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(54) Title: USE OF KINASE DOMAIN M7CK OF TRPM7 IN PREPARATION OF DRUG FOR TREATING ALZHEIMER'S DISEASE

(57) Abstract: Relating to the technical field of biological medicine, particularly the use of a kinase domain M7CK of TRPM7 in the preparation of a drug for treating Alzheimer's disease. The over-expression of M7CK can relieve cognitive impairment of a mouse model with AD by increasing an MMP14 phosphorylation level, improving learning and memory ability, increasing synaptic density and density of a presynaptic vesicle protein synaptophysin and a postsynaptic density protein PSD-95, promoting degradation of A $\beta$  in soluble and insoluble components, and reducing accumulation of A $\beta$  amyloid plaques. Therefore, the kinase domain of TRPM7 provided has a great application prospect in gene therapy of a patient with Alzheimer's disease



## USE OF KINASE DOMAIN M7CK OF TRPM7 IN PREPARATION OF DRUG FOR TREATING ALZHEIMER'S DISEASE

### FIELD

[0001] The present disclosure relates to the technical field of biological medicine, particularly relating to the use of a kinase domain M7CK of TRPM7 in the preparation of a drug for treating Alzheimer's disease.

### BACKGROUND

[0002] Alzheimer's disease (AD) is a neurodegenerative disease with a wide range of effects. Among the population over 60 years old in China, about 10 million people are affected. According to the report of the World Health Organization; there are World Health Organization, there will be about 55 million patients with Alzheimer's disease worldwide in 2021. This number is expected to reach 78 million by 2030. Pathogenesis is highly complex, mainly manifested by loss of neuronal cell synapses in the brain, amyloid deposition, neurofibrillary tangles, and the like. The clinical manifestations are progressive memory and cognitive decline, language function and personality change and difficulty in daily activities. The disease brings significant mental and economic burden to the patients themselves, their families and even the whole of society. To date, no effective radical treatment has been available. One of the critical targets for the development of drugs for Alzheimer's disease is directed at amyloid deposition. Therefore, targeting the elimination of amyloid to relieve the pathological characteristics of Alzheimer's disease such as synapse loss, cognitive impairment and the like has a great application prospect.

[0003] TRPM7 is a member of the M-type transient receptor potential family, a divalent ion channel protein widely expressed in various cell types and contains 6 transmembrane channel domains and a C-terminal kinase domain (M7CK) with both N-terminal and C-terminal directed to the cytoplasm. TRPM7 is selectively permeable to divalent cations such as calcium ion, magnesium ion and zinc ion, has important effects on maintaining ion homeostasis inside and outside cells and regulating downstream signaling pathways, and is expressed in various nerve cells such as the

hippocampus, cerebral cortex, cerebellum and brainstem. TRPM7 cleaved kinase fragments (M7CK) can exist alone after being cleaved and are involved in critical cellular processes such as regulating chromatin remodeling and synaptic plasticity. Its unique dual function as an ion channel with kinase domain enables TRPM7 to be involved in a variety of cellular functions. TRPM7 is involved in neurodegenerative diseases, including Alzheimer's disease by a variety of means. However, some studies suggest that inhibition of TRPM7 may be a therapeutic strategy, while other studies suggest that activation of TRPM7 may have a protective effect. In 2005, a study found that missense mutation of TRPM7 can change the sensitivity of cells to magnesium ions, thereby affecting magnesium ion homeostasis and further causing neurodegenerative diseases and dementia. In 2010, a study found that presenilin protein mutants related to familial Alzheimer's disease can inhibit the channel function of TRPM7. In 2017, a study found that pharmacological activation of the channel function of TRPM7 can promote the clearance of A $\beta$  by activating autophagy. In 2024, a genetic screening study in human subjects found three mutations in TRPM7 in Chinese patients with sporadic Alzheimer's and frontotemporal dementia. To date, there has been no study on the protective effect of the kinase domain M7CK of TRPM7 on Alzheimer's disease.

## SUMMARY

**[0004]** In view of the shortcomings of the prior art described above, the present disclosure aims to provide the use of a kinase domain M7CK sequence of TRPM7 in the preparation of a drug for treating Alzheimer's disease and aims to solve the problems in the prior art.

**[0005]** In order to realize the objective and other related objectives, the present disclosure provides the use of a kinase domain M7CK of TRPM7 in the preparation of a drug for treating Alzheimer's disease.

**[0006]** Preferably, the M7CK has the amino acid sequence selected from the following:

**[0007]** 1) a polypeptide sequence coded by the nucleotide sequence of SEQ ID No. 1; and

**[0008]** 2) a polypeptide fragment having the sequence identity of at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% and at

least 99.5% to the polypeptide of 1) on the basis of having the same biological functions as the polypeptide of 1).

[0009] Preferably, the polypeptide sequence is shown in SEQ ID No. 3.

[0010] The present disclosure further provides a pAAV2/9-M7CK plasmid capable of over-expressing M7CK at sequence positions 1299-1863 of TRPM7 and constructed on the basis of a kinase domain M7CK sequence of TRPM7.

[0011] The present disclosure further provides a virus capable of over-expressing M7CK at sequence positions 1299-1863 of TRPM7 and constructed based on a kinase domain M7CK sequence of TRPM7, and the virus is obtained by virus packaging of the pAAV2/9-M7CK plasmid.

[0012] The present disclosure further provides a method for preventing or treating Alzheimer's disease, wherein the method includes administering to a subject an effective dose of the kinase domain M7CK sequence of TRPM7, the pAAV2/9-M7CK plasmid or the virus.

[0013] As mentioned above, the use of a kinase domain M7CK of TRPM7 in the preparation of a drug for treating Alzheimer's disease of the present disclosure has the following beneficial effects:

[0014] The over-expression of M7CK in the present disclosure can relieve cognitive impairment of a mouse model with AD by increasing an MMP14 phosphorylation level, improving learning and memory ability, increasing synaptic density and density of a presynaptic vesicle protein synaptophysin and a postsynaptic density protein PSD-95, promoting degradation of A $\beta$  in soluble and insoluble components, and reducing accumulation of A $\beta$  amyloid plaques. Therefore, the kinase domain of TRPM7 provided by the present disclosure has a great application prospect in gene therapy of a patient with Alzheimer's disease.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIGs. 1A-1C show the results of analyzing the expression levels of TRPM7 in the hippocampus of patients with Alzheimer's disease, 5XFAD mice at 12 months of age and APP/PS1 mice at 15 months of age in the present disclosure;

[0016] FIGs. 2A-2B show the immunofluorescence signals and statistical results of synaptophysin/PSD95 in primary hippocampal neuron over-expressing M7CK and treated with A $\beta$  in the present disclosure;

[0017] FIG. 3 shows that AAV2/9-M7CK or AAV2/9-EGFP is injected into the hippocampus of WT and 5XFAD mice at 5 months of age, and then learning and memory functions thereof are tested at 6/10/15 months of age by using a novel object recognition test, a cognition wall test and a Y-maze test in the present disclosure;

[0018] FIGs. 4A-4F show the results of a behavioral test of mice at 6/10/15 months of age in the present disclosure;

[0019] FIGs. 5A-5B show that before injection of M7CK in the present disclosure, 5XFAD mice at 14 months of age do not have the ability to recognize novel objects, and after over-expressing M7CK, the novel object recognition index is significantly increased in the 5XFAD mice;

[0020] FIGs. 6A-6B show the immunofluorescence staining and statistical results of synaptophysin and PSD95 in the hippocampus of 5XFAD mice at 15 months of age over-expressing M7CK in the present disclosure;

[0021] FIG. 7 shows immunohistochemical staining and statistical results of A $\beta$  plaques in the hippocampus of 5XFAD mice over-expressing M7CK in the present disclosure;

[0022] FIG. 8 shows the quantitative results of ELISA of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> in soluble and insoluble protein components in the hippocampus of 5XFAD mice over-expressing M7CK in the present disclosure;

[0023] FIG. 9 shows that over-expressing M7CK in the hippocampus of 5XFAD mice increases the phosphorylation level of MMP14 in the present disclosure;

[0024] FIGs. 10A-10C show that M7CK can bind and phosphorylate MMP14 in the present disclosure;

[0025] FIGs. 11A-11B show that knock-out of TRPM7 reduces the phosphorylation level of MMP14 in the present disclosure;

[0026] FIG. 12 shows that M7CK promotes the degradation of A  $\beta$  by MMP14 in the present disclosure;

- [0027] FIG. 13 shows after knock-down of MMP14, M7CK fails to restore down-regulation of synaptic density caused by A  $\beta$  in the present disclosure;
- [0028] FIG. 14 shows after knock-down of MMP14, M7CK fails to promote the degradation of A  $\beta$  in the present disclosure;
- [0029] FIG. 15 shows the structure of a TRPM7 protein in the present disclosure;
- [0030] FIG. 16 shows a pAAV2/9-EGFP plasmid in the present disclosure;
- [0031] FIGs. 17A-17F show the results of a study of the activity and specificity of a substance promoting TRPM7 ion channel part activity to promote TRPM7 cleavage to produce a free and active kinase domain (M7CK) in the present disclosure;
- [0032] FIGs. 18A-18D show the results that a chronic treatment with the TRPM7 modulator ATT which promotes the cleavage of TRPM7 to produce free and active M7CK can rescue cognitive and memory function in 5XFAD mice in the present disclosure; and
- [0033] FIGs. 19A-19C show the results that a chronic treatment with the TRPM7 modulator ATT which promotes the cleavage of TRPM7 to produce free and active M7CK can protect the synaptic density and reduce A  $\beta$  plaques in 5XFAD mice in the present disclosure.
- [0034] FIGs. 20A-20C show the results of altering the M7CK/TRPM7 ratio with a substance that promotes the cleavage of TRPM7 to produce M7CK in the present disclosure.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

- [0035] The present disclosure provides use of a kinase domain M7CK of TRPM7 in preparing a drug for treating Alzheimer's disease. The kinase domain M7CK is a polypeptide.
- [0036] In some specific embodiments, the amino acid sequence of the M7CK is a polypeptide sequence coded by the nucleotide sequence of SEQ ID No. 1.
- [0037] In some specific embodiments, the M7CK is: a polypeptide fragment having the sequence identity of at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% and at least 99.5% to the polypeptide coded by the nucleotide sequence SEQ ID No. 1 on the basis of having the same biological functions as the polypeptide coded by the nucleotide sequence SEQ ID No. 1.

**[0038]** In some specific embodiments, the amino acid sequence of the kinase domain M7CK of TRPM7 includes the amino acid sequence at positions 1299-1863 of TRPM7. Preferably, the amino acid sequence of the M7CK is shown in SEQ ID No. 3.

**[0039]** In some specific embodiments, the kinase domain M7CK of TRPM7 may be an amino acid sequence containing a secretory tag. The secretory tag directs the kinase domain of TRPM7 to the periplasmic space of a host cell. In particular embodiments, a signal peptide is derived from DsbA, PelB, OmpA, TolB, MalE, Ipp, TorA or HylA. In one aspect, the secretory tag is attached to the kinase domain of TRPM7. In another aspect, the secretory tag is cleaved from the kinase domain of TRPM7.

**[0040]** In some specific embodiments, the kinase domain M7CK of TRPM7 includes a histidine tag. The histidine tag or a polyhistidine tag is a sequence of 2 to 20 histidine residues attached to collagen. The histidine tag includes 2 to 20 histidine residues, 5 to 15 histidine residues, 5 to 18 histidine residues, 5 to 16 histidine residues, 5 to 15 histidine residues, 5 to 14 histidine residues, 5 to 13 histidine residues, 5 to 12 histidine residues, 5 to 11 histidine residues, 5 to 10 histidine residues, 6 to 12 histidine residues, 6 to 11 histidine residues or 7 to 10 histidine residues. The histidine tag can be used to purify proteins by a chromatographic method using a nickel-based chromatographic medium.

**[0041]** In some specific embodiments, the drug for treating Alzheimer's disease includes an M7CK accelerator.

**[0042]** In some specific embodiments, the M7CK accelerator is selected from one or more of the following: a plasmid or a virus effectively over-expressing M7CK; and/or a substance promoting the cleavage of TRPM7 to produce M7CK.

**[0043]** In some specific embodiments, the plasmid or viral vector over-expressing M7CK includes a coding nucleic acid fragment of M7CK.

**[0044]** In some specific embodiments, the substance promoting the cleavage of TRPM7 to produce M7CK is selected from one or more of the following compounds: resveratrol, zafirlukast, TPCA-1, ATT and glutamic acid or glutamate.

**[0045]** Further, the coding nucleic acid fragment is shown in SEQ ID No. 1. Preferably, the coding nucleic acid fragment is obtained by optimizing the nucleic acid molecule shown in SEQ ID No. 1 by any one or more of the following modes : 1) compared with the nucleic acid molecule

shown in SEQ ID No. 1, increasing the content of G/C; and 2) codon-optimizing the nucleic acid molecule shown in SEQ ID No. 1 to suit the species acts thereon, wherein after the codon-optimizing of the nucleic acid fragment or the content of G/C is increased, the sequence of the encoded amino acid is not altered.

[0046] In some specific embodiments, the plasmid is selected from an adenovirus packaging plasmid, an adeno-associated virus packaging plasmid and a lentivirus packaging plasmid; and the virus is selected from an adenovirus, an adeno-associated virus and a lentivirus.

[0047] In some specific embodiments, the plasmid is a pAAV2/9-M7CK plasmid, preferably, the sequence of the pAAV2/9-M7CK plasmid is shown in SEQ ID No. 2.

[0048] In some specific embodiments, the pAAV2/9-M7CK plasmid is used for virus packaging of an AAV to obtain a packaged AAV.

[0049] In some specific embodiments, a use method of the drug for treating Alzheimer's disease is injecting the packaged AAV into the brain by brain stereotaxic localization, specifically into an intracerebral hippocampus region.

[0050] In some specific embodiments, the drug for treating Alzheimer's disease has one or more of the following functions:

[0051] 1) increasing neuronal synaptic density;

[0052] 2) improving learning and memory ability;

[0053] 3) reducing A $\beta$  plaques or A $\beta$  proteins; and

[0054] 4) activating the activity of MMP14. Increasing neuronal synaptic density refers to an increase in neuronal synaptic density relative to that of patients with Alzheimer's disease; improving learning and memory ability refers to an increase in learning and memory ability relative to that of patients with Alzheimer's disease; and reducing A $\beta$  plaques refers to a decrease in A $\beta$  plaques relative to that of patients with Alzheimer's disease.

[0055] Further, the A $\beta$  plaque is a protein clump formed by aggregating A $\beta_{1-40}$  proteins or A $\beta_{1-42}$  proteins.

[0056] Further, the activating the activity of MMP14 is phosphorylating the MMP14 protein.

[0057] In some specific embodiments, the drug for treating Alzheimer's disease further includes a pharmaceutically acceptable excipient. Particularly, the excipient includes various excipients and diluents. These excipients are not essential active ingredients and not excessively

toxic after administration. The excipients include sterile water or normal saline, a stabilizer, an excipient, an antioxidant (ascorbic acid, etc.), a buffer (phosphoric acid, citric acid, other organic acids, etc.), a preservative, a surfactant (PEG, Tween, etc.), a chelating agent (EDTA, etc.) or a binder. The excipients further include other low molecular weight polypeptides, serum albumin, glycine, glutamine, asparagine, arginine, polysaccharides, monosaccharides, mannitol or sorbitol. The excipients are selected from normal saline, a glucose isotonic solution, a D-sorbitol isotonic solution, a D-mannose isotonic solution, a D-mannitol and sugar alcohol isotonic solution when used in an injectable aqueous solution. The injectable aqueous solution includes a solubilizer. The solubilizer is selected from alcohol (ethanol), polyalcohol (propylene glycol or PEG) and a nonionic surfactant (Tween 80 or HCO-50). In the drug for treating Alzheimer's disease, the kinase domain M7CK sequence of TRPM7 may be a single effective component or may be combined with one or more other active ingredients useful for treating Alzheimer's disease to form a combined preparation. The active ingredients are other various drugs for treating Alzheimer's disease. The content of the active ingredients in a pharmaceutical composition is a safe and effective amount which can be adjusted by those skilled in the art. For example, the administration amount of the kinase domain M7CK sequence of TRPM7 and the active ingredients of a therapeutic product of Alzheimer's disease depends on the body weight of patients, the type of application and the condition and severity of the disease, for example, the administration amount of the pharmaceutical composition as an active component is 1-1,000 mg/kg/day, 1-3 mg/kg/day, 3-5 mg/kg/day, 5-10 mg/kg/day, 10-20 mg/kg/day, 20-30 mg/kg/day, 30-40 mg/kg/day, 40-60 mg/kg/day, 60-80 mg/kg/day, 80-100 mg/kg/day, 100-200 mg/kg/day, 200-500 mg/kg/day, or greater than 500 mg/kg/day.

**[0058]** The present disclosure further provides a pAAV2/9-M7CK plasmid capable of effectively over-expressing M7CK at sequence positions 1299-1863 of TRPM7 and constructed on the basis of a sequence of kinase domain M7CK of TRPM7.

**[0059]** In some specific embodiments, the amino acid sequence of the M7CK is a polypeptide sequence encoded by the nucleotide sequence of SEQ ID No. 1.

**[0060]** In some specific embodiments, the M7CK is: a polypeptide fragment having the sequence identity of at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% and at least 99.5% to the polypeptide coded by the nucleotide

sequence SEQ ID No. 1 on the basis of having the same biological functions as the polypeptide coded by the nucleotide sequence SEQ ID No. 1.

**[0061]** In some specific embodiments, the amino acid sequence of the M7CK includes the amino acid sequence at positions 1299-1863 of TRPM7. Preferably, the amino acid sequence of the M7CK is shown in SEQ ID No. 3.

**[0062]** In some specific embodiments, the plasmid or viral vector over-expressing M7CK includes a coding nucleic acid fragment of M7CK.

**[0063]** Further, the coding nucleic acid fragment is shown in SEQ ID No. 1. Preferably, the coding nucleic acid fragment is obtained by optimizing the nucleic acid molecule shown in SEQ ID No. 1 by any one or more of the following modes: 1) compared with the nucleic acid molecule shown in SEQ ID No. 1, increasing the content of G/C; and 2) codon-optimizing the nucleic acid molecule shown in SEQ ID No. 1 to suit the species acts thereon, wherein after the codon-optimizing of the nucleic acid fragment or the content of G/C is increased, the sequence of the encoded amino acid is not altered.

**[0064]** In some specific embodiments, the nucleotide sequence of the pAAV2/9-M7CK plasmid is shown in SEQ ID No. 2.

**[0065]** The present disclosure further provides a virus capable of over-expressing M7CK at sequence positions 1299-1863 of TRPM7 and constructed on the basis of a sequence of kinase domain M7CK of TRPM7, which is obtained by virus packaging of the pAAV2/9-M7CK plasmid.

**[0066]** In some specific embodiments, the virus may be selected from one or more of an adenovirus, an adeno-associated virus and a lentivirus.

**[0067]** Further, the plasmid backbone of the virus may be any one or more of pLKO.1-CMV-tGFP, pLKO.1-puro-CMV-tGFP, pLKO.1-CMV-Neo, pLKO.1-Neo, pLKO.1-Neo-CMV-tGFP, pLKO.1-puro-CMV-TagCFP, pLKO.1-puro-CMV-TagYFP, pLKO.1-puro-CMV-TagRFP, pLKO.1-puro-CMV-TagFP635, pLKO.1-puro-UbC-TurboGFP, pLKO.1-puro-UbC-TagFP635, pLKO-puro-IPTG-1xLacO, pLKO-puro-IPTG-3xLacO, pLP1, pLP2, pLP/VSV-G, pENTR/U6, pLenti6/BLOCK-iT-DEST, pcDNA1.2/V5-GW/lacZ, pLenti6.2/N-Lumio/V5-DEST, pGCSIL-GFP and pHR-SFFV-Puro.

**[0068]** In some specific embodiments, the pAAV2/9-M7CK plasmid or virus is obtained by introducing the M7CK sequence into an engineered cell and performing intracellular replication or

assembly. Specifically, the engineered cell may be one or more of *escherichia coli*, *lactobacillus*, *bacillus licheniformis*, *bacillus subtilis*, *streptomyces*, *saccharomyces cerevisiae*, *candida*, *torulopsis*, *rhodotorula*, *hansenula*, *pichia*, *kluyveromyces*, 293, 293T, HUCCT1, HCCC-9810 and RBE.

[0069] The present disclosure further provides a method for preventing or treating Alzheimer's disease, specifically a method for preventing or treating a subject suffering from Alzheimer's disease, including administering to a subject an effective dose of TRPM7 modulator, M7CK accelerator, pAAV2/9-M7CK plasmid or the virus in the use.

[0070] In some specific embodiments, the administration amount is 1-1,000 mg/kg/day. Specifically, the administration amount is 1-3 mg/kg/day, 3-5 mg/kg/day, 5-10 mg/kg/day, 10-20 mg/kg/day, 20-30 mg/kg/day, 30-40 mg/kg/day, 40-60 mg/kg/day, 60-80 mg/kg/day, 80-100 mg/kg/day, 100-200 mg/kg/day, 200-500 mg/kg/day, or 500-1,000 mg/kg/day.

[0071] In some specific embodiments, the subject of the method may be a mammal, preferably a human.

[0072] In the present disclosure, the term “identity” is generally a relationship between the sequences of two or more polypeptides or polynucleotides, as determined by comparing the sequences. In the art, the identity also means the degree of sequence correlation between them, as determined by the number of matches between series sections of two or more amino acid residues or nucleotide residues. The identity measures the percentage of identical matches between two or more sequences compared to a smaller sequence, wherein gap alignments are processed by a particular mathematical model or computer program (e.g., an “algorithm”). The identity of the related peptides may be readily calculated by a known method. When applied to a polypeptide or polynucleotide sequence, “identity%” is defined as the percentage of residues (amino acid residues or nucleic acid residues) in a candidate amino acid or nucleic acid sequence that are identical to the residues in an amino acid sequence or nucleic acid sequence of a second sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity. Methods and computer programs for alignment are well-known in the art. It should be understood that identity depends on the calculation of percent identity but can differ in value due to gaps and penalties introduced in the calculation. Generally, variants of specific polynucleotides or peptides have at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% but less than 100% of sequence identity with that specific reference polynucleotides or peptides, as

determined by sequence alignment programs and parameters described herein and known to those skilled in the art.

[0073] In the present disclosure, the term “phosphorylation” generally refers to a process in which a phosphate group is added to a specific amino acid residue of a protein in an enzymatic reaction, such as serine, threonine, tyrosine or histidine. Such modifications may result in an increase or decrease in the activity of the protein or alter its interaction with other molecules.

[0074] The following contents describe implementations of the present disclosure through specific embodiments, and those skilled in the art can easily understand other advantages and effects of the present disclosure from the contents disclosed in this description. The present disclosure can also be implemented or applied through other embodiments. On the basis of different viewpoints and applications, various modifications or amendments can be made to various details of the description without departing from the spirit of the present disclosure.

[0075] Before further describing the specific embodiments of the present disclosure, it should be understood that the protection scope of the present disclosure is not limited to the following specific implementations; it should also be understood that the terms used in the examples of the present disclosure are intended to describe specific implementations and not to limit the protection scope of the present disclosure; and in the description and claims of the present disclosure, unless otherwise explicitly indicated, the singular forms "a", "one" and "this" include plural forms.

[0076] When numerical ranges are given in the examples, it should be understood that, unless otherwise specified in the present disclosure, two endpoints of each numerical range and any value between the two endpoints may be selected. Unless otherwise defined, all technical and scientific terms used in the present disclosure have the same meaning as commonly understood by those skilled in the art. In addition to the specific methods, devices and materials used in the examples, the present disclosure may be realized by further using any method, device and material that is similar or equivalent to the methods, devices and materials described in the examples of the present disclosure according to grasp of the prior art by those skilled in the art and the record of the present disclosure.

[0077] The specific nucleotide or amino acid sequences used in this application are as follows:

[0078] SEQ ID NO. 1 (M7CK sequence):

[0079] atgtctcttctcaaggtgatcgggaaagtaataatcctttctttgtaatatatttatgaaagatgaaaaagaccccaatataat  
ctgtttggacaagatttggcctgatacccccagagaaaaaattcaacattccagaggctggttctcctgtggtgccttattcccaagtgtgtttct  
ccccagaattacgacagagacgacatggggtagaaatgttaaaaatatttaaaaaatcaaaaattaggcagttcacctaatagttcaccacata  
tgtcctccccaccaaccaaattttctgtgagtagcccatccagccaagttgcaaaagtcacttggaatccacaaccaaagatcaagaacccattt  
ctataaagctgcagaaggggataacatagaatttggagcatttggggacacagagatagtaggacttacagaggtttaagaaacatcaaaca  
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ctcgggtgcaagaaagctgtagtagaagagcgtcgacggaagactctccagaagtcgattctaaagcagctttgtaccggattggttacgagata  
gaccatcaaacagagaaatgccatctgaaggagggaacattaaatggcttctcctcatttaagcccgtttggatacaaaattactatttcagctg  
tggaagaaataacctgatgaggtgtcacagagtagtcccttctgtacccacagaggcgagcctgtcacagtgtaccgtctggaggaga  
gttctcccagtatactgaataacagcatgtcttcatggctcagctaggcctctgtgccaaaattgagttttaagtaaaggagaaaggagggtgtt  
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[0080] SEQ ID NO. 2 (pAAV2/9-M7CK plasmid sequence)

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**[0082]** SEQ ID No. 3

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 LASPFKPVLDTNYYYSAVERNLMRLS QSIPFVPVPPRGEPVTVYRLEESSPSILNNSMSSWS  
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 KYNNNNGDEIIPNTLEEIMLAFSHWTYEYTRGELLVLDLQGVGENLTDPSVIKAE EKRS CD  
 MVFGPANLGEDAIKNFRAKHHCNSSCCRKLKLPDLKRNDYTPDKIIFPQDESSDLNLQSGNST  
 KESEATNSVRLML

**[0084]** Example 1

**[0085]** The expression level of TRPM7 in the hippocampus of patients with Alzheimer's disease and mice in transgenic models of Alzheimer's disease (5XFAD and APP/PS1) was significantly reduced.

**[0086]** The inventors of the present application observed that the expression level of a TRPM7 protein in the hippocampus of patients with Alzheimer's disease was obviously reduced compared with that of a control group by using a western blotting method. Further, a significant reduction in the expression level of TRPM7 was observed in the hippocampus of 5XFAD mice at 12 months of age and APP/PS1 mice at 15 months of age compared to the same-age wild-type mice by using the same method as shown in FIGs. 1A-1C.

**[0087]** The method used in this example was as follows:

**[0088]** The hippocampal sample of the patients with Alzheimer's disease used in this example was from the National Health And Disease Human Brain Tissue Resource Center (China Human Brain Center, School of Medicine, Zhejiang University). The 5XFAD and APP/PS1 mice (purchased from Nanjing university&Model Animal Research Center, Nanjing Biomedical Research

Institute) were widely used mouse models with Alzheimer's disease. In this example, the method of western blotting analysis was as follows:

**[0089]** Cryopreserved hippocampal tissues of the patients with Alzheimer's disease, fresh hippocampal tissues of the 5XFAD and APP/PS1 mice, and hippocampal tissues of the control group were taken, placed in EP tubes, proteins were extracted by using a RIPA lysate (100  $\mu$ L lysate/10 mg tissue) containing a phosphatase inhibitor and a protease inhibitor, and protein concentration was quantified by using a BCA kit. A sampling buffer solution was added into the protein and the protein was fully denatured at 100°C for 5 min. Sampling was performed on a 10% SDS-PAGE gel in the amount of 20  $\mu$ g. Electrophoresis was started for 30 min at 80 V on an upper layer glue and 50 min at 120 V on a lower layer glue. A PVDF membrane was cut into the same size as the SDS-PAGE gel and put into methanol for activation for several seconds, then the gel blocks and the PVDF membrane were clamped between multiple layers of filter paper of a transfer clamp in a membrane transfer liquid after precooled at 4°C (the gel faced a black cathode and the membrane faced a red anode) to perform membrane transfer at a constant pressure of 100 V for 70 min. After the membrane transfer was completed, the membrane was blocked in a blocking solution made of 5% skim milk powder for 2 h and then transferred to a primary antibody (TRPM7 antibody, Alomone, ACC-047, 1:750; GAPDH antibody, Proteitech, 1:10, 000) diluted with the blocking solution for overnight incubation, and the membrane was washed three time with PBST, incubated with a secondary antibody for 1.5 h and was washed three times with PBST. A freshly prepared ECL developing solution was dripped for developing in a Tanon developing instrument, proper exposure time was selected and pictures were stored. The strips were subjected to an optical density quantitative analysis by using an Image Pro Plus software. The relative expression level of TRPM7 was calculated by using GAPDH as an internal control.

**[0090] Example 2**

**[0091]** An AAV2/9-M7CK adeno-associated virus capable of efficiently over-expressing M7CK at positions 1299-1863 of TRPM7 was constructed.

**[0092]** Vector construction:

**[0093]** 1) Mouse hippocampal cDNA was taken as a template and Forward-ggatccatgtctcttctcaaggtgat and Reverse-accggtcgtaacatcagacgaacagaa were taken as primers (two ends of the primers included two enzyme cutting sites BamHI and AgeI and

corresponding protection bases) for cloning the 1299th-1863rd sequence of TRPM7. The PCR reaction system was as follows:

[0094] PRIME STAR MAX 12.5  $\mu$ L

[0095] Primers F/R 1  $\mu$ L/1  $\mu$ L (0.2  $\mu$ M)

[0096] cDNA 100 ng

[0097] ddH<sub>2</sub>O supplemented to 25  $\mu$ L

[0098] PCR reaction conditions:

[0099] 98°C for 5 min

[0100] 98°C for 10 s

[0101] 55°C for 15 s 35 cycles

[0102] 72°C for 30 s

[0103] 72°C for 10 min

[0104] 2) After the PCR reaction, the PCR product was subjected to electrophoresis by using 1% agarose gel, a strip of about 1.7 kb was cut, and the M7CK fragment was collected according to instructions of a gel recovery kit: the gel at the position of the target strip was cut in a nucleic acid developer, collected in an EP tube and weighed, a PN solution (1  $\mu$ L/mg gel block) was added, the gel was dissolved in water bath at 50°C and shaken continuously until the gel was completely dissolved; 500  $\mu$ L of a BL solution was added into an adsorption column in advance at 12,000 rpm for 1 min, and waste liquid in a collection tube was discarded; the completely dissolved gel block solution was restored to room temperature, added into the adsorption column, stood at room temperature for 2 min and centrifuged at 12,000 rpm for 1 min, and waste liquid was removed; 600  $\mu$ L of PW was added, the solution was stood for 4 min and centrifuged at 12,000 rpm for 1 min, the waste liquid was discarded, and the step was repeated twice; the residue was subjected to air centrifugation at 12,000 rpm for 2 min and then air-dried for 9 min while the cover was opened; the adsorption column was placed into a new EP tube, 30  $\mu$ L of ddH<sub>2</sub>O was added, the solution was stood for 2 min and centrifuged at 12,000 rpm for 2 min, and the collected liquid was loaded on the column again and centrifuged at 12,000 rpm for 2 min; and the concentration and purity of the resulting DNA fragment were measured by using a photometer.

[0105] 3) The M7CK fragment and a vector pAAV2/9-EGFP (AAV2/92/9) were cut and the system was as follows:

- [0106] Plasmid 3  $\mu\text{g}$  or M7CK fragment 1  $\mu\text{g}$
- [0107] BamHI 1  $\mu\text{L}$
- [0108] AgeI 1  $\mu\text{L}$
- [0109] 10X CutSmart Buffer 5  $\mu\text{L}$
- [0110] dd H<sub>2</sub>O supplemented to 50  $\mu\text{L}$
- [0111] 37°C water bath for 1-2 h
- [0112] 4) The enzymes were inactivated in 65°C water bath for 20 min, a sampling buffer was added, the solution was subjected to agarose gel electrophoresis for 30 min at 120 V, and the gels of the fragment and vector of corresponding size were recovered.
- [0113] 5) Ligation
- [0114] 10X ligase buffer 2  $\mu\text{L}$
- [0115] Vector: 50 ng
- [0116] T4 ligase 1  $\mu\text{L}$
- [0117] Target gene fragment (~50 ng/ $\mu\text{L}$ ): supplemented to 20  $\mu\text{L}$
- [0118] 6) Ligation was performed overnight at room temperature (about 25°C).
- [0119] 7) Transformation: 5  $\mu\text{L}$  of the ligation product was added into 50  $\mu\text{L}$  of DH5 $\alpha$  competent cells just taken out from a refrigerator at -80°C and stood on ice for half an hour; the competent cells were subjected to heat shock in 42°C water bath for 45 s and immediately transferred on ice for 2 min, this operation is required to be mild and a centrifugal tube cannot be shaken; then 900  $\mu\text{L}$  of an antibiotic-free LB culture medium was added and the solution was shaken for one hour at 37°C and 150 rpm to resuscitate the bacteria; the solution was centrifuged at 4,500 rpm at room temperature for 5 min, 900  $\mu\text{L}$  of the supernatant was discarded, 100  $\mu\text{L}$  of the remaining supernatant and the bacterial precipitate on the bottom were pipetted to resuspend and poured onto a flat plate containing ampicillin, a glass rod was sterilized with an alcohol burner and restored to room temperature, and evenly spread the bacterial liquid, the flat plate was placed in a 37°C incubator until the liquid was absorbed, the flat plate was inverted, and the bacteria were cultured overnight.
- [0120] 8) On the next day, single colonies were picked up using a 10  $\mu\text{L}$  pipette tip, added to a liquid medium containing ampicillin, and shaken at 220 rpm and 37°C for 8-12 h. Then 500  $\mu\text{L}$  of the bacterial liquid was taken and sent to sequencing (Sangon). If the sequencing was correct, the

nucleotide sequence of the plasmid was shown as SEQ ID No. 2 and the structure was shown in FIG. 16. The corresponding bacterial liquid was added into a 300-mL culture flask for amplification culture for 12 h and then the plasmid was extracted according to the plasmid extraction steps (Vazyme, #DC202-01): the overnight cultured bacterial liquid was transferred into a 50-mL centrifuge tube and centrifuged at 11,000 rpm for 2 min, and the supernatant was discarded; 7.5 mL of a P1 solution was added into the bacterial precipitate and evenly mixed by vortex oscillation; 7.5 mL of a P2 solution was continuously added and mixed evenly by gently turning upside down, and the solution was stood at room temperature for 5 min; 7.5 mL of a P4 solution was added, the solution was immediately turned upside down for about 8 times, a white flocculent precipitate generated at this time, and the solution was centrifuged at 11,000 rpm for 10 min, and the supernatant (avoiding sucking the white precipitate) was sucked to a new 50-mL round bottom tube; an endotoxin-removing agent at the volume of 0.1 time was added, the solution was mixed evenly by turning upside down, the solution was stood on ice for 5 min and at room temperature for 10 min, and mixed evenly by turning upside down; the solution was centrifuged at 11,000 rpm and at room temperature for 10 min, the upper layer water phase (containing DNA) was taken in a new tube, isopropanol at the volume of 0.5 time was added, and the solution was transferred into the adsorption column in multiple times and centrifuged at 11,000 rpm for 1 min, and the waste liquid in the collection tube was discarded; the residue was washed by using 10 mL of a rinsing liquid PW and the solution was centrifuged at 11,000 rpm for 1 min, the waste liquid was discarded, and the operation was repeated twice; the residue was subjected to air centrifugation at 11,000 rpm for 3 min; the adsorption column was put into a new centrifuge tube, 1 mL ddH<sub>2</sub>O was added in the middle of the membrane, and the solution was stood for 3 min at room temperature and centrifuged at 11,000 rpm for 3 min; and the concentration and purity of the resulting plasmid were measured by using a photometer.

**[0121]** AAV2/9 virus packaging: 293 cells (six 15-cm dishes) transfected (VigoFect transfection reagent) with helper and target plasmids 60 hours were taken out from an incubator, the culture solution was removed, the cells were washed once by gently adding PBS, then fresh PBS (25 mL/dish) was added and the cells were collected in three 50-mL tubes by using a cell scraper; the cells were centrifuged at 400 g at room temperature for 10 min; the supernatant was removed, 10 mL of a salt solution (150 mM NaCl and 20 mM Tris-HCl) was added into each tube of the

precipitate, the cell precipitate was shaken and resuspended, and collected in a 50-mL tube; 1.5 mL of 10% sodium deoxycholate (prepared when used and working concentration of 0.5%) was prepared and all added into 30 mL of the cell suspension, a Benzonase nuclease (working concentration of 50 U/mL) was added, and the cells were lysed in water bath at 37°C for 2 h; the lysed cells were centrifuged at 3,000 g and 4°C for 15 min, the supernatant was taken, and cell debris was filtered by using a 0.45- $\mu$ m filter membrane; then a heparin tube (taken from the refrigerator 2 h in advance and placed under ultraviolet at room temperature) was connected to a 50-mL syringe and pre-equilibrated with 10 mL of a saline solution (150 mM NaCl and 20 mM Tris-HCl), and air bubbles in the tube were avoided; the liquid containing the viruses (30 mL in total) was sucked by a syringe, a syringe pump was adjusted to 0.7 mL/min, and the viruses were subjected to column chromatography; after the viruses were subjected to column chromatography, the column was rinsed by sucking 20 mL of a saline solution (100 mM NaCl and 20 mM Tris-HCl) by using a syringe; the column was rinsed by sucking 1 mL of a saline solution (200 mM NaCl and 20 mM Tris-HCl) using a 5-mL syringe; then the column was rinsed by sucking 1 mL of a saline solution (300 mM NaCl and 20 mM Tris-HCl); the viruses were eluted by sucking 1.5 mL of a saline solution (400 mM NaCl and 20 mM Tris-HCl), 3 mL of a saline solution (450 mM NaCl and 20 mM Tris-HCl) and 1.5 mL of a saline solution (500 mM NaCl and 20 mM Tris-HCl), and the liquid was collected in a 15-mL tube; the viruses were filtered with a 0.22- $\mu$ m filter membrane, the liquid was added into an Amicon Ultra 4-mL filter, centrifuged at 2,000 g and 4°C for 2 min until the liquid in the tube is less than 200  $\mu$ L, washed with 3.5 mL of PBS for 2 times, 5  $\mu$ L of the liquid was subpackaged each tube and stored at -80°C; the virus titer was quantified by using real-time quantitative PCR and the viruses were diluted to approximately  $3\text{-}5\times 10^{12}$  genomes/mL; and the viruses were added to primary neurons or injected into the mouse brain to verify the infection efficiency.

**[0122] Example 3**

**[0123]** The M7CK at positions 1299-1863 of TRPM7 over-expressed by primary hippocampal neurons had a protective effect on synaptic toxicity induced by A $\beta$ .

**[0124]** AAV2/9-M7CK or AAV2/9-EGFP was added by the inventors of the present application when the primary hippocampal neurons were cultured in vitro to the 7th day and A $\beta$  was added on the 12nd day. After 48 h, through an immunofluorescence staining technology, the synaptic

density marked by synaptophysin and PSD95 was observed to be remarkably reduced in an A $\beta$  group and significantly recovered in an A $\beta$  group over-expressing the M7CK (FIGs. 2A-2B). Therefore, over-expressing the M7CK had a protective effect on synaptic toxicity induced by A $\beta$ .

[0125] The method used in this example was as follows:

[0126] Culture of primary hippocampal neurons: young mice within 3 days of birth were taken, the heads were quickly cut off, the brain tissues were stripped off and placed in a dissection buffer on ice, the hippocampal tissues at two sides were carefully separated out under a stereoscope, and blood cells on the surfaces of the hippocampal tissues were stripped off as much as possible; after the dissection was finished, the hippocampus tissues were cut into pieces while tearing was avoided; the cut hippocampus tissues were washed with the dissection buffer for three times; a pancreatin lysate was added, the tissues were shaken gently for 1-2 times at 37°C for 5 min during the period and washed with the dissection buffer once; a dissociation solution containing a DNA enzyme was added and the tissues were repeatedly pipetted with a pipette tip until no obvious tissue block existed; the obtained solution was centrifuged at 4°C and 1,500 rpm for 15 min; the supernatant was discarded, the precipitate was resuspended (400  $\mu$ L culture medium/pair hippocampus) in a plating culture medium, and a cell suspension was added to a slide at the bottom of a four-well plate (100  $\mu$ L cell suspension/1.8-mm diameter well and the slide required a pre-coating treatment with matrigel (matrigel diluted 1:100 with buffer) at 37°C for 1 h). After cultured for 1.5-2 h in a constant-temperature incubator, the cells basically adhered to the wall, at this time, 1 mL of the plating culture medium/well was added, the culture medium was changed into a serum-free culture medium after two days, and half of the culture medium was changed at an interval of 3-4 days.

[0127] Cellular immunofluorescence staining and quantification of neuronal synaptic density: the neurons cultured in the four-well plate (16-mm wells and a cell-growing slide paved at the bottom) were taken out from an incubator, the culture solution was discarded, the neurons were quickly washed with PBS once gently, and then 1 mL/well of 4%PFA was added for fixing for 45 min; the neurons were washed with PBS for three times with 5 min/time; the neurons were blocked for 2 h on a shaker at room temperature using 5% goat serum diluted with 0.2% PBS-Triton at 0.2 mL/well; a primary antibody was diluted to desired concentration (synaptophysin, SYSY, 101011, 1:500; and PSD95, SYSY, 124003, 1:200) with a blocking liquid and the neurons were incubated

overnight in a refrigerator at 4°C; the neurons were washed with PBS for three times with 5 min/time; the neurons were incubated 2 h at room temperature with a fluorescent secondary antibody diluted with 0.2% PBS-Triton on a shaker; the neurons were washed with PBS for three times with 5 min/time; the cell-growing slide was carefully clamped out from the bottom of the well with forceps, covered on a slide dripped with a mounting medium (containing dapi dye), and after the mounting medium was dried for 10 min, the cell-growing slide was carefully coated with nail polish. The cell-growing slide was air-dried. After immunofluorescent staining of the in-vitro cultured neurons was completed, the slide was placed under a confocal microscopy and shot using a 60× oil immersion lens (digital magnification 3 times at the same time). During the shooting, the body of the neurons was arranged at the edge of a visual field and then synaptophysin or PSD-95 signals on dendrites were shot with the shooting thickness of 7 μm and the interval of 1 μm/layer. The number of synaptophysin/PSD-95 puncta on the dendrites 10 μm away from the cell body was selected for counting and the number of the puncta on the dendrites per 10 μm was taken as the dendrite density.

**[0128] Example 4**

**[0129]** A substance promoting the cleavage of TRPM7 to produce M7CK was identified and its activity and specificity were studied.

**[0130]** 80,000 compounds were screened in this example by using a high throughput screening platform and four compounds/regulators (resveratrol, zafirlukast, TPCA-1 and ATT) were identified to be capable of increasing the activity of a TRPM7 channel. To confirm the effects of these regulators, this example studied their effects on a current of the TRPM7 channel by using a whole-cell patch clamp technology in TRPM7-transfected HEK cells. Once the stable TRPM7 current was obtained, this example used these four compounds by perfusion. The results were shown in FIGs. 17A-17F that these four compounds (resveratrol, zafirlukast, TPCA-1 and ATT: 30 μM) significantly increased the current amplitude of the TRPM7 (resveratrol:  $1.75 \pm 0.24$  fold,  $p = 0.015$ ; zafirlukast:  $1.44 \pm 0.12$  fold,  $p = 0.007$ ; TPCA-1:  $1.34 \pm 0.07$  fold,  $p = 0.002$ ; ATT:  $1.41 \pm 0.11$  fold,  $p < 0.001$ ) FIG. 17A). In addition, this example determined the median effective concentration (EC<sub>50</sub>) of these compounds (resveratrol: 28.1 μM; zafirlukast: 1.4 μM; TPCA-1: 9.0 μM; ATT: 15.7 μM, FIG. 17C). Thus, this example identified four substances that promote the cleavage of TRPM7 to produce M7CK and confirmed their ability to activate the TRPM7 channel. Next, this example

examined substances that have been determined to promote the cleavage of TRPM7 to produce M7CK. This example evaluated their effects on other TRPM ion channels, such as TRPM2, TRPM6 and TRPM8. With regard to the TRPM2, only the TPCA-1 showed an excitatory effect on the activity of an ion channel (FIG. 17D). With regard to the TRPM6, the resveratrol and zafirlukast activated the ion channel; and at the same time, the ATT inhibited the activity of the channel (FIG. 17E). With regard to the TRPM8, the resveratrol, zafirlukast and ATT inhibited the activity of the ion channel (FIG. 17F). Therefore, in the determined regulators, only ATT appeared to specifically activate the TRPM7; and at the same time, other determined regulators activated other TRPM ion channels. In contrast, the ATT may inhibit two TRPM ion channels, TRPM6 and TRPM8.

**[0131]** An electrophysiological experiment in this example specifically included the following steps: whole-cell patch clamp recording was performed at room temperature by using an EPC-10 amplifier and a Patch Master software (HEKA, Germany). The current was digitized at a sampling rate of 10 kHz and low-pass filtered at 2.0 kHz. Needle tubes were prepared from glass capillaries (World Precision Instruments, USA) with the electrode resistance between 2 M $\Omega$  and 4 M $\Omega$ . A needle tube solution free of Mg<sup>2+</sup> contained (in mM): 145 CsCl, 8 NaCl, 10 HEPES and 10 EGTA (adjusted to pH 7.2 with CsOH). A bath solution contained (in mM): 140 NaCl, 5 KCl, 2 CaCl<sub>2</sub>, 20 HEPES and 10 glucose (adjusted to pH 7.4 with NaOH). With the holding potential of 0 mV, a 300 ms voltage ramp injection from -100 mV to +100 mV was performed in a manner repeated every 5 s, and the TRPM7 current was recorded. When the current reached a steady state (no change in at least 30 ramps), the TRPM7 channel current at +100 mV and -100 mV were obtained.

**[0132]** In addition, this Example found that promoting neuronal activity by increasing the extracellular glutamate concentration (for 1 hr) promotes the cleavage of TRPM7 in a dose-dependent manner, as indicated by the gradual increase in the M7CK/TRPM7 ratio (FIG. 20A). The minimum effective glutamate concentration (40  $\mu$ M) was used to determine the time course of the cleavage process. This embodiment found that glutamate-induced cleavage started after 30 min of incubation with glutamate, peaked after 1 h, and returned to baseline after 3 h (FIG. 20B and FIG. 20C).

**[0133]** The items in FIGs. 17A-17F were specifically described as follows:

[0134] (FIG. 17A) Left figures: Quantitative analysis of the effect of increasing the TRPM7 channel current amplitude by the substances promoting the cleavage of TRPM7 to produce M7CK (resveratrol: n = 5, zafirlukast: n = 5, TPCA-1: n = 6 and ATT: n = 8). They were analyzed by using an unpaired t test (resveratrol and zafirlukast) and a Mann-Whitney test (TPCA-1 and ATT). Right figures: A representative current-voltage relationship of the substances promoting the cleavage of TRPM7 to produce M7CK.

[0135] (FIG. 17B) Representative time course of the substances promoting the cleavage of TRPM7 to produce M7CK (resveratrol, zafirlukast, TPCA-1 and ATT) for the TRPM7 channel.

[0136] (FIG. 17C) A dose-response curve of the substances promoting the cleavage of TRPM7 to produce M7CK (resveratrol, zafirlukast, TPCA-1 and ATT).

[0137] (FIG. 17D) Left figures: Quantitative analysis of the effect of the substances promoting the cleavage of TRPM7 to produce M7CK (resveratrol: n = 4, zafirlukast: n = 7, TPCA-1: n = 5 and ATT: n = 5) on the activity of the TRPM2 channel. They were analyzed by an unpaired t test. Right figures: a representative current-voltage relationship of the substances promoting the cleavage of TRPM7 to produce M7CK.

[0138] (FIG. 17E) Left figures: Quantitative analysis of the effect of the substances promoting the cleavage of TRPM7 to produce M7CK (resveratrol: n = 6, zafirlukast: n = 6, TPCA-1: n = 6 and ATT: n = 6) on the activity of the TRPM6 channel. The analytical methods were the Mann-Whitney test (resveratrol) and the unpaired t test (zafirlukast, TPCA-1 and ATT). Right figures: A representative current-voltage relationships of the effect of a TRPM7 agonist.

[0139] (FIG. 17F) Left figures: Quantitative analysis of the effect of the substances promoting the cleavage of TRPM7 to produce M7CK (resveratrol: n = 7, zafirlukast: n = 6, TPCA-1: n = 6 and ATT: n = 7) on the activity of the TRPM8 channel. They were analyzed by an unpaired t test. Right figures: a representative current-voltage relationship of the substances promoting the cleavage of TRPM7 to produce M7CK. All the recording experiments were performed in the transfected HEK cells.

[0140] \*P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001.

[0141] **Example 5**

[0142] The AAV2/9-M7CK adeno-associated virus constructed by the present disclosure can effectively restore the impaired learning and memory functions of 5XFAD mice, restore the synaptic density and reduce the A $\beta$  level.

[0143] The constructed AAV2/9-M7CK virus was injected into the bilateral hippocampus of 5XFAD mice at 5 months of age by using a mouse brain stereotaxic localization technology by the inventors of the present application. It was discovered that over-expressing M7CK can restore the impaired learning and memory functions of the 5XFAD mice, by behavioral analysis (novel object recognition, Y-maze and cognition wall tests, and the results were shown in FIGs. 3-5B) at 6 months of age, 10 months of age and 15 months of age. Compared with wild-type mice of the same age, the synaptic density marked by synaptophysin and PSD95 in the CA1 region of the hippocampus of the 5XFAD mice were significantly reduced, while over-expressing the M7CK can significantly restore the synaptic density, and the results were shown in FIGs. 6A-6B. A $\beta$  plaques within the hippocampus of the 5XFAD mice over-expressing the M7CK were significantly down-regulated and the results were shown in FIG. 7. In insoluble protein components of the hippocampus of the 5XFAD mice, over-expressing the M7CK significantly down-regulated the concentration of A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub>. In soluble protein components, over-expressing the M7CK significantly down-regulated the concentration of the A $\beta$ <sub>1-42</sub> and the results were shown in FIG. 8.

[0144] This example included the following specific steps:

[0145] AAV2/9-M7CK and AAV2/9-EGFP viruses (as controls) were prepared and respectively injected into the bilateral hippocampus of the WT and 5XFAD mice at 5 months of age. The experiments were divided into 4 groups: WT mice given AAV2/9-EGFP; WT mice given AAV2/9-M7CK; 5XFAD mice given AAV2/9-EGFP; and 5XFAD mice given AAV2/9-M7CK. One month after the injection was completed, the behavioral test was started. After the behavioral tests at 6/10/15 months of age were finished, these mice were perfused and the brains were cryosectioned.

[0146] Hippocampal brain stereotaxic localization injection: the mice were anesthetized with 2% isoflurane; after the mice were successfully anesthetized, the heads of the mice were firmly fixed by using ear rods and nose rods; the left, right, front and back were adjusted to enable the back sides of the brains of the mice to be positioned on the same horizontal plane; after the surface skin of the heads of the mice were wiped with alcohol and disinfected, the skin was cut with scissors to fully expose the skull, and surface bloodstains were wiped off with a cotton swab stained with iodophors;

and holes were drilled according to the position of the hippocampus in the brain atlas of the mice and the following coordinates. 2 holes were drilled on the left side and the right side respectively.

[0147] CA1: AP = -2.06 mm, ML =  $\pm 1.4$  mm, DV = 1.4 mm

[0148] DG: AP = -2.06 mm, ML =  $\pm 1.4$  mm, DV = 2.0 mm

[0149] CA3: AP = -2.06 mm, ML =  $\pm 2.25$  mm, DV = 2.1 mm

[0150] After the drilling, an injection needle was slowly inserted into the brain region corresponding to the coordinates, the viruses were started to be injected at the speed of 1 nl/s at the volume of 300 nl per point. After the injection, the needle was kept for 10 min and slowly leaved. The scalp was sutured and the mice were placed in a warm environment to wait for recovery from anesthesia.

[0151] Novel object recognition test: the mice were changed from being fed in one cage with multiple mice into one cage with one mouse in one week before the start of the test until the end of the test. A test device consisted of a colorless, tasteless and opaque box body with the top hung with a camera and the length, width and height of 50 cm, a computer connected with the camera and objects A, B and C. At 9:00 am on the first day of the test, a mouse was placed in a box (without any object) and allowed to explore freely for 10 min to adapt to the environment. Before the next mouse was placed in the box, the box body required to be cleaned with a 30% odorless diluted hand sanitizer to avoid the smell left by the last mouse to affect the behaviors of the next mouse. At 9:00 am on the second day of the test, the mouse was again placed in the same box (without any object) and allowed to explore freely for 5 min. At 9:00 am on the third day of the test, four same objects A were placed at equal intervals on the bottom of the box body, then the mouse was placed and allowed to explore freely for 5 min, and at this time, a tester observed and recorded the exploration times and time of each mouse on the four objects through the top camera. At 9:00 am on the fourth day, the object A was replaced with a novel object B, and the mouse was placed in the environment and allowed to explore for 5 min. This period was referred to as a 24-h memory test. During the memory test phase, novel object recognition index = number of explores of novel objects/number of explores of all objects $\times 100\%$ .

[0152] Cognition wall test: the test was performed by using a PhenoTyper model 3000 device from the Noldus company and a fully automated record analysis software. At 21:00 in the evening before the test, a mouse was put into a PhenoTyper cage to adapt for 11 h, fasted, but free to

water. At 8:00 a.m, a feeding device was communicated with the cage and placed in a learning box, about 10 grains of sugar beans were manually given to the mouse and then a video tracking software was started. In the process, the software tracked the shuttling times of the mouse in the learning box in real time and the sugar beans were awarded according to the following rules. A single sugar bean was given as a reward if the mouse entered the learning box from a left hole 5 consecutive times without interruption. If the mouse entered the left hole 24 out of 30, it can be considered that the mouse had reached a learning standard, i.e. 80% criteria. A learning curve of each group of mice can be drawn according to the required hole-entering times when the mice reached the 80% criteria. The number of the remaining sugar beans in the feeding device was counted before and after the test, and was compared with the feeding number automatically calculated by the software. Whether software or hardware faults occurred or not was checked in time.

**[0153]** Y-maze test: the mice were respectively placed in the middle of a Y-maze and allowed to explore freely for 8 min. The whole process was recorded as a video for archiving by using a camera. The order in which the mice entered three arms of the Y-maze (one time was recorded when the hind feet of the mice fully entered the arms) during this process was recorded. A spontaneous alternation (e.g., ABC/BCA/CAB, etc.) was recorded when the mice continuously entered into three different arms without repeating. Percent spontaneous alternation = number of combinations of successive entries into three different arms/total number of entries into the arms-2×100%. A larger percent indicated a better spatial memory ability of the mice.

**[0154]** Quantification of synaptic density of mouse brain slices: the mice were anesthetized with 2% isoflurane; after the mice entered a complete anesthesia state, four limbs of the mice were fixed on a foam board, the chest skin was cut open to fully expose the heart, the right auricle was cut open, and the needle tip of an injector was inserted into the left ventricle, which was perfused once with 20 mL of precooled normal saline firstly and then perfused with 20 mL of 4% PFA once. After the perfusion was finished, the brain tissues of the mice were carefully peeled off, soaked in 4% PFA overnight, transferred to 20% and 30% sucrose solutions in sequence until the brain tissues were dehydrated and sunk to the bottom of a tube; and the dehydrated brain tissues were embedded in an OCT embedding medium, placed in a freezing microtome, and started to perform frozen sectioning after the brain tissues were completely frozen on a freezing head. The slice thickness was 16 μm. The cut brain slices were stored in PBS, carefully stuck on a glass slide by using a brush, and

air-dried at room temperature for 2-4 h until the brain slices were firmly stuck on the glass slide; closed circles were coated around the brain slices with an immunohistochemical pen and air-dried for 30 min; the brain slices were washed in PBS for 3 times with 5 min/time; the brain slices were blocked in a 5% goat serum (in 0.2% Triton-PBS) for 2 h in a wet box at room temperature; a primary antibody (synaptophysin, SYSY, 101011, 1:500; PSD95, CST, 3450, 1:200) was diluted in a blocking liquid and the brain slices were incubated overnight at 4°C; the brain slices were washed in PBS for 3 times with 5 min/time; a secondary antibody was diluted in 0.2% Triton-PBS (1:500) and the brain slices were incubated at room temperature for 2 h; the brain slices were washed in PBS for 3 times with 5 min/time; a plate was mounted by using an anti-quenching mounting medium, a cover glass was covered, and the edge of the cover glass was coated with nail polish to fix the cover glass; and images were taken using a confocal laser microscope. After the brain slices were subjected to immunofluorescent staining, the slide was placed under the confocal microscope and photographed using a 60× lens (digital magnification 3 times at the same time). The shooting thickness was 9 μm at the interval of 1 μm per layer. The three layers of brightest signals in the photographed pictures were synthesized into a picture of a tiff format with a derived resolution of 1,024\*1,024. The derived pictures were subjected to puncta automatic counting by using Image Pro Plus: firstly, a picture was firstly subjected to a HiGauss enhanced filter twice; most typical puncta areas in the picture were measured and the appropriate threshold range (puncta greater than the maximum threshold will be segmented; puncta less than the minimum threshold will not be counted) was set; and finally, a measurement button was clicked to obtain the number of the puncta in the picture. Finally, according to the scale, the number of puncta contained in each 1,000 μm<sup>2</sup> was calculated to be used as the synaptic density index.

Immunohistochemical staining and quantification of amyloid plaques: the mouse brain slices soaked in PBS were carefully stuck on a glass slide by using a brush and air-dried for 2-3 h; circles were drawn around the brain slices and air-dried for half an hour; the brain slices were washed in PBS for 3 times; the brain slices were blocked using a 5% goat serum diluted by 0.3% PBS-Triton for 2 h in a shaker at room temperature at about 40 μL/brain slices; an Aβ antibody (CST, 9888S, 1:1,600) was diluted by a blocking liquid and the brain slices were incubated overnight at 4°C; the brain slices were washed three times with PBS with 5 min/time; a biotinylated secondary antibody (Vector Laboratories, PK-4000) was diluted by PBS at 1:200 and the brain slices were

incubated at room temperature for 30 min; the brain slices were washed in PBS for three times with 5 min/time; an ABC reagent (reagent A of 1:100; reagent B of 1:100, prepared half an hour in advance) was diluted with PBS and the brain slices were incubated at room temperature for 30 min; the brain slices were washed three times with PBS with 5 min/time; a DAB reagent (prepared when used) was added, the color development was observed (about 5-10 min) and the reaction was terminated by rinsing with running water; a plate was mounted by using a mounting medium, the edge was fixed, and a picture was taken by using an Olympus vs120 microscope under a 10× lens in a bright field; the picture was imported into the Image Pro Plus software, a hippocampal region was manually circled to be used as a region of interest (ROI), a uniform color-taking standard was set (to ensure that the color-taking standard can cover all A $\beta$  plaque signals), and the software automatically calculated the area occupied by the A $\beta$  signals and the optical density.

**[0155]** A $\beta$  ELISA: the soluble fraction of an A $\beta$  protein was extracted in the same method as described above for western blotting. 5 M guanidine hydrochloride (dissolved in 50 mM Tris-HCl, pH 8.0) was added into the precipitate after RIPA lysis and centrifugation, the precipitate was dissolved in a shaker at room temperature for 4 h, the solution was centrifuged at 12,000 rpm at 4°C for 30 min, at this time, almost no precipitate was seen, and the supernatant was taken as an insoluble fraction; a soluble fraction of the protein was diluted by 100 times and 50 times by using a dilution buffer in a kit for detecting A $\beta_{1-42}$  and A $\beta_{1-40}$  respectively; an insoluble fraction of the protein was diluted 20,000 times and 2,000 times respectively for detecting A $\beta_{1-42}$  and A $\beta_{1-40}$ ; the ELISA kit (Wako, 292-62301, 298-62401) and the related liquid were taken out from a 4°C refrigerator and restored to room temperature for a period of time; standard substances at a series of concentration and samples to be detected were respectively added into an ELISA plate (the total volume of the liquid in each well was 100  $\mu$ L), and 3 multiple wells for each unknown sample were respectively made; the ELISA plate was sealed with a sealing film and the samples were incubated overnight at 4°C; and all the liquid in the ELISA plate was discarded and washed 5 times with a rinsing solution (200  $\mu$ L/well). The liquid in the wells needed to be poured as completely as possible (the plate was patted on absorbent paper) during each washing; and an HRP-conjugated secondary antibody was added at 100  $\mu$ L/well. The samples were incubated for 1 h at the 4°C refrigerator; the secondary antibody in the ELISA plate was discarded, and the samples were washed 5 times with

the rinsing solution (200  $\mu$ L/well); 100  $\mu$ L/well of a TMB solution (the samples were ensured to be added into all the wells in short time as much as possible), and the samples were incubated at room temperature in a dark place for 30 min; a stop solution was added to stop the reaction and the light absorption value of 450 nm was read (the reading needed to be finished within 30 min after the stop solution was added); and standard curves were drawn according to the concentrations of the standard substances and the corresponding absorbance values, and thus the concentrations of the A $\beta$ <sub>1-42</sub> and the A $\beta$ <sub>1-40</sub> in the unknown samples were calculated.

**[0156] Example 6**

**[0157]** ATT treatment can protect memory, preserve synaptic density, and reduce A $\beta$  plaques in a 5XFAD AD mouse model.

**[0158]** This example investigated whether the treatment with the substances promoting the cleavage of TRPM7 to produce M7CK can prevent AD pathology in the 5XFAD mouse model. Pharmacological and high performance liquid chromatography studies showed that TPCA-1 cannot cross the blood-brain barrier, since no TPCA-1 or its metabolites can be detected from the brain tissue and cerebrospinal fluid samples of the treated mice. Therefore, ATT was selected for *in-vivo* studies in this example. This example prepared two groups of to-be-treated mice injected daily (i.p.) with two doses (50 mg/kg or 75 mg/kg) of ATT. Another group of control mice was provided to receive a similar treatment regimen using the equal amount of a vehicle. All the treatments were initiated when the mice were at 5 months of age and a behavioral test was performed after 1 month of treatments. When the mice were at 6 months of age, an open field test showed that the drug treatments did not affect the movement or exploratory activity of the mice (FIG. 18A). The nesting behavior of the 5XFAD mice treated by the vehicle was impaired. The nesting behavior of the mice treated by the low dose (50 mg/kg) of ATT was also affected. In contrast, the high dose (75 mg/kg) of ATT successfully rescued the nesting behavior of the mice (FIGs. 18B and C). The memory function was assessed by a novel object recognition test (NORT). This example found that the short-term memory was normal in the all groups, including the 5XFAD mice treated by the vehicle. However, the 5XFAD mice treated by the vehicle exhibited long-term memory impairment (FIG. 18D). The memory impairment was alleviated in the 5XFAD mice after one-month treatment with two doses (50 mg/kg and 75 mg/kg) of ATT (FIG. 18D). These research results indicated that the TRPM7 regulator ATT can address memory and other cognitive deficits of the 5XFAD mice. The

data indicated that the activation of TRPM7 can prevent learning and memory impairment in this mouse model.

**[0159]** This example further investigated whether the substances promoting the cleavage of TRPM7 to produce M7CK, namely ATT, can protect the synaptic density and reduce A $\beta$  plaques in the brain of the 5XFAD mice. The mice were perfused in this example and the brains were collected for histological analysis. This example showed that the synaptophysin puncta density of in a CA1 stratum radiatum of the hippocampus of the 5XFAD mice without ATT treatment was significantly reduced. The ATT treatment counteracted the reduction of the synaptic density in a dose-dependent manner. The synaptic density remained unchanged only at the dose of 75 mg/kg, and changed at the dose of 50 mg/kg (FIGs. 19A and B). In addition, both doses of the ATT treatment significantly reduced the A $\beta$  plaque density in the hippocampus and cortex in the brain sections of the 5XFAD mice (FIG. 19C). Therefore, the substance promoting the cleavage of TRPM7 to produce M7CK, namely ATT, can retain the synaptic density and reduce A $\beta$  plaque deposition in the 5XFAD mice. The data of this example showed that the activation of TRPM7 may mitigate AD pathology by protecting synapses and through a disease regulatory mechanism.

**[0160]** The steps of the behavioral experiment or histological staining in this example can be referred to other examples of the present disclosure that include the behavioral experiment or histological staining.

**[0161]** The detail description of FIGs. 18A-18D was as follows:

**[0162]** (FIG. 18A) Measurement of the total distance that the wild type (WT) mice and 5XFAD mice treated by the vehicle and ATT (50 mg/kg and 75 mg/kg) were free to move for 5 min in an open field. N = at least 10 mice per group, and the data was analyzed by a one-way analysis of variance and a Kruskal-Wallis test.

**[0163]** (FIG. 18B) Representative images of the nesting social behavior of the WT and 5XFAD mice at 6 months of age, wherein 5XFAD mice were respectively treated by the vehicle and ATT (50 mg/kg and 75 mg/kg).

**[0164]** (FIG. 18C) Nesting scores based on the nesting social behavior of the WT and 5XFAD mice at 6 months of age, wherein 5XFAD mice were respectively treated by the vehicle and ATT (50 mg/kg and 75 mg/kg). N = at least 10 mice per group, and the data was analyzed by a one-way analysis of variance and a Kruskal-Wallis test.

[0165] (FIG. 18D) The upper figure was a schematic diagram of an experimental design of a novel object recognition test. The lower figure showed the recognition index of the familiar and novel objects of the WT mice and 5XFAD mice, wherein 5XFAD mice were treated by the vehicle and ATT (50 mg/kg and 75 mg/kg), the left drawing corresponded to an NORT training period, the middle drawing corresponded to a short-term memory test two hours after the NORT training, and the right drawing corresponded to a long-term memory test twenty-four hours after the NORT training. N = at least 8 mice per group, and the data was analyzed by a two-way analysis of variance, followed by a Bonferroni post-test.

[0166] \*P < 0.05 and \*\*\*P < 0.001.

[0167] The detail description of FIGs. 19A-19C was as follows:

[0168] (FIG. 19A) Representative images of the synaptophysin puncta density of a stratum radiatum in a CA1 region of the wild type (WT) and 5XFAD mice at 6 months of age, wherein 5XFAD mice were treated by the drug and ATT (50 mg/kg and 75 mg/kg).

[0169] (FIG. 19B) Quantitative analysis of the synaptophysin puncta density of a stratum radiatum in a CA1 region of the WT and 5XFAD mice at 6 months of age, wherein 5XFAD mice were treated by the drug and ATT (50 mg/kg and 75 mg/kg). N = at least 22 brain sections of 6 mice per group, and the data was analyzed by a one-way analysis of variance, followed by a Bonferroni post-test.

[0170] (FIG. 19C) The upper figure was representative images of hippocampal and cortical amyloid plaques of the 5XFAD mice treated by the vehicle and ATT (50 mg/kg and 75 mg/kg). The lower figure was quantitative analysis of the hippocampal and cortical amyloid plaques of the 5XFAD mice treated by the vehicle and ATT (50 mg/kg and 75 mg/kg). N = at least 18 brain sections of 6 mice per group, and the data was analyzed by a one-way analysis of variance, followed by a Bonferroni post-test. At least three brain sections per mouse were used for immunohistochemical quantitation.

[0171] \*P < 0.05 and \*\*\*P < 0.001.

[0172] **Example 7**

[0173] In this example, the content of MMP14 in the WT and 5XFAD mice in example 4 was detected. It was observed that the expression level of the A $\beta$ -degrading enzyme MMP14 was up-regulated in the 5XFAD mice compared with the WT mice, and the expression level was not

affected by over-expressing M7CK. Compared with the 5XFAD-EGFP group, the phosphorylation level of MMP14 was significantly up-regulated in the brains of the 5XFAD-M7CK mice. The results were shown in FIG. 9. The results of protein co-immunoprecipitation in this example illustrated that MMP14 had an interaction with M7CK. Besides, when MMP14 coexisted with M7CK, the kinase activity color-rendering index was significantly higher of than that of MMP14 and M7CK present alone, and M7CK can bind and phosphorylate MMP14. The results were shown in FIGs. 10A-10C. Knock-out of TRPM7 in primary hippocampal neurons and HT22 cell line did not affect the expression level of MMP14 itself, but decreased the level of p-MMP14/MMP14. Therefore, M7CK had an important role in regulating the phosphorylation level of MMP14. The results were shown in FIGs. 11A-11B. An *in-vitro* A $\beta$  degradation experiment in this example showed that M7CK phosphorylated MMP14 to increase the efficiency of MMP14 in degrading A $\beta$ . When MMP14 was added alone to A $\beta$ , A $\beta$  degradation (about 40%) can occur. However, when MMP14+M7CK were added simultaneously to A $\beta$ , the degradation level of A $\beta$  was significantly increased (about 70%), i.e., M7CK promoted the degradation level of A $\beta$  by MMP14. The results were shown in FIG. 12. The experiment of knockdown of MMP14 in this example showed that M7CK no longer had a protective effect against the reduction in the synaptic density induced by A $\beta$  in the case of knockout of MMP14. Regardless of whether an exogenous A $\beta$  treatment was used, the knockout of MMP14 alone resulted in the reduction in the synaptic density, indicating MMP14 itself also had an important regulatory role in the synaptic function. Therefore, the loss of synapse protective effect of M7CK after the knockout of MMP14 was also likely due to the effect of the knockout of MMP14 itself on synapses. The results were shown in FIG. 13. In addition, the experiment of knockdown of MMP14 in this example also showed an increase in the A $\beta$  concentration of about 21% when MMP14 alone was knocked out and an increase in the A $\beta$  concentration of about 43% when TRPM7 alone was knocked out. When MMP14 was knocked out while M7CK was over-expressed, M7CK failed to reduce the A $\beta$  concentration level. The results indicated that the knockout of TRPM7 had a greater effect on the metabolic level of A $\beta$ . The results were shown in FIG. 14.

[0174] This example included the following specific steps:

[0175] construction of recombinant MMP14-EGFP vector

[0176] 1) Mouse hippocampal cDNA was used as a template and a Forward-CCGCTCGAGATGTCTCCCGCCCCTCGACC and a

Reverse-TGCTCTAGAGACCTTGTCCAGCAGCGAACG were used as primers (two ends of the primers included two restriction enzyme sites XhoI and XbaI and corresponding protection bases) for cloning a coding (CDS) region of MMP14. The PCR reaction system was as follows:

[0177] PRIME STAR MAX 12.5  $\mu$ L

[0178] Primers F/R 1  $\mu$ L/1  $\mu$ L (0.2  $\mu$ M)

[0179] cDNA 100 ng

[0180] ddH<sub>2</sub>O (used to make a final volume of 25  $\mu$ L)

[0181] PCR reaction conditions:

[0182] 98°C for 5 min

[0183] 98°C for 10 s

[0184] 55°C for 15 s 35 cycles

[0185] 72°C for 30 s

[0186] 72°C for 10 min

[0187] 2) After the PCR reaction, the PCR product was subjected to electrophoresis by using 1% agarose gel, a band of about 1.8 kb was excised, and the MMP14 fragment was collected according to instructions of a gel extraction kit:

[0188] a) the gel at the position of the target band was cut into small pieces in a nucleic acid developer, collected in an EP tube, and weighed, a PN solution (1  $\mu$ L/mg gel ) was added, the gel was placed in a water bath at 50°C and shaken continuously until the gel was completely dissolved;

[0189] b) 500  $\mu$ L of a BL solution was added into an adsorption column in advance, a centrifugation was performed at 12,000 rpm for 1 min, and waste liquid in a collection tube was discarded;

[0190] c) the solution containing the completely dissolved gel was heated to room temperature, added into the adsorption column, the obtained mixture was placed at room temperature for 2 min and centrifuged at 12,000 rpm for 1 min, and waste liquid was removed;

[0191] d) 600  $\mu$ L of PW was added, allowed to stand for 4 min, and centrifuged at 12,000 rpm for 1 min, the waste liquid was discarded, and the step was repeated twice;

[0192] e) the column was subjected to centrifugation at 12,000 rpm for 2 min to remove any remaining liquid and then air-dried at room temperature for 9 min with the lid was opened;

[0193] f) the adsorption column was placed into a new EP tube, 30  $\mu$ L of ddH<sub>2</sub>O was added, allowed to stand for 2 min and centrifuged at 12,000 rpm for 2 min, and the collected liquid was loaded on the column again and centrifuged at 12,000 rpm for 2 min; and

[0194] g) the concentration and purity of the resulting DNA fragment were measured by using a photometer.

[0195] 3) The MMP14 fragment and a vector Pegfp-N1 were digested with restriction enzyme and the system was as follows:

[0196] Plasmid 3  $\mu$ g or MMP14 fragment 1  $\mu$ g

[0197] XhoI 1  $\mu$ L

[0198] XbaI 1  $\mu$ L

[0199] 10X CutSmart Buffer 5  $\mu$ L

[0200] ddH<sub>2</sub>O (used to make a final volume of 25  $\mu$ L)

[0201] 37°C water bath for 1-2 h

[0202] 4) The reaction were inactivated in 65°C water bath for 20 min, a sampling buffer was added, the mixture was subjected to agarose gel electrophoresis for 30 min at 120 V, and the fragment and vector of corresponding size were recovered from the gel.

[0203] 5) Ligation

[0204] 10X ligase buffer 2  $\mu$ L

[0205] Vector: 50 ng

[0206] T4 ligase 1  $\mu$ L

[0207] Target gene fragment (~50 ng/ $\mu$ L): up to 20  $\mu$ L

[0208] 6) Ligation was performed overnight at room temperature (about 25°C).

[0209] 7) Transformation: 5  $\mu$ L of the ligation product was added into 50  $\mu$ L of DH5 $\alpha$  competent cells just taken out from a refrigerator at -80°C and incubated on ice for half an hour; the competent cells were subjected to heat shock in 42°C water bath for 45 s and immediately transferred on ice for 2 min, this operation is required to be mild and the centrifugal tube cannot be shaken; then 900  $\mu$ L of an antibiotic-free LB culture medium was added and the mixture was shaken for one hour at 37°C and 150 rpm to resuscitate the bacteria; the mixture was centrifuged at 4,500 rpm at room temperature for 5 min, 900  $\mu$ L of the supernatant was discarded, 100  $\mu$ L of the remaining supernatant and the bacterial precipitate at the bottom were pipetted for resuspension and

poured onto a flat plate containing ampicillin, a glass rod was sterilized with an alcohol burner and restored to room temperature and used to evenly spread the bacterial liquid, the flat plate was placed in a 37°C incubator until the liquid was absorbed, the flat plate was inverted, and the bacteria were cultured overnight.

[0210] 8) On the next day, single colonies were picked up using a 10 µL pipette tip, added to a liquid medium containing ampicillin, and shaken at 220 rpm and 37°C for 8-12 h. Then 500 µL of the bacterial liquid was sent for sequencing (Sangon). If the sequencing result was correct, the corresponding bacterial liquid was added into a 300-mL culture flask for amplification culture for 12 h and then the plasmid was extracted according to the plasmid extraction steps (Vazyme, #DC202-01):

[0211] a) the overnight cultured bacterial liquid was transferred into a 50-mL centrifuge tube and centrifuged at 11,000 rpm for 2 min, and the supernatant was discarded;

[0212] b) 7.5 mL of a P1 solution was added into the bacterial precipitate and evenly mixed by vortex oscillation;

[0213] c) 7.5 mL of a P2 solution was then added and mixed evenly by gently inverting the tube, and the solution was left at room temperature for 5 min;

[0214] d) 7.5 mL of a P4 solution was added, the tube was immediately inverted for about 8 times, a white flocculent precipitate generated at this time, and the mixture was centrifuged at 11,000 rpm for 10 min, and the supernatant (avoiding sucking the white precipitate) was transferred to a new 50-mL round bottom tube;

[0215] e) an endotoxin-removing agent at the volume of 0.1 time was added, the solution was mixed evenly by inversion, left on ice for 5 min and then at room temperature for 10 min, and mixed evenly by inversion;

[0216] f) the mixture was centrifuged at 11,000 rpm and at room temperature for 10 min, the upper layer water phase (containing DNA) was transferred to a new tube, isopropanol at the volume of 0.5 time was added, and the solution was transferred into the adsorption column in multiple times and centrifuged at 11,000 rpm for 1 min, and the waste liquid in the collection tube was discarded;

[0217] g) the column was washed by using 10 mL of a rinsing liquid PW and the obtained solution was centrifuged at 11,000 rpm for 1 min, the waste liquid was discarded, and the operation was repeated twice;

[0218] h) the column was subjected to centrifugation at 11,000 rpm for 3 min to remove any remaining liquid;

[0219] i) the adsorption column was put into a new centrifuge tube, 1 mL ddH<sub>2</sub>O was added in the middle of the membrane, allowed to stand for 3 min at room temperature and centrifuged at 11,000 rpm for 3 min; and

[0220] j) the concentration and purity of the resulting plasmid were measured by using a photometer.

[0221] Recombinant protein purification

[0222] 1) 1 mL of a glycerol strain expressing MMP-14-EGFP or M7CK was added to 1 L of an LB medium and shaken at 37°C and 220 rpm overnight. The amplified bacterial liquid was centrifuged for many times at 4,200 rpm and 4°C until the bacterial precipitate was concentrated in a single 50-mL tube.

[0223] 2) A bacterial lysate (50 mM Tris HCl pH 8.0, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 1 mM EDTA, 10 mM mercaptoethanol, 20% glycerol, 1 mg/μL lysozyme, 8 M urea, one tablet of a Roche protease inhibitor) was added into the bacterial precipitate and incubated on ice for 30 min.

[0224] 3) The mixture was centrifuged at 28,000 rpm and 4°C for 30 min.

[0225] 4) The supernatant was added to an amicon ultra 15 ML-30 K protein ultrafiltration centrifuge tube to be centrifuged at 4,200 rpm and 4°C for 60 min, and this operation was repeated several times until the remaining liquid was less than 1 mL. The liquid contained a concentrated protein and was used for a subsequent *in-vitro* enzyme activity test.

[0226] Protein co-immunoprecipitation (Co-IP)

[0227] 1) 293 cells transfected with M7CK and MMP14-EGFP for 48 hours were collected, the surface culture medium was washed away by PBS, and then the PBS was added into a culture dish and repeatedly pipetted using a pipette tip to enable adherent cells to be suspended in the PBS, so as to obtain a cell suspension. The cell suspension was centrifuged at 4°C and 12,000 rpm for 5 min, the supernatant was removed, an IP special RIPA buffer containing a protease inhibitor and a phosphatase inhibitor was added into the cell precipitate, then vigorously vortexed, and evenly mixed, so as to fully lyse cells, and later incubated on ice for 30 min. The mixture was centrifuged at 4°C and 12,000 rpm for 30 min and the supernatant was transferred into a new EP tube to obtain a total protein extraction solution.

[0228] 2) 50  $\mu$ L of evenly mixed magnetic beads were transferred into the 2-mL EP tube and placed on a magnetic frame, and the supernatant was removed.

[0229] 3) 200  $\mu$ L of an antibody binding solution was added to the magnetic beads, then an antibody M7CK (dilution ratio of 1:100) or MMP14 (dilution ratio of 1:200) or control group IgG was added, and the mixture was rotated at room temperature for 30-60 min.

[0230] 4) The EP tube was placed on the magnetic frame, the supernatant was discarded, and at this time, the antibody was firmly bonded on the magnetic beads.

[0231] 5) The antibody binding solution was added and evenly mixed by inversion, then the tube was placed on the magnetic frame, the supernatant was discarded, and the unbound antibody was washed away.

[0232] 6) The cell total protein extraction solution was added to the magnetic beads and subjected to rotary incubation at 4°C for 42-48 h.

[0233] 7) The tube was placed on the magnetic frame to remove the supernatant, a cleaning solution was added, the mixture was rotated at room temperature for 1 min, the supernatant was discarded on the magnetic frame, and this operation was repeated for three times.

[0234] 8) 20  $\mu$ L of an eluent was added to the magnetic beads, evenly mixed, and transferred to 600  $\mu$ L of an EP tube, then 5  $\mu$ L of a sampling buffer was added, and the solution was boiled at 100°C for 5 min.

[0235] 9) The relevant proteins in the solution was detected by western blotting.

[0236] In-vitro A $\beta$  degradation test:

[0237] MMP14 and M7CK recombinant protein were purified from *escherichia coli* by the above method. 80 nM MMP14/M7CK was mixed with 2  $\mu$ M A $\beta$ <sub>1-42</sub>, a zymogen buffer (50 mM tris HCl, 200 mM NaCl, 5 mM CaCl<sub>2</sub>, pH 7.5) and 10 mM ATP were added, and a total volume of the system was 50  $\mu$ L, and the system was incubated at 37°C for 10 h. The remaining A $\beta$  content was detected by using western blotting. Lower residual A $\beta$  content indicated higher degradation efficiency.

[0238] The specific experimental steps of knocking out TRPM7 by AAV-TRPM7 shRNA were as follows:

[0239] 1. Primary hippocampal neuronal culture

[0240] 1) newborn mice within 3 days of birth were taken, the heads were quickly cut off, the brain tissues were dissected and placed in a dissection buffer on ice, the hippocampal tissues at two sides were carefully separated out under a stereoscope, and blood cells on the surfaces of the hippocampal tissues were removed as much as possible;

[0241] 2) after the dissection was finished, the hippocampus tissues were cut into pieces while tearing was avoided;

[0242] 3) the cut hippocampus tissues were washed with the dissection buffer for three times;

[0243] 4) a pancreatin lysate was added and the tissues were incubated at 37°C for 5 min with gentle shaking for 1-2 times during the period;

[0244] 5) the tissues were washed with the dissection buffer once;

[0245] 6) a dissociation solution containing DNA enzymes was added and the tissues were repeatedly pipetted with a pipette tip until no obvious tissue block existed;

[0246] 7) the obtained mixture was centrifuged at 4°C and 1,500 rpm for 15 min; and

[0247] 8) the supernatant was discarded, the precipitate was resuspended (400 µL culture medium/pair hippocampus) in a plating culture medium, and the cell suspension was added to a slide at the bottom of a four-well plate (100 µL cell suspension/1.8-mm diameter well, and the slide required a pre-coating treatment with matrigel (matrigel was diluted with DM at a ratio of 1:100) at 37°C for 1 h). After cultured for 1.5-2 h in a constant-temperature incubator, the cells basically adhered to the wall, at this time, 1 mL of the plating culture medium/well was added, the culture medium was changed into a serum-free culture medium after two days, and half of the culture medium was changed at an interval of 3-4 days.

[0248] 2. A synthesized TRPM7-shRNA sequence (coding sequence 5153-5172 5'-AAT GCA TGA CTG GGG AAT TTC AAG AGA ATT CCC CAG TCA TGC at-3') was inserted into an AAV-CAG-mCherry plasmid, and the plasmid was packaged into an AAV virus.

[0249] 3. The AAV virus (1 µl/ml of a culture medium) was added when the primary hippocampal neurons of the mice were cultured to the seventh day.

[0250] 4. When the primary hippocampal neurons of the mice were cultured to the fourteenth day, the protein was extracted. It can be detected that compared with the control group, the expression amount of TRPM7 was significantly down-regulated.

[0251] The specific experimental steps of knocking down MMP14 were as follows:

[0252] 1. Primary hippocampal neuronal culture

[0253] 1) newborn mice within 3 days of birth were taken, the heads were quickly cut off, the brain tissues were dissected and placed in a dissection buffer on ice, the hippocampal tissues at two sides were carefully separated out under a stereoscope, and blood cells on the surfaces of the hippocampal tissues were removed as much as possible;

[0254] 2) after the dissection was finished, the hippocampus tissues were cut into pieces while tearing was avoided;

[0255] 3) the cut hippocampus tissues were washed with the dissection buffer for three times;

[0256] 4) a pancreatin lysate was added and the tissues were incubated at 37°C for 5 min with gentle shaking for 1-2 times during the period;

[0257] 5) the tissues were washed with the dissection buffer once;

[0258] 6) a dissociation solution containing DNA enzymes was added and the tissues were repeatedly pipetted with a pipette tip until no obvious tissue block existed;

[0259] 7) the obtained mixture was centrifuged at 4°C and 1,500 rpm for 15 min; and

[0260] 8) the supernatant was discarded, the precipitate was resuspended (400 µL culture medium/pair hippocampus) in a plating culture medium, and the cell suspension was added to a slide at the bottom of a four-well plate (100 µL cell suspension/1.8-mm diameter well and the slide required a pre-coating treatment with matrigel (matrigel was diluted with DM at a ratio of 1:100) at 37°C for 1 h). After cultured for 1.5-2 h in a constant-temperature incubator, the cells basically adhered to the wall, at this time, 1 mL of the plating culture medium/well was added, the culture medium was changed into a serum-free culture medium after two days, and half of the culture medium was changed at an interval of 3-4 days.

[0261] 2. A synthesized MMP14-shRNA sequence (#1 coding sequence 1026-1046, 5'-ACC GGG GGG GTG AGG AAT AAC CAA GTC TCG AGA CTT GGT TAT TCC TCA CCC GCT TTT T-3'. # 2 coding sequence 788-808, 5'-ACC GGG GAT GGA CAC AGA GAA CTT CGC TCG AGC GAA GTT CTC TGT GTC CAT CCT TTT T-3') was inserted into an AAV-CAG-mCherry plasmid, and the plasmid was packaged into an AAV virus.

[0262] 3. The AAV virus (1  $\mu$ l/ml of a culture medium) was added when the primary hippocampal neurons of the mice were cultured to the seventh day.

[0263] 4. When the primary hippocampal neurons of the mice were cultured to the fourteenth day, the protein was extracted. It can be detected that compared with the control group, the expression amount of MMP14 was significantly down-regulated.

[0264] It can be seen from examples 1-5 above that the expression level of TRPM7 was down-regulated in the hippocampus of the patients with AD and both AD mouse models; over-expressing the kinase domain of TRPM7 in the primary hippocampal neurons cultured *in vitro* can counteract the reduction in the synaptic density induced by A $\beta$ , whereas the channel domain of TRPM7 had no such protective effect. In addition, over-expressing M7CK in the hippocampus of the 5XFAD mice can relieve cognitive impairment, increase the synaptic density and reduce the accumulation of amyloid plaques induced by A $\beta$ . Meanwhile, M7CK further promotes the degradation of A $\beta$  in the soluble and insoluble components, and M7CK did not influence the generation process of A $\beta$ . Further studies showed that M7CK can promote the degradation process of A $\beta$  by binding to and activating MMP14, which to some extent explained the mechanism of a protective effect of M7CK on AD. The experimental results of the present disclosure indicated a neuroprotective effect of TRPM7. In the studies of the above examples, it was found that over-expressing the kinase segment of TRPM7 could counteract the synaptic toxicity of A $\beta$  by promoting A $\beta$  degradation, thus improving a cognitive function in the mice with AD. The deletion of TRPM7 may contribute to the pathogenesis of AD, while increasing the expression of a TRPM7 kinase may relieve the pathogenesis of AD. The above examples showed that the protein level and transcription level of TRPM7 in the hippocampus of the patients with AD were down-regulated, the expression level of TRPM7 in the hippocampus of 5XFAD and APP/PS1 mice was also reduced, and the kinase domain M7CK of TRPM7 can increase the synaptic density of the primary hippocampal neurons induced by A $\beta$ . Meanwhile, over-expressing M7CK in the hippocampus of the 5XFAD mice can improve the learning and memory ability of the mice and increase the density of presynaptic vesicle protein synaptophysin and postsynaptic density protein PSD-95. However, the channel domain of TRPM7 did not have this function. The above examples showed that the synaptic proteins of the synaptophysin and PSD-95 were reduced in AD models *in vivo* and *in vitro*.

[0265] MMP14, a member of the MMP family, is a specific MMP located on the membrane and has activity itself. The experimental results of the above examples showed that MMP14 had an interaction with M7CK, while MMP2 and MMP9 had no interaction with M7CK. Besides, when MMP14 coexists with M7CK, the activity of MMP14 was further improved, and the protective effect of M7CK against A $\beta$  toxicity also depended on MMP14 rather than MMP2 and MMP9. The above examples showed that MMP14 can degrade exogenous A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub>: under *in-vitro* suitable conditions, after the purified recombinant MMP14 protein was mixed and incubated with the A $\beta$  peptide fragment for 10 h, the A $\beta$  level was reduced by 50%, which was consistent with the literature report. The above examples showed that in the hippocampus of the 5XFAD mice at 6 months of age, the MMP14 protein level was higher than that of the wild-type mice in the same age, which indicated that merely increasing the MMP14 expression level was not sufficient to alleviate AD-related pathological features. However, over-expressing M7CK can promote degradation of A $\beta$ , increase the synaptic density, and improve the cognitive function of the 5XFAD mice by increasing the MMP14 phosphorylation level, indicating that regulating the MMP phosphorylation level had a more significant impact on relieving the AD pathology compared to regulating the MMP14 expression level, with no change in the production process of APP (APP,  $\beta$ CTF). In addition, the knock-out of MMP14 in the hippocampal primary neurons reduced the synaptic density, suggesting that the MMP14 itself played an important regulatory role in the synaptic function.

[0266] The above examples showed that over-expressing M7CK in the hippocampus of the wild-type mice did not enhance learning and memory ability and synaptic density, and was only effective in the 5XFAD mice. Therefore, an increase in the synaptic density by M7CK occurred under specific pathological conditions, rather than an indiscriminate overall enhancement. It was found by combining preliminary studies with the past published studies, M7CK has the possibility of generating different splicing processes under physiological and pathological conditions, thereby influencing a series of downstream molecular mechanisms. The results of the above examples showed that A $\beta$  accumulation did not affect the splicing process under *in-vitro* conditions. Furthermore, the above examples showed that M7CK, anchored to the cell membrane and free in the cytoplasm, both counteracted reduction in synaptic density induced by A $\beta$  in neurons treated by A $\beta$ , indicating that M7CK splicing did not affect its ability to counteract A $\beta$  toxicity under the pathological conditions of AD.

**[0267]** From the basic scientific point of view, the present disclosure will fill the major gap in the prior art for understanding the mechanism of action of the TRPM7 kinase domain in the central nervous system. This project will reveal new signaling pathways and mechanisms of TRPM7 kinase domain regulation. The present disclosure will demonstrate that TRPM7 uses physical and structural changes (cleavage and transport of peptides) rather than chemical modifications (phosphorylation) as triggers for the release and activation of kinases. Recently, structural changes of CaMKII rather than phosphorylation have been shown to trigger important effects on synaptic and cognitive functions. Therefore, the present disclosure is introducing entirely new concepts and discoveries that will expand the understanding of the prior art in cellular, molecular, and cognitive neuroscience. From the perspective of transformation science, the present disclosure combines computational, molecular, systematic, and biomedical research to achieve a transformation from basic science to transformation science. In the future, the new mechanisms are expected to be converted into potential drug targets so as to develop new drugs for treating neurodegenerative diseases (particularly Alzheimer's disease). In addition, the present disclosure provides the evidence for the feasibility of TRPM7 as a target for treating Alzheimer's disease. A novel gene therapy method for AD is provided: over-expressing the TRPM7 enzymatic domain mediated by viruses.

**[0268]** In conclusion, the present disclosure explores in detail the role that TRPM7 may play in the course of AD and the specific action mechanism thereof. The present disclosure provides a potential target with important value for the research and development of drugs for AD.

**[0269]** The above examples are to illustrate the embodiments disclosed in the present disclosure, which are not to be construed as limiting of the present disclosure. In addition, the various modifications listed herein and the changes of methods in the present disclosure are obvious to those skilled in the art without departing from the scope and spirit of the present disclosure. Although the present disclosure has been specifically described in conjunction with various specific preferred examples of the present disclosure, it should be understood that the present disclosure should not be limited to these specific examples. In fact, the various modifications that are obvious to those skilled in the art as described above shall be included within the scope of the present disclosure.

**What is claimed is:**

1. Use of a kinase domain M7CK of TRPM7 in the preparation of a drug for treating Alzheimer's disease.
2. The use according to claim 1, wherein the amino acid sequence of the M7CK is selected from the following:
  - 1) a sequence of a polypeptide encoded by the nucleotide sequence of SEQ ID No. 1; and
  - 2) a polypeptide fragment having the sequence identity of at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% and at least 99.5% to the polypeptide of 1) on the basis of having the same biological functions as the polypeptide of 1).
3. The use according to claim 2, wherein the sequence of the polypeptide of 1) comprises the amino acid sequence at positions 1299-1863 of TRPM7, preferably, the sequence of the polypeptide is shown in SEQ ID No. 3.
4. The use according to claim 1, wherein the drug for treating Alzheimer's disease comprises an M7CK accelerator.
5. The use according to claim 4, wherein the M7CK accelerator is selected from one or more of the following:
  - a plasmid or a virus effectively over-expressing M7CK; and/or
  - a substance promoting the cleavage of TRPM7 to produce M7CK.
6. The use according to claim 5, wherein the plasmid or viral vector over-expressing M7CK comprises a coding nucleic acid fragment of M7CK; or, the substance promoting the cleavage of TRPM7 to produce M7CK is selected from one or more of the following compounds: resveratrol, zafirlukast, TPCA-1, ATT, and glutamic acid or glutamate.

7. The use according to claim 6, wherein a coding gene of the M7CK has the sequence as shown in SEQ ID No. 1.
8. The use according to claim 6, wherein the coding gene of the M7CK is a nucleic acid fragment obtained by optimizing the nucleic acid molecule shown in SEQ ID No. 1 by any one or more of the following modes: a) compared with the nucleic acid molecule shown in SEQ ID No. 1, increasing the content of G/C; and b) codon-optimizing the nucleic acid molecule shown in SEQ ID No. 1, wherein after the codon-optimizing of the nucleic acid fragment or the content of G/C is increased, a sequence of an encoded amino acid is not altered.
9. The use according to claim 5, wherein the plasmid is selected from an adenovirus packaging plasmid, an adeno-associated virus packaging plasmid, and a lentivirus packaging plasmid; and the virus is selected from an adenovirus, an adeno-associated virus, and a lentivirus.
10. The use according to claim 5, wherein the plasmid is a pAAV2/9-M7CK plasmid, preferably, the pAAV2/9-M7CK plasmid has the sequence shown in SEQ ID No. 2.
11. The use according to claim 10, wherein the pAAV2/9-M7CK plasmid is used for virus packaging of an AAV to obtain a packaged AAV.
12. The use according to claim 11, wherein a use method of the drug for treating Alzheimer's disease comprises: injecting the packaged AAV into a brain by brain stereotaxic localization, specifically into an intracerebral hippocampus region.
13. The use according to any one of claims 1-12, wherein the drug for treating Alzheimer's disease has one or more of the following functions:
- 1) increasing neuronal synaptic density;
  - 2) improving learning and memory ability;
  - 3) reducing A $\beta$  plaques or A $\beta$  proteins; and

4) activating the activity of MMP14, preferably, phosphorylating the MMP14 protein.

14. A pAAV2/9-M7CK plasmid capable of over-expressing M7CK at sequence positions 1299-1863 of TRPM7 and constructed on the basis of a sequence of a kinase domain M7CK of TRPM7.

15. The pAAV2/9-M7CK plasmid according to claim 14, wherein the amino acid sequence of the M7CK is selected from the following:

1) a sequence of a polypeptide encoded by the nucleotide sequence of SEQ ID No. 1; and

2) a polypeptide fragment having the sequence identity of at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% and at least 99.5% to the polypeptide of 1) on the basis of having the same biological functions as the polypeptide of 1).

16. The pAAV2/9-M7CK plasmid according to claim 14, wherein the sequence of the polypeptide of 1) comprises the amino acid sequence at positions 1299-1863 of TRPM7, preferably, the sequence of the polypeptide is shown in SEQ ID No. 3.

17. The pAAV2/9-M7CK plasmid according to claim 14, wherein the pAAV2/9-M7CK plasmid comprises a coding nucleic acid fragment of M7CK.

18. The pAAV2/9-M7CK plasmid according to claim 17, wherein the coding nucleic acid fragment of M7CK is:

1) a sequence shown in SEQ ID No. 1; and

2) a nucleic acid fragment obtained by optimizing the nucleic acid molecule shown in SEQ ID No. 1 by any one or more of the following modes: a) compared with the nucleic acid molecule shown in SEQ ID No. 1, increasing the content of G/C; and b) codon-optimizing the nucleic acid molecule shown in SEQ ID No. 1, wherein after the codon-optimizing of the nucleic acid fragment or the content of G/C is increased, a sequence of an encoded amino acid is not altered.

19. A virus capable of over-expressing M7CK at sequence positions 1299-1863 of TRPM7 and constructed on the basis of a sequence of a kinase domain M7CK of TRPM7, wherein the virus is obtained by virus packaging of the pAAV2/9-M7CK plasmid according to any one of claims 14-18.
20. The virus according to claim 19, wherein the virus is selected from one or more of an adenovirus, an adeno-associated virus, and a lentivirus.
21. A method for preventing or treating Alzheimer's disease, wherein the method comprises administering to a subject an effective dose of the M7CK accelerator in the use according to any one of claims 4-13, the pAAV2/9-M7CK plasmid according to any one of claims 14-18 or the virus according to claim 19 or 20.
22. The method according to claim 21, wherein an administration amount is 1-1,000 mg/kg/day.
23. The method according to claim 21, wherein the subject of the method is a mammal, preferably, a human.

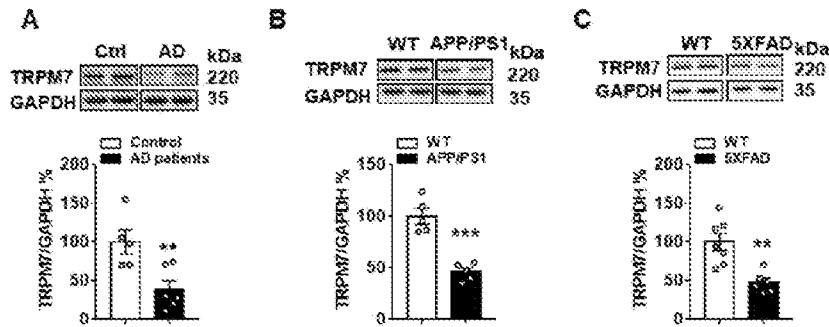


FIG. 1

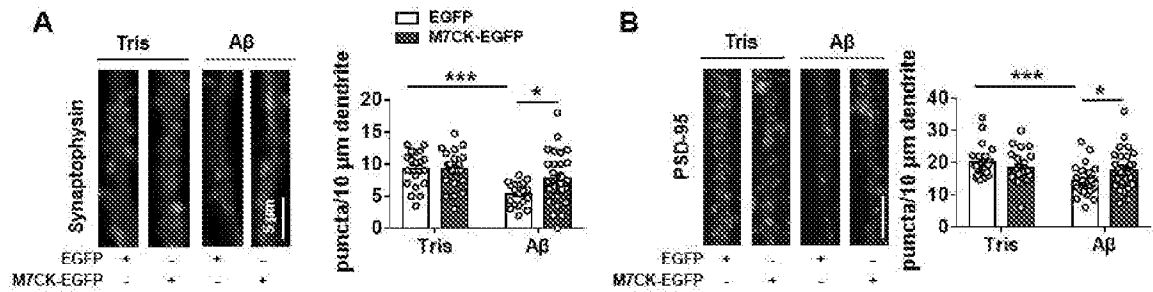


FIG. 2

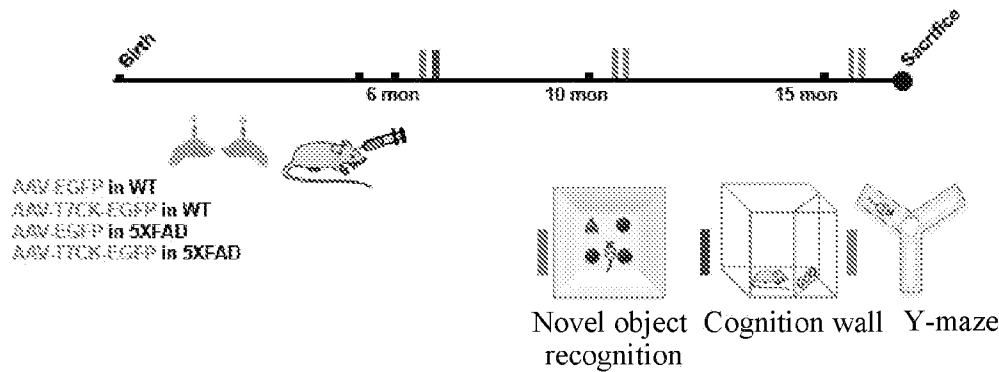


FIG. 3

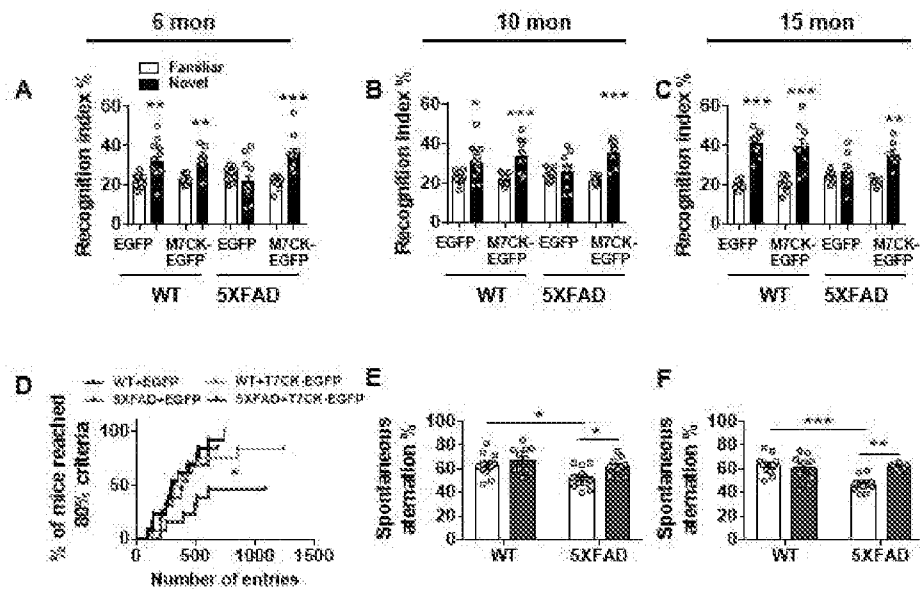


FIG. 4

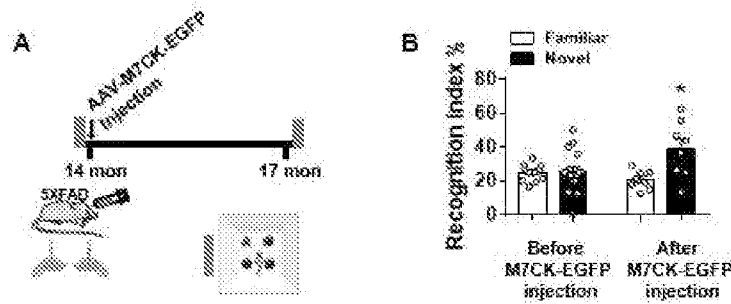


FIG. 5

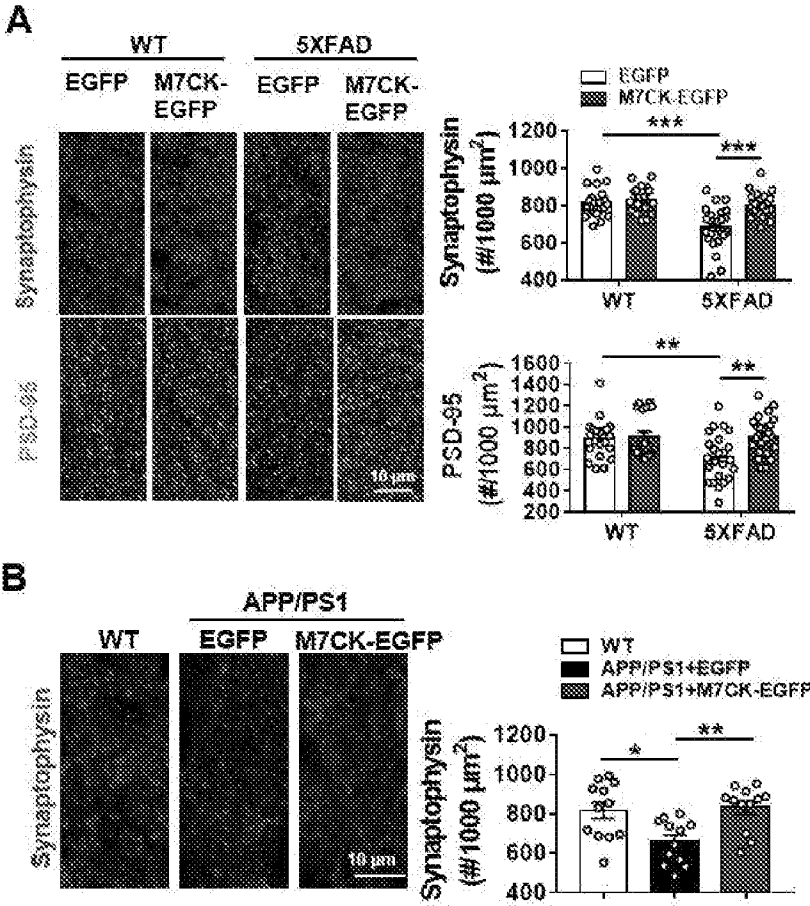


FIG. 6

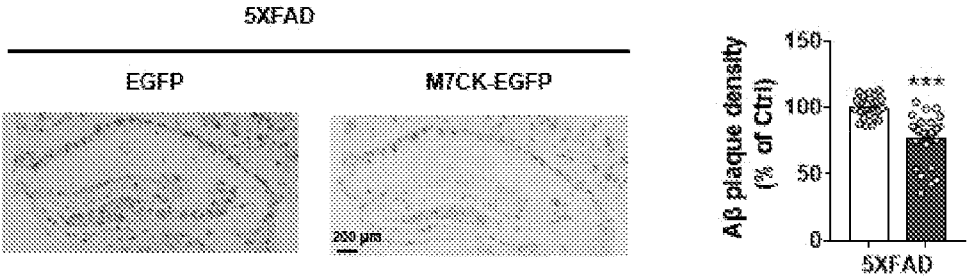


FIG. 7

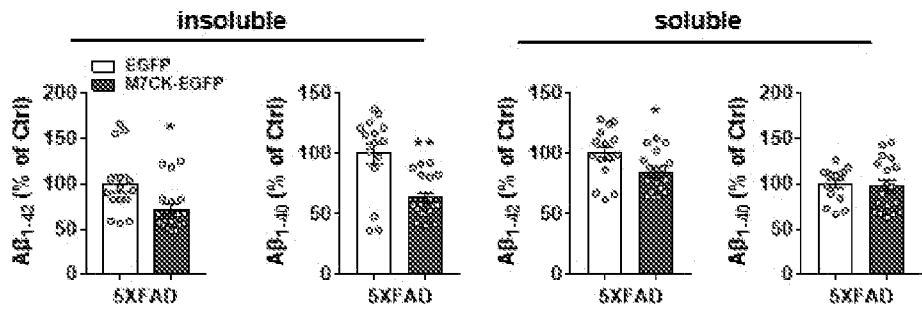


FIG. 8

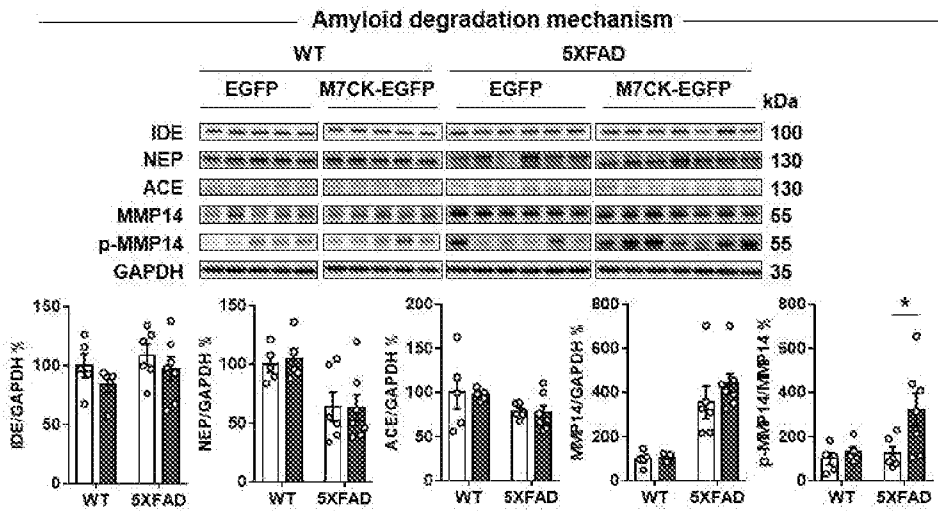


FIG. 9

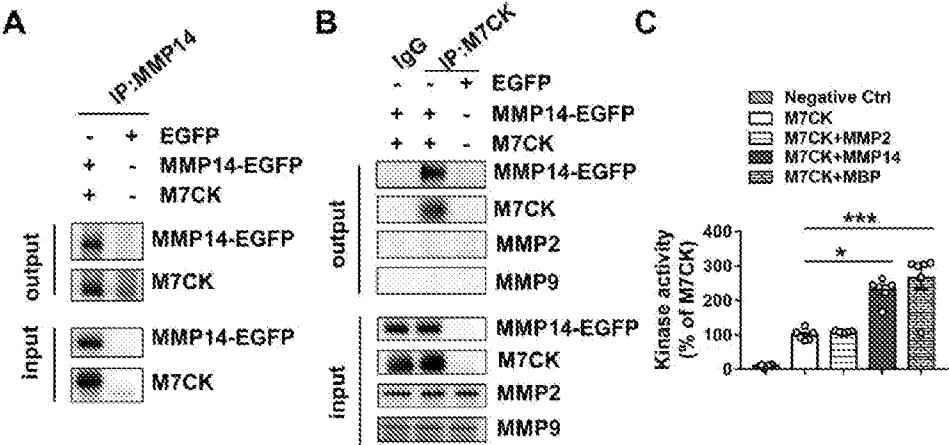


FIG. 10

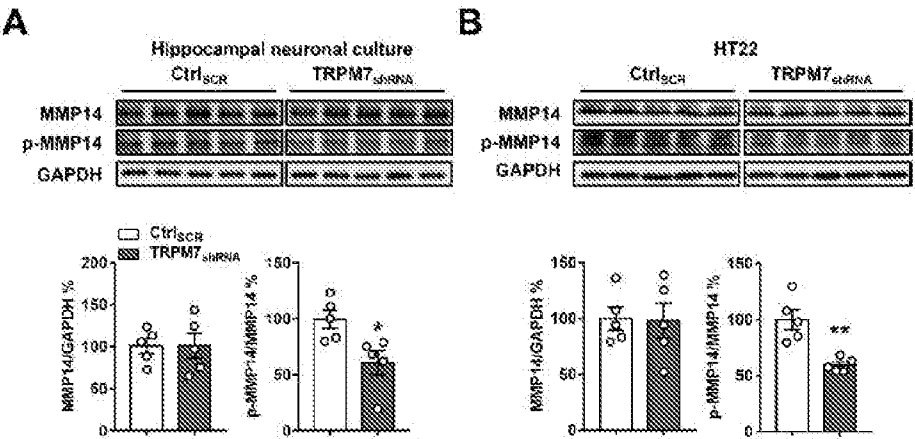


FIG. 11

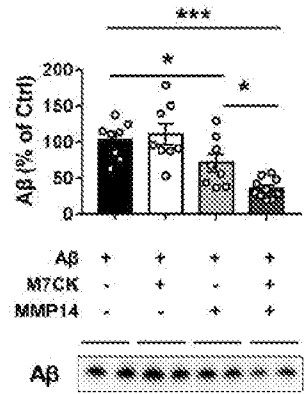


FIG. 12

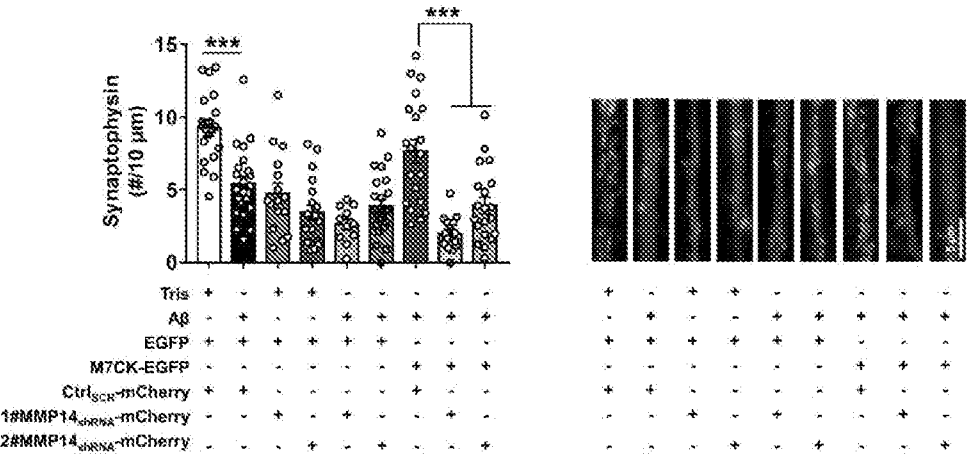


FIG. 13

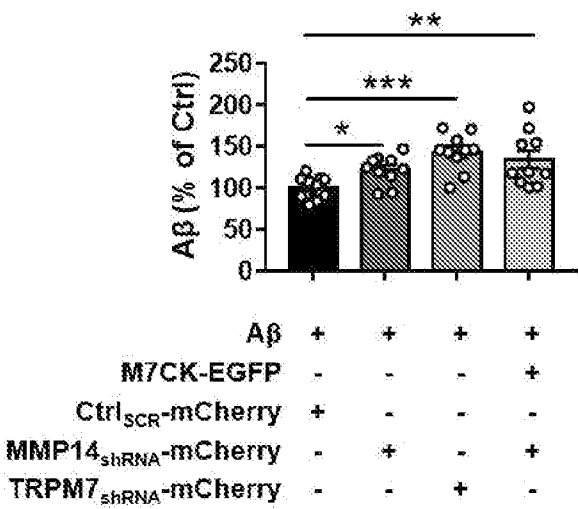


FIG. 14  
TRPM7

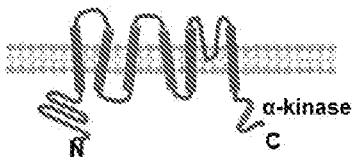


FIG. 15

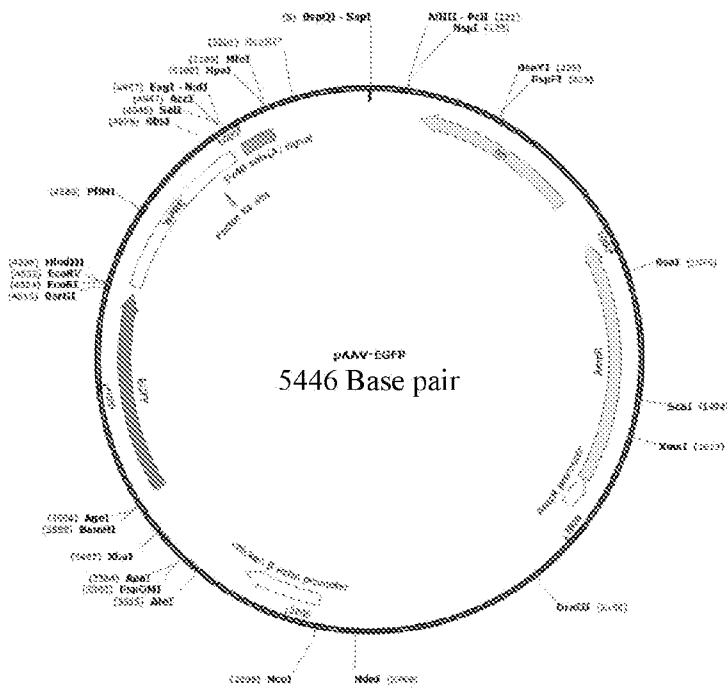


FIG. 16

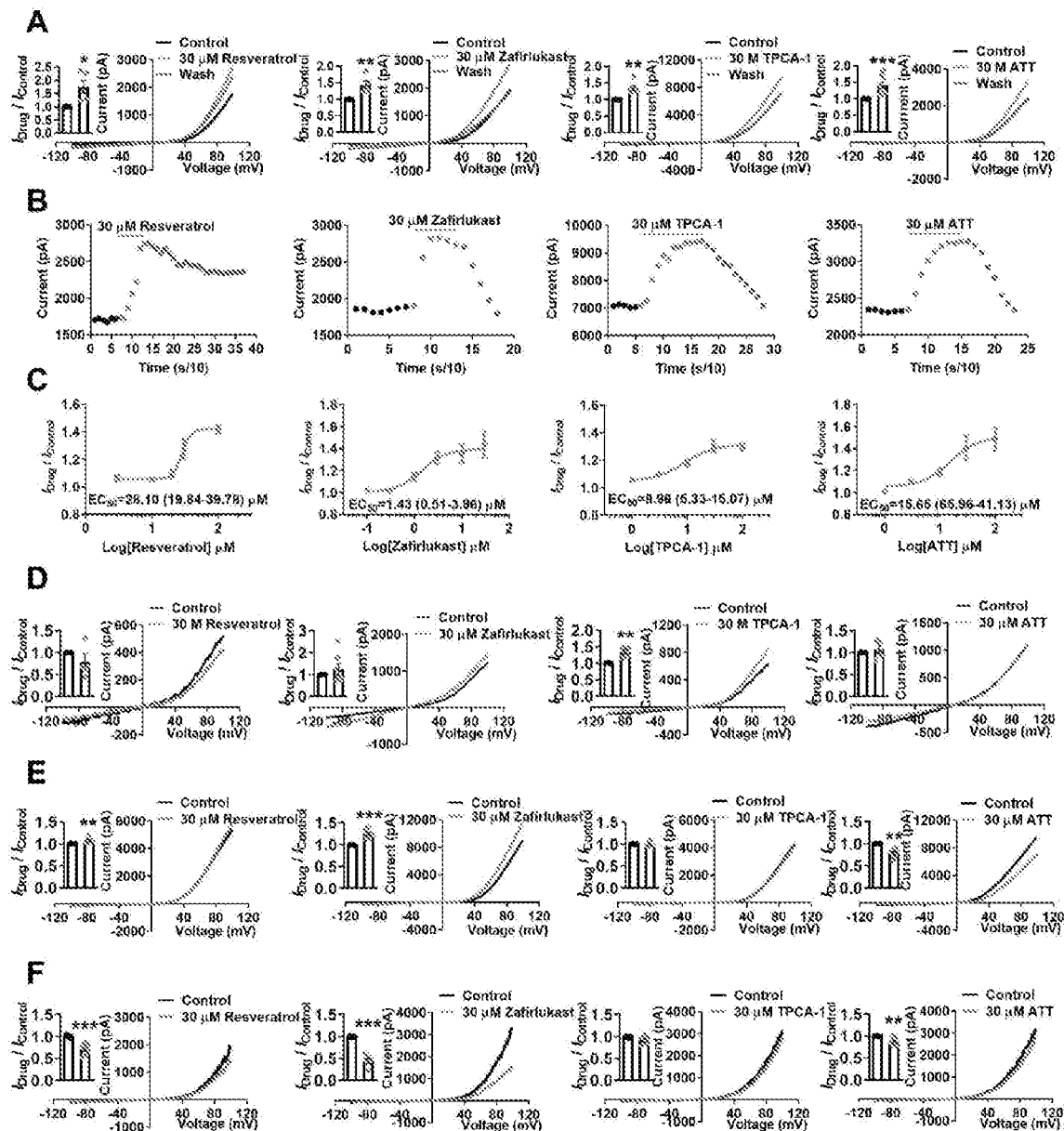


FIG. 17

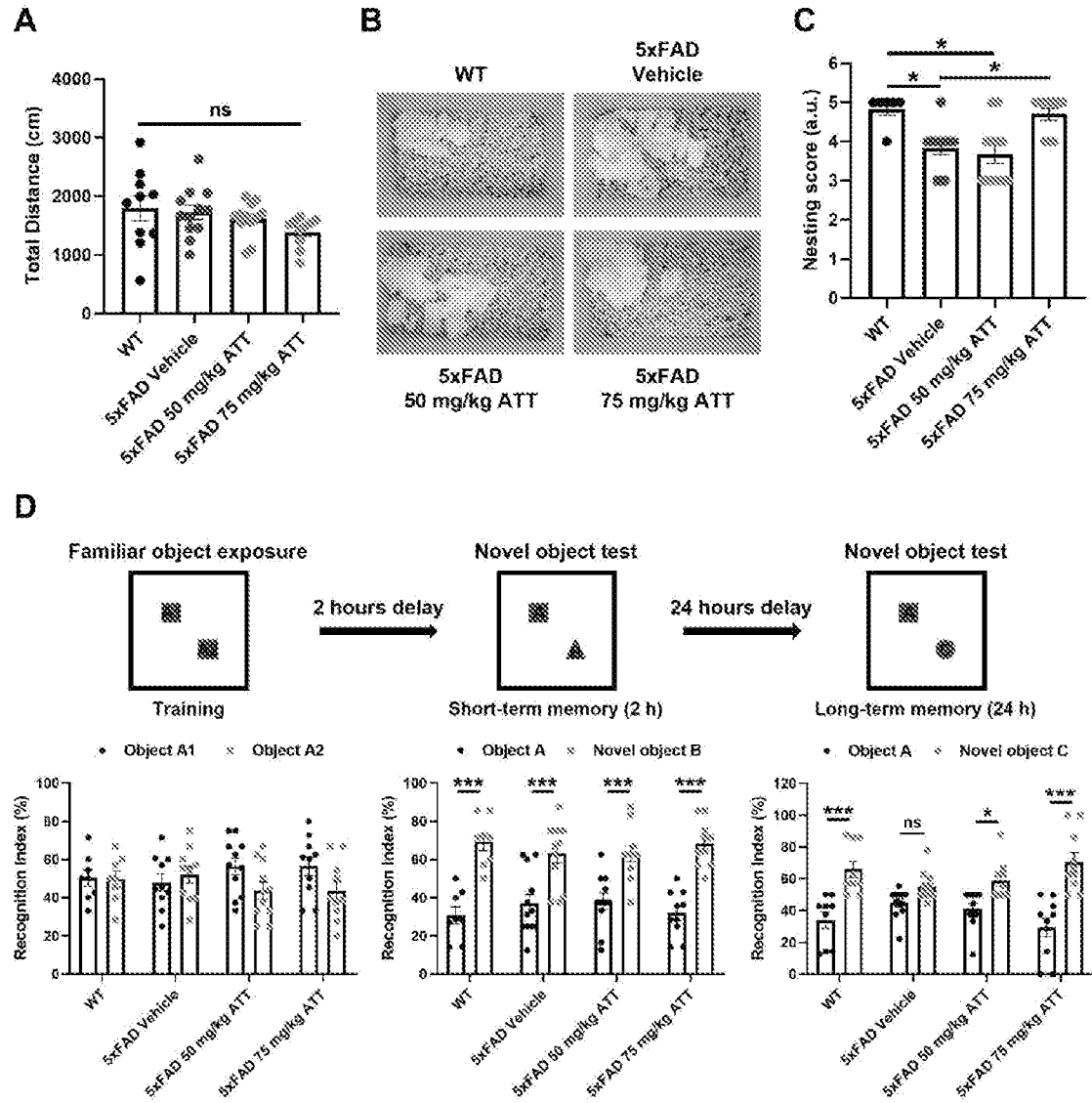


FIG. 18

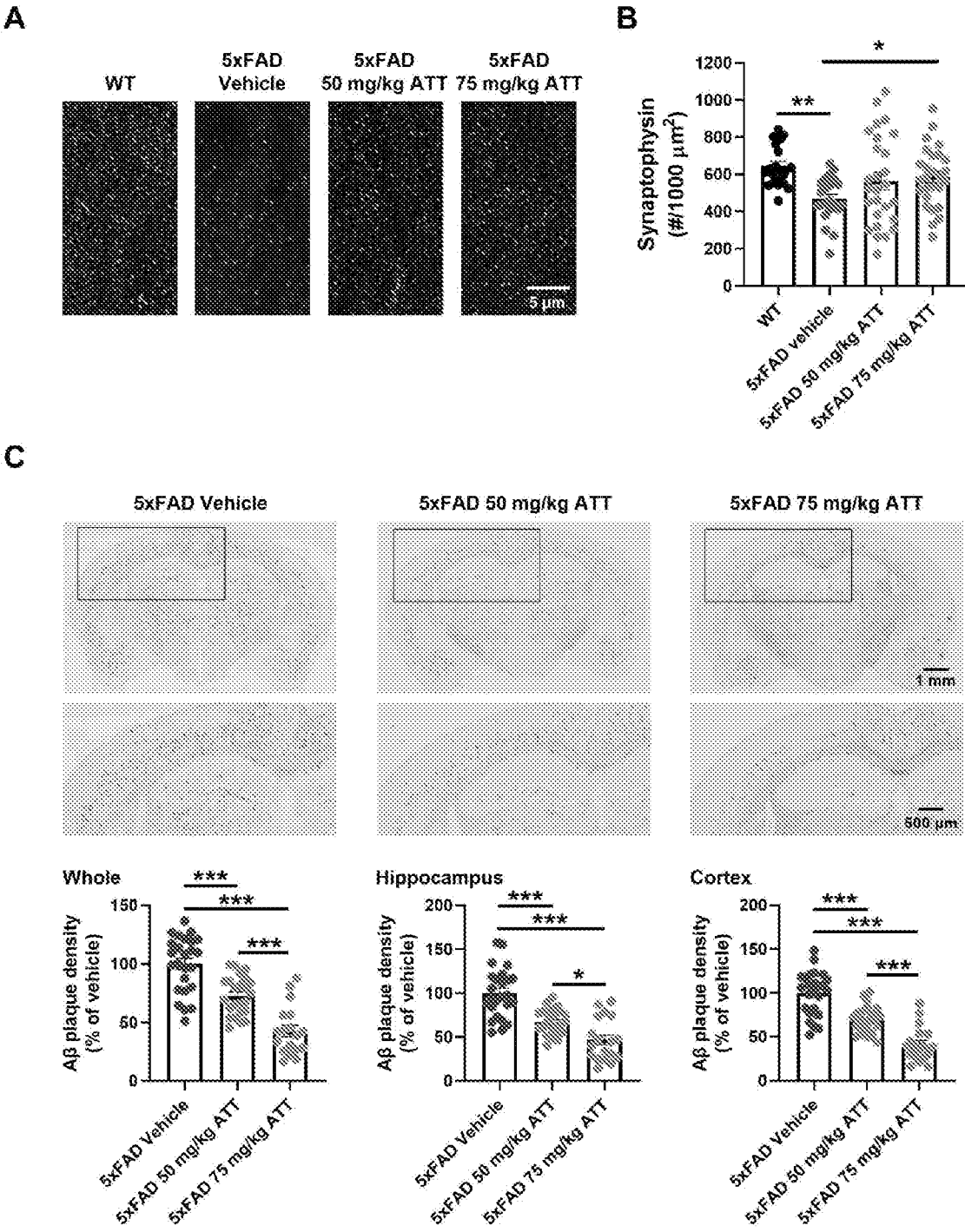


FIG. 19

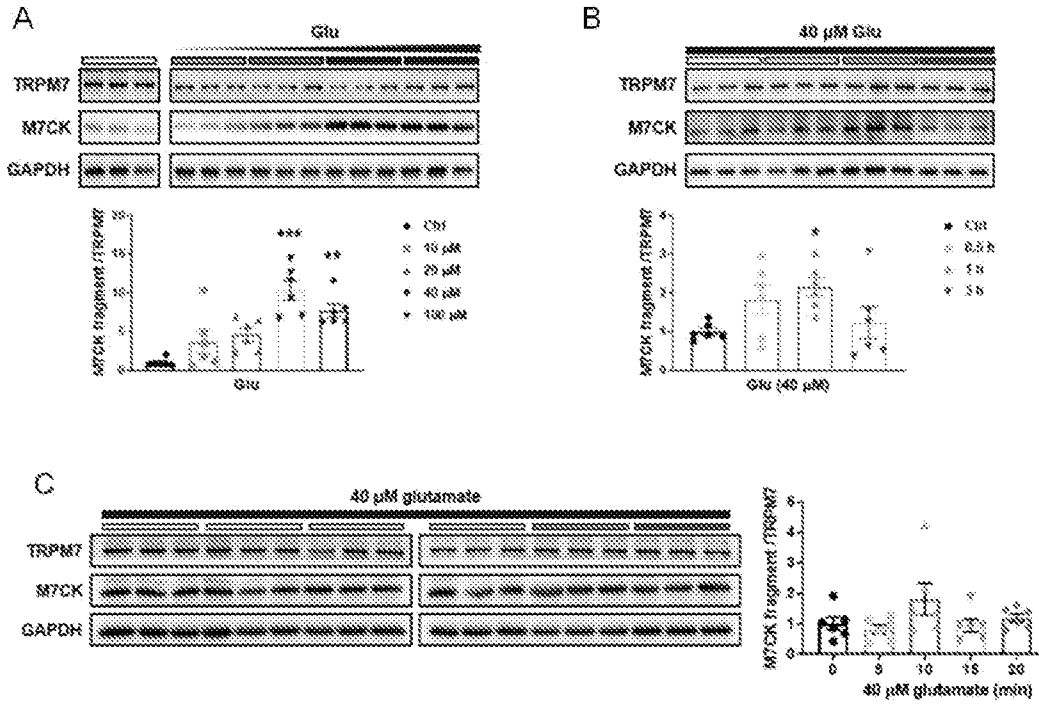


FIG. 20

# INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CN2024/098301**

## Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a. ☒ forming part of the international application as filed.
  - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),  
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CN2024/098301**

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

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

1. ☒ Claims Nos.: **21-23**  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Claims 21-23 relate to a method for treating or preventing a disease and therefore do not warrant an international search according to the criteria set out in PCT Rule 39.1(iv). An international search is still carried out on the basis of the use of M7CK in the manufacture of a medicament for preventing and/or treating a disease or disorder.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2024/098301

**A. CLASSIFICATION OF SUBJECT MATTER**

A61K 38/45(2006.01)i; A61P 25/28(2006.01)i

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K A61P

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

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

SIPOABS,CNXTX,ENTXT,ENTXTC,CNKI,NCBI,PubMed,ISI web of knowledge, baidu scholar, stn:ad,att,ATT,kinase,m7ck, resveratrol,TPCA+,trmp-7,TRPM7,zafirlukast,Alzheimer+,TPCA-1,ALZHEIMER+,anethole trithione,tpca-1,SEQ ID NOs: 1-3

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                    | Relevant to claim No. |
|-----------|-----------------------------------------------------------------------------------------------------------------------|-----------------------|
| PX        | CN 116785413 A (FUDNA UNIVERSITY) 22 September 2023 (2023-09-22)<br>claims 1-5, description, paragraphs 0004-0017     | 1-23                  |
| X         | US 2007083941 A1 (WYETH) 12 April 2007 (2007-04-12)<br>claims 1-37, Figure 7, description, paragraphs 0032, 0185-0187 | 1,4,13,21-22          |
| X         | CN 102304028 A (ZHONGSHAN UNIVERSITY) 04 January 2012 (2012-01-04)<br>description, paragraph 0005                     | 1,4-6                 |
| X         | CN 103505731 A (CHINA PHARMACEUTICAL UNIVERSITY) 15 January 2014<br>(2014-01-15)<br>claims 1-3                        | 1,4-6                 |
| X         | WO 2012156225 A1 (DEL SOLDATO, PIERO et al.) 22 November 2012 (2012-11-22)<br>claims 1-5                              | 1,4-6                 |
| Y         | US 2007083941 A1 (WYETH) 12 April 2007 (2007-04-12)<br>claims 1-37, Figure 7, description, paragraphs 0032, 0185-0187 | 2-3,13,21-23          |



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“D” document cited by the applicant in the international application

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

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

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

08 August 2024

Date of mailing of the international search report

21 August 2024

Name and mailing address of the ISA/CN

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| C. DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                  |                       |
|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                              | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                               | Relevant to claim No. |
| PX                                     | ZHANG, S.M. et al. "TRPM7 kinase activity induces amyloid- $\beta$ degradation to reverse synaptic and cognitive deficits in mouse models of Alzheimer's disease"<br><i>Sci. Signal.</i> , 11 July 2023 (2023-07-11),<br>abstract                                                                                                                                                                | 1-23                  |
| PX                                     | SIVAMARUTHI, B.S. et al. "NF-kB Pathway and Its Inhibitors: A Promising Frontier in the Management of Alzheimer's Disease"<br><i>Biomedicines</i> , 21 September 2023 (2023-09-21),<br>Table 1                                                                                                                                                                                                   | 1,4-6                 |
| X                                      | LU, B.F. et al. "Study on correlation between glutamate and alzheimer's disease"<br><i>Chinese journal of coal industry medicine</i> , Vol. 26, No. 2, 30 April 2023 (2023-04-30),<br>page 223, left column, paragraph 4                                                                                                                                                                         | 1,4-6                 |
| X                                      | LIU, Y.Q. et al. "TRPM7 Is Required for Normal Synapse Density, Learning, and Memory at Different Developmental Stages"<br><i>Cell Reports</i> , Vol. 23, No. 12, 19 June 2018 (2018-06-19),<br>page 3484, left column, paragraph 2, page 3485, right column, paragraph 4, to page 3488, right column, paragraph 1, page 3490, left column, paragraphs 2-3, Supplemental Experimental Procedures | 14-23                 |
| Y                                      | LIU, Y.Q. et al. "TRPM7 Is Required for Normal Synapse Density, Learning, and Memory at Different Developmental Stages"<br><i>Cell Reports</i> , Vol. 23, No. 12, 19 June 2018 (2018-06-19),<br>page 3484, left column, paragraph 2, page 3485, right column, paragraph 4, to page 3488, right column, paragraph 1, page 3490, left column, paragraphs 2-3, Supplemental Experimental Procedures | 2-3,13                |
| A                                      | ABUMARIA, N. et al. "Role of the chanzyme TRPM7 in the nervous system in health and disease"<br><i>Cellular and Molecular Life Sciences</i> , 09 May 2019 (2019-05-09),<br>Abstract                                                                                                                                                                                                              | 1-23                  |
| A                                      | SUN, Y.Y. et al. "TRPM7 and its role in neurodegenerative diseases"<br><i>Channels</i> , 01 September 2015 (2015-09-01),<br>abstract                                                                                                                                                                                                                                                             | 1-23                  |
| A                                      | FAYEZ, A.M. et al. "Neuroprotective effects of zafirlukast, piracetam and their combination on L-Methionine-induced vascular dementia in rats"<br><i>Fundamental &amp; Clinical Pharmacology</i> , 18 April 2019 (2019-04-18),<br>abstract                                                                                                                                                       | 1-23                  |
| A                                      | WALTON, H.S. et al. "Glutamate-glutamine cycling in Alzheimer's disease"<br><i>Neurochemistry International</i> , 01 December 2006 (2006-12-01),<br>abstract                                                                                                                                                                                                                                     | 1-23                  |
| A                                      | DONG, Y. et al. "Hyperphosphorylated tau mediates neuronal death by inducing necroptosis and inflammation in Alzheimer's disease"<br><i>Journal of Neuroinflammation</i> , Vol. 19, 15 August 2022 (2022-08-15),<br>abstract                                                                                                                                                                     | 1-23                  |

INTERNATIONAL SEARCH REPORT  
Information on patent family members

International application No.  
**PCT/CN2024/098301**

| Patent document cited in search report |            |    | Publication date (day/month/year) | Patent family member(s) |            |    | Publication date (day/month/year) |
|----------------------------------------|------------|----|-----------------------------------|-------------------------|------------|----|-----------------------------------|
| CN                                     | 116785413  | A  | 22 September 2023                 | None                    |            |    |                                   |
| US                                     | 2007083941 | A1 | 12 April 2007                     | CA                      | 2623485    | A1 | 12 April 2007                     |
|                                        |            |    |                                   | EP                      | 1931707    | A1 | 18 June 2008                      |
|                                        |            |    |                                   | JP                      | 2009511015 | A  | 19 March 2009                     |
|                                        |            |    |                                   | WO                      | 2007041687 | A1 | 12 April 2007                     |
| CN                                     | 102304028  | A  | 04 January 2012                   | None                    |            |    |                                   |
| CN                                     | 103505731  | A  | 15 January 2014                   | None                    |            |    |                                   |
| WO                                     | 2012156225 | A1 | 22 November 2012                  | ITMI                    | 20110847   | A1 | 14 November 2012                  |
|                                        |            |    |                                   | ITMI                    | 20110847   | A1 | 14 November 2012                  |