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(54) **Title:** KLOTHO FUSION PROTEIN AND USES THEREOF

(57) **Abstract:** The present invention relates to a fusion protein comprising sTREM2 or a functional variant thereof; TIMP2 or a functional variant thereof; and sKL or a functional variant thereof. The invention also relates to a polynucleotide, an expression vector, a host cell, and a pharmaceutical composition encoding or comprising the fusion protein. Furthermore, the invention relates to the fusion protein for use as a medicament, particularly for use in the prevention or treatment of an age-related disease or disorder, and more particularly an age-related neurodegenerative disease or disorder.

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Klotho fusion protein and uses thereof

This application claims the benefit of European Patent Application EP23382593.4 filed on June 15th, 2023.

5 Technical Field

The present invention relates to the field of medicine, in particular, it relates to medical approaches for preventing and treating age-related disorders. The compounds of the invention are particularly useful for the treatment of age-related neurodegenerative disorders.

10

Background Art

By 2050, the number of people in the world over the age of 65 will have grown exponentially to over 1.5 billion, reaching as much as 20% in several developed countries. This is undoubtedly an unprecedented
15 challenge, with profound social, economic, and health impacts, which is why the search for strategies to address aging has become a key strategic issue in National Health Systems worldwide. From a health perspective, aging is a multifactorial process that results in the progressive deterioration of biological functions that typically keep organs and tissues healthy. In the brain, aging is associated with increased fragility of neuronal physiology. This increased fragility leads not only to the development of cognitive deficits and mild
20 cognitive impairment (MCI), but also to a greater predisposition to neurodegenerative diseases, some of which are as paradigmatic as Alzheimer's disease (AD), which is characterized by severe deterioration of the same cognitive domains, with an additional progressive decline of other cognitive functions.

At the cellular and tissue level, cognitive decline is linked to a widespread decrease in synaptic plasticity, a
25 reduction in specific neuronal subpopulations, dysregulation of epigenetic and metabolic pathways, and an increase in neuroinflammation due to increased activation of astrocytes and microglia. Unfortunately, the molecular mechanisms underlying these functional changes during normal aging and neuropathological conditions remain poorly understood and require new in-depth studies. The lack of effective treatments calls for the development of new therapeutic strategies, and in this context, chronokines have become very
30 promising tools.

Chronokines are proteins that play a role in the aging process and the pathologies associated with it, mainly by modulating metabolism, oxidative stress, and inflammation. Different chronokines have shown some beneficial effects in diseases associated with aging, such as Alzheimer's disease. Secreted isoform Klotho
35 (sKL) has been recently drawn a lot of attention as a chronokine potentially useful in the treatment of diverse age-related disorders. However, there are currently no available treatments based on chronokines, probably due to their limited therapeutic activity.

Thus, in spite the efforts made so far, there is still a need for improved treatments for age-related diseases or

disorders.

Summary of Invention

- 5 The present inventors have designed fusion proteins comprising the sequence of three known chronokines — sKL, sTREM2, and TIMP2—, which provides synergistic anti-aging effects compared to the use of the free chronokines.

- 10 As shown in the Examples below, seeking to improve the therapeutic efficacy of known chronokines, the present inventors have devised fusion proteins with strong anti-aging effects. Surprisingly, the inventors found that by fusing the proteins sKL, sTREM2, and TIMP2 into a single chimeric protein, their individual activities were synergistically increased (see, for example, Figure 5 and Figure 6).

- 15 Moreover, from the data provided below in an animal model of Alzheimer's disease, it is apparent that the fusion proteins of the invention can be used as a medicament for treating age-related human diseases, in particular neurodegenerative diseases (see Figure 7).

- 20 Thus, in a first aspect, the invention provides a fusion protein comprising (i) sTREM2 or a functional variant thereof; (ii) TIMP2 or a functional variant thereof; and (iii) secreted splicing isoform of Klotho (sKL) or a functional variant thereof.

In a second aspect, the invention provides a polynucleotide that encodes the fusion protein as defined in the first aspect.

- 25 In a third aspect, the invention provides an expression vector comprising the polynucleotide as defined in the second aspect.

- 30 In a fourth aspect, the invention provides a host cell comprising the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, or the expression vector as defined in the third aspect.

In a fifth aspect, the invention provides a cell culture comprising the host cell as defined in the fourth aspect.

- 35 In a sixth aspect, the invention provides a pharmaceutical composition comprising a therapeutically effective amount of the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, or the host cell as defined in the fourth aspect, with at least one pharmaceutically acceptable excipient, diluent, or carrier.

In a seventh aspect, the invention provides a kit of parts comprising the fusion protein as defined in the first

aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, the host cell as defined in the fourth aspect, or the pharmaceutical composition as defined in the sixth aspect, and instructions for its use.

- 5 In an eighth aspect, the invention provides the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, the host cell as defined in the fourth aspect, or the pharmaceutical composition as defined in the sixth aspect, for use a medicament.

- 10 In a ninth aspect, the invention provides the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, the host cell as defined in the fourth aspect, or the pharmaceutical composition as defined in the sixth aspect, for use in the prevention or treatment of an age-related disease or disorder; particularly an age-related neurodegenerative disease or disorder.

- 15 This aspect can also be formulated as the use of the fusion protein, polynucleotide, expression vector, or host cell of the invention for the manufacture of a medicament for the prevention or treatment of an age-related disease or disorder; particularly an age-related neurodegenerative disease or disorder. This aspect can also be formulated as a method for the prevention or treatment of an age-related disease or disorder, particularly an age-related neurodegenerative disease or disorder, the method comprising administering a therapeutically effective amount of the fusion protein, polynucleotide, expression vector, or host cell of the invention together with at least one pharmaceutically acceptable excipient, diluent, or carrier to a subject in need thereof.

- 20 In a tenth aspect, the invention provides a process for the production of a fusion protein as defined in the first aspect, the process comprising (i) culturing the host cell as defined in the fourth aspect; or, alternatively, (ii) *in vitro* transcription and/or translation of the polynucleotide as defined in the second aspect.

- 25 In an eleventh aspect, the invention provides the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, the host cell as defined in the fourth aspect, or the pharmaceutical composition as defined in the sixth aspect, for use in extending the lifespan of a subject. In a further aspect, the invention provides a non-therapeutic method for extending the lifespan of a subject, the method comprising administering to a subject the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, the host cell as defined in the fourth aspect, or the pharmaceutical composition as defined in the sixth aspect. And in a further aspect, the invention provides the use of the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, the host cell as defined in the fourth aspect, or the pharmaceutical composition as defined in the sixth aspect, for extending the lifespan of a subject.

Brief Description of Drawings

Fig. 1 shows (A) Structure of the protein sKL. (B) Principal Component Analysis (PCA). Projection of the trajectory of sKL in the space of the first two principal components. The square denotes the crystal structure, used as the starting conformation of the simulation. Conformations visited in the trajectory are represented by dots.

Fig. 2 shows (A) Structure of the protein sTREM2. (B) Principal Component Analysis (PCA). Projection of the trajectory of sTREM2 in the space of the first two principal components. The square denotes the crystal structure, used as the starting conformation of the simulation. Conformations visited in the trajectory are represented by dots.

Fig. 3 shows (A) Structure of the protein TIMP2. (B) Principal Component Analysis (PCA). Projection of the trajectory of TIMP2 in the space of the first two principal components. The square denotes the crystal structure, used as the starting conformation of the simulation. Conformations visited in the trajectory are represented by dots.

Fig. 4 shows a Western Blot of HEK293 cells transfected with a pGG2 plasmid encoding a fusion protein of the invention HEBE (SEQ ID NO: 9). 10µg of cell lysates and 16µl of cell media were loaded on the electrophoresis gel. Antibodies against each of the subunits were used for the detection of HEBE. The protein presents an estimated molecular weight of ~100kDa

Fig. 5 shows the β -glucuronidase activity assay of the recombinant proteins. 10µg of sKL and equimolar amounts of the rest of the indicated proteins were used. PBS was used as a negative control, while bovine β -glucuronidase was used as a positive control. The activity was measured as the increase in fluorescence of the product resulting from the enzymatic reaction of the protein and the substrate. Data are shown as mean $\pm$ SE, $p < 0.05$ ANOVA

Fig. 6 shows the phagocytosis assay in BV2 cells. Cells were treated for 16h with 2µg of sTREM2 and equimolar amounts of the rest of the indicated proteins, and the 3µg of pHrodo-labeled myelin were added for 3h. The phagocytic activity was measured using a cytometer. A: the percentage of CD45+ cells was increased in HEBE-treated cells, indicating an increase in activated microglial cells. B: a decrease in the mean fluorescence of myelin+ cells can be observed in HEBE-treated cells, indicating a decrease in phagocytic activity after treatment with HEBE. Data are shown as mean $\pm$ SE, $p < 0.05$ ANOVA

Fig. 7 shows the Novel Object Recognition Test in APP/Tau mice. A: the first day of the test mice did not show preference for any of the identical objects. B: the second day of the test, when one of the objects was replaced by a new object, WT mice and HEBE-treated AD mice showed preference for the new object, while Null-treated AD mice did not show any preference. C: comparison of the preference index from the first day to the second for each group, showing an improvement in both WT and HEBE-treated AD mice, while no

differences can be observed in the Null-treated AD group. In C the two left columns refer to "WT Null", the two middle columns refer to "AD Null", and the two right columns refer to "AD HEBE". Data are shown as mean $\pm$ SE, $p < 0.05$ ANOVA

- 5 Fig. 8 shows a Western Blot of HEK293 cells transfected with a pGG2 plasmid encoding HEBE2 (SEQ ID NO: 10). 10 μ g of cell lysates and 16 μ l of cell media were loaded on the electrophoresis gel. Antibodies against each of the subunits were used for the detection of HEBE2. The protein presents an estimated molecular weight of $\sim$ 100kDa. "HEBE", refers to "HEBE2" in this Figure.

10 Detailed description of the invention

- All terms as used herein in this application, unless otherwise stated, shall be understood in their ordinary meaning as known in the art. Other more specific definitions for certain terms as used in the present application are as set forth below and are intended to apply uniformly through-out the specification and claims
15 unless an otherwise expressly set out definition provides a broader definition.

As used herein, the indefinite articles "a" and "an" are synonymous with "at least one" or "one or more". Unless indicated otherwise, definite articles used herein, such as "the" also include the plural of the noun.

- 20 As explained above, the invention provides in a first aspect a fusion protein comprising (i) sTREM2 or a functional variant thereof; (ii) TIMP2 or a functional variant thereof; and (iii) secreted splicing isoform of Klotho (sKL) or a functional thereof. It should be appreciated that the fusion proteins provided herein may be arranged in any configuration, for example, the fusion protein may have the structure sTREM2-TIMP2-sKL, TIMP2-sTREM2-sKL, sTREM2-sKL-TIMP2, TIMP2-sKL-sTREM2, sKL-sTREM2-TIMP2, or sKL-TIMP2-
25 sTREM2. The order of linkage of the proteins is not meant to be particularly limiting so long as the particular arrangement of the proteins produces a functional fusion protein. The proteins may be fused directly or through a linker, as disclosed below.

- As used herein, "fusion protein" refers to a polypeptide which comprises sequences from at least two different
30 proteins. The fusion protein, per definition, is never found in nature as such. As used herein, the terms "protein", "polypeptide," and "peptide" are used interchangeably, and refer to a compound comprised of two or more amino acid residues covalently linked by peptide bonds. It should be appreciated that any of the protein sequences provided herein are provided with or without an N-terminal methionine (M) residue, and with or without the N-terminal signal peptide.

35

The term "sTREM2" or "soluble triggering receptor expressed on myeloid cells 2" refers to a soluble version of the protein TREM2. The protein sequence of TREM2 from various species is available in several protein databases, such as Uniprot Q9NZC2 Homo sapiens (2000-10-01 update); and Q99NH8 Mus musculus (2001-06-01 update). TREM2 is an innate immune receptor expressed by myeloid cells including macrophages,

dendritic cells, osteoclasts and, in the central nervous system (CNS), by microglia. TREM2 is a type I transmembrane glycoprotein with an extracellular V-type immunoglobulin (Ig) ectodomain, a connecting stalk followed by a transmembrane region and a C-terminal tail. Secretase shedding of the receptor ectodomain gives rise to sTREM2 (corresponding to amino acids 1-157 of TREM2, in humans), which can be detected in the peripheral blood and cerebrospinal fluid (CSF). sTREM2 may also be derived by translation of an alternative transcript that is lacking the transmembrane domain (J.L. Del-Aguila, et al. "TREM2 brain transcript-specific studies in AD and TREM2 mutation carriers", *Mol. Neurodegener.*, 14 (2019), p. 18). The term "sTREM2" embraces any naturally occurring sTREM2 from any organism, any naturally occurring sTREM2 equivalent or functional fragment thereof, any sTREM2 homolog, ortholog, or paralog from any organism. In particular, the term "sTREM2" encompasses the full-length protein with the signal peptide attached (e.g. SEQ ID NO: 1) or the mature full-length protein with the signal peptide removed (e.g. SEQ ID NO: 2).

The term "TIMP2" or "tissue inhibitor of metalloproteinases 2" refers to the protein named TIMP metalloproteinase inhibitor 2, which is a natural inhibitor of the matrix metalloproteinases (MMP), a group of peptidases involved in degradation of the extracellular matrix, in particular MMP1. The protein sequence from various species is available in several protein databases, such as Uniprot P16035 *Homo sapiens* (1990-11-01 update); and P25785 *Mus musculus*, (1993-04-01 update). The term "TIMP2" embraces any naturally occurring TIMP2 from any organism, any naturally occurring TIMP2 equivalent or functional fragment thereof, any TIMP2 homolog, ortholog, or paralog from any organism. In particular, the term "TIMP2" encompasses the full-length protein with the signal peptide attached (e.g. SEQ ID NO: 3) or a shorter version of the mature full-length protein without the signal peptide and a C-terminal segment (e.g. SEQ ID NO: 4).

The term "sKL" or "secreted splicing variant of Klotho" or "secreted splicing isoform of Klotho" refers to the protein resulting from the alternative splicing transcript of the klotho gene, which generates a truncated form of the klotho protein (sKL) that is formed by the KL1 domain, with an approximate weight of 70 kDa, together with a specific secretion signal consisting of 15 amino acid tail that is not found in the m-KL transcript, and for this reason is also called the secreted isoform of klotho, sKL, or the secreted splicing isoform of klotho protein. The protein sequence of sKL from various species is available in several protein databases, such as Uniprot Q9UEF7-2 *Homo sapiens* (2005-10-11 update); and O35082-2 *Mus musculus* (2011-07-27 update). sKL is different from other forms of soluble klotho, namely p-KL, p-KL1 and p-KL2. In the present description, m-KL stands for the full-length transmembrane form; p-KL stands for the soluble proteolyzed klotho, which is generated by cleavage of the m-KL; and p-KL1 and p-KL2 stand for the soluble klotho forms consisting of on the KL1 domain and the KL2 domain of p-KL, respectively. m-KL comes from the full-length transcript encoding a single pass transmembrane protein with a molecular weight of approximately 130 kDa (m-KL). The protein contains three domains: a short transmembrane domain at the C-terminal, an extracellular domain composed of two internal repeated sequences of about 550 amino acids called KL1 and KL2 respectively, and a very short intracellular domain of 10 amino acids. The extracellular domain of the transmembrane form can be cleaved by metalloproteinases ADAM10 and ADAM17 resulting in another form of soluble Klotho of about

130 kDa (abbreviated p-KL for proteolyzed membrane isoform. Moreover, there is a second recognition site for the proteases ADAM10 and 17 located between the KL1 and KL2 domains, which generates two new 70 kDa isoforms, one contained the KL1 domain only (like the one generated from alternative splicing but without the specific amino acid tail), and the other one contained the KL2 domain. However, it has not been demonstrated *in vivo* that p-KL is proteolyzed into p-KL1 and p-KL2. The term "sKL" embraces any naturally occurring sKL from any organism, any naturally occurring sKL equivalent or functional fragment thereof, any sKL homolog, ortholog, or paralog from any organism. In particular, the term "sKL" encompasses the full-length protein with the signal peptide attached (e.g. SEQ ID NO: 5) or the mature full-length protein with the signal peptide removed (e.g. SEQ ID NO: 6).

The skilled person would appreciate that when sTREM2, TIMP2, or sKL is located at the N-terminal end of the fusion protein, it may encompass the full-length protein with the signal peptide (e.g. SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5, respectively). On the other hand, when sTREM2, TIMP2, or sKL is located in the middle or at the C-terminal end of the fusion protein, it may encompass the mature full-length protein with the signal peptide removed (e.g. SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6, respectively).

Protein sequence variants are well understood to those of skill in the art and can involve amino acid sequence modifications. Amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional, or deletional variants. Substitutional modifications in the sequence of protein variants are typically limited or conservative, so that the sequences of the reference peptide and the variant are closely similar overall and, in many regions, identical. A variant and reference peptide can differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A variant of a protein can be a naturally occurring such as an allelic variant or can be a variant that is not known to occur naturally. Non-naturally occurring variants of proteins may be made by mutagenesis techniques or by direct synthesis following routine methods. The term "functional variant" refers to variants that maintain the biological activity of the protein and is meant to also encompass functional fragments.

In one embodiment, sTREM2 is mammalian sTREM2, TIMP2 is mammalian TIMP2, and/or sKL is mammalian sKL. In another embodiment, sTREM2 is human sTREM2, TIMP2 is human TIMP2, and/or sKL is human sKL.

In one embodiment, sTREM2 comprises or consists of SEQ ID NO: 1 or SEQ ID NO: 2. In another embodiment, the functional variant of sTREM2 comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to wild-type sTREM2, in particular to SEQ ID NO: 1 or SEQ ID NO: 2. In another embodiment, sTREM2 comprises or consists of SEQ ID NO: 1 or SEQ ID NO: 2 and the functional variant thereof comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or

at least 99% identical to SEQ ID NO: 1 or SEQ ID NO: 2.

In one embodiment, TIMP2 comprises or consists of SEQ ID NO: 3 or SEQ ID NO: 4. In another embodiment, the functional variant of TIMP2 comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to wild-type TIMP2, in particular to SEQ ID NO: 3 or SEQ ID NO: 4. In another embodiment, TIMP2 comprises or consists of SEQ ID NO: 3 or SEQ ID NO: 4 and the functional variant thereof comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO:3 or SEQ ID NO: 4.

In one embodiment, sKL comprises or consists of SEQ ID NO: 5 or SEQ ID NO: 6. In another embodiment, the functional variant of sKL comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to wild-type sKL, in particular to SEQ ID NO: 5 or SEQ ID NO: 6. In another embodiment, sKL comprises or consists of SEQ ID NO: 5 or SEQ ID NO: 6 and the functional variant thereof comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 5 or SEQ ID NO: 6.

In one embodiment, sKL comprises or consists of SEQ ID NO: 11. In another embodiment, sKL comprises or consists of SEQ ID NO: 11 and the functional variant thereof comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 11.

In some embodiments, the functional variant of sTREM2 comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acid changes compared to a wild type sTREM2, particularly compared to SEQ ID NO: 1 or SEQ ID NO: 2.

In some embodiments, the functional variant of TIMP2 comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acid changes compared to a wild type TIMP2, particularly compared to SEQ ID NO: 3 or SEQ ID NO: 4.

In some embodiments, the functional variant of sKL comprises up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acid changes compared to a wild type sKL, particularly compared to SEQ ID NO: 5 or SEQ ID NO: 6.

In some embodiments, the functional variant of sTREM2 comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, or at least 150 identical contiguous amino acid residues of wild type sTREM2, particularly of SEQ ID NO: 1 or SEQ ID NO: 2.

In some embodiments, the TIMP2 variant comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues of wild type TIMP2, particularly of SEQ ID NO: 3 or SEQ ID NO: 4.

In some embodiments, the functional variant of sKL comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues of wild type sKL, particularly of SEQ ID NO: 5 or SEQ ID NO: 6.

In one embodiment, the fusion protein comprises, in the N-terminal to C-terminal direction:

- sTREM2 or a functional variant thereof; TIMP2 or a functional variant thereof; and sKL or a functional variant thereof; or, alternatively
- sTREM2 or a functional variant thereof; sKL or a functional variant thereof; and TIMP2 or a functional variant thereof; or, alternatively
- TIMP2 or a functional variant thereof; sTREM2 or a functional variant thereof; and sKL or a functional variant thereof; or, alternatively
- TIMP2 or a functional variant thereof; sKL or a functional variant thereof; and sTREM2 or a functional variant thereof; or, alternatively
- sKL or a functional variant thereof; TIMP2 or a functional variant thereof; and sTREM2 or a functional variant thereof; or, alternatively
- sKL or a functional variant thereof; sTREM2 or a functional variant thereof; and TIMP2 or a functional variant thereof.

In one embodiment, the fusion protein comprises, in the N-terminal to C-terminal direction:

- sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 1; TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 4; and sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 6; or, alternatively,
 - sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 1; sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 6; and TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 4; or, alternatively,
 - TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 3; sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 2; and sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 6; or, alternatively,
 - TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 3; sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 6; and sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 2; or, alternatively,
 - sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 5; TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 4; and sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 2; or, alternatively,
 - sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 5; sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 2; and TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 4.
- 20 In the present invention the term "identical" or "identity" refers to the percentage of positions that are identical in the two sequences when the sequences are optimally aligned. If, in the optimal alignment, a position in a first sequence is occupied by the same nucleotide or amino acid as the corresponding position in the second sequence, the sequences exhibit identity with respect to that position. The percentage of identity determines the number of identical nucleotides or amino acids over a defined length in a given alignment. Thus, the level
- 25 of identity between two sequences or ("percent sequence identity") is measured as a ratio of the number of identical positions shared by the sequences with respect to the number of positions compared (i.e., percent sequence identity = (number of identical positions/total number of positions compared) x 100). A gap, i.e., a position in an alignment where a nucleotide or amino acid is present in one sequence but not in the other, is regarded as a position with non-identical nucleotide or amino acid and is counted as a compared position.
- 30 A number of mathematical algorithms for rapidly obtaining the optimal alignment and calculating identity between two or more sequences are known and incorporated into a number of available software programs. For purposes of the present invention, the sequence identity between two nucleic acid or amino acid sequences is preferably determined using algorithms based on global alignment, such as the Needleman-
- 35 Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453), preferably implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends Genet. 16: 276-277); or the BLAST Global Alignment tool (Altschul et al., "Basic local alignment search tool", 1990, J. Mol. Biol. v. 215, pages 403-410), using default settings. Local alignment also can be used when the sequences being compared are substantially the same length.

The skilled person can readily identify variants of sTREM2, TIMP2, and sKL that are functional following routine methods known in the art, for example those disclosed in the examples below, without the need of inventive skill.

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In a particular embodiment, the functional variant of sTREM2 maintains phagocytosis-inhibiting activity. The skilled person can readily produce and test the phagocytosis-inhibiting activity of variants following routine methods known in the art, for example those disclosed in the examples below, without the need of inventive skill. For example, a way to test if a variant maintains the phagocytosis-inhibiting activity is by a phagocytosis assay as explained in the examples below. Briefly, BV2 cells are contacted with the variant of sTREM2 for 16 hours and then pHrodo-labeled myelin is added. Phagocytic activity is then measured as the increase in intracellular fluorescence by flow cytometry. The assay is repeated with sTREM2. If the sTREM2 variant treatment produces a phagocytosis-inhibiting effect equal to or higher than sTREM2 treatment, it is indicative that the variant maintains the phagocytosis-inhibiting activity.

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In a particular embodiment, the functional variant of TIMP2 maintains MMP1-inhibiting activity. The skilled person can readily produce and test the MMP1-inhibiting activity of variants following routine methods known in the art, for example those disclosed in the examples below, without the need of inventive skill. For example, a way to test if a variant maintains the MMP1-inhibiting activity is by a MMP1 inhibition activity assay as explained in the examples below. Briefly, MMP1 is incubated with a substrate and the variant of TIMP2 for 30 minutes at room temperature and fluorescence is then measured with an excitation of 324nm and an emission of 400nm. The MMP1-inhibiting activity is measured as the reduction of fluorescence due to the inhibition of MMP1 enzymatic activity on the substrate that yields the fluorescent product. The assay is then repeated incubating with TIMP2. If the TIMP2 variant treatment produces a MMP1-inhibiting effect equal to or higher than MMP1 treatment, it is indicative that the variant maintains the MMP1-inhibiting activity.

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In one embodiment, the functional variant of sKL maintains β -glucuronidase activity. The skilled person can readily generate and test the β -glucuronidase activity of variants following routine methods known in the art, for example those disclosed in the examples below, without the need of inventive skill. For example, a way to test if a variant maintains the β -glucuronidase activity is by a β -glucuronidase assay as explained in the examples below. Briefly, the variant of sKL diluted in citrate buffer is incubated with substrate 4-methylumbelliferyl β -D-glucuronide hydrate for 2 hours at 37°C, and the fluorescence of the reaction product is then measured with an excitation of 360nm and an emission of 470nm. The assay is repeated with sKL. If the sKL variant treatment generates a fluorescence signal equal to or higher than sKL treatment, it is indicative that the variant maintains the β -glucuronidase activity.

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In one embodiment, the fusion protein comprises improved phagocytosis-inhibiting activity compared to isolated sTREM2; improved MMP1-inhibiting activity compared to isolated TIMP2; and/or improved β -glucuronidase activity compared to isolated sKL.

Linkers may be optionally used to link any of the proteins comprised in the fusion protein of the invention. As used herein, the term "linker" refers to a chemical group or a molecule linking two moieties, in particular two proteins. Typically, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond, thus connecting the two.

Thus, in a particular embodiment of the first aspect, the fusion protein further comprises a linker between at least two of the proteins comprised in the fusion protein (i.e., sTREM2, TIMP2, and sKL). It would be appreciated that the linker could be present between any two of the proteins comprised in the fusion protein.

10 In a more particular embodiment, the fusion protein further comprises a linker between each protein comprised in the fusion protein. Thus, in this latter case, the fusion protein would contain two linkers, which could be the same or different.

In one embodiment, the linker is a peptide linker. In some embodiments, the peptide linker is 5-50 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 30-35, 35-40, 40-45, or 45-50 amino acids in length. In a more particular embodiment, the peptide linker is from 10 to 30 amino acids in length. In a more particular embodiment, the peptide linker is of 15 or 25 amino acids in length. Longer or shorter linkers are also contemplated.

20 In a particular embodiment, the peptide linker is a rigid peptide linker. In an even more particular embodiment, the peptide linker comprises or consists of the sequence (EAAAK)_n, (XP)_n, or a combination thereof. In a more particular embodiment, the peptide linker comprises or consists of (EAAAK)₁₋₁₀, (XP)₁₋₂₅, or a combination thereof.

25 In some embodiments, the linker is a non-peptide linker. In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In certain embodiments, the linker is a covalent bond (e.g., a carbon-carbon bond, disulfide bond, carbon-heteroatom bond, etc.). In certain embodiments, the linker is a carbon-nitrogen bond of an amide linkage. In certain embodiments, the linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or hetero aliphatic linker. In certain embodiments, the linker is polymeric (e.g., polyethylene, polyethylene glycol, polyamide, polyester, etc.). In certain

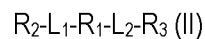
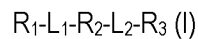
30 In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In certain embodiments, the linker comprises an aminoalkanoic acid (e.g., glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, etc.). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminohexanoic acid (Ahx). In certain embodiments, the linker is

35 based on a carbocyclic moiety (e.g., cyclopentane, cyclohexane). In other embodiments, the linker comprises a polyethylene glycol moiety (PEG). In certain embodiments, the linker comprises an aryl or heteroaryl moiety. In certain embodiments, the linker is based on a phenyl ring. The linker may include functionalized moieties to facilitate attachment of a nucleophile (e.g., thiol, amino) from the peptide to the linker. Any electrophile may be used as part of the linker. Exemplary electrophiles include, but are not limited to, activated esters, activated

amides, Michael acceptors, alkyl halides, aryl halides, acyl halides, and isothiocyanates. Additional suitable linker motifs and linker configurations will be apparent to those of skill in the art.

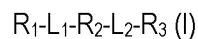
5 In one embodiment of the first aspect, sKL or a functional variant thereof is located at the C-terminal end of the fusion protein. In a more particular embodiment, the fusion protein comprises a linker between each protein comprised in the fusion protein, and sKL or a functional variant thereof is located at the C-terminal end of the fusion protein.

10 In one embodiment of the first aspect, the fusion protein is of formula (I) or (II):



wherein R_1 is sTREM2 or a functional variant thereof; L_1 is a linker; R_2 is TIMP2 or a functional variant thereof; L_2 is a linker; and R_3 is sKL or a functional variant thereof.

15 In one embodiment of the first aspect, the fusion protein is of formula (I):



20 wherein R_1 is sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 1; L_1 is a peptide linker; R_2 is TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 4; L_2 is a peptide linker; and R_3 is sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 6.

or, alternatively,

the fusion protein is of formula (II):



wherein R_1 is sTREM2 or a functional variant thereof, wherein sTREM2 comprises or consists of SEQ ID NO: 2; L_1 is a peptide linker; R_2 is TIMP2 or a functional variant thereof, wherein TIMP2 comprises or consists of SEQ ID NO: 3; L_2 is a peptide linker; and R_3 is sKL or a functional variant thereof, wherein sKL comprises or consists of SEQ ID NO: 6.

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The skilled person would appreciate that the above formulas (I) and (II) correspond to $\text{NH}_2-R_1-L_1-R_2-L_2-R_3-\text{COOH}$ and $\text{NH}_2-R_2-L_1-R_1-L_2-R_3-\text{COOH}$, respectively, wherein NH_2 is the N-terminus of the fusion protein, and COOH is the C-terminus of the fusion protein.

35 In one embodiment of the first aspect, L_1 comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to the sequence EAAAKEAAAKEAAK (i.e., $[\text{EAAAK}]_3$) (SEQ ID NO: 7); and/or L_2 comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least

83%, at least 84%, 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%, or 100 % identical to the sequence (i.e., [EAAAK]₅) EAAAKEAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 8).

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In one embodiment of the first aspect, the fusion protein comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to SEQ ID NO: 9 or SEQ ID NO: 10.

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In one embodiment, the fusion protein is of a length equal to or lower than 1500, equal to or lower than 1400, equal to or lower than 1300, equal to or lower than 1200, equal to or lower than 1100, or equal to or lower than 1000 amino acids.

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In another embodiment of the first aspect of the invention, the C-terminal and N-terminal ends of the fusion protein are derivatized. It is well-known in the state of the art how to derivatize the terminal ends of a protein, for example, to improve its stability. In one embodiment, the C-terminal is amidated (-C(O)NH₂) and/or the N-terminal is acetylated.

20

Any of the proteins or polynucleotides provided herein may be produced by any method known in the art. For example, the proteins provided herein may be produced via recombinant protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known in the art, and belong to the common general knowledge of the skilled person.

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The fusion proteins of the present disclosure may comprise one or more additional features. For example, in some embodiments, the fusion protein further comprises a cell-penetrating peptide (CPP) or a blood-brain barrier penetrating peptide (BBP) for improving its functionalities. In the present invention the term "cell penetrating peptide" or "CPP" refers to short peptides that facilitate cellular uptake of various molecular

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cargos, in particular polypeptides. The term "blood-brain barrier penetrating peptide" or "BPP" refers to short peptides that facilitate cellular the transport of various molecular cargos, in particular polypeptides, through the blood-brain barrier. The CPP or BPP can be located at any one of the (amino or carboxyl) terminal ends of the fusion protein of the invention.

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Other exemplary features that may be present in the fusion protein are localization sequences, such as cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the fusion proteins. Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags, hemagglutinin (HA)-tags, polyhistidine

tags, also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (e.g., Softag 1, Softag 3), strep-tags, biotin ligase tags, FIAsh tags, V5 tags, and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the fusion protein comprises one or more His tags.

In a particular embodiment of the first aspect, the fusion protein is an isolated, synthetic, or recombinant protein. "Isolated" means altered or removed from its natural state.

10 In a further aspect, the invention provides a fusion protein comprising at least two of (i) sTREM2 or a functional variant thereof; (ii) TIMP2 or a functional variant thereof; and (iii) sKL or a functional variant thereof.

The fusion protein of the invention may be administered directly, or it can be expressed inside target cells of interest by means of gene therapy. To this aim the invention also provides, in a second aspect, a polynucleotide that encodes the fusion protein of the invention. The term "polynucleotide" or "nucleic acid", are used herein interchangeably, and refer to a chain comprising two or more individual nucleotide residues linked to each other via a phosphodiester linkage.

20 In a particular embodiment of the second aspect, the polynucleotide is DNA (single or double stranded) or RNA.

In a particular embodiment of the second aspect, the polynucleotide is an isolated, synthetic, or recombinant polynucleotide.

25 In some embodiments, the polynucleotide comprises a sequence optimized for expression in one or more cell types. For example, the polynucleotide sequence may be optimized for expression in a mammalian cell (e.g., a HEK 293T cell). The polynucleotide sequence may be codon optimized for expressing in a mammalian cell using Integrated DNA Technologies (IDT), GeneArt, Celler, and GenScript.

30 Polynucleotides may be naturally occurring, for example, in the context of a genome, a transcript, an mRNA, tRNA, rRNA, siRNA, snRNA, a plasmid, cosmid, chromosome, chromatid, or other naturally occurring nucleic acid molecule. On the other hand, a nucleic acid molecule may be a non-naturally occurring molecule, e.g., a recombinant DNA or RNA, an artificial chromosome, an engineered genome, or fragment thereof, or a synthetic DNA, RNA, DNA/RNA hybrid, or including non-naturally occurring nucleotides or nucleosides.

35 Furthermore, the terms "nucleic acid" and "polynucleotide" include nucleic acid analogs, e.g., analogs having other than a phosphodiester backbone. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, and backbone modifications. A nucleic acid sequence is

presented in the 5' to 3' direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (e.g. adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, 2- aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7- deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5'-N-phosphoramidite linkages).

In a particular embodiment of the second aspect, the polynucleotide comprises or consists of SEQ ID NO: 12 or SEQ ID NO: 13, or a functional variant thereof at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 12 or SEQ ID NO: 13.

As above indicated, the invention also provides in a third aspect an expression vector comprising the polynucleotide of the second aspect. Examples of suitable expression vectors include those conventionally used in biomedicine and known to the skilled person.

Vectors can be designed for expression in prokaryotic or eukaryotic cells. For example, vectors can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Alternatively, expression vectors can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

In some embodiments, the expression vector is a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 and pMT2PC. When used in mammalian cells, the expression vector's control functions are typically provided by one or more regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, simian virus 40, and others disclosed herein and known in the art. In a particular embodiment of the third aspect, the expression vector further comprises an expression promoter operatively linked to the polynucleotide of the second aspect

In a particular embodiment, the expression vector is a viral vector. The use of RNA or DNA viral based systems for the delivery of nucleic acids take advantage of highly evolved processes for targeting a virus to specific cells in the body and trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (in vivo) or they can be used to treat cells in vitro, and the modified cells may optionally be administered to patients (ex vivo). Conventional viral based systems could include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often

resulting in long term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

- The tropism of a viruses can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof. In applications where transient expression is preferred, adenoviral based systems may be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors may also be used to transduce cells with target nucleic acids, e.g., in the in vitro production of nucleic acids and peptides, and for in vivo and ex vivo gene therapy procedures. There are several methods known in the art for constructing recombinant AAV vectors.
- Thus, in a particular embodiment of the third aspect, the viral vector is an adeno-associated virus (AAV) of serotype selected from the group consisting of AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, PHPeB, and 9P31.

- The invention also provides in a fourth aspect a host cell. In a particular embodiment, the host cell is transformed or transfected with the polynucleotide or the expression vector of the invention. The skilled person would know which host cells are suitable for the synthesis of the fusion protein of the invention.

- In a particular embodiment of the fourth aspect, the host cell is a eukaryotic host cell. In an alternative embodiment of the fourth aspect, the host cell is a prokaryotic host cell. Examples of host cells include, but are not limited to, C8161, CCRF-CEM, MOLT, mIMCD-3, NHDF, HeLa- S3, Huh1, Huh4, Huh7, HUVEC, HASMC, HEK293, HEK293A, MiaPaCell, Panel, PC-3, TF1, CTLL-2, C1R, Rat6, CV1, RPTE, A10, T24, J82, A375, ARH-77, Calu3, SW620, SKOV3, SK-UT, CaCo2, P388D1, SEM-K2, WEHI-231, HB56, TIB55, Jurkat, J45.01, LMB, Bcl-1, BC-3, IC21, DLD2, Raw264.7, NRK, NRK-52E, MRC5, MEF, Hep G2, HeLa B, HeLa T4, COS, COS-1, COS-6, COS-M6A, BS-C-1 monkey kidney epithelial, BALB/3T3 mouse embryo fibroblast, 3T3 Swiss, 3T3-L1, I32-d5 human fetal fibroblasts; 10.1 mouse fibroblasts, 293-T, 3T3, 721, 9L, A2780, 2780ADR, A2780cis, A 172, A20, A253, A431, A-549, ALC, B16, B35, BCP-1 cells, BEAS-2B, bEnd.3, BHK-21, BR 293, BxPC3, C3H-10T1/2, C6/36, Cal-27, CHO, CHO-7, CHO-IR, CHO-K1, CHO-K2, CHO-T, CHO Dhfr -/-, COR-L23, COR-L23/CPR, COR-L23/5010, COR-L23/R23, COS-7, COV- 434, CML TI, CMT, CT26, D17, DH82, DU145, DuCaP, EL4, EM2, EM3, EMT6/AR1, EMT6/AR10.0, FM3, H1299, H69, HB54, HB55, HCA2, HEK-

293, HeLa, Hepalcl7, HL- 60, HMEC, HT-29, Jurkat, JY cells, K562 cells, Ku812, KCL22, KG1, KY01, LNCap, Ma- Mel 1-48, MC-38, MCF-7, MCF-10A, MDA-MB-231, MDA-MB-468, MDA-MB-435, MDCK II, MDCK 11, MOR/0.2R, MONO-MAC 6, MTD-1A, MyEnd, NCI-H69/CPR, NCI- H69/FX10, NCI-H69/FX20, NCI-H69/FX4, NIH-3T3, NAFM-I, NW-145, OPCN/OPCT cell lines, Peer, PNT-1A/PNT 2, RenCa, RIN-5F, RMA/RMAS, 5 Saos-2 cells, Sf-9, SkBr3, T2, T-47D, T84, THP1 cell line, U373, U87, U937, VCaP, Vero cells, WM39, WT-49, X63, YAC-I, YAR, and transgenic varieties thereof. Cell lines are available from a variety of sources known to those with skill in the art (see, e.g., the American Type Culture Collection (ATCC)).

As mentioned before, in a fifth aspect the invention provides a cell culture comprising the host cell of the 10 fourth aspect. Examples of suitable cell culture mediums and conditions include those conventionally used in cell biology and known to the skilled person.

In a sixth aspect, the invention provides a pharmaceutical composition. The expression "pharmaceutical composition" encompasses both compositions intended for human as well as for non-human animals. The 15 skilled in the art understands that a pharmaceutical composition must comprise a therapeutically effective amount of the active compound. The expression "therapeutically effective amount" as used herein, refers to the amount of fusion protein, polynucleotide, expression vector, or host cell that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of the disease which is addressed. The particular dose of compound administered according to this invention will of course 20 be determined by the particular circumstances surrounding the case, including the compound administered, the route of administration, the particular condition being treated, and the similar considerations.

The expression "pharmaceutically acceptable excipient, diluent or carrier" refers to pharmaceutically acceptable materials, compositions or vehicles. Each component must be pharmaceutically acceptable in the 25 sense of being compatible with the other ingredients of the pharmaceutical composition. It must also be suitable for use in contact with the tissue or organ of humans and non-human animals without excessive toxicity, irritation, allergic response, immunogenicity or other problems or complications commensurate with a reasonable benefit/risk ratio.

30 Examples of suitable pharmaceutically acceptable excipients are solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical 35 composition, its use is contemplated to be within the scope of this invention.

The relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be

administered.

Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or
5 emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Excipients such as coloring agents, coating agents, sweetening, and flavoring agents can be present in the composition, according to the judgment of the formulator.

The pharmaceutical compositions of the invention can be presented in any dosage form, for example, solid or
10 liquid, and can be administered by any suitable route, for example, oral, parenteral, rectal, topical, intranasal, intraocular, intraperitoneal, sublingual, intraventricular route, for which they will include the pharmaceutically acceptable excipients necessary for the formulation of the desired dosage form.

In one embodiment, the pharmaceutical composition is for being administered to the patient via mucosa (e.g.,
15 nasal, sublingual, vaginal, buccal, or rectal), parenterally (e.g., subcutaneous, intravenous, intramuscular, or intraarterial injection, either bolus or infusion), orally, transdermally or via inhalation by means e.g. of an aerosol. Formulations suitable for parenteral administration, such as, for example, by intraarticular, intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes
20 that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Injection solutions and suspensions can also be prepared from sterile powders, granules, and tablets. In some embodiments, the composition is administered by injection e.g subcutaneous, intraperitoneal, intravesically, intravenous, intracerebroventricular, by infusion, e.g., using a reservoir or osmotic minipump or
25 intramuscular. The formulation can be provided in unit-dose or multi-dose sealed containers, such as ampoules and vials. In an even more particular embodiment, the pharmaceutical composition is for intraventricular administration or for intravenous administration; even more particularly systemic intravenous administration.

30 Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, corn-starch, powdered sugar, and combinations thereof.

35 Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked polyvinylpyrrolidone (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose,

pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, and combinations thereof.

- 5 Exemplary binding agents include, but are not limited to, starch (e.g., corn-starch and starch paste); gelatin; sugars (e.g., sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol); natural and synthetic gums (e.g., acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, polyvinylpyrrolidone), magnesium
- 10 aluminium silicate (Veegum), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; and combinations thereof.

- Exemplary preservatives may include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives. Exemplary antioxidants
- 15 include, but are not limited to, alpha tocopherol, ascorbic acid, ascorbyl palmitate, ascorbyl stearate, ascorbyl oleate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate,
- 20 tartaric acid, and trisodium edetate.

- Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium
- 25 glubionate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine,
- 30 magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and combinations thereof.

- Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate,
- 35 sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and combinations thereof.

In a particular embodiment, the pharmaceutical composition further comprises a nanoparticle encapsulating the fusion protein, the polynucleotide, the expression vector, or the host cell. In a more particular embodiment,

the nanoparticle is a lipid nanoparticle. The skilled person would appreciate that the nanoparticle is to be biocompatible and protect the active ingredient from degradation. The encapsulation in the nanoparticle can be performed using well-known methods in the state of the art.

- 5 The present invention also provides the fusion protein as defined in the first aspect, the polynucleotide as defined in the second aspect, the expression vector as defined in the third aspect, the host cell as defined in the fourth aspect, or the pharmaceutical composition as defined in the sixth aspect, for use a medicament e.g. in a subject, particularly for use in the prevention or treatment of an age-related disease or disorder in a subject, and even more particularly an age-related neurodegenerative disease or disorder in a subject.

10

- The term "treatment", as used herein, unless otherwise indicated, refers to any type of therapy which is aimed at terminating, preventing, ameliorating or reducing the susceptibility to a clinical condition or existing disease as described herein, including complete curing of a disease as well as amelioration or alleviation of said disease. Thus, "treatment," "treating," and their equivalent terms refer to obtaining a desired pharmacologic or physiologic effect, covering any treatment of a pathological condition or disorder in a subject. The effect may be prophylactic in terms of completely or partially preventing a disorder or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disorder and/or adverse effect attributable to the disorder. That is, "treatment" includes preventing the disorder from occurring or recurring in a subject, inhibiting the disorder, such as arresting its development, stopping or terminating the disorder or, at least, clinical signs associated therewith, so that the host no longer suffers from the disorder or its clinical signs, such as causing regression of the disorder or its clinical signs, for example, by restoring or repairing a lost, missing or defective function, or stimulating an inefficient process, or relieving, alleviating, or ameliorating the disorder, or clinical signs associated therewith, where ameliorating is used in a broad sense to refer to at least a reduction in the magnitude of a clinical sign parameter. The term "prevention" or "preventing", as used herein, means, but is not limited to, a process of prophylaxis in which a subject is exposed to the fusion protein of the invention prior to the induction or onset of the disease process. Altogether, such treatment results in prevention or reduction of the clinical signs of the disease or disorder.

- 30 A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

- 35 As used herein, "age-related disease or disorder" refers to diseases and disorders often associated with aging, that is, diseases or disorders in which aging is a major risk factor, and include cancers (e.g., gliomas, leukemia, lymphoma, breast cancer, prostate cancer, lung cancer, etc.), neurodegenerative diseases (e.g., Parkinson's disease, Alzheimer's disease, Huntington's disease, dementia, etc.), sarcopenia, osteopenia, osteoporosis, arthritis, atherosclerosis, cardiovascular disease, hypertension, cataracts, presbyopia,

glaucoma, type 2 diabetes, metabolic syndrome, alopecia, chronic inflammation, immunosenescence, and the like.

Thus, in one embodiment, the age-related disease or disorder is selected from the group consisting of cancer, neurodegenerative disease, sarcopenia, osteopenia, osteoporosis, arthritis, skin atrophy, atherosclerosis, cardiovascular disease, hypertension, cataracts, presbyopia, glaucoma, type 2 diabetes, metabolic syndrome, chronic obstructive pulmonary disease (COPD), alopecia, hearing loss, impaired kidney function, chronic inflammation, and immunosenescence.

In a more particular embodiment, the age-related disease or disorder is an age-related neurodegenerative disease or disorder. In an even more particular embodiment, the age-related neurodegenerative disease or disorder is selected from the group consisting of Parkinson's disease, Alzheimer's disease, Huntington's disease, and dementia.

In a particular embodiment of the eighth and ninth aspects, the subject is a mammal, particularly a human or a non-human mammal. In another embodiment, the subject is a non-human primate. In some embodiments, the subject is a rodent. In some embodiments, the subject is a sheep, a goat, a cattle, a cat, or a dog. In some embodiments, the subject is a vertebrate, an amphibian, a reptile, a fish, an insect, a fly, or a nematode. In some embodiments, the subject is a research animal. In some embodiments, the subject is genetically engineered, e.g., a genetically engineered non-human subject. The subject may be of either sex and at any stage of development.

In another embodiment, the polypeptide, the nucleic acid, the expression vector, the host cell, or the pharmaceutical composition is administered in combination with another active agent. Suitable active agents to be administered in combination with a compound of the invention are, for example, drugs for treating neurodegenerative diseases.

As above mentioned, in a seventh aspect the invention provides process for the production of the compound according to the first aspect. The skilled person is familiar with several standard methods to isolate the resulting compound from the cell culture or after *in vitro* transcription and/or translation, for instance, Protein A purification column purification.

Sequences disclosed in the present invention are shown in Table 1 below:

Table 1

SEQ ID NO: 1 (sTREM2)	MEPLRLLILLFVTELSGAHNNTTVFQGQVAGQSLQVSCPYDSMKHWGRRKAWCRQLGEK GPCQRVVSTHNLWLLSFLRRWNGSTAITDDTLGGTLTITLRNLQPHDAGLYQCQSLHG SEADTLRKVLVEVLADPLDHRDAGDLWFPGESESFEDAHVEH
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SEQ ID NO: 2 (sTREM2 w/o signal peptide)	HNTTVFQGVAGQSLQVSCPYSMKHWGRRKAWCRQLGEKGPCQRVWSTHNLWLLS FLRRWNGSTAITDDTLGGTLTITLRNLQPHDAGLYQCQSLHGSEADTLRKVLVEVLAD PLDHRDAGDLWFPGESESFEDAHVEH
SEQ ID NO: 3 (TIMP2)	MGAAARTLRLALGLLLLATLLRPADACSCSPVHPQQAFCNADVIRAKAVSEKEVDSG NDIYGNPIKRIQYEIKQIKMFKGPEKDIEFIYTAPSSAVCGVSLDVGGKKEYLIAGKAEGD GKMHITLCDFIVPWDLTSTTQKKSLNHRYQMGCECKITRCPMIPCYISSPDECLWMDW VTEKNINGHQAKFFACIKRSDGSCAWYRGAAPPKQEFLDIEDP
SEQ ID NO: 4 (TIMP2 w/o signal peptide and C-terminal fragment)	CSCSPVHPQQAFCNADVIRAKAVSEKEVDSGNDIYGNPIKRIQYEIKQIKMFKGPEKDI EFIYTAPSSAVCGVSLDVGGKKEYLIAGKAEGDGKMHITLCDFIVPWDLTSTTQKKSLN HRYQMGCECKITRCPMIPCYISSPDECLWMDWVTEKNINGHQAKFFACIKRSDGSCA WYRGAA
SEQ ID NO: 5 (sKL)	MPASAPRRRPRPPPSLSLLLVLGLGGRRRLRAEPGDGAQTWARFSRPPAPEAAGLF QGTFPDGFLWAVGSAAYQTEGGWQQHKGKASIWDFTFHHPLAPPGDSRNASLPLGA PSPLQPATGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGL RYYRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRH FGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHL YNT SFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAKPVFIDGDYPESMK NNLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWID LEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLM DGF EWHRGYSIRRGFLFYVDFLSQDKMLLPKSSALFYQKLIKNGFPPLPENQPLEGTF PCDFAWGVDNYIQVSQLTKPISSLTKPYH
SEQ ID NO: 6 (sKL w/o signal peptide)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGFLWAVGSAAYQTEGGWQQHKGKAS IWDFTFHHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNNVFRDTEALRELGVTH YRFSISWARVLPNGSAGVPNREGLRYYRLLERLRELGVQPVTLYHWDLPQRLQDA YGGWANRALADHFRDYAELCFRHFGGQVKYWITIDNPYVVAWHGYATGRLAPGIRGS PRLGYLVAHNLLLAHAKVWHL YNTSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQK SLDFVLGWFAKPVFIDGDYPESMKNNLSSILPDFTESEKKFIKGTADFFALCFGPTLSF QLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKK KFIMETLKAIKLDGVDVIGYTAWSLMDGF EWHRGYSIRRGFLFYVDFLSQDKMLLPKSSA LFYQKLIKNGFPPLPENQPLEGTFPCDFAWGVDNYIQVSQLTKPISSLTKPYH
SEQ ID NO: 7 (L1)	EAAAKEAAAKEAAK
SEQ ID NO: 8 (L2)	EAAAKEAAAKEAAAKEAAAKEAAK
SEQ ID NO: 9 (HEBE)	MEPLRLLILLFVTELSGAHNTTVFQGVAGQSLQVSCPYSMKHWGRRKAWCRQLGEK GPCQRVWSTHNLWLLSFLRRWNGSTAITDDTLGGTLTITLRNLQPHDAGLYQCQSLHG SEADTLRKVLVEVLADPLDHRDAGDLWFPGESESFEDAHVEHEAAAKEAAAKEAAK

	CSCSPVHPQQAFCNADVIRAKAVSEKEVDSGNDIYGNPIKRIQYEIKQIKMFKGPEKDI EFIYTAPSSAVCGVSLDVGGKKEYLIAGKAEGDGKMHITLCDFIVPWDTLSTTQKKS LN HRYQMGCECKITRCPMIPCYISSPDECLWMDWVTEKNINGHQAKFFACIKRSDGSCA WYRGAAEAAAKEAAAKEAAAKEAAAKEAAAKEPGDGAQTWARFSRPPAPEAAGLFQ GTFPDGF LWAVGSAAYQTEGGWQQHKGASIWDFTTHPLAPPGDSRNASLPLGAP SPLQPATGDVASDSYNNVFRDTEALRELGVTHYRFSISWARVLPNGSAGVPNREGLR YYRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWANRALADHFRDYAELCFRHF GGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGYLVAHNLLLAHAKVWHLYN TS FRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFVLGWFAPVFDGDYPESMKN NLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDPHMKFRQLESPNLRQLLSWIDL EFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFIMETLKAIKLDGVDVIGYTAWSLMD GFEWHRGYSIRRGIFYVDFLSQDKMLLPKSSALFYQK LIEKNGFPPLPENQPLEGTFP CDFAWGVVDNYIQVSQLTKPISSLTKPYH
SEQ ID NO: 10 (HEBE2)	MGAAARTLRLALG LLLLATLLRPADACSCSPVHPQQAFCNADVIRAKAVSEKEVDSG NDIYGNPIKRIQYEIKQIKMFKGPEKDIEFIYTAPSSAVCGVSLDVGGKKEYLIAGKAEGD GKMHITLCDFIVPWDTLSTTQKKS LN HRYQMGCECKITRCPMIPCYISSPDECLWMDW VTEKNINGHQAKFFACIKRSDGSCAWYRGAAAPPKQEF LDIEDPEAAAKEAAAKEAAAK HNTTVFQGVAGQSLQVSCPYDSMKHWGRRKAWCRQLGEKGPCQRV VSTHNLWLLS FLRRWNGSTAITDDTLGGTLTITLRNLQPHDAGLYQCQSLHGSEADTLRKVLVEVLAD PLDHRDAGDLWFPGESESFEDAHVEHEAAAKEAAAKEAAAKEAAAKEEPGDGA QTWARVSRPPAPEAAGLFQGTFPDGF LWAVGSAAYQTEGGWQQHKGASIWDFTT HHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNNVFRDTEALRELGVTHYRFSIS WARVLPNGSAGVPNREGLRYYRLLERLRELGVQPVTLYHWDLPQRLQDAYGGWA NRALADHFRDYAELCFRHF GGQVKYWITIDNPYVVAWHGYATGRLAPGIRGSPRLGY LVAHNLLLAHAKVWHLYN TSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQKSLDFV LGWFAKPVFDGDYPESMKN NLSSILPDFTESEKKFIKGTADFFALCFGPTLSFQLLDP HMKFRQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYLKKFIMET LKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLLPKSSALFYQK LIEKNGFPPLPENQPLEGTFPCDFAWGVVDNYIQVSQLTKPISSLTKPYH
SEQ ID NO: 11 (KL1)	EPGDGAQTWARFSRPPAPEAAGLFQGTFPDGF LWAVGSAAYQTEGGWQQHKGAS IWDFTTHPLAPPGDSRNASLPLGAPSPLQPATGDVASDSYNNVFRDTEALRELGVTH YRFSISWARVLPNGSAGVPNREGLRYYRLLERLRELGVQPVTLYHWDLPQRLQDA YGGWANRALADHFRDYAELCFRHF GGQVKYWITIDNPYVVAWHGYATGRLAPGIRGS PRLGYLVAHNLLLAHAKVWHLYN TSFRPTQGGQVSIALSSHWINPRRMTDHSIKECQK SLDFVLGWFAPVFDGDYPESMKN NLSSILPDFTESEKKFIKGTADFFALCFGPTLSF QLLDPHMKFRQLESPNLRQLLSWIDLEFNHPQIFIVENGWVFSGTTKRDDAKYMYL K KFIMETLKAIKLDGVDVIGYTAWSLMDGFEWHRGYSIRRGIFYVDFLSQDKMLLPKSSA LFYQK LIEKNGFPPLPENQPLEGTFPCDFAWGVVDNYIQV

Throughout the description and claims the word "comprise" and variations of the word, are not intended to exclude other technical features, additives, components, or steps. Furthermore, the word "comprise" encompasses the case of "consisting of". Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples and drawings are provided by way of illustration, and they are not intended to be limiting of the present invention. Furthermore, the present invention covers all possible combinations of particular and preferred embodiments described herein.

10 Examples

1. Materials and methods

All-atom models

For sKL, sTREM2 and TIMP2, all-atom models were built from the crystal structures in PDB entries 5W21, 5UD7 and 1BR9, respectively. The model for Klotho included residues E34-L515 of chain A of 5W21.pdb. A stretch of 18 residues (L98-S115) absent from the crystal was included using Modeller. For sTREM2, all residues (T21-A130) of the chain F of 5UD7.pdb were retained. For TIMP2, all residues (C1-A182) in 1BR9.pdb were included (12 C-terminal residues not seen in the crystal structure were not added to the model).

All-atom MD simulations

For each protein, titrable residues were attributed their dominant protonation state at pH 7.4. Neutral groups were added to cap the N- and C-termini of each protein chain. The software HTMD was employed to solvate and ionize the proteins. Each protein was inserted in a water box whose edges were at a minimum distance of 12 Å from the solute. Ions were added to neutralize charges and up to a concentration of 0.15 M NaCl.

After 500 steps of energy minimization with the conjugated gradient method, systems were equilibrated for 3 ns in the NPT ensemble, with temperature of 300 K and pressure of 1 atm. Harmonic position restraints of 1.0 kcal·mol⁻¹·Å⁻² were applied to the Cα atoms, while restraints of 0.1 kcal·mol⁻¹·Å⁻² were applied to the remaining heavy atoms of the protein. These restraints were progressively released during equilibration. Production simulations of 520 ns were performed in the NVT ensemble, at 300 K. In all stages, temperature was controlled by a Langevin thermostat, a 9.0 Å cut-off was used for non-bonded interactions, and long-range electrostatic interactions were calculated with the particle-mesh Ewald (PME) method. Hydrogen mass repartitioning allowed a timestep of 4 fs. The ff14SB force field was used for proteins, and water was described by the TIP3P model. The Acemd 2 software was employed to run the trajectories.

Analysis of all-atom MD simulations

Principal Component Analysis (PCA) consisted in the diagonalization of the covariance matrix, whose elements are:

$$C_{ij} = \langle (r_i - \langle r_i \rangle) \cdot (r_j - \langle r_j \rangle) \rangle$$

- 5 where i and j denote all pairs of the 3N Cartesian coordinates of the N C α atoms in the protein. The vector r_i indicates the instantaneous value of coordinate i and $\langle r_i \rangle$ is the average value of this coordinate in the ensemble of conformations. The coordinates of the C α atoms in each individual trajectory were projected on the space of the respective first two principal components. These analyses were performed with the Gromacs software.

10

Root-mean-square fluctuations (RMSF) were also calculated with Gromacs, according to the expression:

$$\text{RMSFi} = [\langle (r_i - \langle r_i \rangle)^2 \rangle]^{1/2}$$

where r_i denotes the coordinates of atom i .

15 Construction of models of the united proteins

These models were built by homology modeling using Modeller. A single sequence, consisting of the sequences of the three proteins and the linkers arranged in the desired order, was defined as the target, and the structures of sKL, sTREM2 and TIMP2 were used as templates. Additional restrictions were added to enforce the helical secondary structure of the linkers.

20

Coarse-grained MD simulations

- The all-atom models of the united proteins were converted to CG representation using the "Martini Maker" functionality of the Charmm-gui webserver. The systems were described by the Martini 2.2 force field, with the addition of an elastic network composed of harmonic springs between interacting sites (elndyn) to preserve
- 25 the secondary and overall tertiary structures of each protein. However, it is important to note that there were no inter-protein springs, so the relative motions of the three components were not restrained. The CG models were solvated and ionized up to a 0.15 M NaCl concentration. Each system was submitted to two independent simulation replicas. In each replica, after energy minimization with the steepest descent algorithm, equilibration in the NPT ensemble, with progressive release of position restraints on the protein, was run for
- 30 20 ns. Equilibration was followed by a 10 μ s production in the NPT ensemble, with temperature of 300 K and pressure of 1 atm. Temperature was controlled with the v-rescale thermostat, and pressure was controlled with the Berendsen (during equilibration) and Parrinello-Rahman (during production) barostats. A dielectric constant of 15 was adopted, and electrostatic interactions as well as van der Waals interactions were shifted to zero between 0 and 11 Å. In equilibration and production the timestep was 20 fs. All CG simulations were
- 35 performed with the Gromacs software (version 2019). Trajectories were visually inspected for an assessment on protein aggregation.

Production and purification of recombinant proteins

The proteins were generated using adenoviral vectors (Ad5) that expressed each protein with a histidine tag for purification. CHO cells (ATCC CCL-61) were used to produce the proteins by infecting them with the viral vector as previously described by Liu et al, 2003. After 48 hours of infection, the cellular media was collected and the soluble proteins were purified using affinity chromatography (Maertens et al., 2015).

Western Blot

Cell lysates with RIPA (50 mM Tris HCl, 150 mM NaCl, 1.0% (v/v) NP-40, 0.5% (w/v) Sodium Deoxycholate, 1.0 mM EDTA, 0.1% (w/v) SDS, 0.01% (w/v) sodium azide, pH 7.4) and cellular media were resolved on an SDS-PAGE gel and transferred to a PVDF membrane. After blocking, the proteins were detected with appropriate primary antibodies. Then, the membranes were incubated with HRP-conjugated antibodies and visualized with a Chemidoc (Bio-Rad). Primary antibodies used were: TREM2 (RD Systems, AF1828, 1:1000), TIMP2 (RD Systems, AF971, 1:1000), Klotho (Merck, MABN1807, 1:1000)

β-glucuronidase assay

10 μg of sKL (SEQ ID NO: 5) and equimolar amounts of the other indicated proteins diluted in 0.1M citrate buffer were incubated with 0.5mM of substrate 4-methylumbelliferyl β-D-glucuronide hydrate (Sigma M9130). The reaction was allowed to take place for 2 hours at 37°C, and the fluorescence of the reaction product was measured with an excitation of 360nm and an emission of 470nm in a Spark plate reader following manufacturer's instructions as previously described by Clerin et al., 2020 . Bovine β-glucuronidase (Sigma G0251) was used as a positive control, and PBS as a negative control.

Phagocytosis assay

BV2 cells (CLS 305156) were seeded in 96-well plates. After 16 hours of incubation with 2 μg/ml of sTREM2 and equimolar concentrations of the other indicated proteins, 3 μg of pHrodo-labeled myelin was added. After an incubation with an antibody against CD45 to label activated microglial cells, phagocytic activity was measured as the increase in intracellular fluorescence by flow cytometry in a CytoFLEX cytometer following manufacturer's instructions.

MMP1 inhibition activity assay

In a 96-well plate, 1.6 μg/ml of MMP1 (Peprtech 420-01, NCBI accession NP_002412, and version NP_002412.1) was incubated with 30 μM substrate (Sigma SCP0193), 1 μg/ml of TIMP2, and equimolar concentrations of the other indicated proteins. The mixture was incubated for 30 minutes at room temperature and fluorescence was measured with an excitation of 324nm and an emission of 400nm in a Spark plate reader following manufacturer's instructions. The activity of TIMP2 was measured as the reduction of fluorescence due to the inhibition of MMP1 enzymatic activity on the substrate that yields the fluorescent product. TIMP2 was omitted as a positive control, and MMP1 was not added as a negative control.

Animals

Double mutant APP/Tau mice (Flinn et al., 2018) were used as models of Alzheimer's disease, as well as WT controls. Both male and female mice at 24 weeks of age were injected intravenously with AAV9P31 (Hou et al., 2021) adeno-associated vectors that expressed HEBE (SEQ ID NO: 9) or Null as a control. Dose was $5 \cdot 10^{11}$ vg/mouse.

5 Novel Object Recognition Test

The test consisted of 3 days. On the first day, the mouse was familiarized with a 40x40cm box for 5 minutes. On the second day, 2 identical objects were placed inside the box, and the mouse had 10 minutes to explore them, expecting it to explore each object for the same amount of time. On the third day, one of the objects was replaced with a new one, and the mouse had 10 minutes to explore them. The time that the mouse spent
10 exploring each object was analyzed, wherein a longer time exploring the new object was indicated of a better memory.

2. Results

15 Bioinformatics study

In order to get insights into the conformational dynamics of sKL, sTREM2 and TIMP2, molecular dynamics (MD) simulations were performed separately for each one of them. Starting from the crystal structures (see above), all-atom models were built and a 520 ns trajectory was calculated for each protein. While this limited sampling was not enough for a detailed evaluation of the proteins' dynamical behavior, the short trajectories
20 nevertheless revealed that sKL (Figure 1) and TIMP2 (Figure 3) were flexible. This conformational flexibility could be evaluated through a Principal Component Analysis (PCA), whose results are illustrated in Figures 1B, 2B and 3B. In each of these illustrations, the square indicates the initial conformation in the simulation (corresponding to the crystal structure), and the dots represent the conformations visited along the trajectory. While sTREM2 (Figure 2) exhibited a more restricted dynamics, the other two proteins explored a larger
25 conformational space and therefore adopted distinct conformations during the simulations (Figure 1 and 2).

Calculation of root-mean-square fluctuations (RMSF) allowed to identify the most flexible regions of each structure.

30 It was concluded that the proteins – particularly sKL and TIMP2 – were flexible, and this flexibility could be potentially associated to functional motions. Therefore, any attempt to unite these proteins should preserve their conformational freedom, to avoid disturbing their function.

Then, models of the three proteins united in a single polypeptide chain were built as above indicated. Different
35 orders of the proteins in the sequence, as well as different linkers, were tested. New MD simulations of these models were performed to verify that proteins would not aggregate, and that each one would remain relatively distant from the others in order to preserve its conformational freedom. However, given the size of the system composed by the three proteins and the possibility that aggregation could occur on long timescales, a coarse-

grained (CG) representation was used. Despite the simplifications of this model (which prevents large scale conformational changes of individual proteins), it allowed to reach longer simulation times and interrogate the interactions between the three proteins; in particular, it was possible to monitor protein aggregation in the different constructs.

5

The most satisfactory results were obtained with rigid, helical linkers. The model in which inter-protein contacts were less frequently observed was constituted by: sTREM2 + Linker 1 + TIMP2 + Linker 2 + sKL.

10 In this construct (named HEBE = SEQ ID NO: 9), Linker 1 was attached to the C-terminal of sTREM2 and the N-terminal of TIMP2; Linker 2 was connected to the N-terminal of sKL. The signaling peptides of TIMP2 and sKL were not included in the sequence. Linker 1 was composed of 3 repeats of the sequence EAAAK; Linker 2 was composed of 5 repeats of the sequence EAAAK.

In vitro characterization

15 To verify that HEBE could be produced and was stable, HEK293 cells were transfected *in vitro*.

The protein (~101kDa) was detected by Western Blot using antibodies against each of the subunits, both in the cell extract and in the media (Figure 4). This indicated that the protein was expressed and secreted correctly and did not break down. The secreted protein had a higher molecular weight than the intracellular one, probably due to post-translational modifications.

20

Both the HEBE protein (SEQ ID NO: 9) and the three individual proteins (sTREM2 = SEQ ID NO: 1; TIMP2 = SEQ ID NO: 3; and sKL = SEQ ID NO: 5) were produced in *in vitro* cultures to obtain recombinant protein and test the biological activity of HEBE against the rest.

25

The first activity analyzed was β -glucuronidase. sKL has this activity, so it was expected that HEBE would also have it.

30 In this case, as shown in Figure 5, although sTREM2 or TIMP2 alone did not exhibit any β -glucuronidase on their own, they strongly potentiated the β -glucuronidase activity of sKL when incorporated into HEBE, clearly demonstrating an unexpected synergistic effect.

The phagocytic activity of BV2 cells was also analyzed *in vitro*. sTREM2 modulates the phagocytic activity of microglia, so one would expect HEBE to maintain this function.

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As it can be observed in Figure 6, the fusion protein HEBE reduced phagocytic activity to a much greater extent than any of the individual proteins, including sTREM2. Demonstrating, again, an unexpected synergistic effect of the fusion protein of the invention.

With these data, it can be concluded that HEBE (SEQ ID NO: 9), a fusion protein according to the invention, presents *in vitro* biological activity, and according to the results, even greater than the individual proteins.

5 In vivo activity

To test whether HEBE has *in vivo* activity in improving pathologies, a study was conducted by injecting AAV viral vectors into APP/Tau mice, which are models of Alzheimer's disease.

To analyze the effects on memory, the Novel Object Recognition Test was performed.

10

On the first day, when the mice were presented with two identical objects, they showed no preference for either, as expected (Figure 7 A). However, on the second day, when one of the two objects was replaced with a new one, it can be observed that WT mice had a greater preference for the new object (Figure 7 B). Diseased control mice (AD Null) did not remember the objects, so they showed no preference for the new object. However, when these mice were treated with HEBE, they appeared to remember the objects, and their preference for the new object increased to levels similar to those of the WT mice.

15

These results clearly indicates that HEBE, a fusion protein according to the invention, could be useful in the treatment of conditions associated with aging and neurodegenerative diseases like Alzheimer's.

20

Design of a new version of HEBE

Next, a second fusion protein according to the invention was designed, produced, and tested (hereafter referred to as HEBE2 = SEQ ID NO: 10) by placing TIMP2 at the N-terminal end, therefore obtaining a fusion protein with the following structure —TIMP2-sTREM2-sKL. In this protein, the signaling peptides of sTREM2 and sKL were not included.

25

Using a Western blot, the protein (~101kDa) was detected with antibodies against each of the subunits, both in the cell extract and in the media (Figure 8). This indicates that, as observed in the first version of HEBE, the HEBE2 version was expressed and secreted correctly, was stable and not broken. It was also observed that the secreted HEBE2 protein had a higher molecular weight than the intracellular one, probably due to post-translational modifications. Finally, it was confirmed by *in vitro* and *in vivo* assays that HEBE2 showed a biological activity comparable to that observed for HEBE.

30

For reasons of completeness, various aspects of the invention are set out in the following numbered clauses:

35

1. A fusion protein comprising:

- (i) sTREM2 or a functional variant thereof;
- (ii) TIMP2 or a functional variant thereof; and
- (iii) sKL or a functional variant thereof.

2. The fusion protein according to clause 1, wherein:
- sTREM2 comprises SEQ ID NO: 2, and the functional variant of sTREM2 comprises a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 2;
 - TIMP2 comprises SEQ ID NO: 4, and the functional variant of TIMP2 comprises a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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% identical to SEQ ID NO: 4; and/or
 - sKL comprises SEQ ID NO: 6, and the functional variant of sKL comprises a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 6.
3. The fusion protein according to clause 1 or 2, wherein:
- the functional variant of sTREM2 maintains phagocytosis-inhibiting activity;
 - the functional variant of TIMP2 maintains MMP1-inhibiting activity; and/or
 - the functional variant of sKL maintains β -glucuronidase activity.
4. The fusion protein according to any one of clauses 1-3, wherein the fusion protein further comprises a linker between at least two of the proteins comprised in the fusion protein; optionally between each protein comprised in the fusion protein.
5. The fusion protein according to clause 4, wherein the linker is a peptide linker; optionally of sequence (EAAAK)_n.
6. The fusion protein according to any one of clauses 1-5, which is of formula (I) or (II):
- R₁-L₁-R₂-L₂-R₃ (I)
 R₂-L₁-R₁-L₂-R₃ (II)
- wherein:
- R₁ is sTREM2 or a functional variant thereof;
 - L₁ is a linker;
 - R₂ is TIMP2 or a functional variant thereof;
 - L₂ is a linker; and
 - R₃ is sKL or a functional variant thereof.
7. The fusion protein according to clause 6, wherein:

- L₁ comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to of the sequence EAAAKEAAAKEAAAK (SEQ ID NO: 7); and/or
- 5 - L₂ comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to the sequence EAAAKEAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 8).
- 10 8. The fusion protein according to any one of clauses 1-7, which comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to SEQ ID NO: 9 or SEQ ID NO: 10.
- 15 9. A polynucleotide that encodes the fusion protein as defined in any one of clauses 1-8, optionally wherein the polynucleotide comprises SEQ ID NO: 12 or SEQ ID NO: 13 or a functional variant thereof at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 12 or SEQ ID NO: 13..
- 20 10. An expression vector comprising the polynucleotide as defined in clause 9.
- 11. The expression vector according to clause 10, which is a viral vector; optionally wherein the viral vector is an adeno-associated virus of serotype selected from the group consisting of AAV1, AAV2, AAV4, AAV5,
- 25 AAV6, AAV7, AAV8, AAV9, AAVrh10, PHPeB, and 9P31.
- 12. A host cell comprising the fusion protein as defined in any one of clauses 1-8, the polynucleotide as defined in clause 9, or the expression vector as defined in clause 10 or 11.
- 30 13. A pharmaceutical composition comprising a therapeutically effective amount of the fusion protein as defined in any one of clauses 1-8, the polynucleotide as defined in clause 9, the expression vector as defined in clause 10 or 11, or the host cell as defined in clause 12, with at least one pharmaceutically acceptable excipient, diluent, or carrier.
- 35 14. The fusion protein as defined in any of clauses 1-8, the polynucleotide as defined in clause 9, the expression vector as defined in clause 10 or 11, the host cell as defined in clause 12, or the pharmaceutical composition as defined in clause 13, for use as a medicament.
- 15. The fusion protein as defined in any of clauses 1-8, the polynucleotide as defined in clause 9, the

expression vector as defined in clause 10 or 11, the host cell as defined in clause 12, or the pharmaceutical composition as defined in clause 13, for use in the prevention or treatment of an age-related disease or disorder; particularly an age-related neurodegenerative disease or disorder.

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Claims

1. A fusion protein comprising:
 - (i) sTREM2 or a functional variant thereof that maintains phagocytosis-inhibiting activity;
 - 5 (ii) TIMP2 or a functional variant thereof that maintains MMP1-inhibiting activity; and
 - (iii) secreted splicing isoform of Klotho (sKL) or a functional variant thereof that maintains β -glucuronidase activity.
2. The fusion protein according to claim 1, wherein:
 - 10 - sTREM2 comprises SEQ ID NO: 2, and the functional variant of sTREM2 comprises a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 2;
 - TIMP2 comprises SEQ ID NO: 4, and the functional variant of TIMP2 comprises a sequence at least 80 %,
 - 15 at least 81 %, at least 82%, at least 83%, at least 84%, 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% identical to SEQ ID NO: 4; and/or
 - secreted splicing isoform of Klotho (sKL) comprises SEQ ID NO: 6, and the functional variant of secreted splicing isoform of Klotho (sKL) comprises a sequence at least 80 %, at least 81 %, at least 82%, at least
 - 20 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 6.
3. The fusion protein according to claim 1 or 2, wherein the fusion protein further comprises a linker between
 - 25 at least two of the proteins comprised in the fusion protein; optionally between each protein comprised in the fusion protein.
4. The fusion protein according to claim 3, wherein the linker is a peptide linker; optionally of sequence (EAAAK)_n.
 - 30
5. The fusion protein according to any one of claims 1-4, which is of formula (I) or (II):

$$R_1-L_1-R_2-L_2-R_3 \text{ (I)}$$

$$R_2-L_1-R_1-L_2-R_3 \text{ (II)}$$

- 35 wherein:
 - R_1 is sTREM2 or a functional variant thereof;
 - L_1 is a linker;
 - R_2 is TIMP2 or a functional variant thereof;
 - L_2 is a linker; and

R₃ is secreted splicing isoform of Klotho (sKL) or a functional variant thereof.

6. The fusion protein according to claim 5, wherein:

- 5 - L₁ comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to of the sequence EAAAKEAAAKEAAAK (SEQ ID NO: 7); and/or
- 10 - L₂ comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to the sequence EAAAKEAAAKEAAAKEAAAKEAAAK (SEQ ID NO: 8).

7. The fusion protein according to any one of claims 1-6, which comprises or consists of a sequence at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or 100 % identical to SEQ ID NO: 9 or SEQ ID NO: 10.
- 15

8. A polynucleotide that encodes the fusion protein as defined in any one of claims 1-7, optionally wherein the polynucleotide comprises SEQ ID NO: 12 or SEQ ID NO: 13 or a functional variant thereof at least 80 %, at least 81 %, at least 82%, at least 83%, at least 84%, 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%, or at least 99% identical to SEQ ID NO: 12 or SEQ ID NO: 13..
- 20

9. An expression vector comprising the polynucleotide as defined in claim 8.
- 25

10. The expression vector according to claim 9, which is a viral vector; optionally wherein the viral vector is an adeno-associated virus of serotype selected from the group consisting of AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAVrh10, PHPeB, and 9P31.

- 30 11. A host cell comprising the fusion protein as defined in any one of claims 1-7, the polynucleotide as defined in claim 8, or the expression vector as defined in claim 9 or 10.

12. A pharmaceutical composition comprising a therapeutically effective amount of the fusion protein as defined in any one of claims 1-7, the polynucleotide as defined in claim 8, the expression vector as defined in claim 9 or 10, or the host cell as defined in claim 11, with at least one pharmaceutically acceptable excipient, diluent, or carrier.
- 35

13. The fusion protein as defined in any of claims 1-7, the polynucleotide as defined in claim 8, the expression vector as defined in claim 9 or 10, the host cell as defined in claim 11, or the pharmaceutical composition as

defined in claim 12, for use as a medicament.

14. The fusion protein as defined in any of claims 1-7, the polynucleotide as defined in claim 8, the expression vector as defined in claim 9 or 10, the host cell as defined in claim 11, or the pharmaceutical composition as defined in claim 12, for use in the prevention or treatment of an age-related disease or disorder; particularly an age-related neurodegenerative disease or disorder.

15. The fusion protein, the polynucleotide, the expression vector, the host cell, or the pharmaceutical composition for use according to claim 14, wherein the age-related neurodegenerative disease or disorder is selected from the group consisting of Parkinson's disease, Alzheimer's disease, Huntington's disease, and dementia.

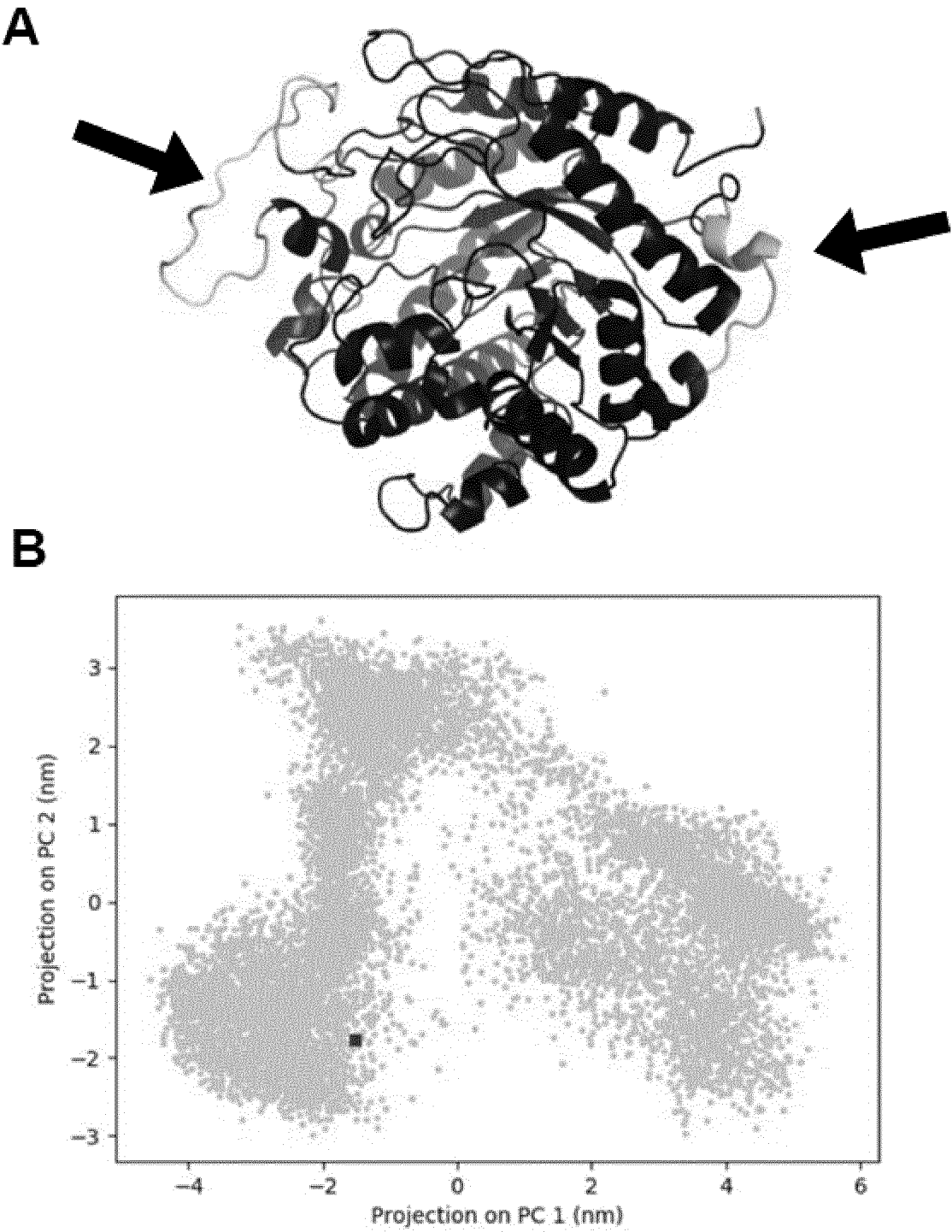


FIG. 1

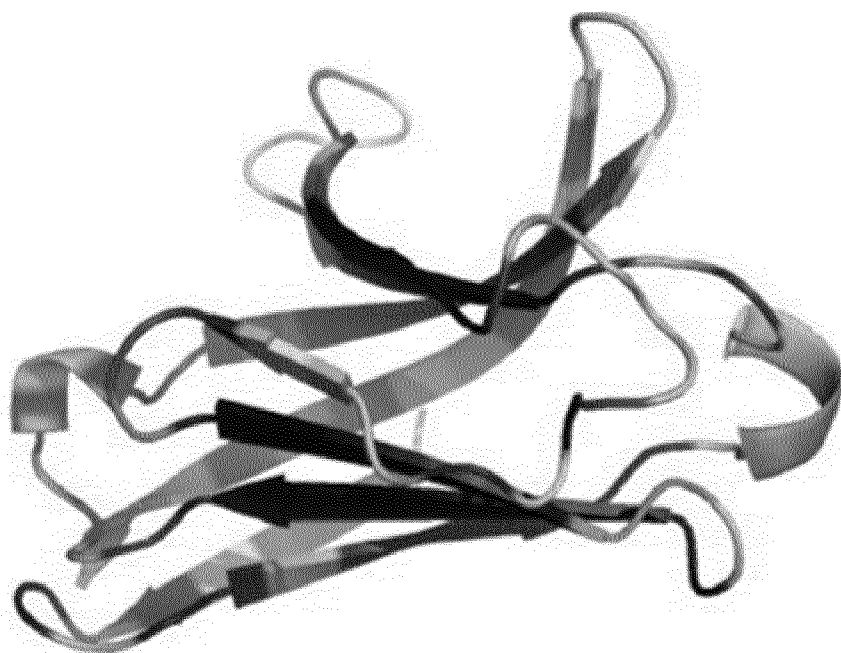
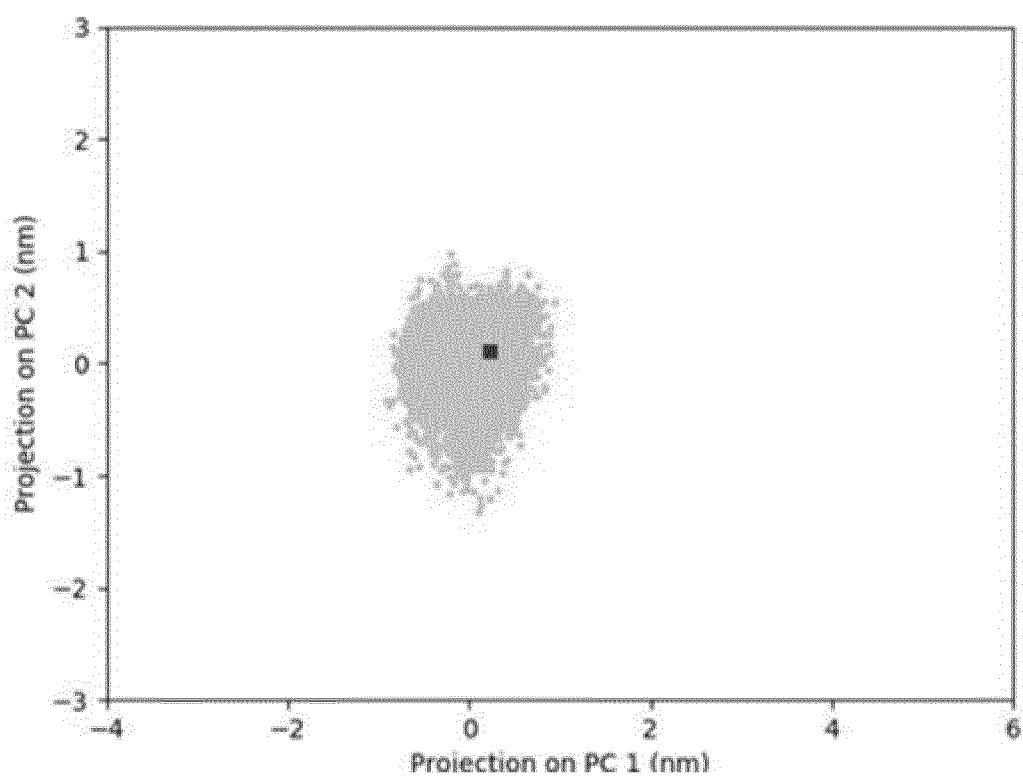
A**B**

FIG. 2

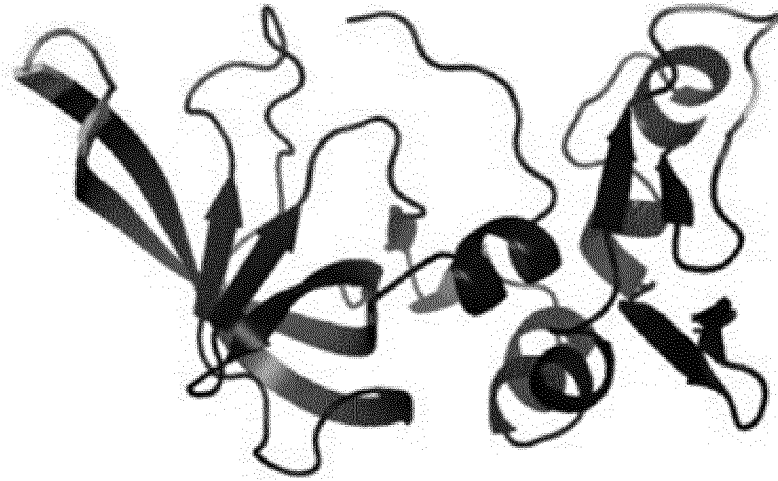
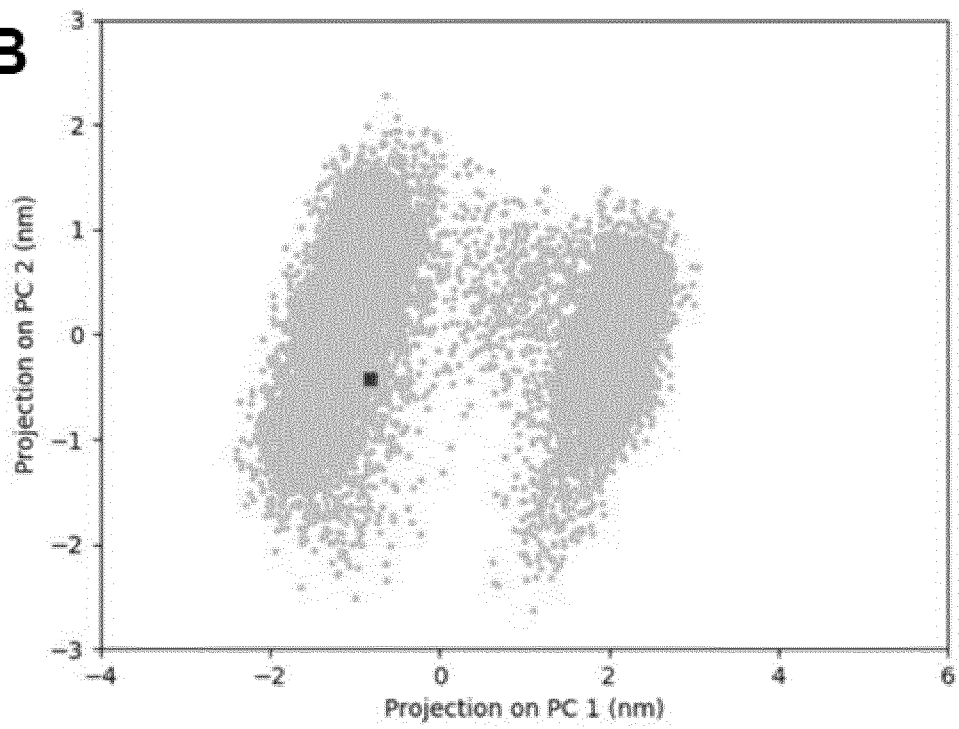
A**B**

FIG. 3

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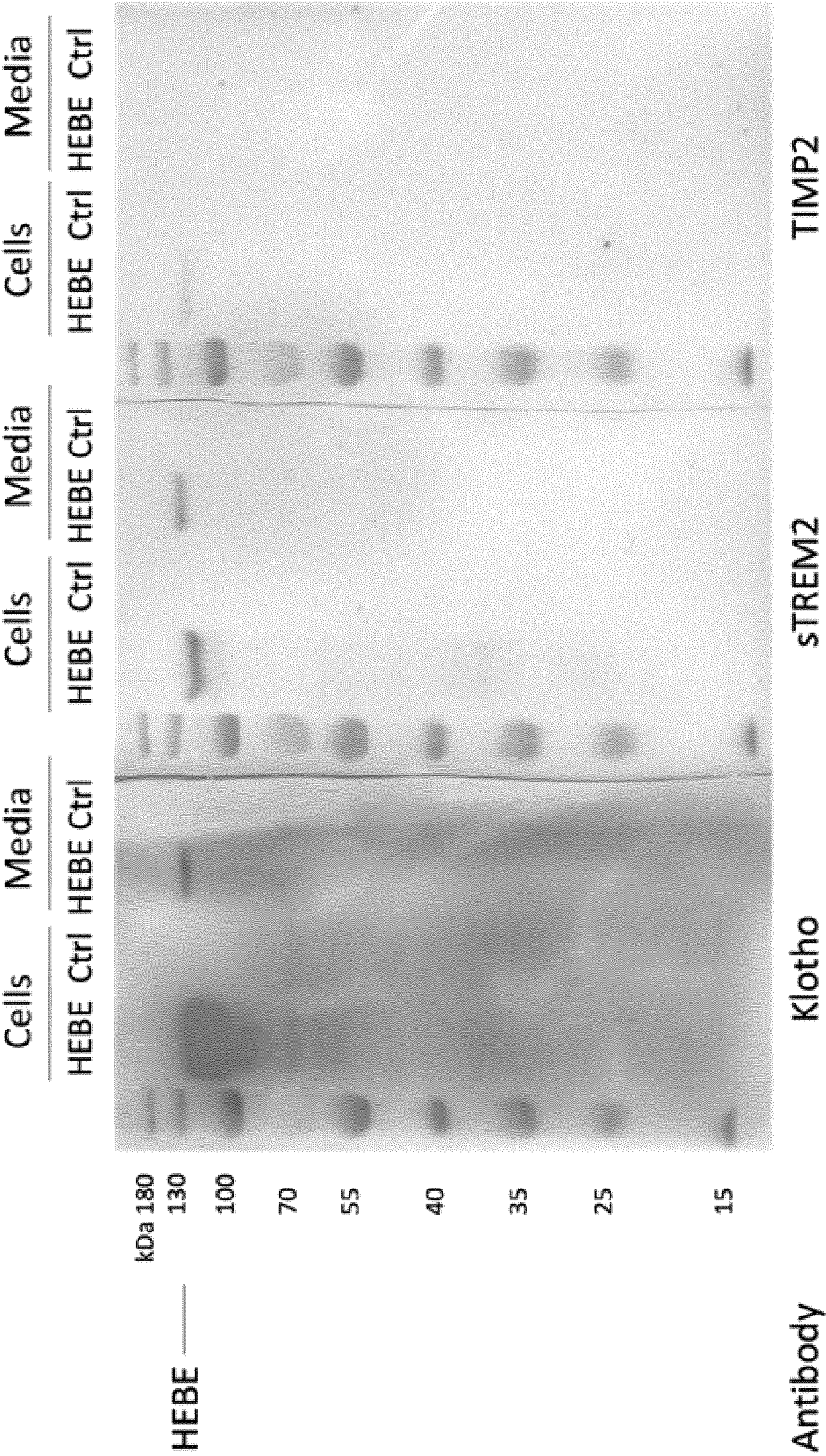


FIG. 4

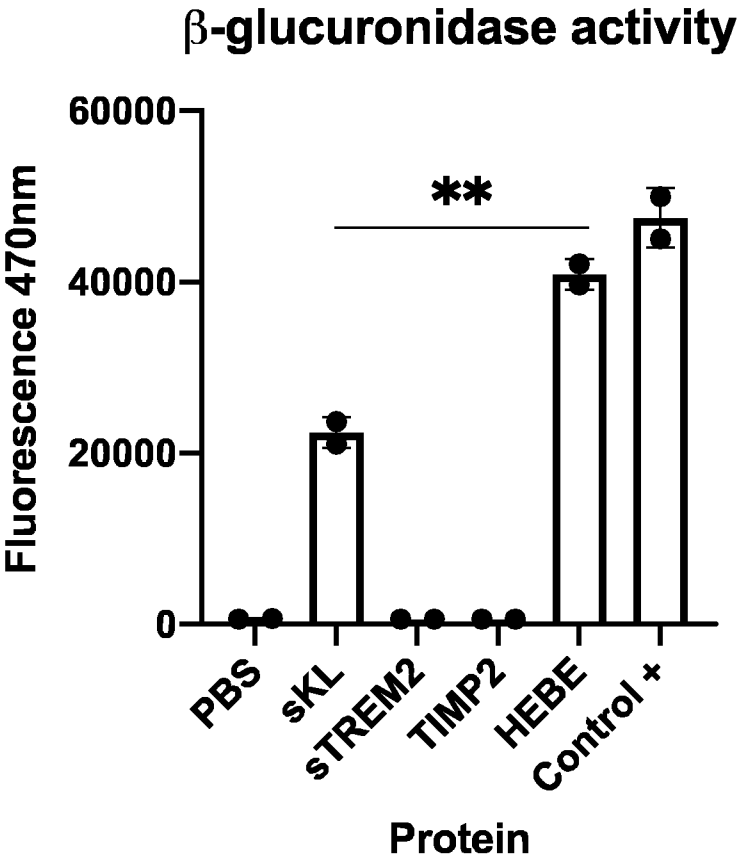
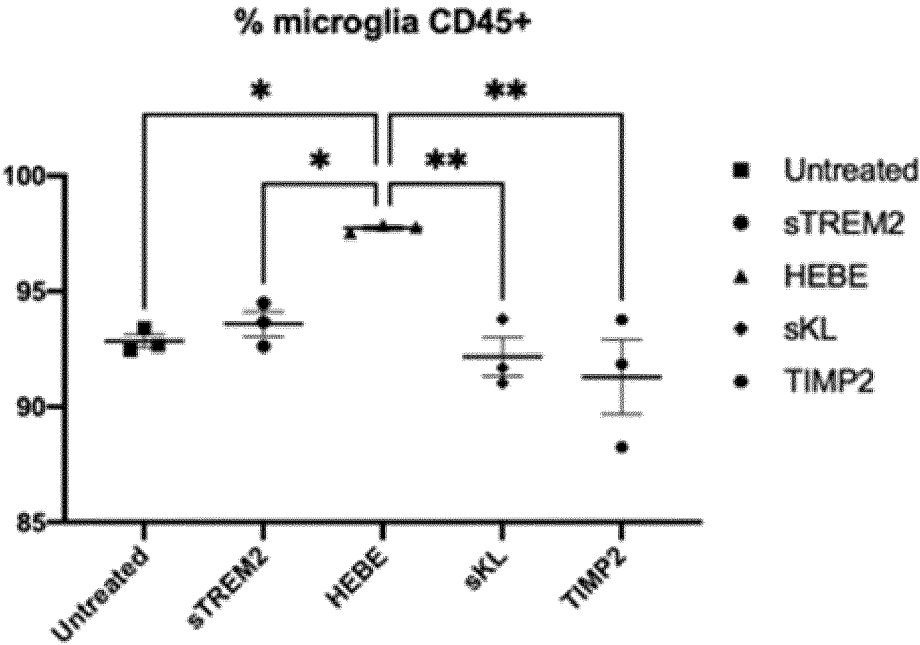


FIG. 5

A



B

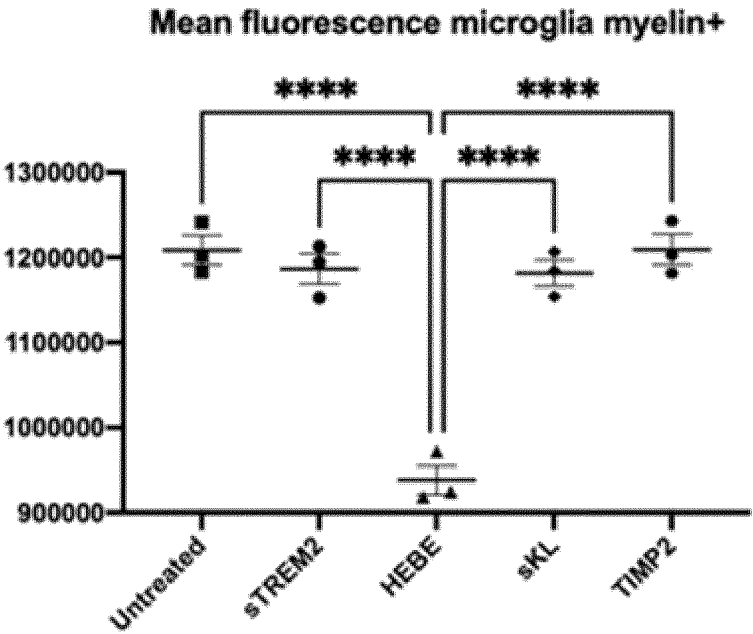


FIG. 6

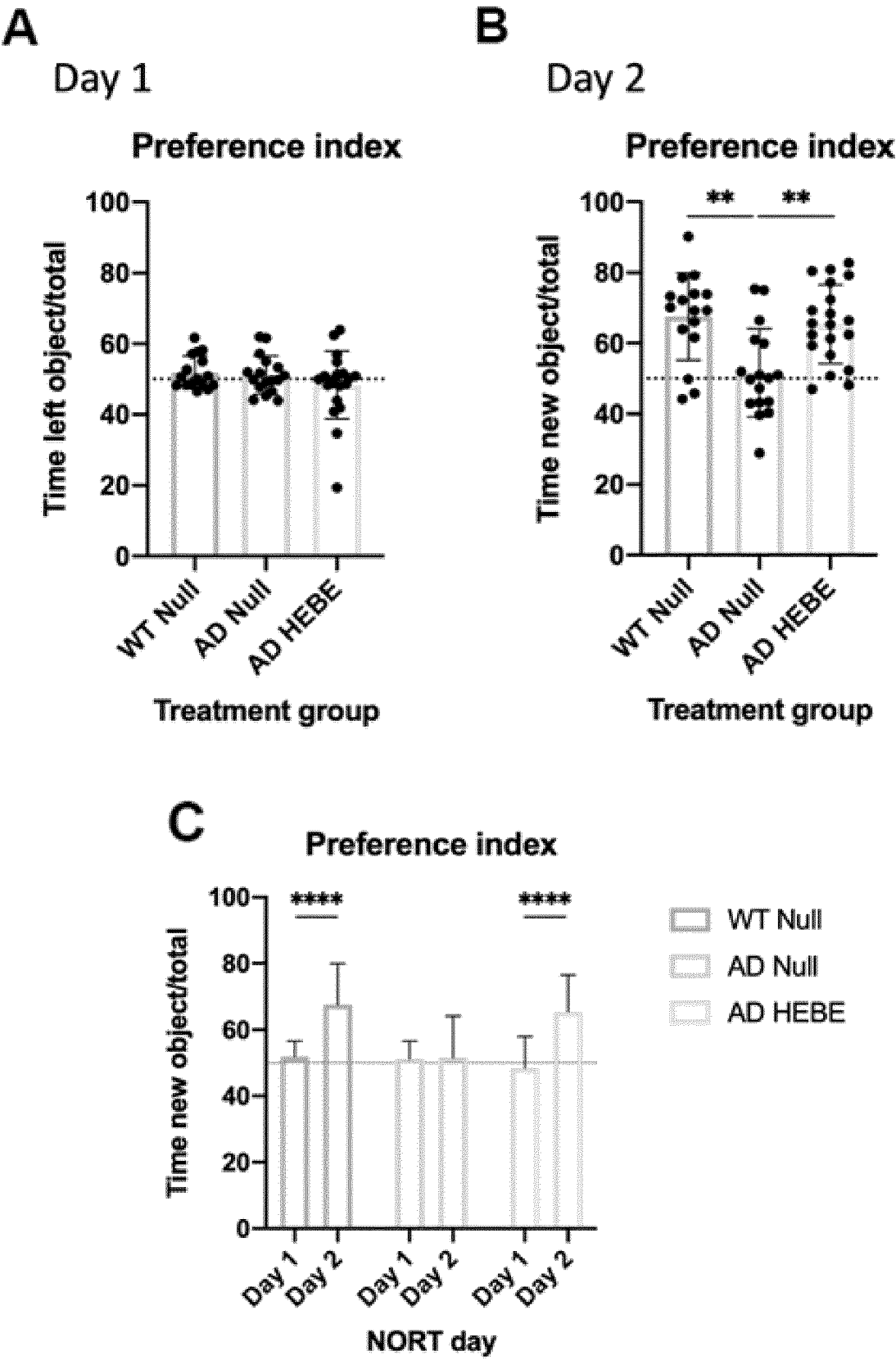


FIG. 7

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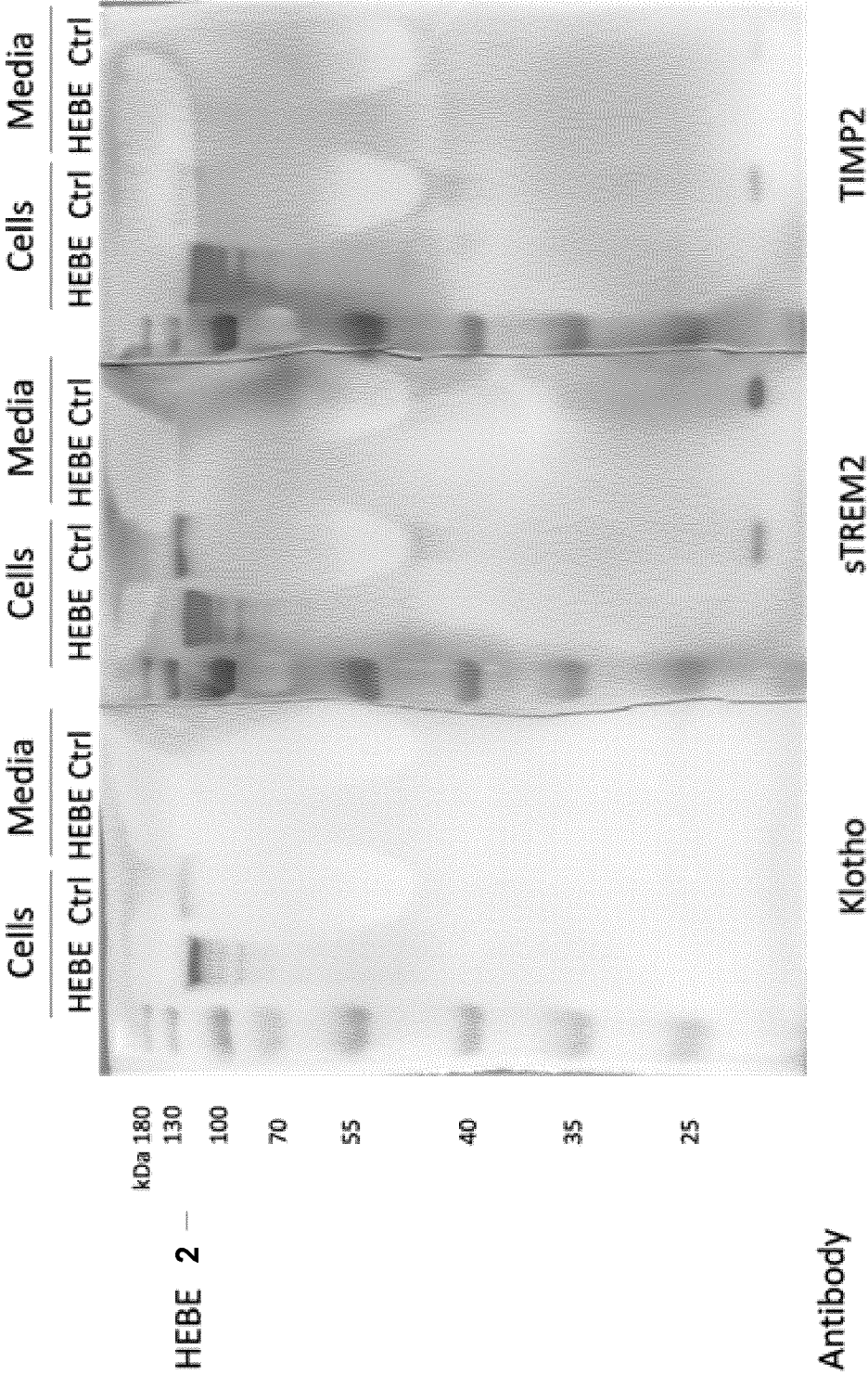


FIG. 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/066642

A. CLASSIFICATION OF SUBJECT MATTER		
INV.	C07K14/705	A61K38/00
	C12N9/24	
ADD.	A61P25/00	A61P25/28
		C07K14/81
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)		
C07K A61P A61K C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HENDRICKX JHANA O. ET AL: "Inflammation, Nitro-Oxidative Stress, Impaired Autophagy, and Insulin Resistance as a Mechanistic Convergence Between Arterial Stiffness and Alzheimer's Disease", FRONTIERS IN MOLECULAR BIOSCIENCES, vol. 8, 29 March 2021 (2021-03-29), XP093103648, DOI: 10.3389/fmolb.2021.651215 Abstract; page 2, left-hand column, line 47 to right-hand column, line10; page 8, right-hand column, line 4; page 6, left-hand column, line 14 and page 14, reference Chen, K., and Sun, Z. (2019) ----- -/-	1 - 15
☒ 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		"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
"E" earlier application or patent but published on or after the international filing date		"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
"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)		"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
"O" document referring to an oral disclosure, use, exhibition or other means		"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search		Date of mailing of the international search report
12 August 2024		18/09/2024
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Benzerari, Maëlis

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/066642

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	PRUD'HOMME GÉRALD J. ET AL: "Pathobiology of the Klotho Antiaging Protein and Therapeutic Considerations", FRONTIERS IN AGING, vol. 3, 12 July 2022 (2022-07-12), XP093103640, DOI: 10.3389/fragi.2022.931331 Abstract; section 3.1 Membrane-Bound and Soluble Forms -----	1 - 15
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1 A	CN 109 206 503 A (UNIV XIAMEN; SHENZHEN RESEARCH INSTITUTE OF XIAMEN UNIV) 15 January 2019 (2019-01-15) claims 1-9; SEQ ID NO:2 ----- -/-	1 - 15

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2024/066642

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2024/066642

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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