRDAVM
KIAVAGDLFQPERGLYHLNLTVGGIPFHEKDLVQPINPRLDGCMRSWNWLNGEDTTIQE
TVKVNTRMQCFSVTERGSFYPGSGFAFYSLDYMRTPLDVGTESTWEVEVVAHIRPAADT
GVLFALWAPDLRAVPLSVALVDYHSTKKLKKQLVVLAVEHTALALMEIKVCDGQEHVVT
VSLRDGEATLEVDGTRGQSEVSAAQLQERLAVLERHLRSPVLTFAGGLPDVPVTSAPVT
AFYRGCMTLEVNRRLLDLDEAAYKHSDITAHSCPPVEPAAA*

2. αAβ[Mab]-Gas6 (Aducanumab (Mab)-Gas6-FLAG, nucleotide sequence)

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGG
TGACGAGGTTCAGCTTGTCGAGTCTGGGGGGGGAGTCGTTCAGCCAGGTAGAAGCCTCA
GACTGAGCTGTGCCGCAAGTGGGTTTGCTTTTTCATCTTACGGTATGCACTGGGTGAGA
CAGGCTCCTGGCAAAGGACTCGAGTGGGTCGCTGTAATATGGTTCGATGGTACAAAGAA
ATACTATACCGATAGTGTGAAAGGAAGATTCACCATTTCACGAGACAACAGTAAAAATA
CCTTGTACCTTCAGATGAACACCCTGAGAGCAGAAGACACAGCCGTGTACTACTGCGCC
AGAGATAGAGGTATCGGAGCAAGGCGTGGTCCCTATTATATGGATGTGTGGGGGAAGGG

AACAACAGTGACTGTGAGCTCTGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCAC
CCTCCTCCAAGAGCACCT
CTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG
GTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACA
GTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACTGTGCCCTCTAGCAGCTTGGGCA
CCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAA
GTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACT
CCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCT
CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTC
AAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGA
GGAGCAGTACAACAGCAC
GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGT
ACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAA
GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCT
GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTG
CTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTG
GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACA
CGCAGAA
GAGCCTCTCCCTGTCCCCGGGTAAAGGCGGAGGTGGAAGCGGAGGCGGTGGAAGCGACA
TCTTGCCGTGCGTGCCCTTCAGCGTGGCCAAGAGTGTGAAGTCCTTGTACCTGGGCCGG
ATGTTCAGTGGGACCCCCGTGATCCGACTGCGCTTCAAGAGGCTGCAGCCCACCAGGCT
GGTAGCTGAGTTTGACTTCCGGACCTTTGACCCCGAGGGCATCCTCCTCTTTGCCGGAG
GCCACCAGGACAGCACCTGGATCGTGCTGGCCCTGAGAGCCGGCCGGCTGGAGCTGCAG
CTGCGCTACAACGGTGTCGGCCGTGTCACCAGCAGCGGCCCGGTCATCAACCATGGCAT

GTGGCAGACAATCTCTGTTGAGGAGCTGGCGCGGAATCTGGTCATCAAGGTCAACAGGG
ATGCTGTCATGAAAATCGCGGTGGCCGGGGACTTGTTCCAACCGGAGCGAGGACTGTAT
CATCTGAACCTCACCGTGGGAGGTATTCCCTTCCATGAGAAGGACCTCGTGCAGCCTAT
AAACCCTCGTCTGGATGGCTGTATGAGGAGCTGGAACTGGCTGAACGGAGAAGACACCA
CCATCCAGGAAACGGTGAAAGTGAACACGAGGATGCAGTG
CTTCTCGGTGACGGAGAGAGGCTCTTTCTACCCCGGGAGCGGCTTCGCCTTCTACAGCC
TGGACTACATGCGGACCCCTCTGGACGTCGGGACTGAATCAACCTGGGAAGTAGAAGTC
GTGGCTCACATCCGCCCAGCCGCAGACACAGGCGTGCTGTTTGCGCTCTGGGCCCCCGA
CCTCCGTGCCGTGCCTCTCTCTGTGGCACTGGTAGACTATCACTCCACGAAGAAACTCA
AGAAGCAGCTGGTGGTCCTGGCCGTGGAGCATACGGCCTTGGCC
CTAATGGAGATCAAGGTCTGCGACGGCCAAGAGCACGTGGTCACCGTCTCGCTGAGGGA
CGGTGAGGCCACCCTGGAGGTGGACGGCACCAGGGGCCAGAGCGAGGTGAGCGCCGCGC
AGCTGCAGGAGAGGCTGGCCGTGCTCGAGAGGCACCTGCGGAGCCCCGTGCTCACCTTT
GCCGGCGGCCTGCCAGATGTGCCGGTGACTTCAGCGCCAGTCACCGCGTTCTACCGCGG
CTGCATGACACTGGAGGTCAACCGGAGGCTGCTGGACCTGGACGAGGCGGCGTACAAGC
ACAGCGACATCACGGCCCACTCCTGCCCCCCCGTGGAGCCCGCCGCAGCCtga

[00138]

[00139] **Experimental Example 1. Gas6-based fusion molecule targeting beta-amyloid (I): beta-amyloid binding domain in scFv form**

[00140] **1-1. Analysis of expression of fusion molecule in transfected cells**

[00141] After plasmid transfection into HEK293 cells, the expression of the fusion molecule containing the Flag tag according to Preparation Example 1 analyzed by Western blot

analysis using the Flag tag, and the results are shown in FIG. 2.

[00142]

[00143] 1-2. Analysis of beta-amyloid specific binding affinity of prepared fusion molecules

[00144] To verify whether each of αAβ-Gas6(E), αAβ-Gas6, αFITC-Gas6(E), and αFITC-Gas6 can selectively recognize beta-amyloid and FITC, the culture broth secreted from the HEK293 transfected with each plasmid was collected and subjected to an experiment using beta-amyloid oligomer and FITC-conjugated beads. The results showed that αAβ-Gas6 (E) and αAβ-Gas6 recognized only beta-amyloid oligomer beads, and αFITC-Gas6 (E) and αFITC-Gas6 recognized only FITC beads, thereby inducing phagocytosis, as shown in FIG. 4.

[00145] Although αAβ-Gas6 (E) and αAβ-Gas6 were shown to exhibit similar activities, it was found that αAβ-Gas6 obtained by additionally removing the EGF domain of Gas6 could be obtained in high yield without aggregation in the protein purification process. Thus, αAβ-Gas6 was used in subsequent experiments.

[00146]

[00147] 1-3. Analysis of mechanism of action of prepared fusion molecule

[00148] (1) Analysis using cell line

[00149] An *in vitro* Aβ engulfment assay was developed, in which

beta-amyloid oligomers are conjugated with a pH indicator and hence can emit red fluorescence in intracellular lysosomes when they are uptaken by phagocytosis.

[00150] As a result of performing the *in vitro* Aβ engulfment assay with HMC3 cells, a human microglial cell line expressing TAM receptors, it was shown that beta-amyloid oligomers were selectively cleared by αAβ-Gas6 (FIGS. 5 and 6).

[00151] In particular, in an experiment where cells were treated additionally with an antibody that interferes with the function of TAM receptors, it was confirmed that αAβ-Gas6 cleared beta-amyloid oligomers mainly through Axl among Tyro3, Mertk, and Axl (FIGS. 7 to 9). In fact, when Axl was removed from HMC3 cells, the activity of αAβ-Gas6 significantly decreased. In addition, THP-1, which is a human monocyte cell line that does not express TAM receptors, did not show an increase in beta-amyloid clearance by αAβ-Gas6, while THP-Axl cells overexpressing Axl exhibited a significantly increased ability to clear beta-amyloid fibrils in a manner dependent on αAβ-Gas6.

[00152] Next, since THP-Axl cells express both Axl and Fc receptors, the degree of inflammatory response induced upon beta-amyloid uptake by each of αAβ-Gas6 and aducanumab was analyzed in those cells. To this end, the NF-kB reporter was first expressed in THP-Axl cells, and each of a control, αAβ-

Gas6 and aducanumab was added to the cells together with beta-amyloid oligomers. As a result, it was confirmed that, when aducanumab was added, the expression of the NF-kB reporter significantly increased, but when αAβ-Gas6 was added, the NF-kB reporter was expressed at or below the control level (FIG. 10). In addition, as a result of measuring the secreted protein levels of IL-1b, IL-6 and TNF, which are the three most representative inflammatory cytokines, it was shown that, when THP-Axl cells were treated with aducanumab, the protein levels of the inflammatory cytokines in the treated cells significantly increased compared to those in the control group (FIG. 11). In contrast, importantly, the levels of these inflammatory cytokines in the cells treated with αAβ-Gas6 did not increase compared to those in the control group. This is a key result that, as our hypothesis suggests, the αAβ-Gas6 fusion phagocytosis-inducing protein does not induce an inflammatory response when phagocytosing a target substance through a TAM receptor, which is similar to recognition and efferocytosis of naturally apoptotic cells.

[00153] In addition, unlike aducanumab, αAβ-Gas6 increased the expression of Twist1/2 gene, which is known as a mechanism of suppressing inflammatory responses (FIG. 12).

[00154]

[00155] (2) Analysis using astrocytes and microglia

[00156] To examine whether astrocytes and microglia, which are

cells expressing TAM receptors in the brain, can clear beta-amyloid through αAβ-Gas6, primary astrocytes and microglia obtained from mouse brains were separately purified and then cultured. Then, each of purified αAβ-Gas6 and aducanumab was added to the cells together with beta-amyloid fibrils, and the degree of clearance of beta-amyloid fibrils was measured in real time.

[00157] The results showed that αAβ-Gas6 increased the beta-amyloid clearing ability of microglia in a concentration-dependent manner, which is similar to the results obtained in HMC3 which is a cell line expressing Axl (FIG. 13). Importantly, it was shown that, when aducanumab was added, the beta-amyloid clearing ability of astrocytes did not change at all, but when αAβ-Gas6 was added, the beta-amyloid clearing ability of astrocytes significantly increased in a concentration-dependent manner (FIG. 14). This suggests that αAβ-Gas6 significantly enhances the beta-amyloid clearing ability of astrocytes, which was previously insignificant, because astrocytes do not express Fc receptors but express TAM receptors.

[00158]

[00159] Each of αAβ-Gas6 and aducanumab was added to astrocytes and the microglia cell line BV2 together with beta-amyloid fibrils to increase beta-amyloid uptake, and then the mRNA levels of TNF, IL-1a and IL-1b in each cell line were measured

to determine the degree of inflammatory responses (FIGS. 15 and 16). As a result, similar to the results obtained in the cell lines, it was shown that, when the cells were treated with aducanumab, the levels of transcripts and proteins of the above inflammatory cytokines in the astrocytes and BV2 cells significantly increased compared to those in the control group, but when the cells were treated with αAβ-Gas6, the levels of these inflammatory cytokines in the cells did not increase compared to those in the control group.

[00160] As described above, it has been found that the use of the αAβ-Gas6 fusion phagocytosis inducer may be a groundbreaking method of effectively clearing beta-amyloid plaques accumulated in the patient's brain, through astrocytes and microglia without causing an inflammatory response, which is a serious side effect of existing monoclonal antibody therapeutics. This could be a very encouraging result that can significantly improve current treatment strategies.

[00161]

[00162] 1-4. Evaluation of *in vivo* efficacy

[00163] (1) Efficacy according to introduction of fusion molecule or expression vector containing the same

[00164] 5XFAD mice were used as Alzheimer's disease model mice. Since 5XFAD mice simultaneously express 5 genes with mutations, the onset at which beta-amyloid plaques are

generated in the mice is early, and pathological symptoms caused by beta-amyloid plaques can be studied from 3 to 4 months of age regardless of aging.

[00165] To verify the effect of αAβ-Gas6 *in vivo* through the 5XFAD model, αAβ-Gas6 was delivered to the brain in two different ways. Through previous studies, it is known that aducanumab is not delivered well to the brain by intravascular injection or intraperitoneal injection even in Alzheimer's disease model mice. Thus, to accurately compare and analyze the effects of αAβ-Gas6 with aducanumab, 1) direct cannulation was performed in the mouse brain, and each of purified αAβ-Gas6 and aducanumab was injected once a day into the ventricle of the brain for 3 weeks, and 2) each of αAβ-Gas6 and aducanumab was made in lentiviral form to be expressed in the hippocampus of the mouse through stereotaxic injection. Importantly, it was found that the number of beta-amyloid plaques significantly decreased both when the purified αAβ-Gas6 protein was added and when the gene was expressed in lentiviral form (FIGS. 17 and 18).

[00166] In addition, by quantifying the levels of beta-amyloid in lysosomes of microglia and astrocytes after αAβ-Gas6 was delivered to the brain in the form of protein or virus, it was shown that the ability to clear beta-amyloid significantly increased in both types of cells (FIGS. 19 to 22).

[00167] This suggests that, since TAM receptors are expressed in both microglia and astrocytes, microglia and astrocytes can recognize and clear beta-amyloid when αAβ-Gas6 is introduced therein, which is similar to the results of the *in vitro* studies.

[00168] (2) Comparison of effects of antibody therapeutics and fusion molecule of the present invention

[00169] It is known that, in Alzheimer's disease, synapses are indiscriminately removed by microglia, resulting in a decrease in the number of synapses. Surprisingly, this phenomenon was aggravated when aducanumab was delivered to Alzheimer's model mice, but when αAβ-Gas6 was expressed in a viral form, abnormal removal of synapses by microglia was restored to a normal level (FIGS. 23 and 24).

[00170] In addition, as in the results from a cognitive and memory test for remembering the shape or location of a new object in Alzheimer's model mice according to the protocol shown in FIG. 25, it was confirmed that the expression of αAβ-Gas6 exhibited significantly superior cognitive and memory recovery effects compared to aducanumab (FIG. 26).

[00171]

[00172] In addition, to verify whether the chimeric phagocytic protein of the present invention is effective in clearing various target substances, phagocytosis-inducing proteins specific for tau and alpha-synuclein (αSyn) in addition to

beta-amyloid were prepared as described in Preparation Examples 2 and 3, and the target substance clearing effects thereof were tested following protocols in Experimental Examples 2 and 3.

[00173]

[00174] Experimental Example 2. Gas6-based fusion molecule targeting tau

[00175] An *in vitro* tau engulfment assay was developed, in which tau oligomers are conjugated with a pH indicator and hence can emit red fluorescence in intracellular lysosomes when they are uptaken by phagocytosis. HMC3 cells, a human microglial cell line expressing TAM receptors, were treated with a culture medium expressing the phagocytosis-inducing protein [αTau-Gas6] according to Preparation Example 2, and *in vitro* tau engulfment assay was performed. As the result shown in FIG. 27, it was confirmed that tau oligomers were selectively cleared by αTau-Gas6.

[00176]

[00177] Experimental Example 3. Gas6-based fusion molecule targeting alpha-synuclein

[00178] An *in vitro* αSyn engulfment assay was developed, in which alpha-synuclein (αSyn) oligomers are conjugated with a pH indicator and hence can emit red fluorescence in intracellular lysosomes when they are uptaken by phagocytosis. HMC3 cells, a human microglial cell line expressing TAM

receptors, were treated with a culture medium expressing the phagocytosis-inducing protein [ααSyn-Gas6] according to Preparation Example 3, and *in vitro* tau engulfment assay was performed. As the result shown in FIG. 27, it was confirmed that αSyn oligomers were selectively cleared by ααSyn-Gas6.

[00179]

[00180] Experimental Example 4. ProS1-based fusion molecule targeting beta-amyloid

[00181] Next, to verify whether the chimeric phagocytosis-inducing protein prepared using a ligand for TAM receptor other than Gas6 is also effective, αAβ-ProS1 was prepared as described in Preparation Example 4 using the ProS1 ligand, and the efficacy thereof was evaluated. To this end, primary-cultured mouse astrocytes expressing TAM receptors were treated with a culture medium expressing αAβ-ProS1, and the *in vitro* Aβ engulfment assay used in Experimental Example 1-3 was performed. As the result shown in FIG. 29, it was confirmed that beta-amyloid oligomers were selectively cleared by αAβ-ProS1.

[00182]

[00183] Experimental Example 5. Gas6-based fusion molecule targeting beta-amyloid (II): beta-amyloid binding regions in the forms of Fab and Mab

[00184] Next, to verify whether various target-binding regions other than scFv may be used as target protein-binding domains

in the preparation of chimeric phagocytosis-inducing proteins, phagocytosis-inducing proteins were prepared according to Preparation Example 5 using an antigen-binding fragment (Fab) or a complete-form monoclonal antibody (Mab) instead of an scFv and were subjected to an experiment (αAβ[Fab]-Gas6 and αAβ[Mab]-Gas6). To this end, HMC3 cells, a human microglial cell line expressing TAM receptors, were treated with a culture medium expressing each of αAβ[Fab]-Gas6 and αAβ[Mab]-Gas6, and the *in vitro* Aβ engulfment assay used in Experimental Example 1-3 was performed. As the results shown in FIGS. 30 and 31, it was confirmed that beta-amyloid oligomers were selectively cleared by each of αAβ[Fab]-Gas6 and αAβ[Mab]-Gas6.

[00185]

[00186] The scope of the present invention is defined by the appended claims, and all changes or modifications derived from the meaning and scope of the claims and equivalents thereto should be construed as being included in the scope of the present invention.

[00187]

Mode for Invention

[00188] Mode for Invention has also been described in Best Mode above.

[00189]

Industrial Applicability

[00190] The fusion molecules having phagocytosis-inducing activity according to the embodiment of the present invention can solve the problem of tissue damage caused by activation of an inflammatory response, which occurs in the prior art. Accordingly, the fusion molecules could effectively clear abnormally accumulated substances such as beta-amyloid, tau, alpha- synuclein, huntingtin or prion protein, and thus may be used to prevent or treat diseases caused by these abnormally accumulated substances, for example, Alzheimer's disease, Parkinson's disease, Huntington's disease, or prion disease. Therefore, it may be used in the therapeutics industry for treatment of the above diseases.

CLAIMS

1. A fusion molecule having phagocytosis-inducing activity, wherein the fusion molecule comprises: a first region that is capable of binding to a TAM receptor; and a second region that specifically binds to a target substance.

2. The fusion molecule according to claim 1, wherein the TAM receptor is at least one selected from the group consisting of Tyro3, Axl, and MerTK.

3. The fusion molecule according to claim 1, wherein the first region comprises Gas6, ProS1, Tubby, Tulp1, or Gal3, or active fragments thereof.

4. The fusion molecule according to claim 1, wherein the first region comprises a laminin G-like domain of Gas6 or ProS1, or an active fragment thereof.

5. The fusion molecule according to claim 1, wherein the first region is a laminin G-like domain comprising the sequences of SEQ ID NOs: 1 and 2, or a laminin G-like domain comprising the sequences of SEQ ID NOs: 3 and 4.

6. The fusion molecule according to claim 1, wherein the target substance is a substance that accumulates in a

living tissue, accumulation of said substance causing a disease.

7. The fusion molecule according to claim 6, wherein the target substance is amyloid.

8. The fusion molecule according to claim 6, wherein the disease is amyloidosis.

9. The fusion molecule according to claim 1, wherein the target substance is selected from among β-amyloid, tau, α-synuclein, huntingtin protein, prion protein, and abnormally accumulated substances listed in Table 1.

10. The fusion molecule according to claim 1, wherein the second region that specifically binds to the target substance is selected from among an antibody, an active fragment thereof, an antibody-like protein, a peptide, an aptamer, and a soluble receptor, which specifically bind to the target substance.

11. The fusion molecule according to claim 1, wherein the phagocytosis is induced in a cell expressing the TAM receptor.

12. The fusion molecule according to claim 11, wherein the cell expressing the TAM receptor is at least one type of professional phagocyte, at least one type of non-professional phagocyte, or a combination thereof.

13. The fusion molecule according to claim 1, wherein induction of the phagocytosis does not involve an inflammatory response.

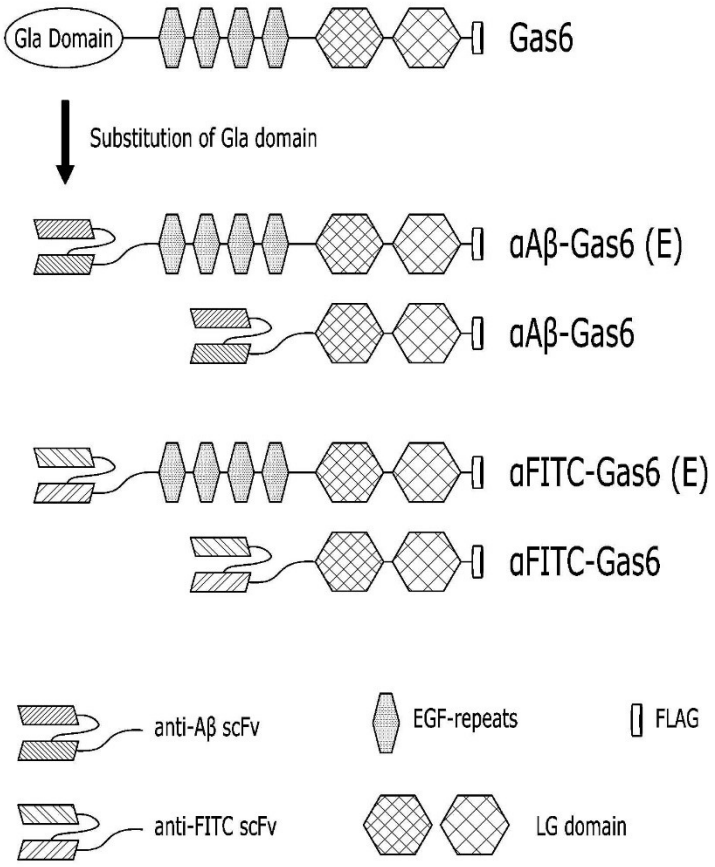
14. A nucleic acid molecule encoding the fusion molecule according to claim 1.

15. An expression vector comprising the nucleic acid molecule according to claim 1.

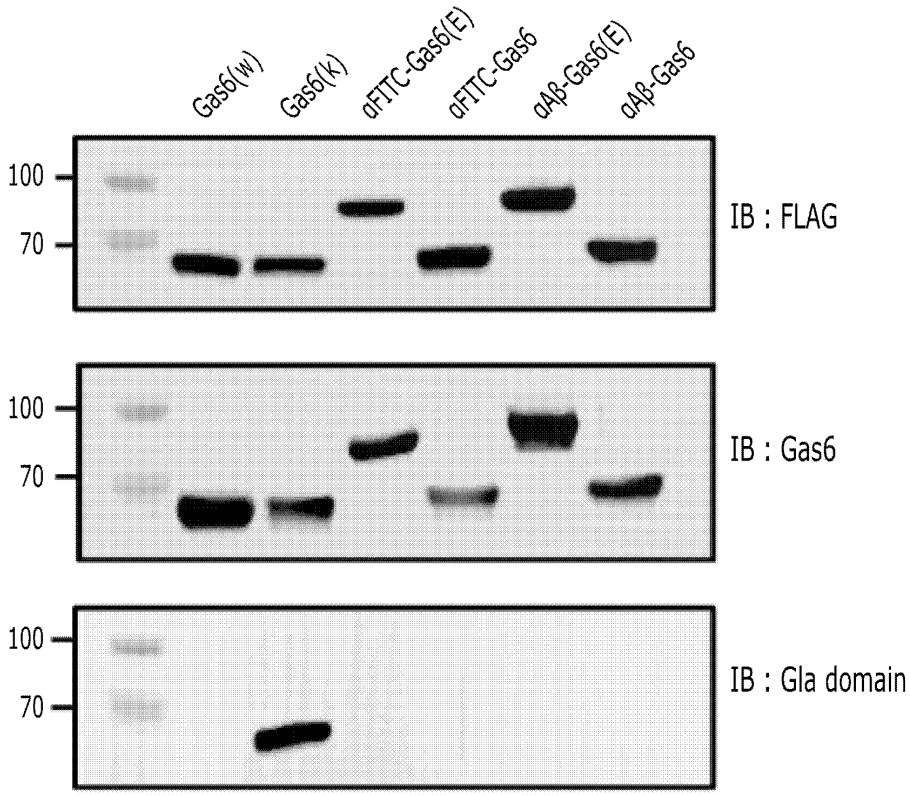
16. A cell expressing the fusion molecule according to claim 1.

17. A pharmaceutical composition for preventing or treating a disease caused by accumulation of a target substance in living tissue, wherein the pharmaceutical composition containing the fusion molecule according to claim 1 or the expression vector according to claim 15.

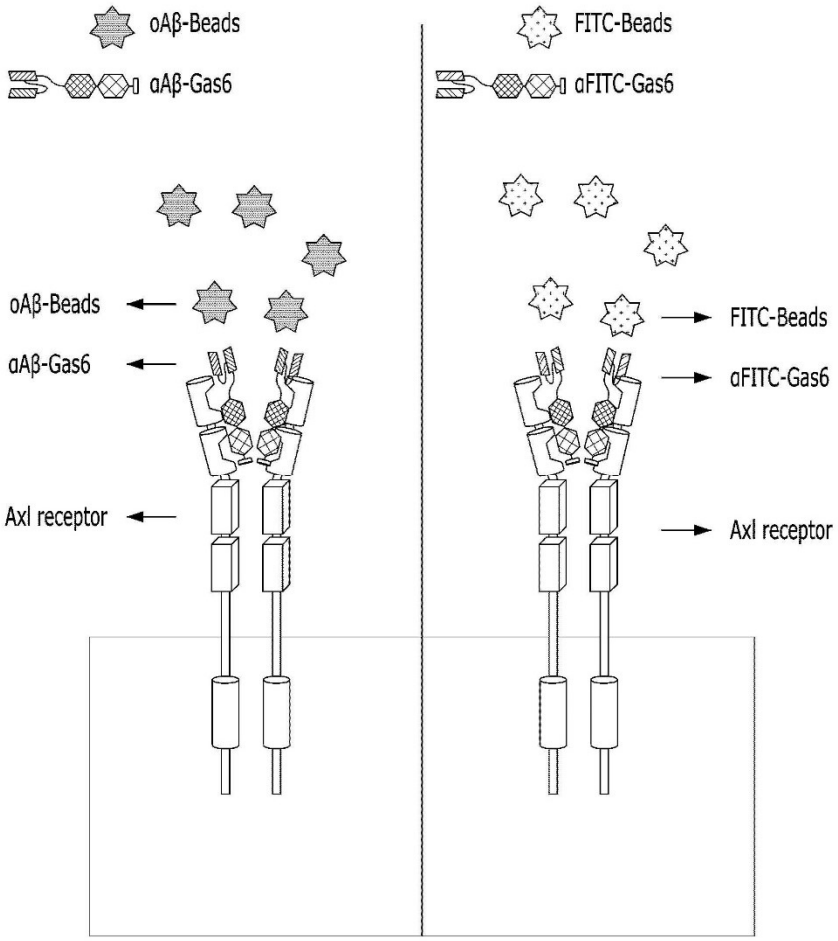
【FIG. 1】


Gla Domain
Gas6
Substitution of Gla domain
αAβ-Gas6 (E)
αAβ-Gas6
αFITC-Gas6 (E)
αFITC-Gas6
anti-Aβ scFv
anti-FITC scFv
EGF-repeats
LG domain
FLAG

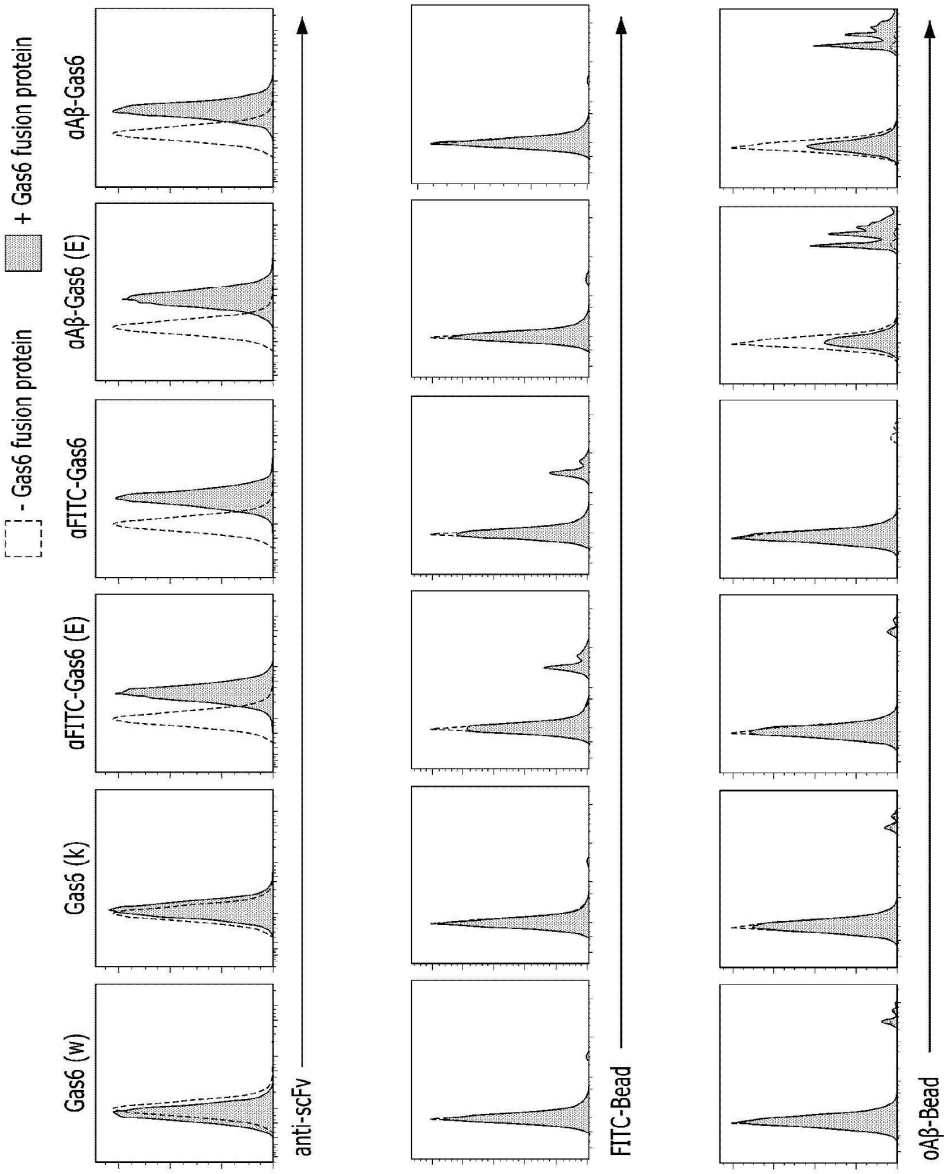
【FIG. 2】


Gas6(w)
Gas6(k)
αFITC-Gas6(E)
αFITC-Gas6
αAβ-Gas6(E)
αAβ-Gas6
100
70
IB : FLAG
100
70
IB : Gas6
100
70
IB : Gla domain

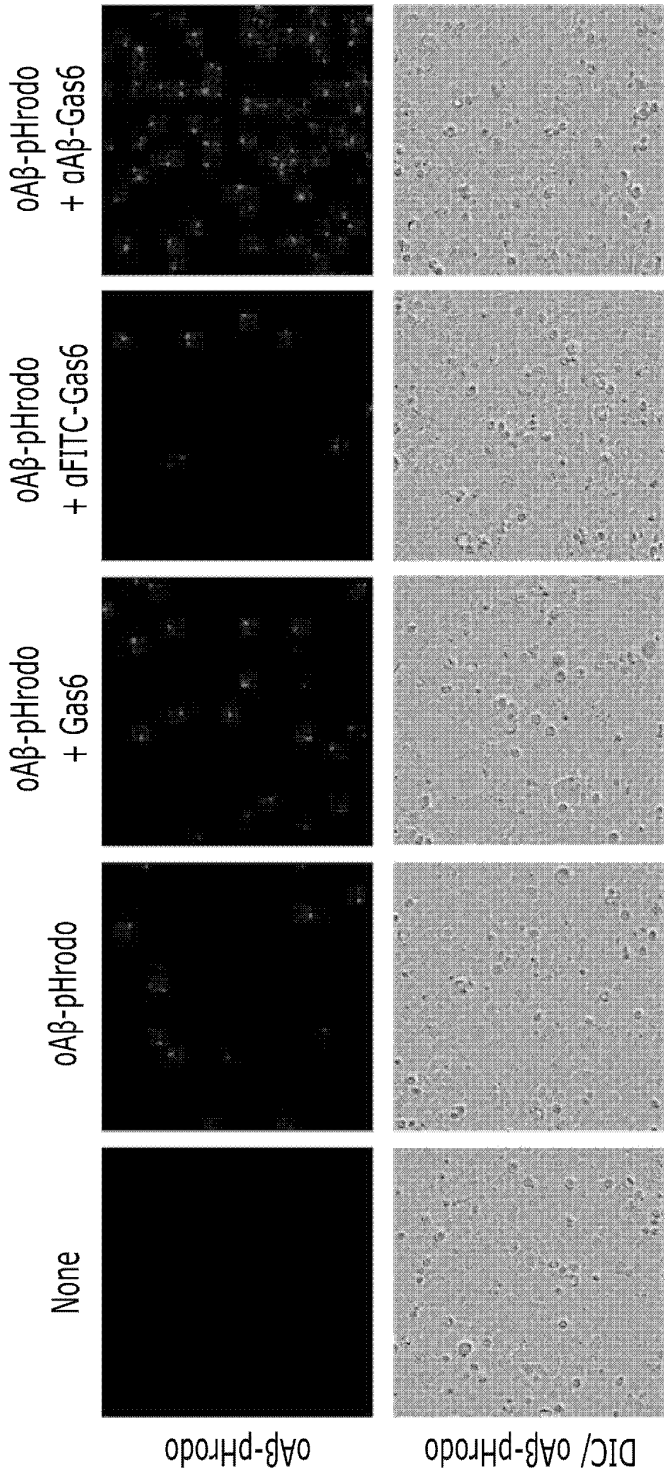
【FIG. 3】


αAβ-Beads
αAβ-Gas6
FITC-Beads
αFITC-Gas6
αAβ-Beads
αAβ-Gas6
Axl receptor
FITC-Beads
αFITC-Gas6
Axl receptor

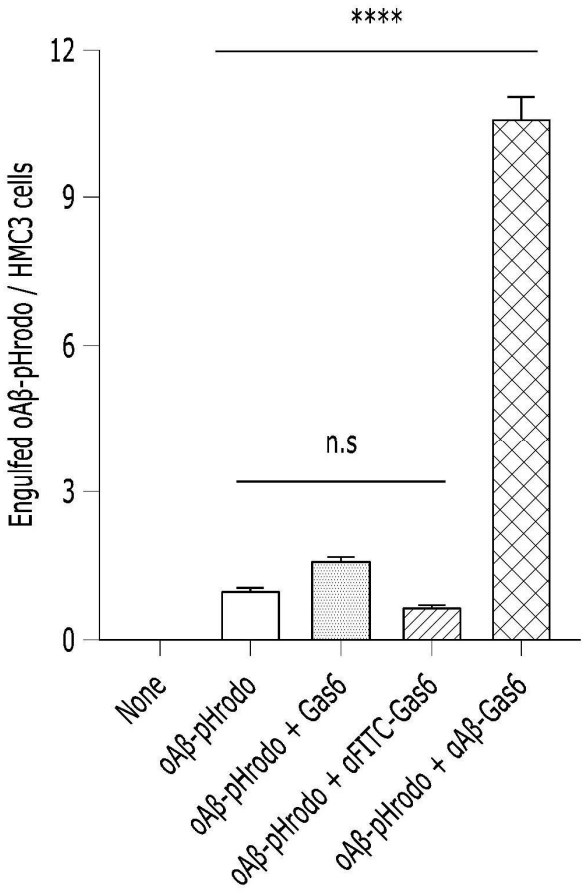
【FIG. 4】


- Gas6 fusion protein
+ Gas6 fusion protein
Gas6 (w)
Gas6 (k)
αFITC-Gas6 (E)
αFITC-Gas6
αAβ-Gas6 (E)
αAβ-Gas6
anti-scFv
FITC-Bead
αAβ-Bead

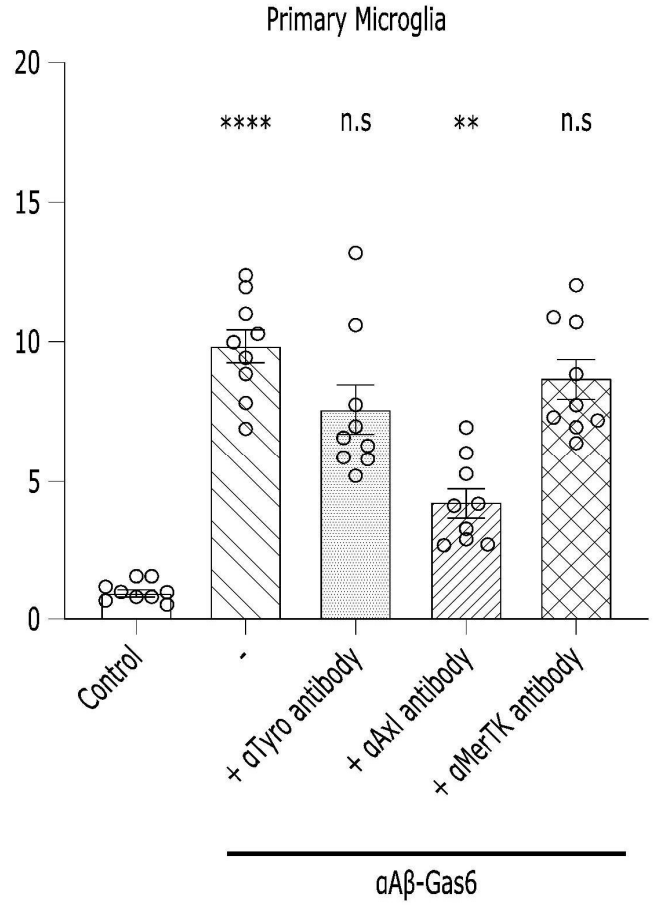
【FIG. 5】


oAβ-pHrodo + αAβ-Gas6
oAβ-pHrodo + αFITC-Gas6
oAβ-pHrodo + Gas6
oAβ-pHrodo
None
oAβ-pHrodo
DIC/ oAβ-pHrodo

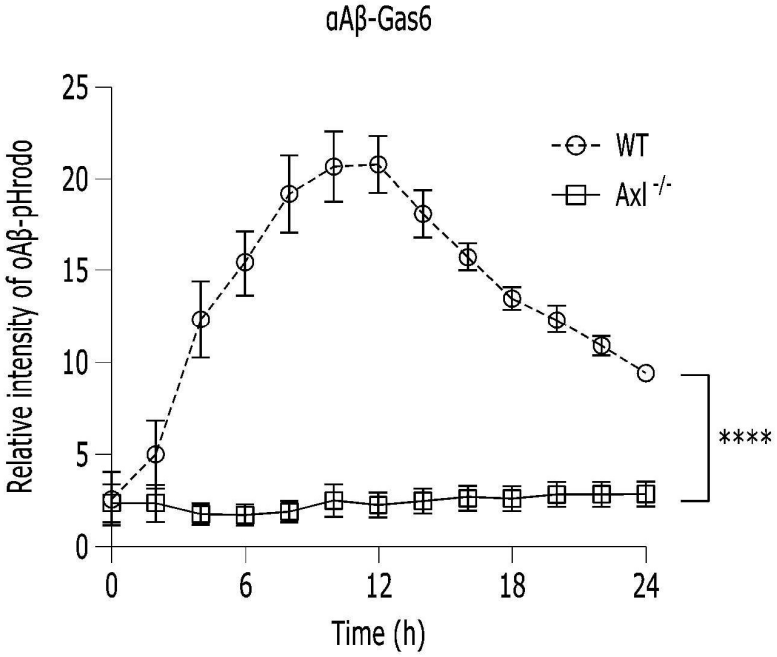
【FIG. 6】


****
n.s
12
9
6
3
0
Engulfed oAβ-pHrodo / HMC3 cells
None
oAβ-pHrodo
oAβ-pHrodo + Gas6
oAβ-pHrodo + αFITC-Gas6
oAβ-pHrodo + αAβ-Gas6

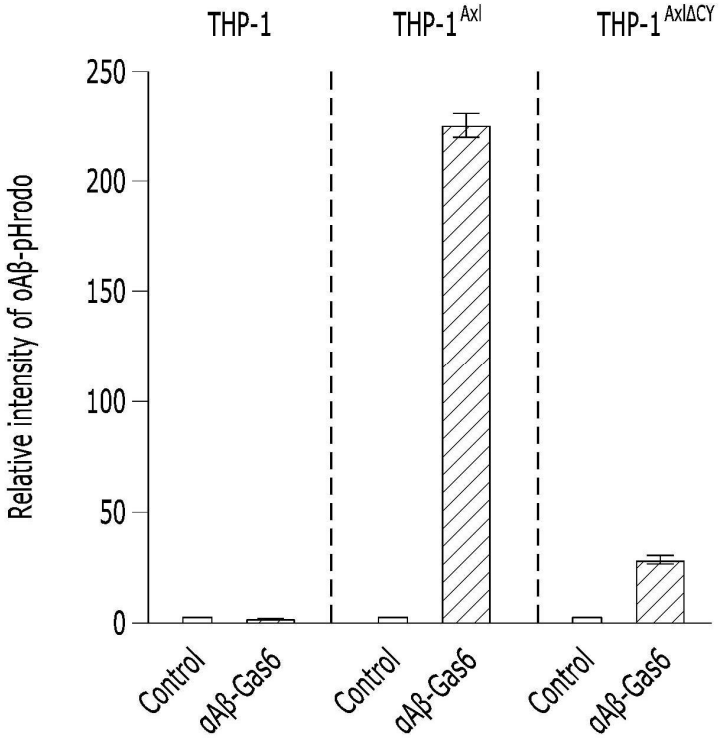
【FIG. 7】


Primary Microglia
20
15
10
5
0
****
n.s
**
n.s
Control
-
+ αTyro antibody
+ αAxl antibody
+ αMerTK antibody
αAβ-Gas6

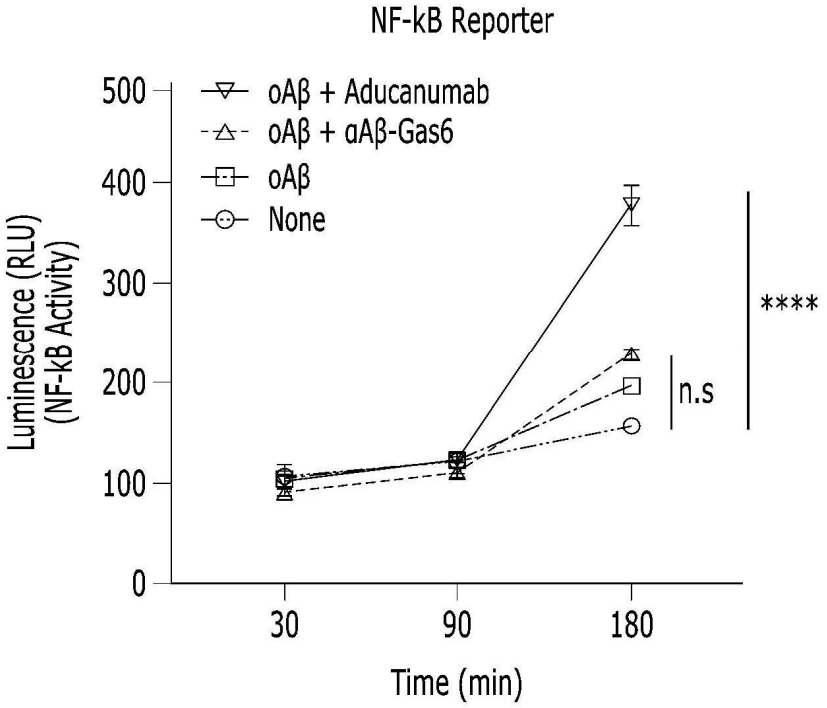
【FIG. 8】


αAβ-Gas6
Relative intensity of oAβ-pHrodo
25
20
15
10
5
0
0
6
12
18
24
Time (h)
WT
Axl -/-
****

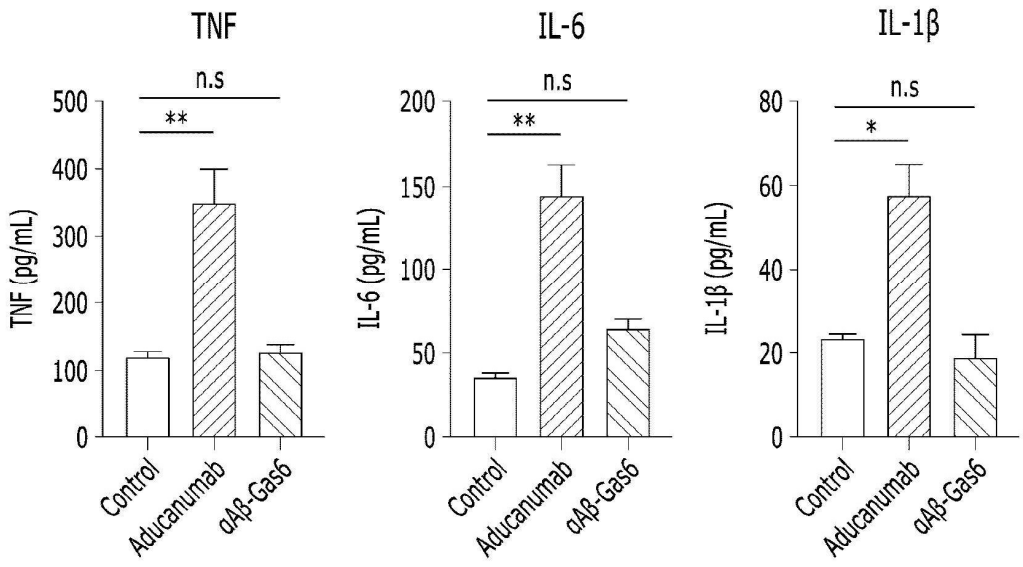
【FIG. 9】


THP-1
THP-1 Axl
THP-1 AxlΔCY
Relative intensity of oAβ-pHrodo
250
200
150
100
50
0
Control
oAβ-Gas6
Control
oAβ-Gas6
Control
oAβ-Gas6

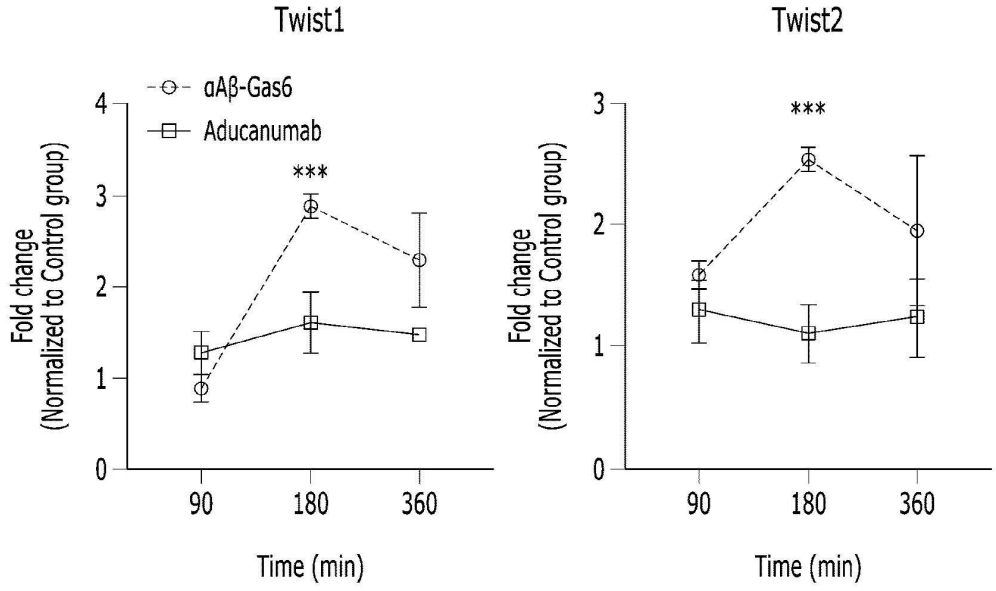
【FIG. 10】


NF-kB Reporter
oAβ + Aducanumab
oAβ + αAβ-Gas6
oAβ
None
Luminescence (RLU)
(NF-kB Activity)
500
400
300
200
100
0
30
90
180
Time (min)
n.s
****

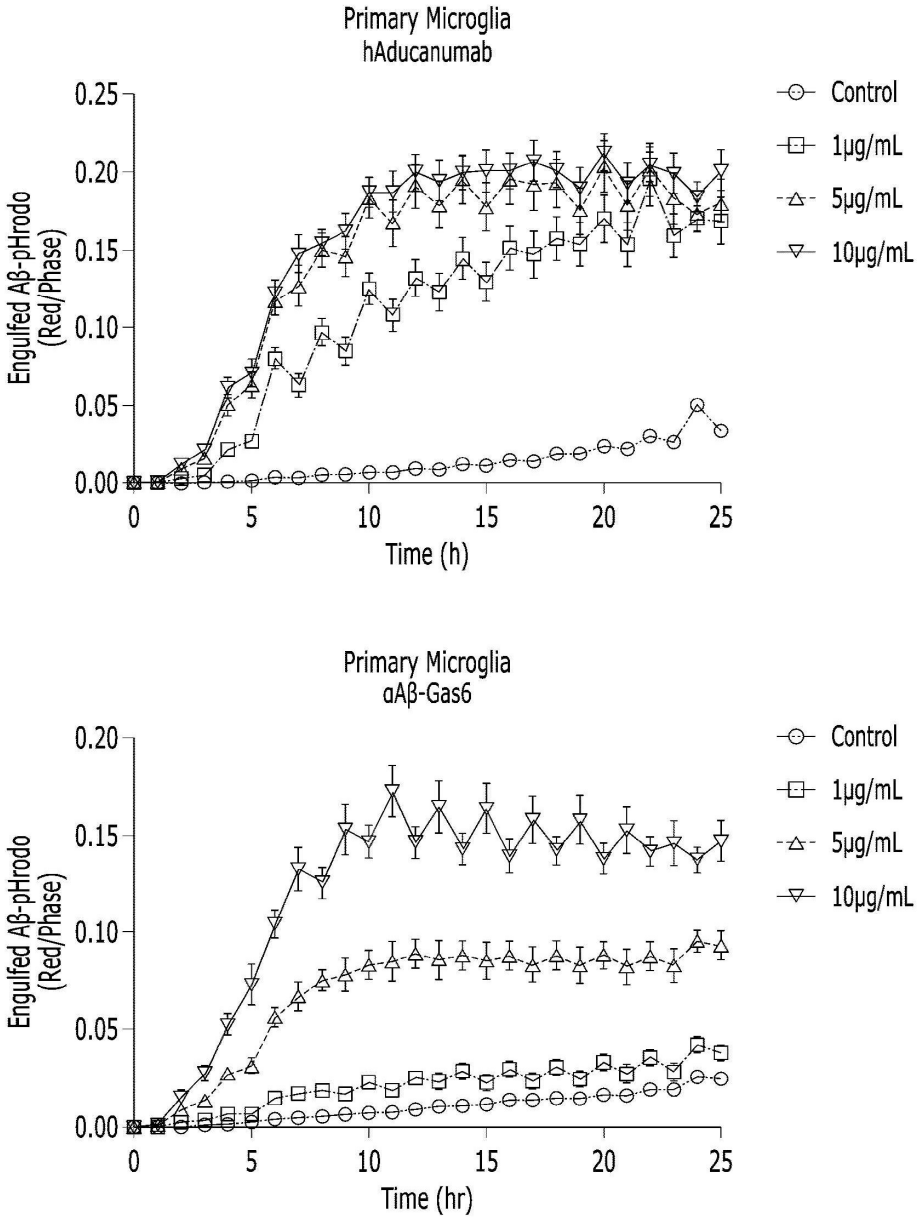
【FIG. 11】


TNF
n.s
**
500
400
300
200
100
0
TNF (pg/mL)
Control
Aducanumab
αAβ-Gas6
IL-6
n.s
**
200
150
100
50
0
IL-6 (pg/mL)
Control
Aducanumab
αAβ-Gas6
IL-1β
n.s
*
80
60
40
20
0
IL-1β (pg/mL)
Control
Aducanumab
αAβ-Gas6

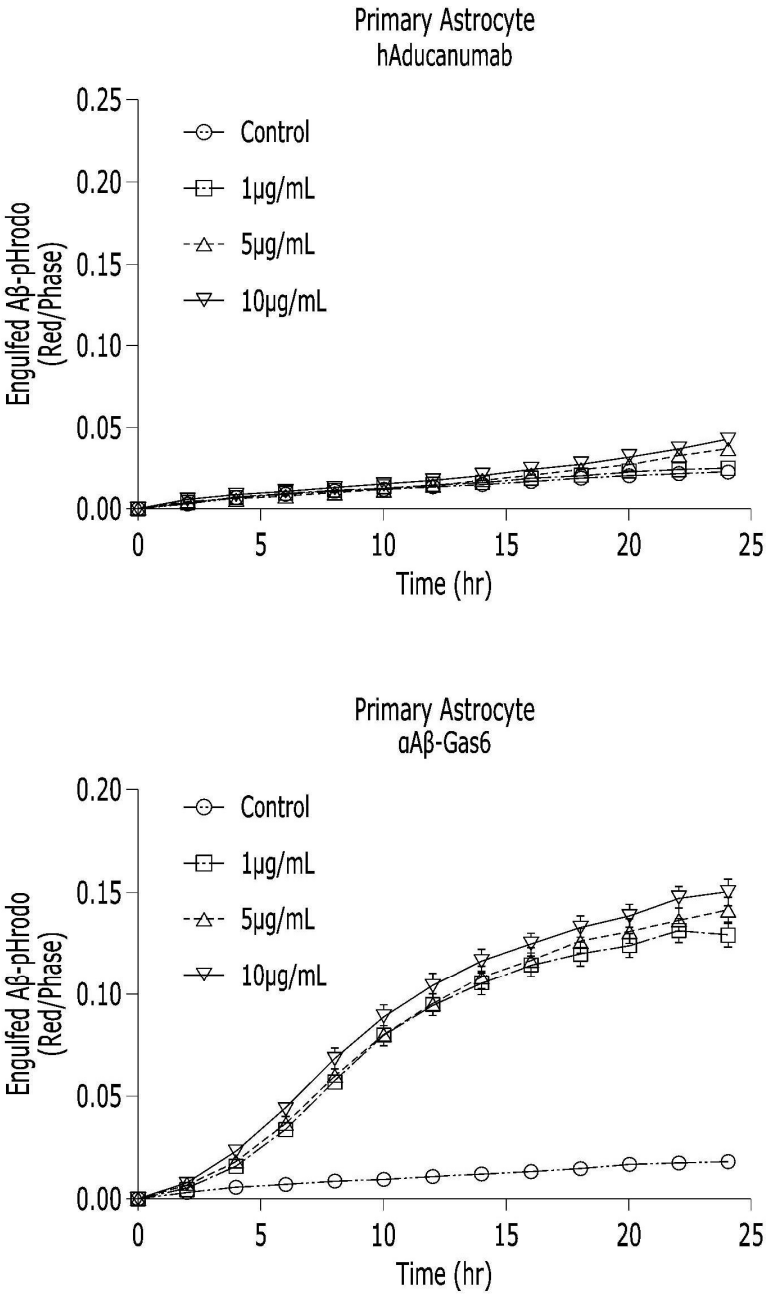
【FIG. 12】


Twist1
αAβ-Gas6
Aducanumab
***
Fold change
(Normalized to Control group)
0
1
2
3
4
90
180
360
Time (min)
Twist2
***
Fold change
(Normalized to Control group)
0
1
2
3
90
180
360
Time (min)

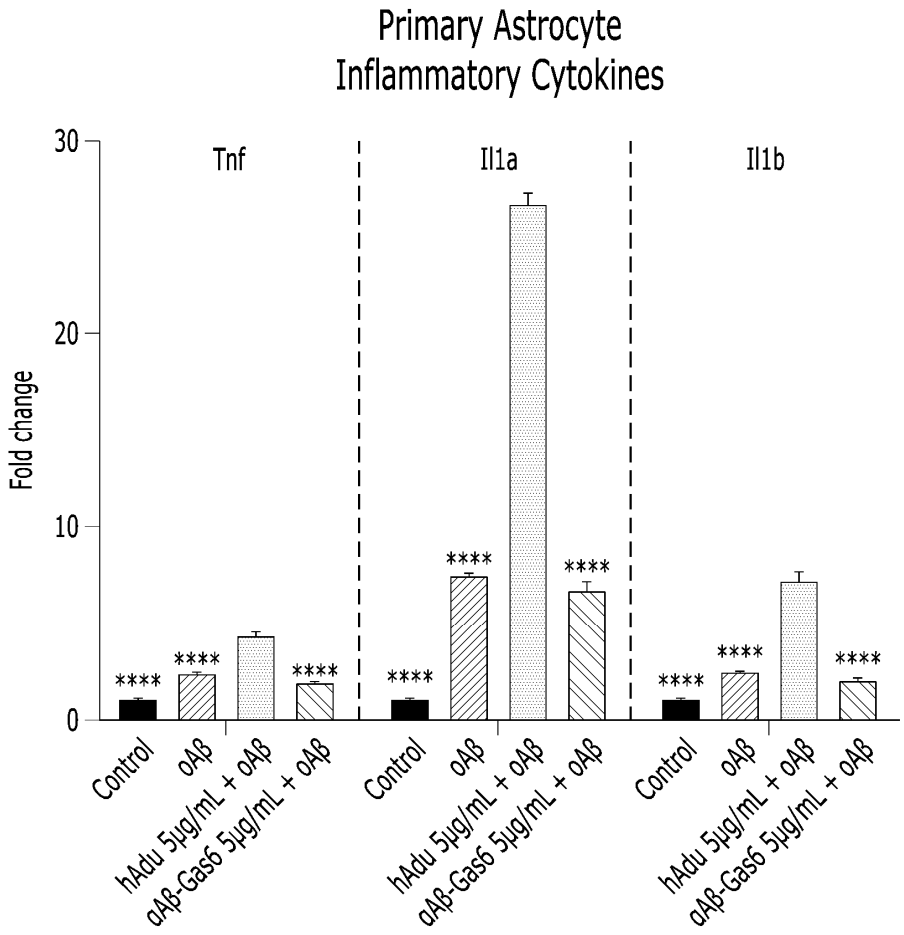
【FIG. 13】


Primary Microglia
hAducanumab
Engulfed Aβ-pHrodo (Red/Phase)
0.25
0.20
0.15
0.10
0.05
0.00
0
5
10
15
20
25
Time (h)
Control
1μg/mL
5μg/mL
10μg/mL
Primary Microglia
αAβ-Gas6
Engulfed Aβ-pHrodo (Red/Phase)
0.20
0.15
0.10
0.05
0.00
0
5
10
15
20
25
Time (hr)
Control
1μg/mL
5μg/mL
10μg/mL

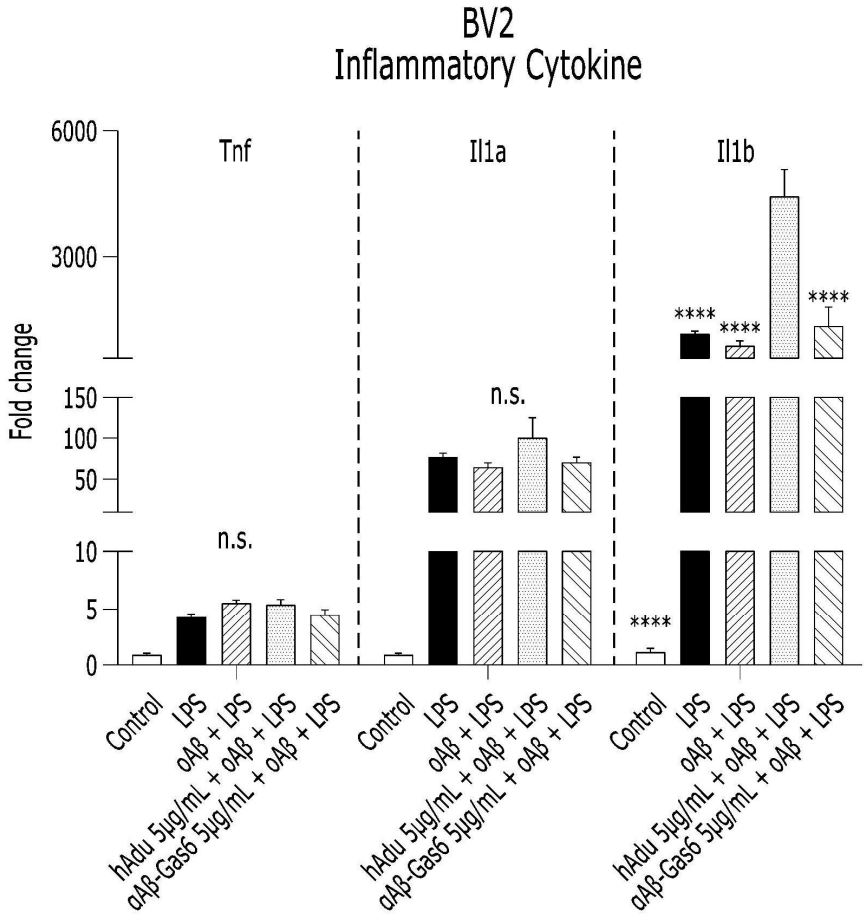
【FIG. 14】


Primary Astrocyte
hAducanumab
Engulfed Aβ-pHrodo (Red/Phase)
0.25
0.20
0.15
0.10
0.05
0.00
Control
1μg/mL
5μg/mL
10μg/mL
0
5
10
15
20
25
Time (hr)
Primary Astrocyte
αAβ-Gas6
Engulfed Aβ-pHrodo (Red/Phase)
0.20
0.15
0.10
0.05
0.00
Control
1μg/mL
5μg/mL
10μg/mL
0
5
10
15
20
25
Time (hr)

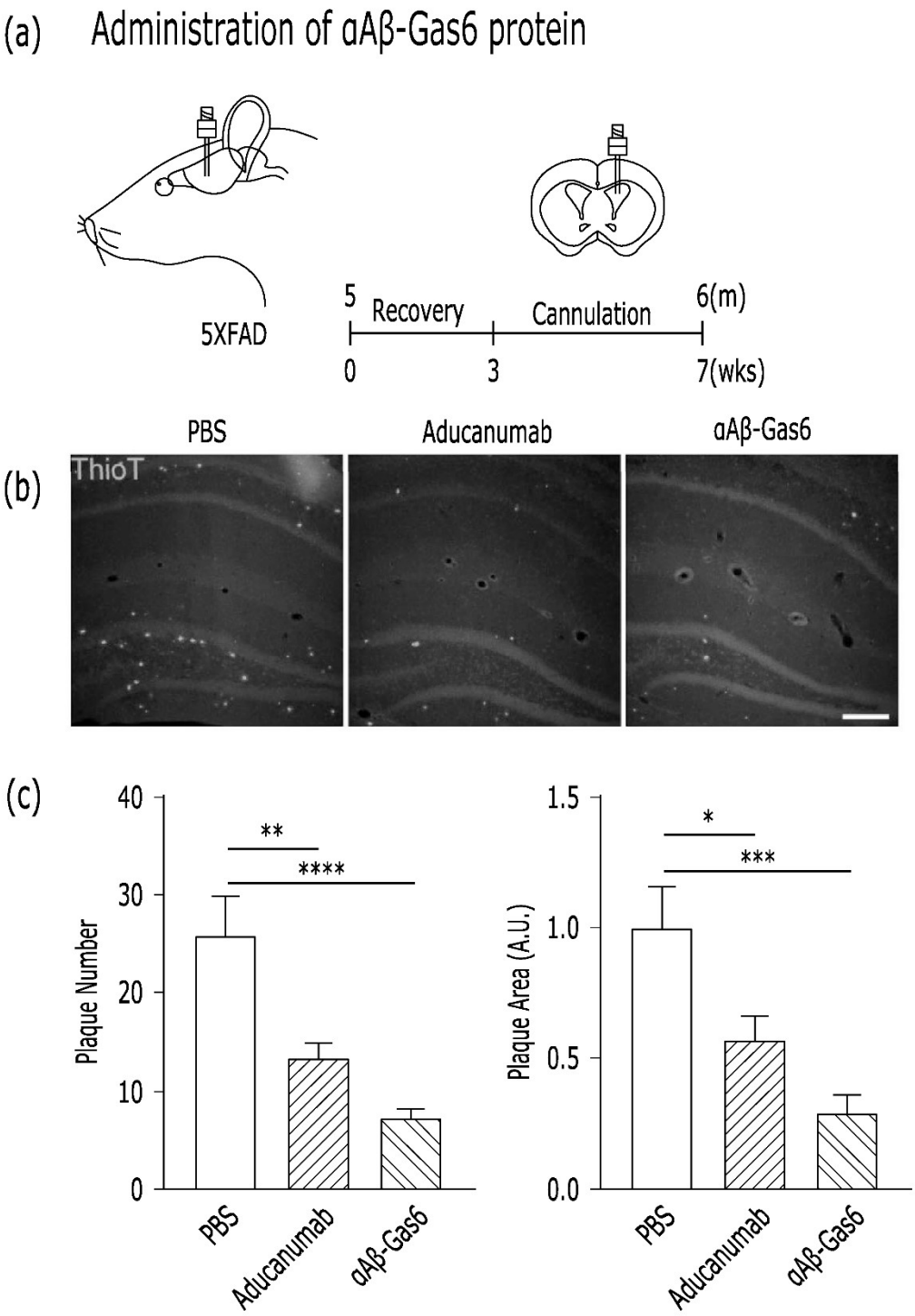
【FIG. 15】


Primary Astrocyte
Inflammatory Cytokines
Tnf
Il1a
Il1b
Fold change
30
20
10
0
****
Control
oAβ
hAdu 5μg/mL + oAβ
oAβ-Gas6 5μg/mL + oAβ

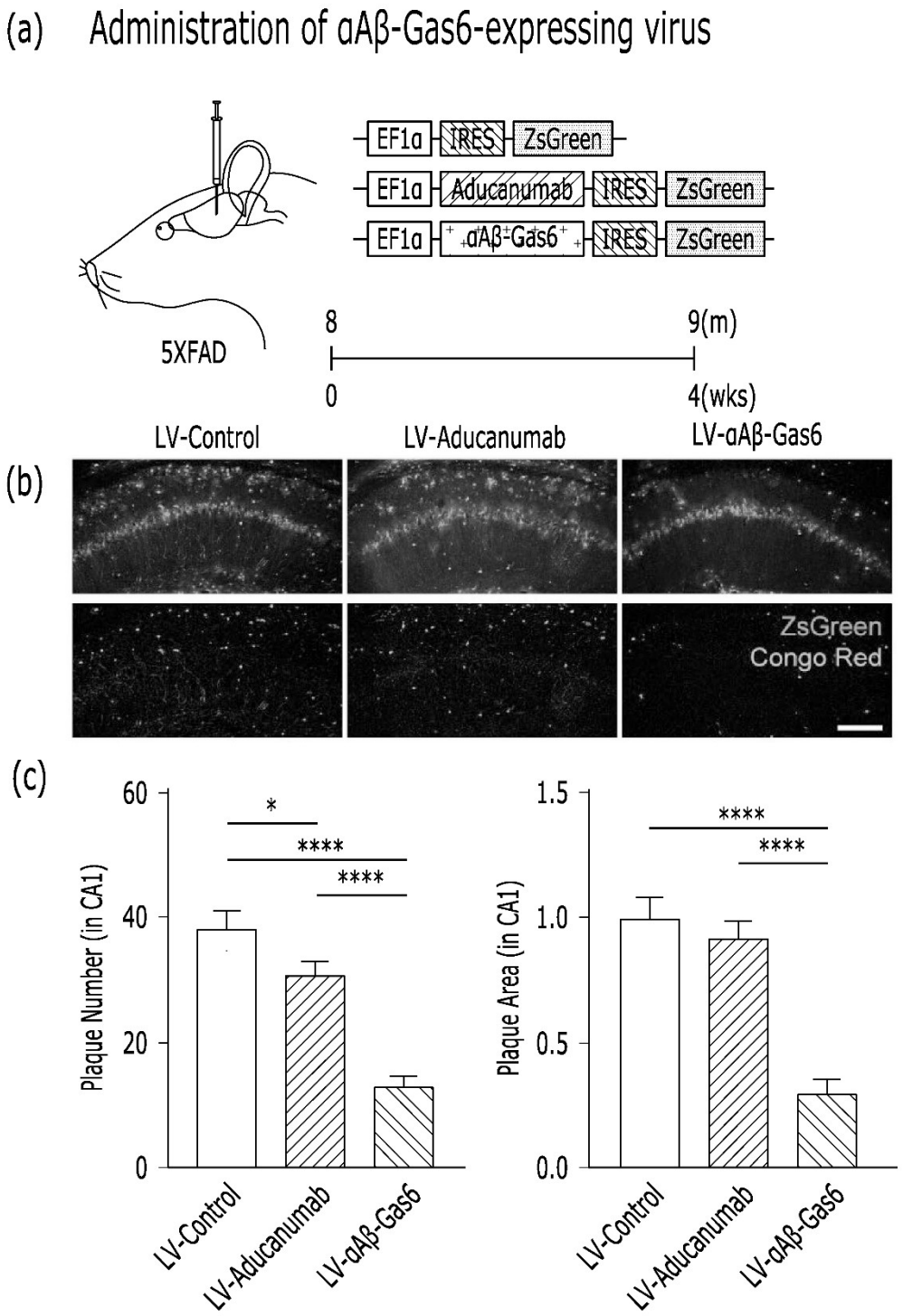
【FIG. 16】


BV2
Inflammatory Cytokine
Fold change
6000
3000
150
100
50
10
5
0
Tnf
Il1a
Il1b
n.s.
n.s.
****
****
****
****
Control
LPS
oAβ + LPS
hAdu 5μg/mL + oAβ + LPS
oAβ-Gas6 5μg/mL + oAβ + LPS

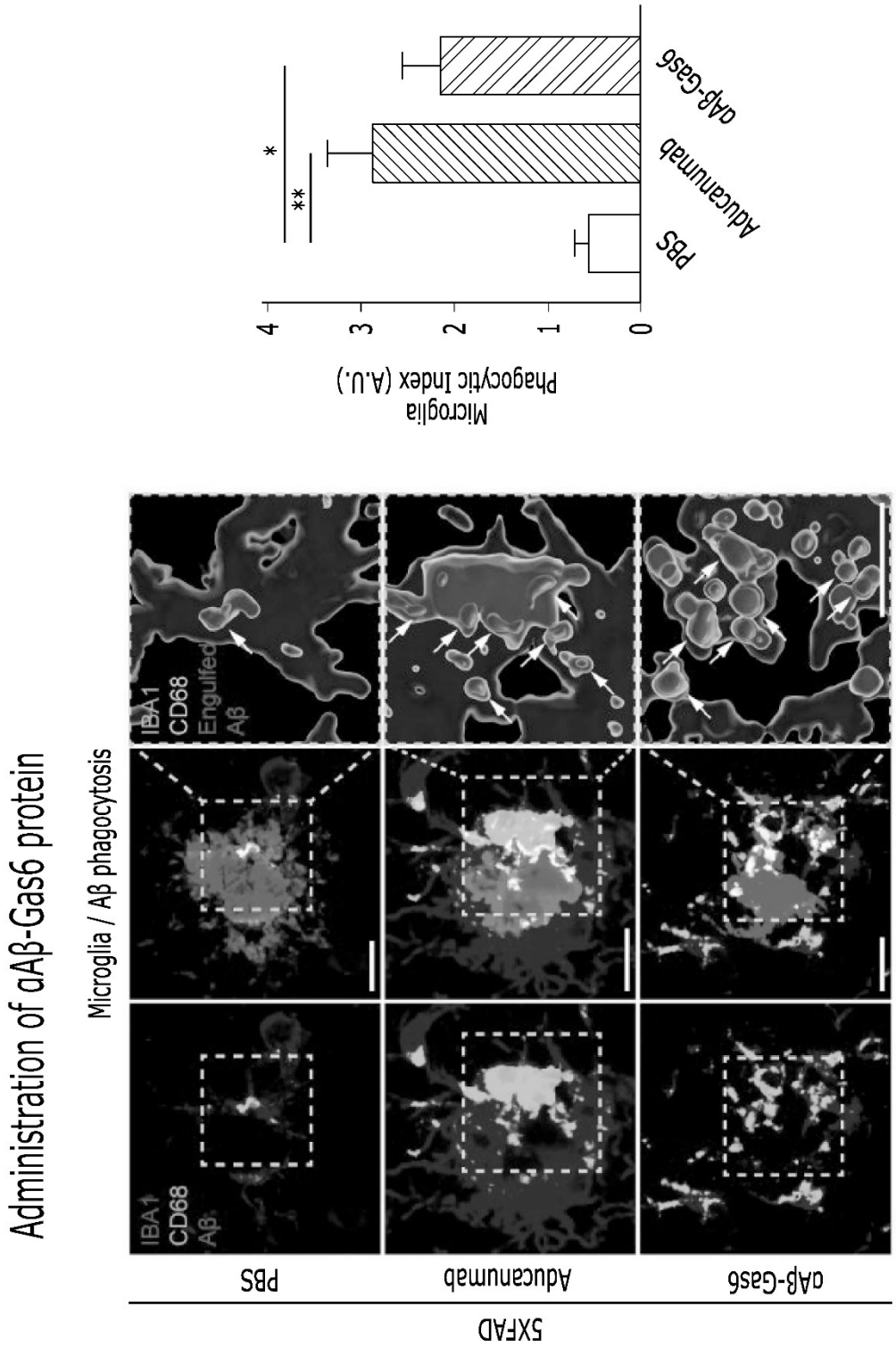
【FIG. 17】


(a) Administration of αAβ-Gas6 protein
5XFAD
5
Recovery
Cannulation
6(m)
0
3
7(wks)
(b)
PBS
Aducanumab
αAβ-Gas6
ThioT
(c)
Plaque Number
40
30
20
10
0
**
****
PBS
Aducanumab
αAβ-Gas6
Plaque Area (A.U.)
1.5
1.0
0.5
0.0
*
***
PBS
Aducanumab
αAβ-Gas6

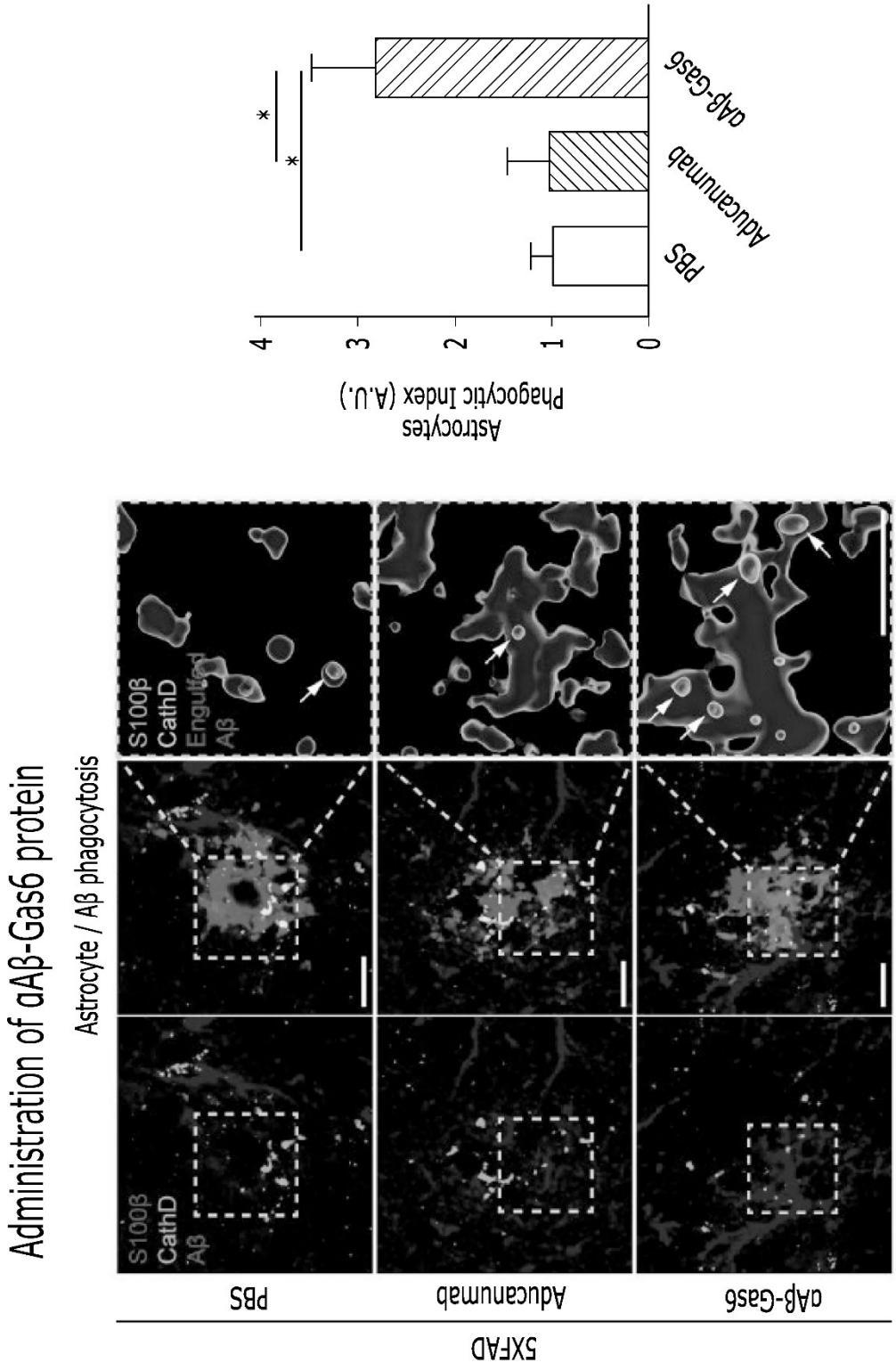
【FIG. 18】


(a) Administration of αAβ-Gas6-expressing virus
EF1α
IRES
ZsGreen
EF1α
Aducanumab
IRES
ZsGreen
EF1α
αAβ-Gas6
IRES
ZsGreen
5XFAD
8
9(m)
0
4(wks)
LV-Control
LV-Aducanumab
LV-αAβ-Gas6
(b)
ZsGreen
Congo Red
(c)
Plaque Number (in CA1)
60
40
20
0
*
****
****
LV-Control
LV-Aducanumab
LV-αAβ-Gas6
Plaque Area (in CA1)
1.5
1.0
0.5
0.0
****
****
LV-Control
LV-Aducanumab
LV-αAβ-Gas6

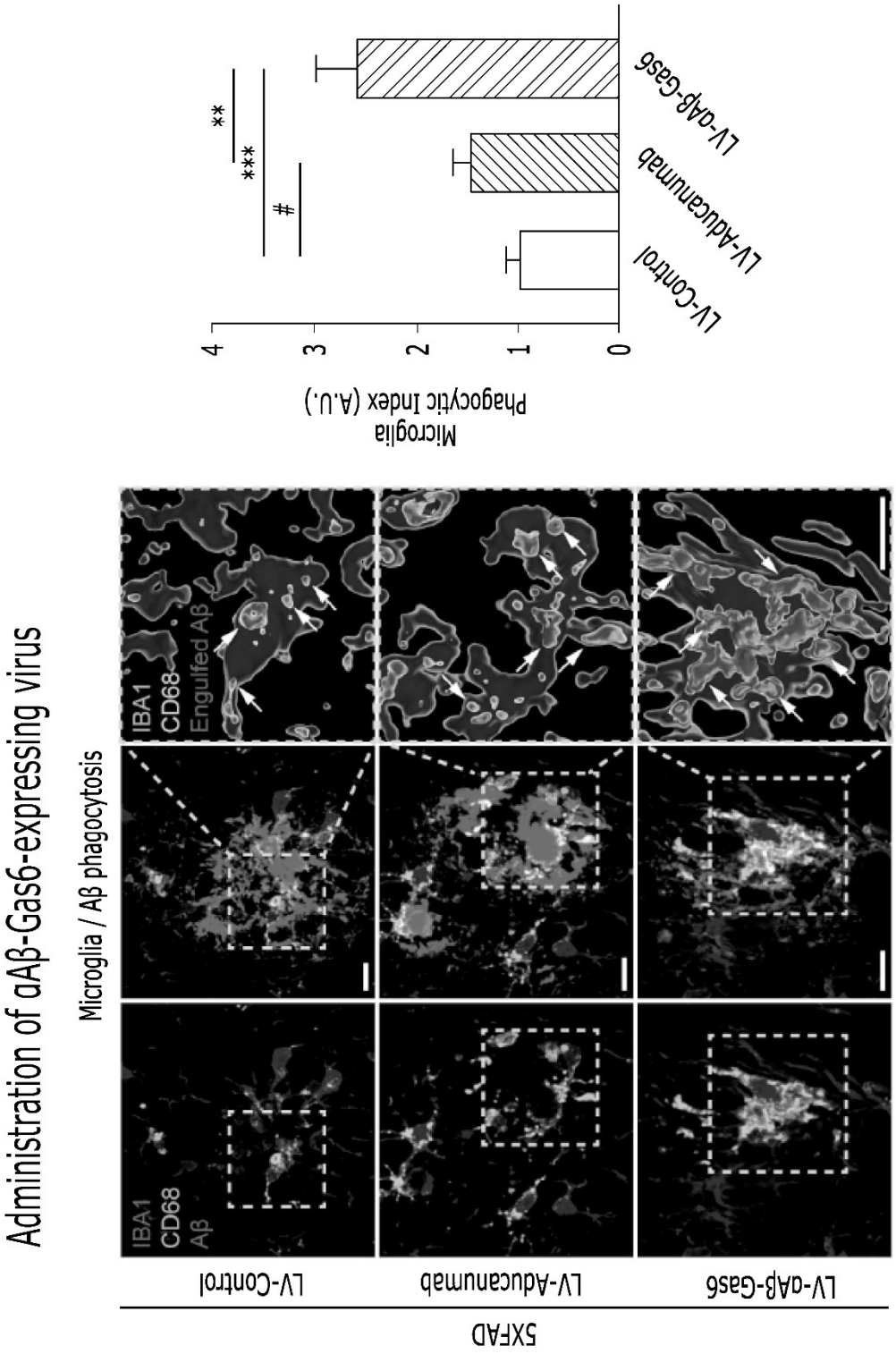
【FIG. 19】


Administration of αAβ-Gas6 protein
Microglia / Aβ phagocytosis
IBA1
CD68
Aβ
IBA1
CD68
Engulfed
Aβ
5XFAD
PBS
Aducanumab
αAβ-Gas6
Microglia
Phagocytic Index (A.U.)
0
1
2
3
4
*
**
PBS
Aducanumab
αAβ-Gas6

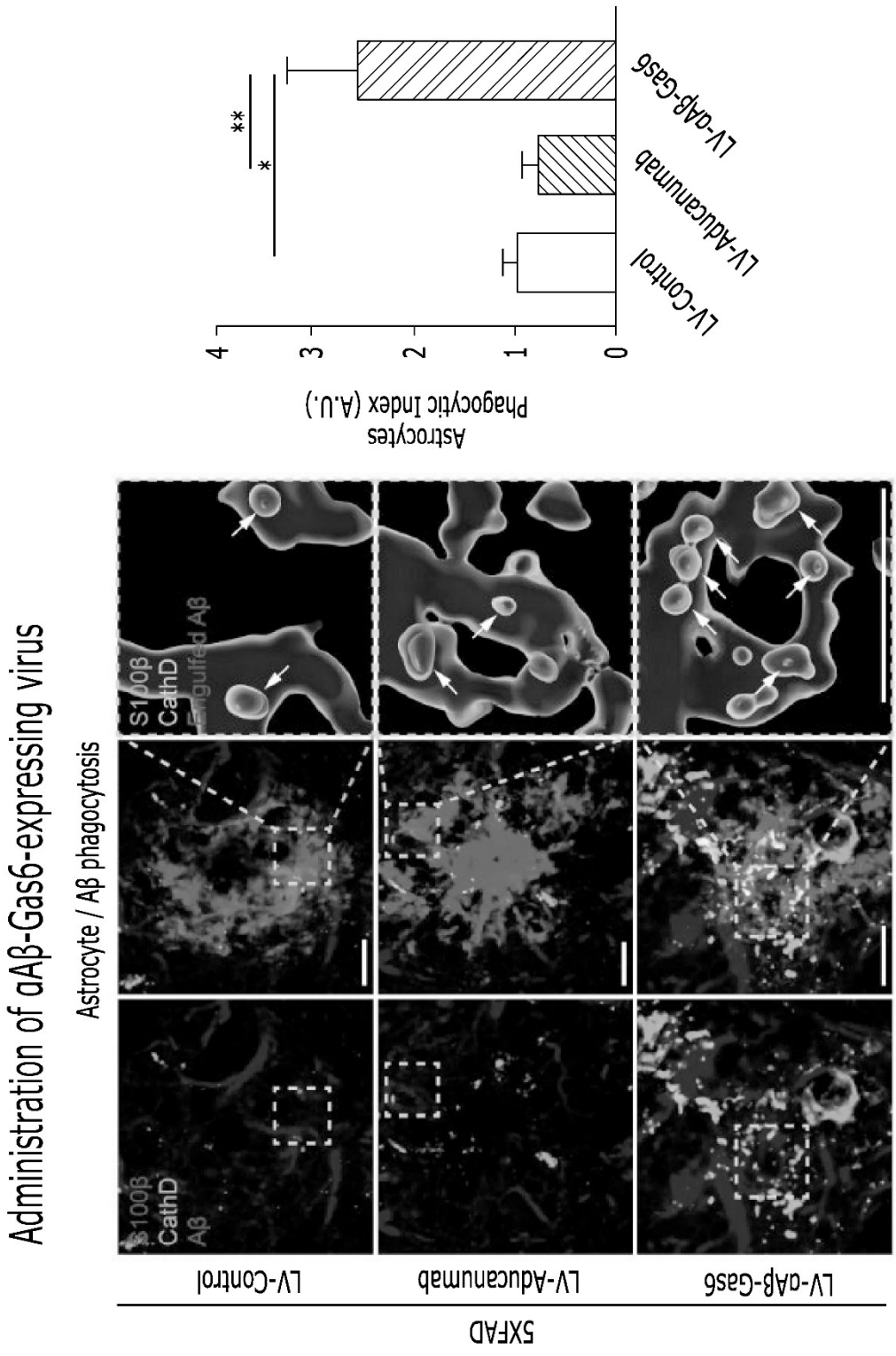
【FIG. 20】


Administration of αAβ-Gas6 protein
Astrocyte / Aβ phagocytosis
5XFAD
PBS
Aducanumab
αAβ-Gas6
S100β
CathD
Aβ
Engulfed Aβ
Astrocytes
Phagocytic Index (A.U.)
0
1
2
3
4
PBS
Aducanumab
αAβ-Gas6
*
*

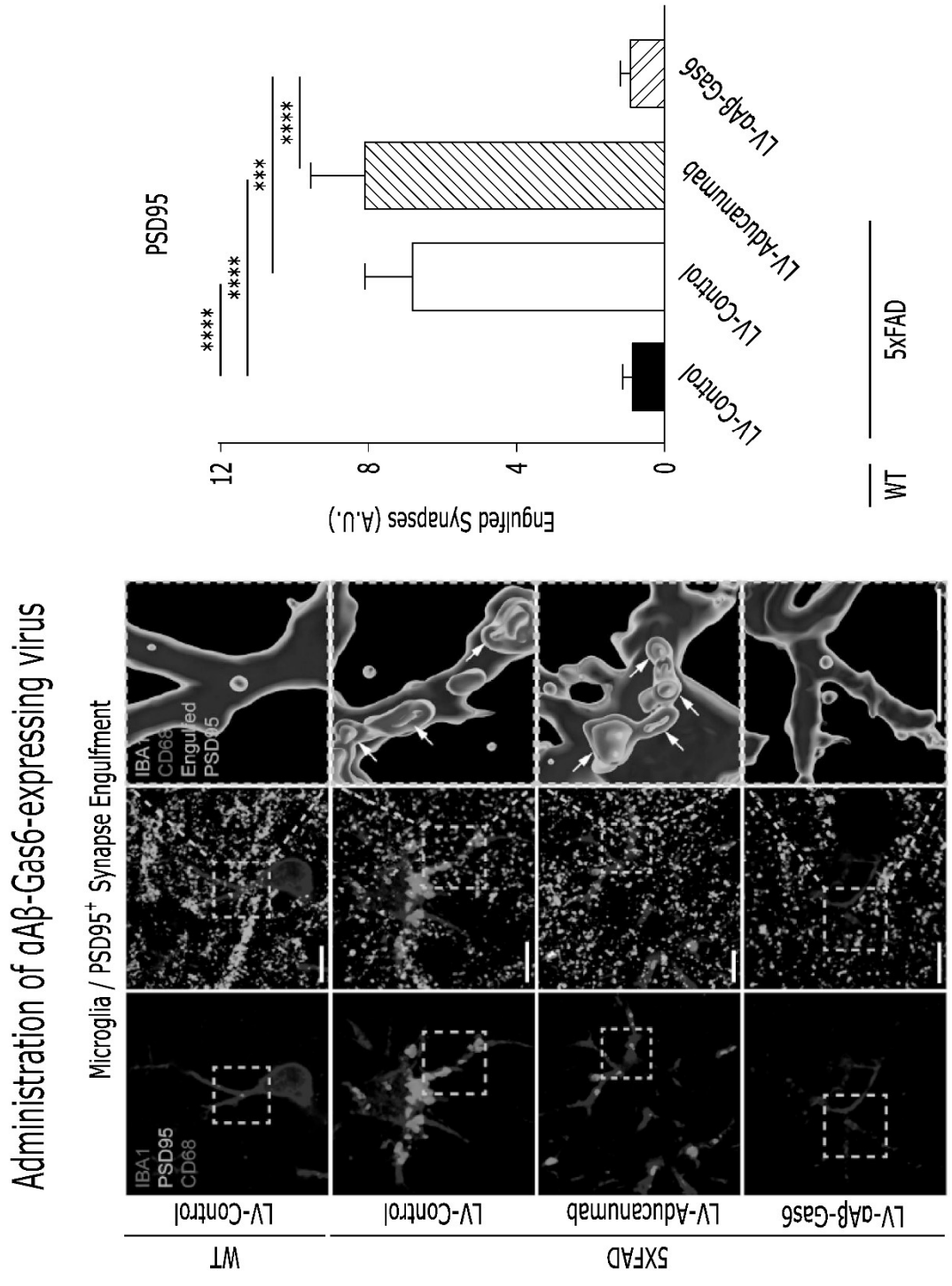
【FIG. 21】


Administration of αAβ-Gas6-expressing virus
Microglia / Aβ phagocytosis
IBA1
CD68
Aβ
IBA1
CD68
Engulfed Aβ
5XFAD
LV-Control
LV-Aducanumab
LV-αAβ-Gas6
Microglia
Phagocytic Index (A.U.)
0
1
2
3
4
#
***
**
LV-Control
LV-Aducanumab
LV-αAβ-Gas6

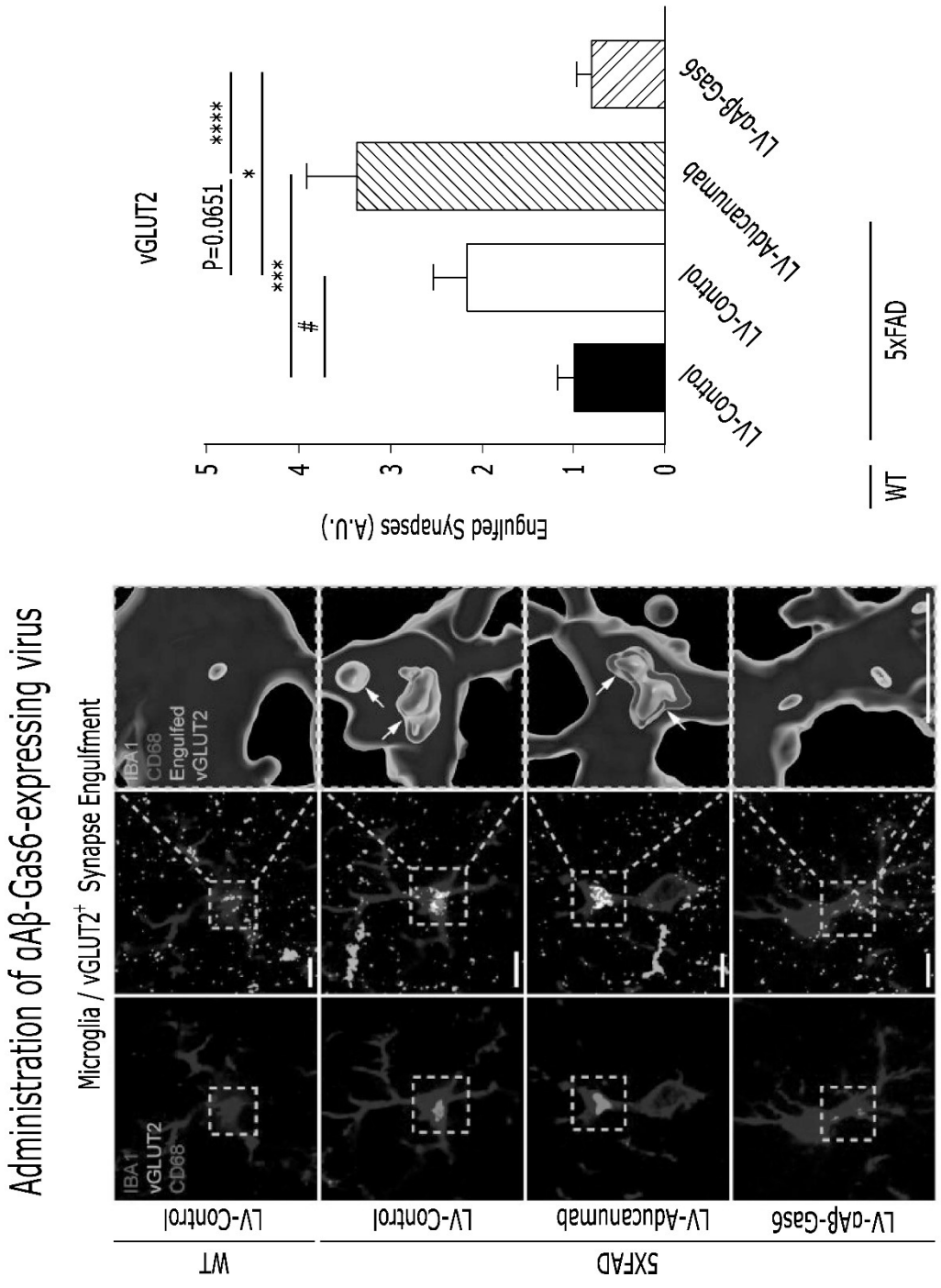
【FIG. 22】


Administration of αAβ-Gas6-expressing virus
Astrocyte / Aβ phagocytosis
5XFAD
LV-Control
LV-Aducanumab
LV-αAβ-Gas6
S100β
CathD
Aβ
Engulfed Aβ
Astrocytes
Phagocytic Index (A.U.)
0
1
2
3
4
*
**
LV-Control
LV-Aducanumab
LV-αAβ-Gas6

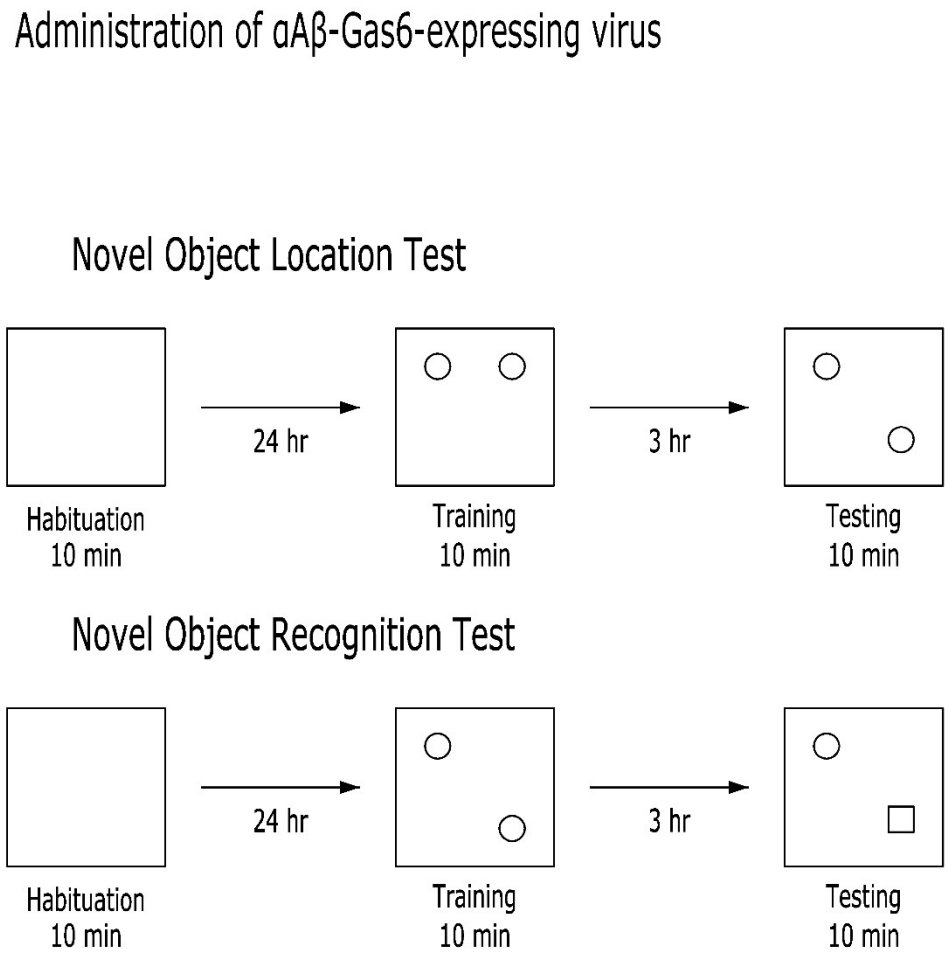
【FIG. 23】


Administration of dAβ-Gas6-expressing virus
Microglia / PSD95+ Synapse Engulfment
IBA1
PSD95
CD68
Engulfed
PSD95
WT
5XFAD
LV-Control
LV-Control
LV-Aducanumab
LV-dAβ-Gas6
PSD95
Engulfed Synapses (A.U.)
12
8
4
0
****
****
***
****
LV-Control
LV-Control
LV-Aducanumab
LV-dAβ-Gas6
WT
5xFAD

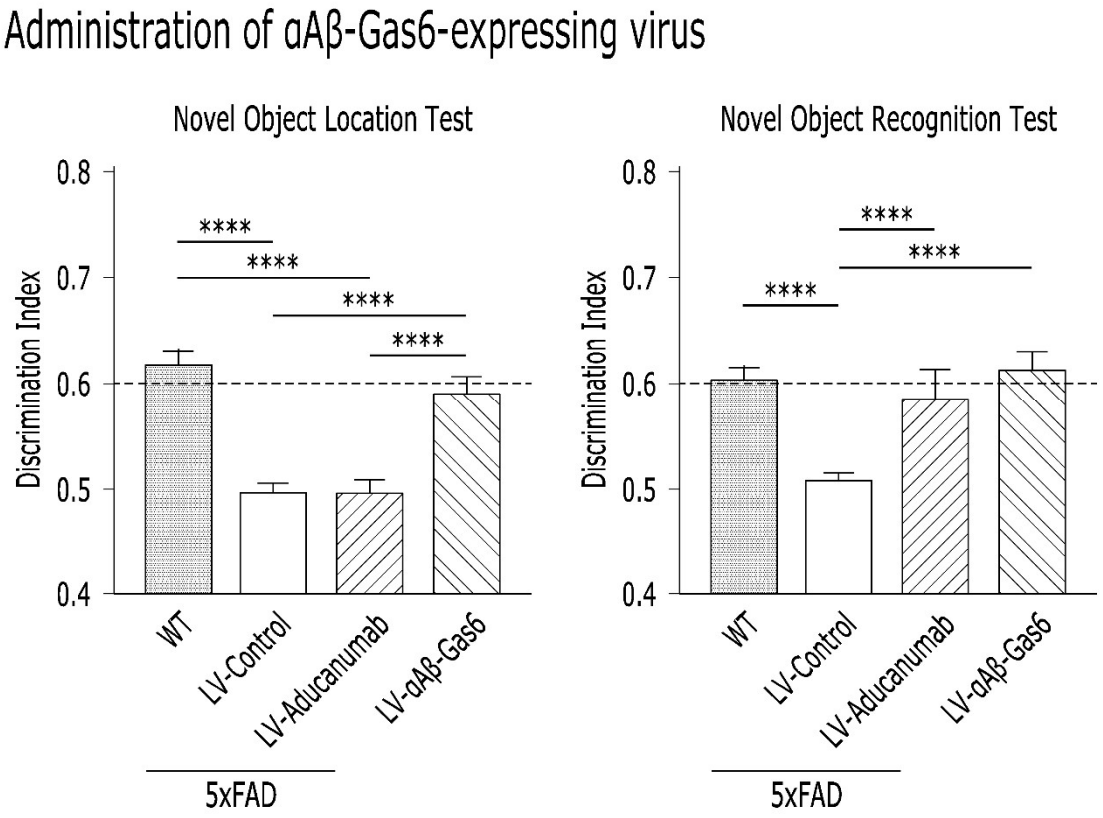
【FIG. 24】


Administration of αAβ-Gas6-expressing virus
Microglia / vGLUT2+ Synapse Engulfment
IBA1
vGLUT2
CD68
IBA1
CD68
Engulfed
vGLUT2
WT
5XFAD
LV-Control
LV-Control
LV-Aducanumab
LV-αAβ-Gas6
vGLUT2
P=0.0651
*
****
***
#
Engulfed Synapses (A.U.)
0
1
2
3
4
5
LV-Control
LV-Control
LV-Aducanumab
LV-αAβ-Gas6
WT
5xFAD

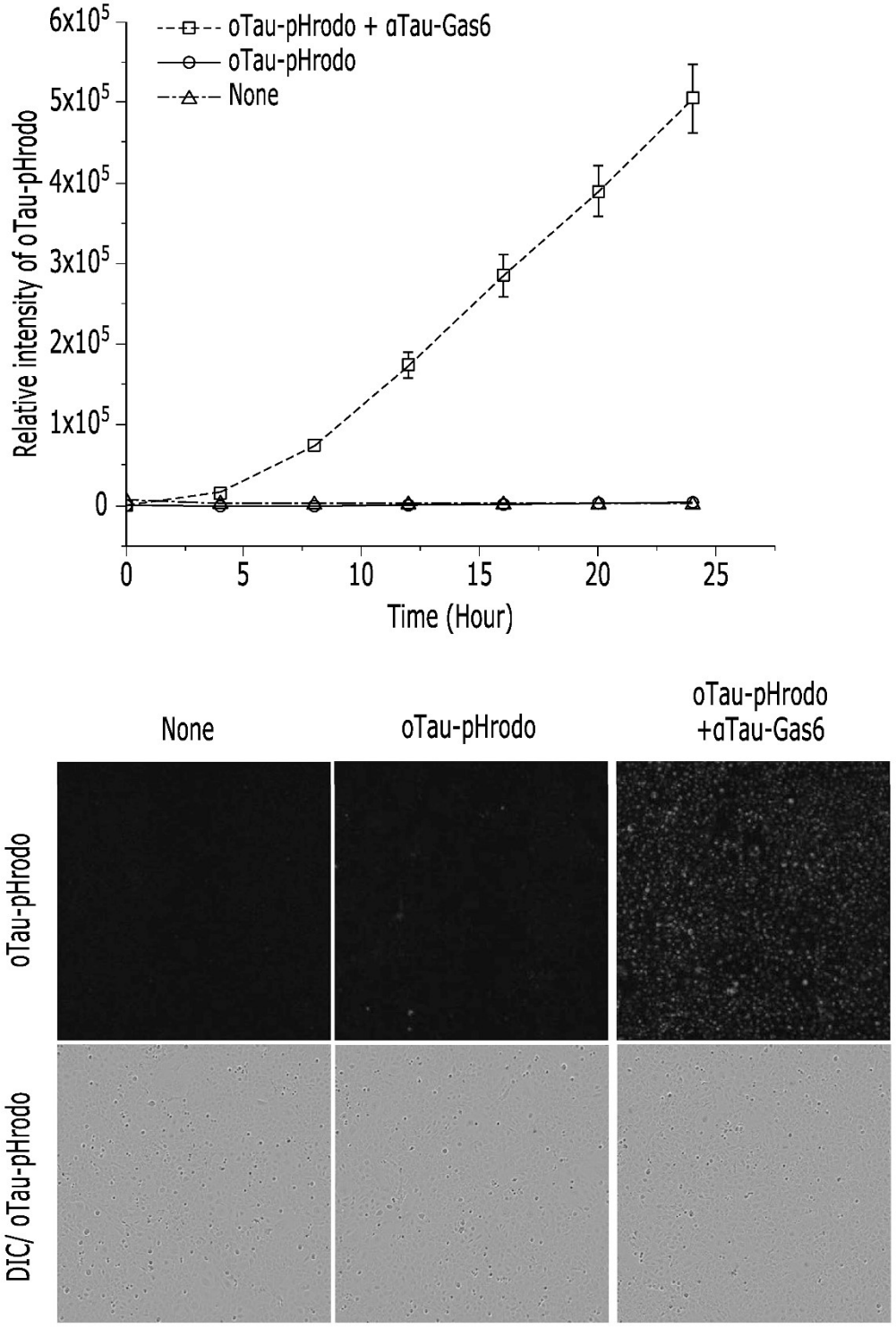
【FIG. 25】


Administration of αAβ-Gas6-expressing virus
Novel Object Location Test
24 hr
3 hr
Habituation
10 min
Training
10 min
Testing
10 min
Novel Object Recognition Test
24 hr
3 hr
Habituation
10 min
Training
10 min
Testing
10 min

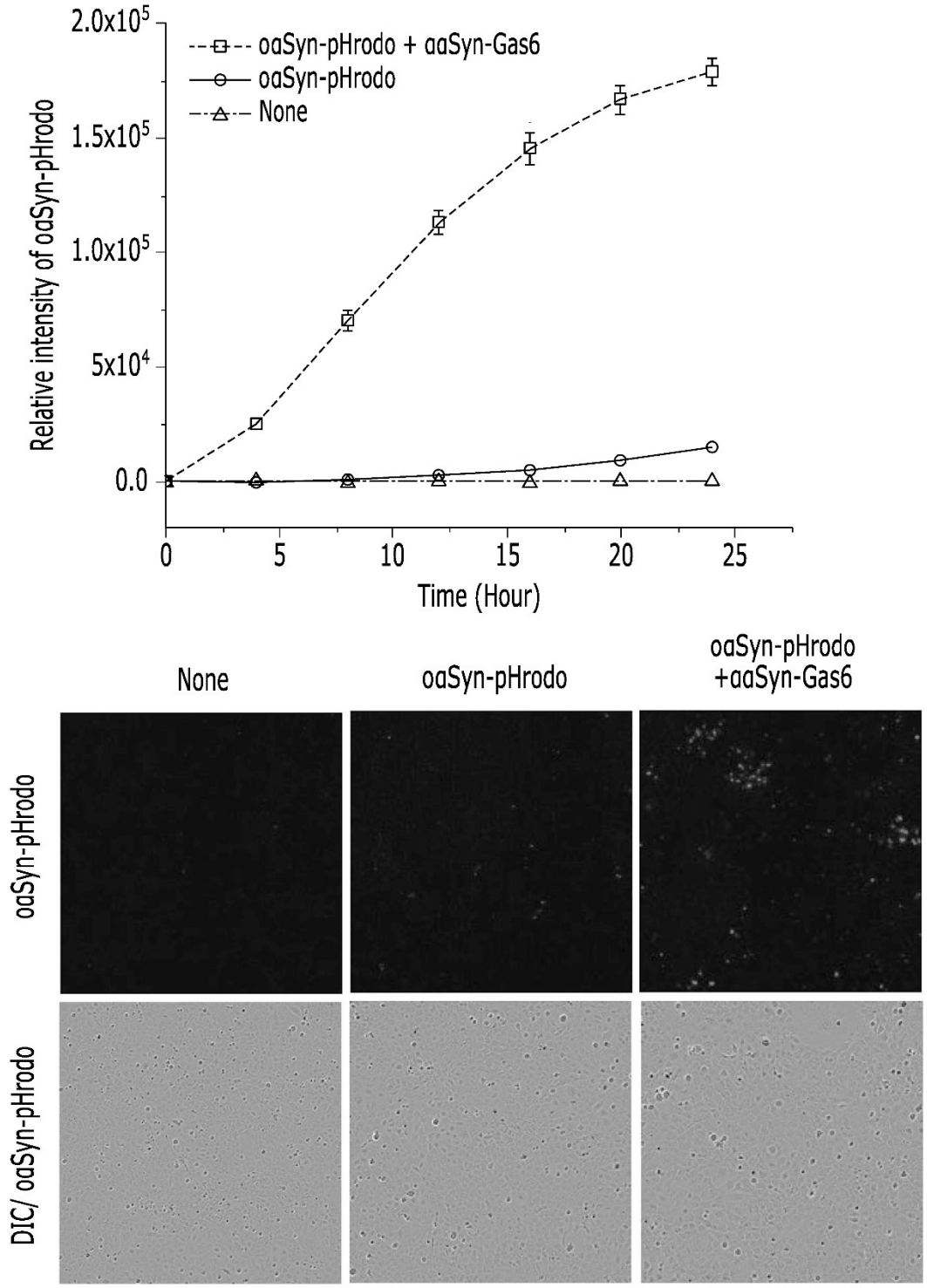
【FIG. 26】


Administration of αAβ-Gas6-expressing virus
Novel Object Location Test
Novel Object Recognition Test
Discrimination Index
0.8
0.7
0.6
0.5
0.4
****
WT
LV-Control
LV-Aducanumab
LV-αAβ-Gas6
5xFAD

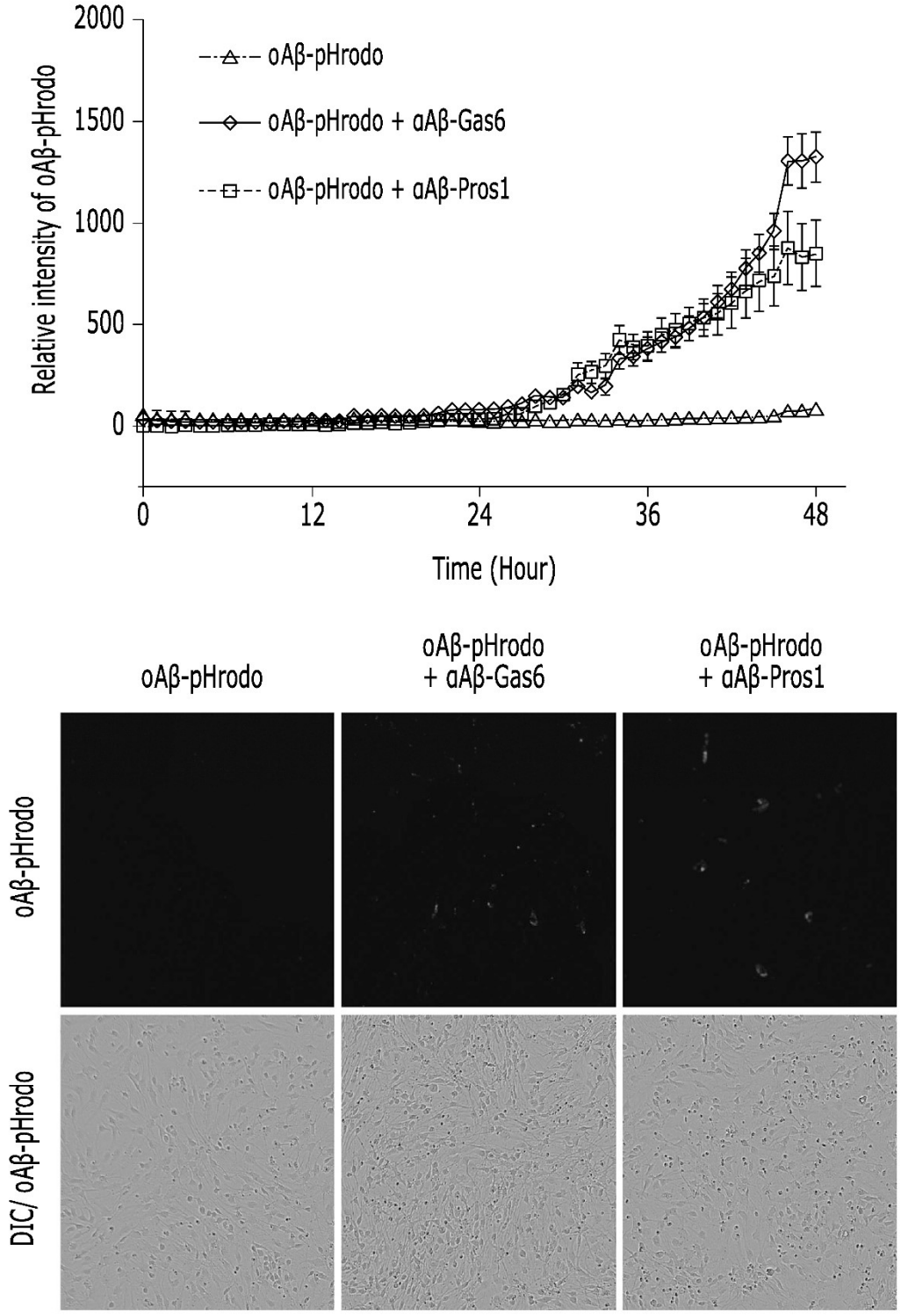
【FIG. 27】


6x10^5
5x10^5
4x10^5
3x10^5
2x10^5
1x10^5
0
Relative intensity of oTau-pHrodo
oTau-pHrodo + αTau-Gas6
oTau-pHrodo
None
0
5
10
15
20
25
Time (Hour)
None
oTau-pHrodo
oTau-pHrodo
+αTau-Gas6
oTau-pHrodo
DIC/ oTau-pHrodo

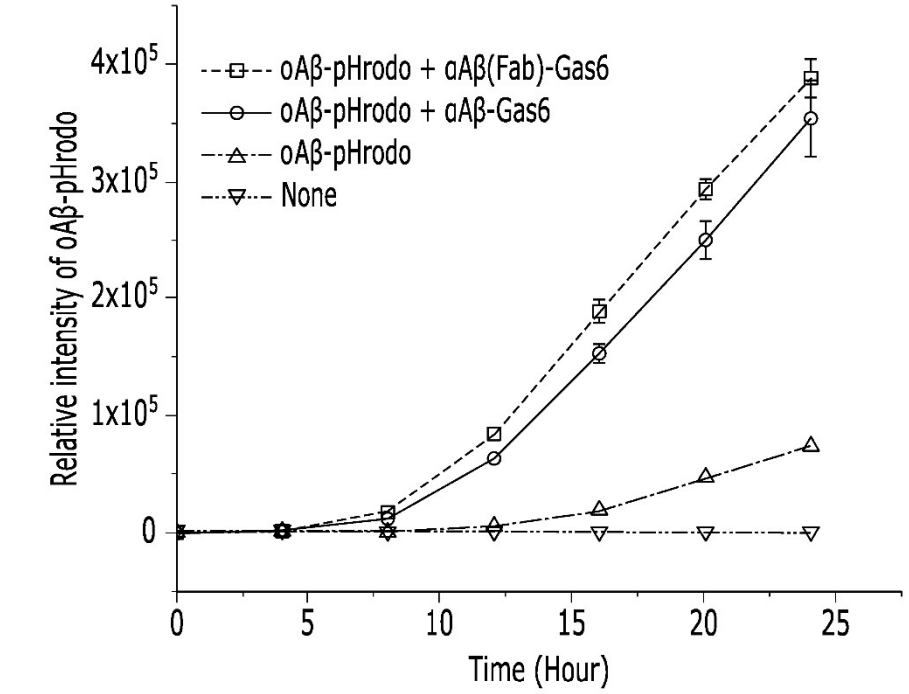
【FIG. 28】

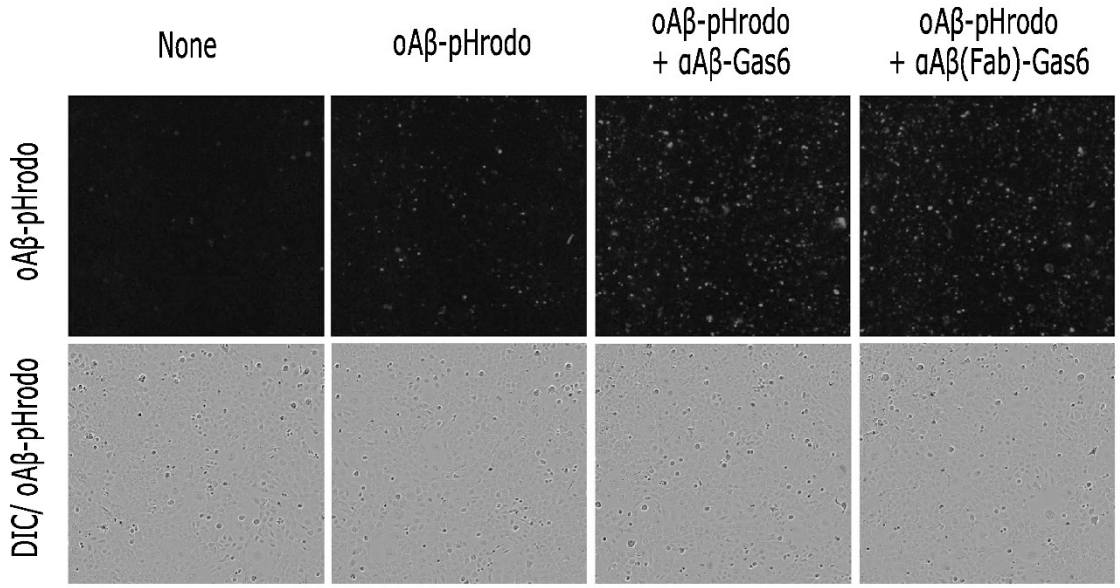

2.0x10^5
1.5x10^5
1.0x10^5
5x10^4
0.0
Relative intensity of αaSyn-pHrodo
αaSyn-pHrodo + αaSyn-Gas6
αaSyn-pHrodo
None
0
5
10
15
20
25
Time (Hour)
None
αaSyn-pHrodo
αaSyn-pHrodo +αaSyn-Gas6
αaSyn-pHrodo
DIC/ αaSyn-pHrodo

【FIG. 29】

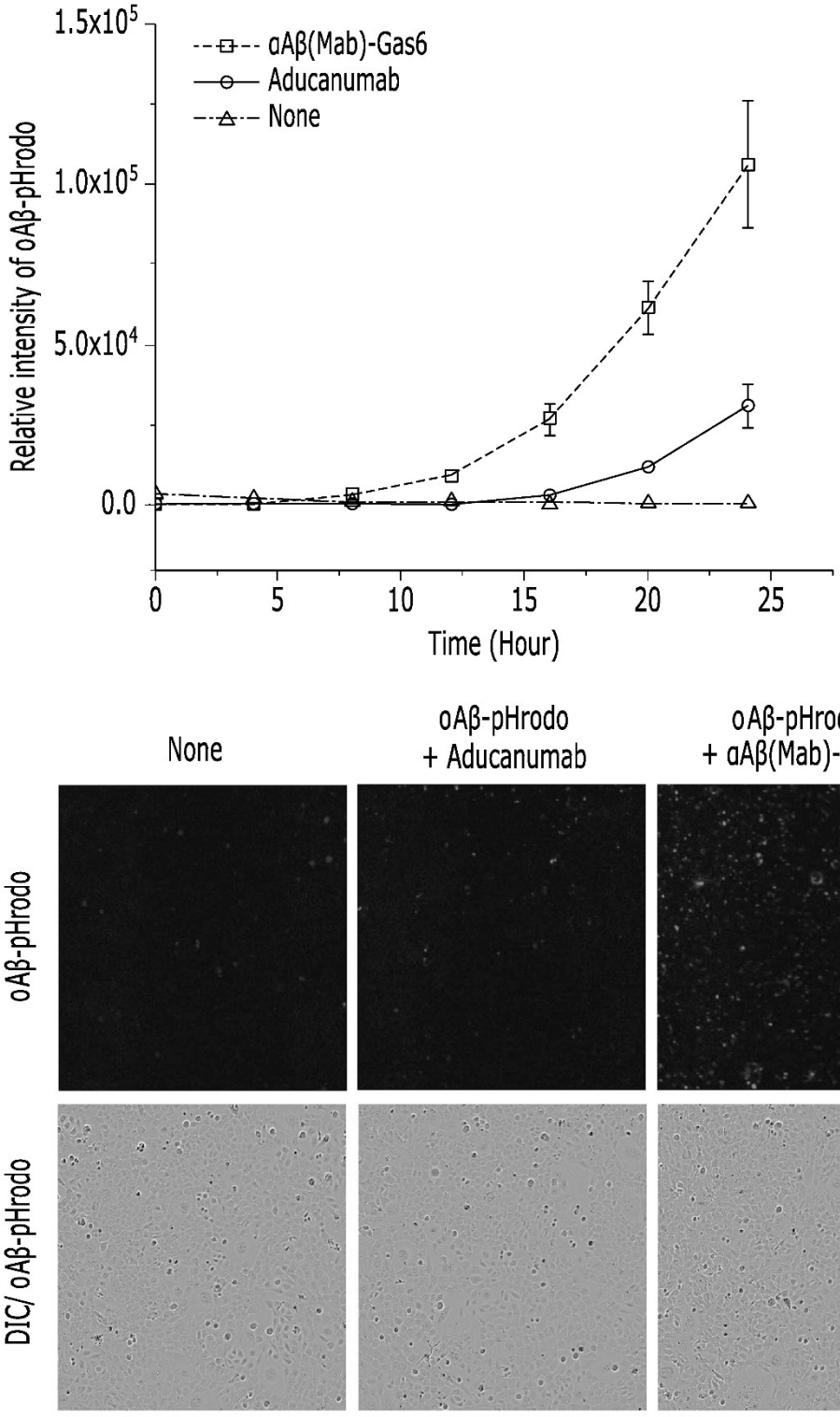

2000
1500
1000
500
0
Relative intensity of oAβ-pHrodo
oAβ-pHrodo
oAβ-pHrodo + αAβ-Gas6
oAβ-pHrodo + αAβ-Pros1
0
12
24
36
48
Time (Hour)
oAβ-pHrodo
oAβ-pHrodo + αAβ-Gas6
oAβ-pHrodo + αAβ-Pros1
oAβ-pHrodo
DIC/ oAβ-pHrodo

【FIG. 30】


4x10^5
3x10^5
2x10^5
1x10^5
0
Relative intensity of oAβ-pHrodo
oAβ-pHrodo + αAβ(Fab)-Gas6
oAβ-pHrodo + αAβ-Gas6
oAβ-pHrodo
None
0
5
10
15
20
25
Time (Hour)


None
oAβ-pHrodo
oAβ-pHrodo + αAβ-Gas6
oAβ-pHrodo + αAβ(Fab)-Gas6
oAβ-pHrodo
DIC/ oAβ-pHrodo

【FIG. 31】


1.5x10^5
1.0x10^5
5.0x10^4
0.0
Relative intensity of oAβ-pHrodo
αAβ(Mab)-Gas6
Aducanumab
None
0
5
10
15
20
25
Time (Hour)
None
oAβ-pHrodo
+ Aducanumab
oAβ-pHrodo
+ αAβ(Mab)-Gas6
oAβ-pHrodo
DIC/ oAβ-pHrodo

<110> KOREA ADVANCED INSTITUTE OF SCIENCE AND TECHNOLOGY

<120> Fusion molecules that induce non-inflammatory phagocytosis

<130> P20220004KR

<150> KR 10/2021/0013045
<151> 2021-01-29

<160> 6

<170> KoPatentIn 3.0

<210> 1
<211> 173
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid sequence

<400> 1

```
Gly Arg Met Phe Ser Gly Thr Pro Val Ile Arg Leu Arg Phe Lys Arg
  1               5                  10                  15

Leu Gln Pro Thr Arg Leu Val Ala Glu Phe Asp Phe Arg Thr Phe Asp
             20                  25                  30

Pro Glu Gly Ile Leu Leu Phe Ala Gly Gly His Gln Asp Ser Thr Trp
         35                  40                  45

Ile Val Leu Ala Leu Arg Ala Gly Arg Leu Glu Leu Gln Leu Arg Tyr
     50                  55                  60

Asn Gly Val Gly Arg Val Thr Ser Ser Gly Pro Val Ile Asn His Gly
 65                  70                  75                  80

Met Trp Gln Thr Ile Ser Val Glu Glu Leu Ala Arg Asn Leu Val Ile
                 85                  90                  95

Lys Val Asn Arg Asp Ala Val Met Lys Ile Ala Val Ala Gly Asp Leu
            100                 105                 110

Phe Gln Pro Glu Arg Gly Leu Tyr His Leu Asn Leu Thr Val Gly Gly
        115                 120                 125

Ile Pro Phe His Glu Lys Asp Leu Val Gln Pro Ile Asn Pro Arg Leu
    130                 135                 140

Asp Gly Cys Met Arg Ser Trp Asn Trp Leu Asn Gly Glu Asp Thr Thr
145                 150                 155                 160

Ile Gln Glu Thr Val Lys Val Asn Thr Arg Met Gln Cys
```

165 170

<210> 2
<211> 194
<212> PRT
<213> Artificial Sequence

<220>
<223> Gas6 derived LG2

<400> 2
Gly Ser Phe Tyr Pro Gly Ser Gly Phe Ala Phe Tyr Ser Leu Asp Tyr
1 5 10 15

Met Arg Thr Pro Leu Asp Val Gly Thr Glu Ser Thr Trp Glu Val Glu
20 25 30

Val Val Ala His Ile Arg Pro Ala Ala Asp Thr Gly Val Leu Phe Ala
35 40 45

Leu Trp Ala Pro Asp Leu Arg Ala Val Pro Leu Ser Val Ala Leu Val
50 55 60

Asp Tyr His Ser Thr Lys Lys Leu Lys Lys Gln Leu Val Val Leu Ala
65 70 75 80

Val Glu His Thr Ala Leu Ala Leu Met Glu Ile Lys Val Cys Asp Gly
85 90 95

Gln Glu His Val Val Thr Val Ser Leu Arg Asp Gly Glu Ala Thr Leu
100 105 110

Glu Val Asp Gly Thr Arg Gly Gln Ser Glu Val Ser Ala Ala Gln Leu
115 120 125

Gln Glu Arg Leu Ala Val Leu Glu Arg His Leu Arg Ser Pro Val Leu
130 135 140

Thr Phe Ala Gly Gly Leu Pro Asp Val Pro Val Thr Ser Ala Pro Val
145 150 155 160

Thr Ala Phe Tyr Arg Gly Cys Met Thr Leu Glu Val Asn Arg Arg Leu
165 170 175

Leu Asp Leu Asp Glu Ala Ala Tyr Lys His Ser Asp Ile Thr Ala His
180 185 190

Ser Cys

<210> 3

<211> 177
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid sequence

<400> 3
Leu Leu Tyr Leu Ala Glu Gln Phe Ala Gly Val Val Leu Tyr Leu Lys
1 5 10 15

Phe Arg Leu Pro Glu Ile Ser Arg Phe Ser Ala Glu Phe Asp Phe Arg
20 25 30

Thr Tyr Asp Ser Glu Gly Val Ile Leu Tyr Ala Glu Ser Ile Asp His
35 40 45

Ser Ala Trp Leu Leu Ile Ala Leu Arg Gly Gly Lys Ile Glu Val Gln
50 55 60

Leu Lys Asn Glu His Thr Ser Lys Ile Thr Thr Gly Gly Asp Val Ile
65 70 75 80

Asn Asn Gly Leu Trp Asn Met Val Ser Val Glu Glu Leu Glu His Ser
85 90 95

Ile Ser Ile Lys Ile Ala Lys Glu Ala Val Met Asp Ile Asn Lys Pro
100 105 110

Gly Pro Leu Phe Lys Pro Glu Asn Gly Leu Leu Glu Thr Lys Val Tyr
115 120 125

Phe Ala Gly Phe Pro Arg Lys Val Glu Ser Glu Leu Ile Lys Pro Ile
130 135 140

Asn Pro Arg Leu Asp Gly Cys Ile Arg Ser Trp Asn Leu Met Lys Gln
145 150 155 160

Gly Ala Ser Gly Ile Lys Glu Ile Ile Gln Glu Lys Gln Asn Lys His
165 170 175

Cys

<210> 4
<211> 183
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid sequence

<400> 4

```
Tyr Tyr Pro Gly Ser Gly Ile Ala Gln Phe His Ile Asp Tyr Asn Asn
  1               5                  10                  15

Val Ser Ser Ala Glu Gly Trp His Val Asn Val Thr Leu Asn Ile Arg
             20                  25                  30

Pro Ser Thr Gly Thr Gly Val Met Leu Ala Leu Val Ser Gly Asn Asn
         35                  40                  45

Thr Val Pro Phe Ala Val Ser Leu Val Asp Ser Thr Ser Glu Lys Ser
     50                  55                  60

Gln Asp Ile Leu Leu Ser Val Glu Asn Thr Val Ile Tyr Arg Ile Gln
 65                  70                  75                  80

Ala Leu Ser Leu Cys Ser Asp Gln Gln Ser His Leu Glu Phe Arg Val
                 85                  90                  95

Asn Arg Asn Asn Leu Glu Leu Ser Thr Pro Leu Lys Ile Glu Thr Ile
            100                 105                 110

Ser His Glu Asp Leu Gln Arg Gln Leu Ala Val Leu Asp Lys Ala Met
        115                 120                 125

Lys Ala Lys Val Ala Thr Tyr Leu Gly Gly Leu Pro Asp Val Pro Phe
    130                 135                 140

Ser Ala Thr Pro Val Asn Ala Phe Tyr Asn Gly Cys Met Glu Val Asn
145                 150                 155                 160

Ile Asn Gly Val Gln Leu Asp Leu Asp Glu Ala Ile Ser Lys His Asn
                165                 170                 175

Asp Ile Arg Ala His Ser Cys
            180
```

<210> 5
<211> 400
<212> PRT
<213> Artificial Sequence

<220>
<223> amino acid sequence

<400> 5

```
Asp Ile Leu Pro Cys Val Pro Phe Ser Val Ala Lys Ser Val Lys Ser
  1               5                  10                  15

Leu Tyr Leu Gly Arg Met Phe Ser Gly Thr Pro Val Ile Arg Leu Arg
             20                  25                  30
```

```
Phe Lys Arg Leu Gln Pro Thr Arg Leu Val Ala Glu Phe Asp Phe Arg
         35                  40                  45

Thr Phe Asp Pro Glu Gly Ile Leu Leu Phe Ala Gly Gly His Gln Asp
     50                  55                  60

Ser Thr Trp Ile Val Leu Ala Leu Arg Ala Gly Arg Leu Glu Leu Gln
 65                  70                  75                  80

Leu Arg Tyr Asn Gly Val Gly Arg Val Thr Ser Ser Gly Pro Val Ile
                 85                  90                  95

Asn His Gly Met Trp Gln Thr Ile Ser Val Glu Glu Leu Ala Arg Asn
            100                 105                 110

Leu Val Ile Lys Val Asn Arg Asp Ala Val Met Lys Ile Ala Val Ala
        115                 120                 125

Gly Asp Leu Phe Gln Pro Glu Arg Gly Leu Tyr His Leu Asn Leu Thr
    130                 135                 140

Val Gly Gly Ile Pro Phe His Glu Lys Asp Leu Val Gln Pro Ile Asn
145                 150                 155                 160

Pro Arg Leu Asp Gly Cys Met Arg Ser Trp Asn Trp Leu Asn Gly Glu
                165                 170                 175

Asp Thr Thr Ile Gln Glu Thr Val Lys Val Asn Thr Arg Met Gln Cys
            180                 185                 190

Phe Ser Val Thr Glu Arg Gly Ser Phe Tyr Pro Gly Ser Gly Phe Ala
        195                 200                 205

Phe Tyr Ser Leu Asp Tyr Met Arg Thr Pro Leu Asp Val Gly Thr Glu
    210                 215                 220

Ser Thr Trp Glu Val Glu Val Val Ala His Ile Arg Pro Ala Ala Asp
225                 230                 235                 240

Thr Gly Val Leu Phe Ala Leu Trp Ala Pro Asp Leu Arg Ala Val Pro
                245                 250                 255

Leu Ser Val Ala Leu Val Asp Tyr His Ser Thr Lys Lys Leu Lys Lys
            260                 265                 270

Gln Leu Val Val Leu Ala Val Glu His Thr Ala Leu Ala Leu Met Glu
        275                 280                 285

Ile Lys Val Cys Asp Gly Gln Glu His Val Val Thr Val Ser Leu Arg
    290                 295                 300

Asp Gly Glu Ala Thr Leu Glu Val Asp Gly Thr Arg Gly Gln Ser Glu
305                 310                 315                 320
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Val Ser Ala Ala Gln Leu Gln Glu Arg Leu Ala Val Leu Glu Arg His
                325                 330                 335

Leu Arg Ser Pro Val Leu Thr Phe Ala Gly Gly Leu Pro Asp Val Pro
            340                 345                 350

Val Thr Ser Ala Pro Val Thr Ala Phe Tyr Arg Gly Cys Met Thr Leu
        355                 360                 365

Glu Val Asn Arg Arg Leu Leu Asp Leu Asp Glu Ala Ala Tyr Lys His
    370                 375                 380

Ser Asp Ile Thr Ala His Ser Cys Pro Pro Val Glu Pro Ala Ala Ala
385                 390                 395                 400


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Val Val Ser Val Cys Leu Pro Leu Asn Leu Asp Thr Lys Tyr Glu Leu
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Leu Tyr Leu Ala Glu Gln Phe Ala Gly Val Val Leu Tyr Leu Lys Phe
             20                  25                  30

Arg Leu Pro Glu Ile Ser Arg Phe Ser Ala Glu Phe Asp Phe Arg Thr
         35                  40                  45

Tyr Asp Ser Glu Gly Val Ile Leu Tyr Ala Glu Ser Ile Asp His Ser
     50                  55                  60

Ala Trp Leu Leu Ile Ala Leu Arg Gly Gly Lys Ile Glu Val Gln Leu
 65                  70                  75                  80

Lys Asn Glu His Thr Ser Lys Ile Thr Thr Gly Gly Asp Val Ile Asn
                 85                  90                  95

Asn Gly Leu Trp Asn Met Val Ser Val Glu Glu Leu Glu His Ser Ile
            100                 105                 110

Ser Ile Lys Ile Ala Lys Glu Ala Val Met Asp Ile Asn Lys Pro Gly
        115                 120                 125

Pro Leu Phe Lys Pro Glu Asn Gly Leu Leu Glu Thr Lys Val Tyr Phe
    130                 135                 140
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Ala Gly Phe Pro Arg Lys Val Glu Ser Glu Leu Ile Lys Pro Ile Asn
145                 150                 155                 160

Pro Arg Leu Asp Gly Cys Ile Arg Ser Trp Asn Leu Met Lys Gln Gly
                165                 170                 175

Ala Ser Gly Ile Lys Glu Ile Ile Gln Glu Lys Gln Asn Lys His Cys
            180                 185                 190

Leu Val Thr Val Glu Lys Gly Ser Tyr Tyr Pro Gly Ser Gly Ile Ala
        195                 200                 205

Gln Phe His Ile Asp Tyr Asn Asn Val Ser Ser Ala Glu Gly Trp His
    210                 215                 220

Val Asn Val Thr Leu Asn Ile Arg Pro Ser Thr Gly Thr Gly Val Met
225                 230                 235                 240

Leu Ala Leu Val Ser Gly Asn Asn Thr Val Pro Phe Ala Val Ser Leu
                245                 250                 255

Val Asp Ser Thr Ser Glu Lys Ser Gln Asp Ile Leu Leu Ser Val Glu
            260                 265                 270

Asn Thr Val Ile Tyr Arg Ile Gln Ala Leu Ser Leu Cys Ser Asp Gln
        275                 280                 285

Gln Ser His Leu Glu Phe Arg Val Asn Arg Asn Asn Leu Glu Leu Ser
    290                 295                 300

Thr Pro Leu Lys Ile Glu Thr Ile Ser His Glu Asp Leu Gln Arg Gln
305                 310                 315                 320

Leu Ala Val Leu Asp Lys Ala Met Lys Ala Lys Val Ala Thr Tyr Leu
                325                 330                 335

Gly Gly Leu Pro Asp Val Pro Phe Ser Ala Thr Pro Val Asn Ala Phe
            340                 345                 350

Tyr Asn Gly Cys Met Glu Val Asn Ile Asn Gly Val Gln Leu Asp Leu
        355                 360                 365

Asp Glu Ala Ile Ser Lys His Asn Asp Ile Arg Ala His Ser Cys Pro
    370                 375                 380

Ser Val Trp Lys Lys Thr Lys Asn Ser
385                 390
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