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(54) **Titre : COMPOSITIONS ET ARTICLES COMPRENANT UN POLYPEPTIDE ADN**
(54) **Title: COMPOSITIONS AND ARTICLES COMPRISING AN ADN POLYPEPTIDE**

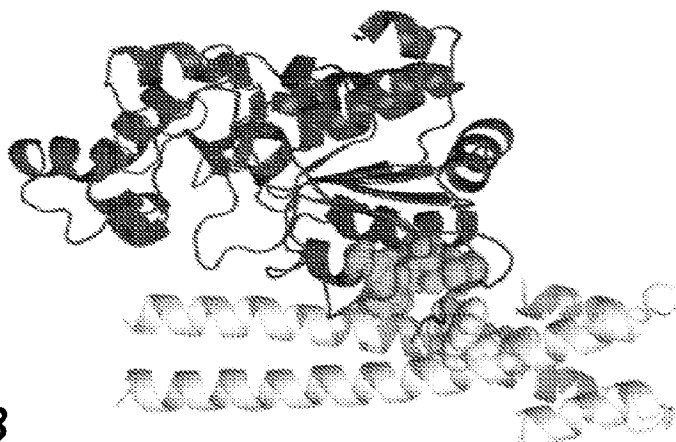


FIG. 1B

(57) **Abrégé/Abstract:**

Compositions and articles comprising an ADNF polypeptide are provided. Accordingly, there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide and a SIRT1 activator. Also provided are methods of treating a disease that can benefit from the article of manufacture.



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Abstract:

Compositions and articles comprising an ADNF polypeptide are provided. Accordingly, there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide and a SIRT1 activator. Also provided are methods of treating a disease that can benefit from the article of manufacture.

COMPOSITIONS AND ARTICLES COMPRISING AN ADNF POLYPEPTIDE

RELATED APPLICATION/S

This application claims the benefit of priority of U.S. Patent Application No. 63/165,801
5 filed on 25 March 2021 and U.S. Patent Application No. 63/178,026 filed on 22 April 2021, the
contents of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING STATEMENT

The ASCII file, entitled 91463SequenceListing.txt, created on March 24, 2022,
10 comprising 23,149 bytes, submitted concurrently with the filing of this application is
incorporated herein by reference.

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions and articles
15 comprising an ADNF polypeptide.

Activity-dependent neuroprotective protein (also referred to as ADNP or ADNF III) is
essential for brain formation and function. ADNP was shown to function in key cellular
activities including embryogenesis, autophagy, dendritic spine plasticity, axonal transport,
alternative RNA-splicing, *wnt* signaling, autism-linked protein translation and chromatin
20 remodeling. *De novo* mutations in ADNP lead to the autistic ADNP syndrome^{18,19} and somatic
ADNP mutations may drive Alzheimer's disease (AD) tauopathy¹⁵. Furthermore, a decrease in
blood ADNP expression was linked to increased inflammation²⁰ and reduced cognitive
functions²¹. ADNP is found in the nucleus being part of the SWItch/Sucrose Non-Fermentable
(SWI/SNF) complex, which constitutes a major part of the chromatin remodeling complexes³³.
25 In mature neurons, ADNP is found in the cytoplasm⁹ in association with microtubules through
interaction with the microtubule end binding proteins, EB1 and EB3¹⁰. In turn, EB1/EB3
interaction with ADNP have been linked to dendritic spine formation^{10,11}, axonal transport¹²,
enhancement of Tau-microtubule binding¹³⁻¹⁵ and protection against tau
hyperphosphorylation/tauopathy¹⁴⁻¹⁶.

30 ADNP polypeptides, including a proline-rich 8-amino acid polypeptide known as NAP
[NAPVSIPQ (SEQ ID NO: 2), also known as Davunetide or CP201] and uses thereof in
neuroprotection and treating multiple disorders are the subject of patents and patent applications
including International Application Publication Nos. WO1/92333, WO98/35042, WO00/27875,

WO00/53217, WO01/12654, WO2004/080957, WO2006/099739, WO2007/096859, WO2008/084483, WO2011/021186, WO2009/026687, WO2011/083461, WO2011/099011, WO2013/171595, WO2017/130190, WO2004/060309, WO2003/022226 and WO2010/075635; and U.S. Patent Nos. US5767240, US6174862 and US6613740; herein each incorporated by
5 reference in their entirety.

Sirtuin 1 (SIRT1) is positively associated with aging. SIRT1 deacetylates many substrates including histones and as such implicated in chromatin remodeling, enhances synaptic plasticity, maintains genomic stability, suppresses inflammation, and protects against neurodegeneration^{24, 59}. SIRT1 deficiency leads to accumulation of hyperphosphorylated Tau,
10 tauopathy and AD²³. High SIRT1 expression was shown to protect aged individuals from dementia²².

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided an
15 article of manufacture comprising as active ingredients an ADNF polypeptide, wherein the ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and a SIRT1 activator.

According to an aspect of some embodiments of the present invention there is provided a method of treating a disease selected from the group consisting of ADNP syndrome, Dravet
20 syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-
25 Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a SIRT1 activator, thereby treating the disease in the subject.

According to some embodiments of the invention, the SIRT1 activator is a small molecule.

30 According to some embodiments of the invention, the SIRT1 activator is selected from the group consisting of Resveratrol, Quercetin, Butein, Beberine, Curcumin, Fisetin, Honokiol, YK 3-237, SRT1720, SRT1460, SRT2183, STAC-5, STAC-9, STAC-10, BML-278 and Piceatannol, or an analog or derivative thereof.

According to some embodiments of the invention, the SIRT1 activator is Resveratrol or an analog or derivative thereof.

According to some embodiments of the invention, the SIRT1 activator is NAD⁺ or an analog or derivative thereof.

5 According to some embodiments of the invention, the SIRT1 activator is nicotinamide riboside (NR).

According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein the ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron
10 culture assay; and an anti-aging agent, wherein the anti-aging agent is not an anti-oxidant.

According to an aspect of some embodiments of the present invention there is provided a method of treating a disease selected from the group consisting of ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXF1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus
15 developmental delay, Angelman syndrome, Noonan syndrome, Kleeftstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an anti-aging agent, thereby treating the disease in
20 the subject.

According to some embodiments of the invention, the anti-aging agent is not an anti-oxidant.

According to some embodiments of the invention, the anti-aging agent is a SIRT1 activator.

25 According to some embodiments of the invention, the anti-aging agent is selected from the group consisting of rapamycin, metformin, melatonin, carnosine, nicotinamide mononucleotide, delta-sleep-inducing-peptide and small molecule Klotho enhancer, or an analog or derivative thereof.

According to some embodiments of the invention, the anti-aging agent comprises a
30 calorie restriction diet.

According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein the ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron

culture assay; and an immune-modulator agent selected from the group consisting of chemokine receptor modulator, immune-check point modulator and a cytokine, wherein the cytokine is not IFN β .

According to an aspect of some embodiments of the present invention there is provided a method of treating a disease selected from the group consisting of ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an immune-modulator agent selected from the group consisting of chemokine receptor modulator, immune-check point modulator and a cytokine, thereby treating the disease in the subject.

According to some embodiments of the invention, the cytokine is not IFN β .

According to some embodiments of the invention, the chemokine receptor is selected from CCR5 and CXCR4.

According to some embodiments of the invention, the modulator is an inhibitor.

According to some embodiments of the invention, the chemokine receptor modulator is selected from the group consisting of maraviroc, leronlimab, aplaviroc, vicriviroc, plerixafor, mavorixafor, BL-8040 and TG-0054, or an analog or derivative thereof.

According to some embodiments of the invention, the chemokine receptor modulator is selected from the group consisting of maraviroc and plerixafor, or an analog or derivative thereof.

According to some embodiments of the invention, the cytokine is selected from the group consisting of IL-6, IL-10 and TNF α .

According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein the ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and bumetanide or an analog or derivative thereof.

According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein the ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and a cannabinoid.

According to some embodiments of the invention, the cannabinoid is selected from the group consisting of THC and CBD.

According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein the ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and ketamine or an analog or derivative thereof.

According to some embodiments of the invention, the method further comprising administering to the subject a therapeutically effective amount of an ADNF polypeptide, wherein the ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay.

According to some embodiments of the invention, the ADNF polypeptide is capable of binding EB1 and or EB3.

According to some embodiments of the invention, the ADNF polypeptide is an ADNF III polypeptide.

According to some embodiments of the invention, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-22.

According to some embodiments of the invention, the polypeptide comprises SEQ ID NO: 2.

According to some embodiments of the invention, the polypeptide has the formula $(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$ (SEQ ID NO: 49), or an analogue thereof, in which R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and x and y are independently selected and are equal to zero or one.

According to some embodiments of the invention, the ADNF polypeptide is an ADNF I polypeptide.

According to some embodiments of the invention, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24-48.

According to some embodiments of the invention, the polypeptide comprises SEQ ID NO: 24.

According to some embodiments of the invention, the polypeptide has the formula $(R^1)_x$ -Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala- $(R^2)_y$ (SEQ ID NO: 50), or an analogue thereof, in which R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and x and y are independently selected and are equal to zero or one.

According to some embodiments of the invention, the polypeptide comprises at least one D-amino acid.

According to some embodiments of the invention, the polypeptide is less than 50 amino acids in length.

According to some embodiments of the invention, the polypeptide is less than 20 amino acids in length.

According to some embodiments of the invention, the polypeptide is attached to a cell penetrating or stabilizing moiety.

According to some embodiments of the invention, the polypeptide and the SIRT1 activator are provided in a co-formulation.

According to some embodiments of the invention, the polypeptide and the agent are provided in a co-formulation.

According to some embodiments of the invention, the polypeptide and the bumetanide are provided in a co-formulation.

According to some embodiments of the invention, the polypeptide and the cannabinoid are provided in a co-formulation.

According to some embodiments of the invention, the polypeptide and the ketamine are provided in a co-formulation.

According to some embodiments of the invention, the polypeptide and the SIRT1 activator are provided in separate formulations.

According to some embodiments of the invention, the polypeptide and the agent are provided in separate formulations.

According to some embodiments of the invention, the polypeptide and the bumetanide are provided in separate formulations.

According to some embodiments of the invention, the polypeptide and the cannabinoid are provided in separate formulations.

According to some embodiments of the invention, the polypeptide and the ketamine are provided in separate formulation. According to some embodiments of the invention, the

subject is a female.

According to some embodiments of the invention, the subject is a male.

According to some embodiments of the invention, the subject is under 18 years old.

According to some embodiments of the invention, the subject is over 60 years old.

According to some embodiments of the invention, the disease is associated with aging.

According to some embodiments of the invention, the disease is an inflammatory disease.

According to some embodiments of the invention, the disease is a neurodegenerative disease or cognitive deficit.

According to some embodiments of the invention, the disease is Alzheimer's disease.

According to some embodiments of the invention, the disease is an autistic spectrum disorder and/or intellectual disability.

According to some embodiments of the invention, the disease is an ADNP syndrome.

According to some embodiments of the invention, the disease is selected from the group consisting of stress, anxiety, bi-polar disease, schizophrenia and aggression.

According to some embodiments of the invention, the disease is selected from the group consisting of high blood pressure, swelling, congestive heart failure, hepatic disease and renal disease.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative

discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-G demonstrate that ADNP interacts with SIRT1. Figure 1A shows a schematic representation of functional protein regions depicted along full-length human ADNP and SIRT1 amino acid coding sequences. The ADNP sequence motifs are extended from the description in ¹⁵. The SSIP (SEQ ID NO: 51) motif that binds to EB1/EB3 is marked on the SIRT1 scheme. Figure 1B shows SIRT1 (pink ribbons) and EB1 homodimer (white ribbons) best docking pose. The binding motif in SIRT1 is shown in cyan spheres. The residues that bind SXIP (SEQ ID NO: 52) motif in EB1 are shown as white spheres (219 of monomer A, and 220, 223 of monomer B). Figure 1C shows images of HEK293T cells immunostained with ADNP (red) and SIRT1 (green) specific antibodies. The cell nuclei are visualized with DAPI (blue). Nuclear co-localization of the two proteins is represented in yellow. Quantitative analysis of ADNP-SIRT1 merged staining co-localizations are presented in the graph; x63 oil immersion lens. Figure 1D shows images of iPSCs from a healthy individual differentiated in suspension into neural progenitors³⁷ and stained as in Figure 1C. Figure 1E shows endogenous Co-IP assay of ADNP and SIRT1 in differentiated SH-SY5Y cells. EB1 or EB3 antibodies were conjugated to the affinity beads and sequential eluted fractions (flow through =FT, wash =W1, W2, W3, elution =E) were further analyzed by immunoblotting (IB) with ADNP, SIRT1, EB1 and EB3 antibodies (IB: ADNP; IB: SIRT1; IB: EB1; IB: EB3). In addition, columns with free agarose beads were used as negative controls (IP: IgG). Figure 1F shows Co-IP assay of a neural progenitor cell extract subjected to the EB1 antibody column. Consecutive antibody reactions (16 hours, 4 °C) were performed on the same blot (after stripping). Blots shown with MW markers (kDa) did not detect SIRT1 in the elution (predicted band size: 81 kDa, or observed band size: 110 kDa [www\(dot\)abcam\(dot\)com/sirt1-antibody-19a7ab4-ab110304\(dot\)html](http://www.abcam.com/sirt1-antibody-19a7ab4-ab110304.html)), lower exposure of the blot (2 minutes, Fusion Fx, Collégien, France) upper panel, higher exposure (5 minutes), lower panel. The blots were further exposed to EB1 antibodies (1 hour, Fuji Medical X ray films, Japan), showing EB1 in the FT as well as in the elution (E) and suggesting column saturation. Potentially non-specific, higher molecular weight bands are observed possibly due to previous antibody exposure of the blots, despite extensive washing. Figure 1G shows the effect of NAP (SEQ ID NO: 2). To enhance EB1 binding to partner proteins, 3 mg NAP were added to the FT and the mixture was incubated with the EB1 agarose-conjugated antibodies. After blotting, SIRT1 expected size band was seen

in the eluted material (E1, EB1 binding), albeit, at low detection level (exposure time to film was as in Figure 1F). The lower panel shows exposure of the blot film to Tau antibodies (two incubation times, 1 hour, 25 °C and 16 hours, 4 °C).

FIGs. 2A-C demonstrate RNA-seq identification of ADNP-SIRT1 co-localization at the human single cell level. Figure 2A shows single cell RNA sequencing data from human cortex specimen (PRJNA295469⁴⁵), visualized using the UCSC Cell Browser ([www\(dot\)cells\(dot\)ucsc\(dot\)edu/?ds=cortex-dev](http://www(dot)cells(dot)ucsc(dot)edu/?ds=cortex-dev)). *ADNP* and *SIRT1* expressing cells are marked in black circles. Figure 2B shows *ADNP* and *SIRT1* total bulk RNA expression levels from human tissues (GTEx)⁴⁸ data, visualized with t-SNE using the UCSC Cell Browser ([www\(dot\)cells\(dot\)ucsc\(dot\)edu/?ds=gtex8](http://www(dot)cells(dot)ucsc(dot)edu/?ds=gtex8)). Figure 2C show single cell RNA sequencing data (GSE36552⁴⁶) from 124 cells of human embryonic stem cells and human pre-implantation embryos. Single cell expression levels of *ADNP*, *SIRT1*, *MAPRE1* and *MAPRE3* were analyzed with Single Cell Expression Atlas⁴⁷.

FIGs. 3A-C demonstrate that ADNP and SIRT1 are co-regulated at the transcriptional level and both control specific histone H3 modifications. Figure 3A shows histone H3 modification screening from 5-month-hippocampus of female *Adnp*^{+/-} and *Adnp*^{+/+} mice (red box H3K79me2). Figure 3B is a WashU Epigenome Browser view of *ADNP*, *SIRT1* and *MAPRE1* showing chip-seq binding peaks of H3K79me2 (GSM733653), ADNP (GSE105573), HDAC2 (GSM1003447), YY1 (GSM803470, GSM803446) and SMARCA4 (GSE91946) in the erythroblast cell line K562. The box shows histone modification adjoining ADNP peaks. The histone peaks are separated for peaks that proximal (<1 kb) to the transcription start site (TSS) and distal to TSS, each is 2 kb around the position with most sequence reads, analyzed and with Factorbook⁵⁰. Figure 3C shows motif enrichment sequences of ADNP, HDAC2, YY1 and SMARCA4.

FIGs. 4A-C demonstrate that ADNP and SIRT1 correlate and interact with histone remodeling complex proteins. Figure 4A shows STRING protein-protein interactions network analysis of histone-interacting proteins network with SIRT1 and ADNP, p-value<1.0e-16; from STRING database, [www\(dot\)string-db\(dot\)org/](http://www(dot)string-db(dot)org/). Figure 4B shows Pearson correlation of *SIRT1* and *ADNP* expression levels (reads per kb of transcript, per million mapped reads, RPKM) in human tissues from 27 normal tissue samples from 95 human individuals public dataset PRJEB4337⁵³. Inset shows Pearson correlation of *Sirt1* and *Adnp* expression levels from *Adnp*^{+/-} and *Adnp*^{+/+} mice hippocampus (GSE72664)¹² (N=23). Figure 4C is a correlation matrix plot of RNA expression levels of key histone-interacting proteins from postmortem brain structures

across human brain development. Data from the BrainSpan atlas⁵¹. Positive correlations are displayed in a blue scale and negative correlations are displayed in a red scale with a significance level of $p < 0.05$. The numbers represent the correlation coefficient values. The blank squares represent insignificant correlation.

FIG. 5 demonstrate marked dysregulation in AD postmortem brains, the ADNP/SIRT1 network. Shown is a correlation matrix plot of postmortem brain expression levels of key histone-interacting proteins from Alzheimer's disease patients or controls (GSE5281). Data was obtained from six postmortem brain regions: entorhinal cortex, hippocampus, medial temporal gyrus, posterior cingulate, superior frontal gyrus and primary visual cortex including 74 samples from control and 87 from AD patients. Positive correlations are displayed in a blue scale and negative correlations are displayed in a red scale. The asterisks represent the significance level *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Figure 6 is an illustration depicting the ADNP-SIRT1 complex network created with the software BioRender(dot)com.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to compositions and articles comprising an ADNF polypeptide.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Whilst investigating the mechanism of action of ADNP gene products, the present inventors have uncovered extensive interaction between activity-dependent neuroprotective factor (ADNF) and the mammalian nicotinamide adenine dinucleotide (NAD⁺) dependent histone deacetylase SIRT1 (Sirtuin 1). Physical interaction between the two polypeptides ADNP and SIRT1 was found to be mediated via mutual binding of the microtubule end binding protein 1 (EB1) (see Fig. 1B and Example 1 hereinbelow). Further investigation uncovered nuclear co-localization, shared histone modification activity and coordinated, tissue-specific expression of ADNP and SIRT1, as well as coordinated expression patterns for a large number of proteins associated with both ADNP and SIRT1 (see Examples 2 and 3 hereinbelow), pointing to their involvement in a variety of signaling pathways. The component factors of these interactions can

now be employed to augment activity of ADNF polypeptides and find previously undisclosed clinical applications.

Considering the importance of ADNP to functions associated with autism spectrum disorders and intellectual disability disorders, the newly recognized physical interaction, co-localization, mutual biochemical functions and coordinated expression and protein interaction between ADNP and SIRT1 suggests a role for SIRT1 and similar molecules in autism spectrum disorders, intellectual disability and certain neurodegenerative disease.

Thus, in some embodiments of the invention there is provided a method of treating a disease selected from the group consisting of Activity-dependent neuroprotective protein (ADNP) syndrome (also known as Helsmoortel-Van der Aa syndrome), Dravet syndrome, fragile X syndrome, Synaptic Ras GTPase Activating Protein 1 (SYNGAP1)-related intellectual disability, Phelan McDermid syndrome, Glutamate Ionotropic Receptor NMDA (GRIN) disorder, Chromodomain Helicase Binding Protein 8 (CHD8)-related disorder, Dual Specificity Tyrosine Phosphorylation-Regulated Kinase 1A (DYRK1A) syndrome, Pogo Transposable Element with ZNF Domain (POGZ) syndrome, Forkhead Box P1 (FOXP1) syndrome, Solute Carrier Family 5 Member 1 (SLC5A1)-related disorder, Coffin-Siris syndrome, AT-Rich Interaction Domain 1B (ARID1B)-related syndrome, Lysine Methyltransferase 5B (KMT5B) syndrome, Phosphatase and tensin homolog (PTEN) autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleeftstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a SIRT1 activator, thereby treating the disease in the subject.

As used herein the term "SIRT1" (also known as Sirtuin 1 and NAD-dependent deacetylase sirtuin-1) refers to the expression product e.g. RNA or protein of the *SIRT1* gene (Gene ID 23411). The SIRT1 protein is a deacetylase, EC No. 2.3.1, that adds the acetyl group from the protein to the ADP-ribose component of NAD⁺ to form O-acetyl-ADP-ribose. Methods of determining the catalytic activity of SIRT1 are well known in the art and include for example isotopic assays such as the charcoal binding assay, which employs a ³H-labeled acetyl-lysine substrate to measure acetate release from hydrolyzed OAADPr; [¹⁴C] NAM release assays and Fluor de Lys assayTM (BIOMOL/Enzo), which measures deacetylation of an acetyl-lysine peptide conjugated to aminomethylcoumarin (AMC). Kits for assaying SIRT1 activity are also commercially available from e.g. BIOMOL/Enzo, Sigma, Biovision and Abcam.

According to specific embodiments, the SIRT1 is the human SIRT1, such as provided in the following Accession Nos. NM_001142498, NM_001314049, NM_012238, NP_001135970, NP_001300978, NP_036370.

Numerous SIRT1 activators are known to the person of ordinary skill in the art. For example SIRT1 activators are described in U.S. Patent Publications 20130085155; 20120197013; 20120165330; 20120108585; 20120022254; 20110306612; 20110306609; 20110263564; 20110257174; 20110152254; 20110130387; 20110077248; 20110039847; 20110015192; 20110009496; 20100215632; 20090163476; 20090105246; 20090099170; 20090069301; 20090012080; 20080249103; 20070043050; 20070037865; 20070037827; 20070037809; 8,343,997; 8,268,862; 8,247,565; 8,178,536; 8,163,908; 8,093,401; 8,088,928; 8,044,198; 7,998,974; 7,893,086; 7,855,289; 7,829,556; 7,345,178, each of which is incorporated by reference herein in its entirety. SIRT1 activators are further described in Dai et al., J Biol Chem, 285 (43): 32695-32703, 2010, which is incorporated by reference herein in its entirety. Additional SIRT1 activators are provided as Formulas I-XXXVIII of U.S. Pat. No. 8,044,198, which is incorporated herein by reference, US20150133527 to London et al, Schiedel et al Med Res Rev 0:1-54, 2017, which are incorporated herein by reference.

Non-limiting examples of SIRT1 activators that can be used with some embodiments of the invention include Resveratrol, Quercetin, Butein, Beberine, Curcumin, Fisetin, Honokiol, YK 3-237, SRT1720, SRT1460, SRT2183, STAC-5, STAC-9, STAC-10, BML-278, SRT 2104 (GSK2245840), SIRT1 Activator 3, STAC 8, MC2563, SCIC2, SCIC2.1, Oxazolo(4,5-b) pyridines, Pyrollo (3-2b) quinoxalines, benzimidazole, pyridoxazole and/or Piceatannol.

Thus, in some embodiments, the SIRT1 activator is selected from the group consisting of Resveratrol, Quercetin, Butein, Beberine, Curcumin, Fisetin, Honokiol, YK 3-237, SRT1720, SRT1460, SRT2183, STAC-5, STAC-9, STAC-10, BML-278 and Piceatannol.

In some embodiments, the SIRT1 activator is a small molecule.

As used herein, the term "small molecule" refers to a low molecular weight (< 900 daltons) organic compound that may regulate a biological process, with a size on the order of 1 nm.

In some embodiments, the SIRT1 activator is selected from the group consisting of Resveratrol, Quercetin, Butein, Beberine, Curcumin, Fisetin, Honokiol, YK 3-237, SRT1720, SRT1460, SRT2183, STAC-5, STAC-9, STAC-10, BML-278 and Piceatannol, or an analog or derivative thereof. In specific embodiments, the SIRT1 activator is Resveratrol, or an analog or derivative thereof.

In still other embodiments, the SIRT1 activator is NAD⁺ or an analog or derivative thereof.

As used herein, "NAD⁺" refers to the oxidized form of nicotinamide adenine dinucleotide (NAD). NAD is a coenzyme central to metabolism consisting of nicotinamide and adenine joined through their phosphate groups.

5 According to some embodiments of the invention, the SIRT1 activator is nicotinamide riboside (NR), a biosynthetic precursor to NAD.

Also envisioned in the present invention is an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and a SIRT1
10 activator. In some embodiments the ADNF polypeptide and the SIRT1 activator are provided in co-formulation in the article of manufacture. In other embodiments, the the ADNF polypeptide and the SIRT1 activator are provided in separate formulations.

The present inventors have uncovered a role for ADNF polypeptides and SIRT1 in chromatin remodeling, and, specifically, in histone modification, e.g. histone methylation. Many
15 of the neurodevelopmental conditions associated with ADNP share components of chromatin reorganization with aging processes, thus, anti-aging agents may be useful in treatment.

Thus, in further embodiments there is provided a method of treating a disease selected from the group consisting of ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-
20 related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of
25 an anti-aging agent, thereby treating the disease in the subject.

Non-limiting examples of anti-aging agents include anti-oxidants such as vitamins (e.g. A, C and B3), polyphenols, flavonoids, cell regulators such as retinols, peptides and Growth Factor. In some embodiments the anti-aging agent is selected from the group consisting of rapamycin, metformin, melatonin, carnosine, nicotinamide mononucleotide, delta-sleep-inducing-peptide
30 and small molecule Klotho enhancer, or an analog or derivative thereof.

Also envisioned in the present invention is an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and an anti-

aging agent, wherein the anti-aging agent is not an anti-oxidant. In some embodiments the ADNF polypeptide and the anti-aging agent are provided in co-formulation in the article of manufacture. In other embodiments, the the ADNF polypeptide and the anti-aging agent are provided in separate formulations.

Also envisioned is the method or article of manufacture, where the anti-aging agent comprises behavior modification, such as a calorie-restriction diet, smoking cessation, exercise and/or improved sleep habits.

In some embodiments, the anti-aging agent is a SIRT1 activator.

The present inventors have identified immune-regulatory factors among the factors expressed in correlation with ADNP and SIRT1 (e.g. HDAC2, SMARCA4 and YY1). Thus, in some aspects of some embodiments there is provided a method of treating a disease selected from the group consisting of ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an immune-modulator agent selected from the group consisting of chemokine receptor modulator, immune-check point modulator and a cytokine, thereby treating the disease in the subject. In some embodiments, the cytokine is not IFN β .

As used herein, the term “chemokine receptor modulator” refers to any agent which modifies the function of a chemokine receptor. Chemokine receptors include, but are not limited to, receptors for the chemokines CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CCR9, CCR10, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CXCR6, CX₃CR1, XCR1, ACKR1, ACKR2, ACKR3, ACKR4 and CCRL2.

In some embodiments, the chemokine receptor is selected from the group consisting of CCR5 and CXCR4.

In some embodiments, the modulator is an inhibitor. In other embodiments, the chemokine receptor modulator is selected from the group consisting of maraviroc, leronlimab, aplaviroc, vicriviroc, plerixafor, mavorixafor, BL-8040 and TG-0054, or an analog or derivative thereof. In a specific embodiment, the chemokine receptor modulator is selected from the group consisting of maraviroc and plerixafor, or an analog or derivative thereof.

Also envisioned in the present invention is an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and an immune-modulator agent selected from the group consisting of chemokine receptor modulator, immune-check point modulator and a cytokine, wherein said cytokine is not IFN β . In some embodiments the ADNF polypeptide and the immune-modulator agent are provided in co-formulation in the article of manufacture. In other embodiments, the ADNF polypeptide and the immune-modulator agent are provided in separate formulations.

As used herein, the term “immune-modulator agent” includes interferons, antigens, tumor phagocytosis-inducing agents, and other immune-enhancing agents (e.g., immune checkpoint inhibitors)

Interferons include, but are not limited to interferon alpha, interferon alpha-2a, interferon alpha-2b, interferon beta, interferon gamma-1a, ACTIMMUNE.RTM. (interferon gamma-1b), or interferon gamma-n1, combinations thereof and the like.

Tumor phagocytosis-inducing agents include, but are not limited to anti-CD47 monoclonal antibodies (e.g., Hu5F9-G4, CC-90002, ZF1, AMMS4-G4, IBI188, SRF231), anti-SIRP.alpha. fusion proteins (e.g., TTI-621, TTI-622), anti-SIRP.alpha. monoclonal antibodies (e.g., OSE-172), anti-CD47/antitumor-associated antigen bispecific antibodies, and inhibitors of leukocyte immunoglobulin-like receptor B1 (LILRB1) binding to major histocompatibility complex class 1.beta.2-microglobulin (MHC class1 .beta.2M).

Anti-CD47/antitumor-associated antigen bispecific antibodies include, but are not limited to anti-CD47/CD19 bispecific antibodies (e.g., TG-1801), anti-CD47/mesothelin bispecific antibodies (e.g., NI-1801), anti-CD47/4-1BB bispecific antibodies (e.g., DSP107), anti-CD47/CD20 bispecific antibodies, anti-CD47/CD33 bispecific antibodies (e.g., HMBD004).

Immune checkpoint inhibitors include, but are not limited to PD-1 inhibitors (e.g. nivolumab, pidilizumab, sintilimab), PD-L1 inhibitors (e.g. atezolizumab, avelumab, durvalumab, BMS-936559), CTLA4 inhibitors (e.g. ipilimumab, tremelimumab) or IDO inhibitors (e.g. indoximod, epacadostat).

Other immune modulating agents include ALFAFERONE.RTM., BAM-002, BEROMUN.RTM. (tasonermin), BEXXAR.RTM. (tositumomab), CamPath.RTM. (alemtuzumab), CTLA4 (cytotoxic lymphocyte antigen 4), decarbazine, denileukin, epratuzumab, GRANOCYTE.RTM. (lenograstim), lentinan, leukocyte alpha interferon, imiquimod, MDX-010, melanomavaccine, mitumomab, molgramostim, MYLOTARG.TM..RTM. (gemtuzumab

ozogamicin). NEUPOGEN.RTM. (filgrastim), OncoVAC-CL, OvaRex.RTM. (oregovomab),
pemtumomab(Y-muHMFG), PROVENGE.RTM., sargaramostim, sizofilan, teceleukin,
TheraCys.RTM., ubenimex, VIRULIZIN, Z-100, WF-10, PROLEUKIN.RTM. (aldesleukin),
ZADAXIN.RTM. (thymalfasin), ZENAPAX.RTM. (daclizumab), ZEVALIN.RTM. (90Y-
5 Ibritumomab tiuxetan) and the like including but not limited to STING (stimulator of interferon
genes) and NOD (nucleotide-binding oligomerization domain-like receptors) Agonists.

As used herein, the term “cytokine” refers to the superfamily of proteins essential to the
signaling network between cells and regulating the immune system. Cytokines include, but are
not limited to interleukins (e.g. IL-1, IL-2, IL-18, IL-4, IL-7, GCSF, etc), interferons (e.g. IFN-
10 alpha, IFN-beta, IFN-gamma, TNF, CD154, etc), TGF and hematopoietins such as Epo, Tpo and
SCF.

Also envisioned herein is the article of manufacture or method of the invention, wherein
the cytokine is selected from the group consisting of IL-6, IL-10 and TNF α .

According to some aspects of the invention, there is provided an article of manufacture
15 comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a
neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and bumetanide
or an analog or derivative thereof.

Bumetanide (also known as Bumdex®) is a drug used to treat hypertension and edema,
edema resulting from heart failure, liver failure and/or renal disorders. Some exemplary
20 bumetanide analogs are described in US Patent No. 9,682,928 to Partridge et al.

In some embodiments the ADNF polypeptide and the bumetanide or analog or derivative
thereof are provided in co-formulation in the article of manufacture. In other embodiments, the
ADNF polypeptide and the bumetanide or analog or derivative thereof are provided in separate
formulations.

25 According to some aspects of the invention, there is provided an article of manufacture
comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a
neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and a
cannabinoid.

As used herein, the term “cannabinoid” refers to a class of diverse chemical compounds
30 that act on cannabinoid receptors on cells that repress neurotransmitter release in the brain.
Ligands for these receptor proteins include the endocannabinoids (produced naturally in the body
by humans and animals), the phytocannabinoids (found in Cannabis and some other plants), and
synthetic cannabinoids (manufactured artificially). There are at least 85 different cannabinoids

isolated from Cannabis, exhibiting varied effects (El-Alfy et al., Pharmacology Biochemistry and Behavior, 2010. Vol. 95(4), pages 434-442). Exemplary cannabinoids include, but are not limited to tetrahydrocannabinolic acid (THCA), cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabivarin (CBV),
5 tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), cannabichromevarin (CBCV), cannabigerovarin (CBGV), cannabigerol monomethyl ether (CBGM). In specific embodiments, the cannabinol is tetrahydrocannabinol (THC) or cannabidiol (CBD).

In some embodiments the ADNF polypeptide and the cannabinoid are provided in co-formulation in the article of manufacture. In other embodiments, the ADNF polypeptide and the
10 cannabinoid are provided in separate formulations.

According to some aspects of the invention, there is provided an article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and ketamine or an analog or derivative thereof.

As used herein, the term "ketamine", "ketamine analog" or "ketamine derivative" refers to ketamine, norketamine, 6-hydroxynorketamine, or pharmaceutically acceptable salts thereof.
15

As used herein, the term "ketamine" includes ketamine in its racemic (R/S) form, in its R-(-) enantiomerically pure form, or in its S-(+) enantiomerically pure form. As used herein, the term "norketamine" includes norketamine in its racemic (R/S) form, in its R-(-) enantiomerically
20 pure form, or in its S-(+) enantiomerically pure form. As used herein, "enantiomerically pure" refers to compositions consisting substantially of a single isomer (i.e., substantially free of the opposite isomer), preferably consisting of 90%, 92%, 95%, 98%, 99%, or 100% (w/w) of a single isomer. For example, when the article of manufacture includes enantiomerically pure R-(-)-ketamine, the article of manufacture can include at least 95% (w/w) S-(+)-ketamine, and less than
25 5% (w/w) R-(-)-ketamine.

In some embodiments the ADNF polypeptide and the ketamine or analog or derivative thereof are provided in co-formulation in the article of manufacture. In other embodiments, the ADNF polypeptide and the ketamine or analog or derivative thereof are provided in separate formulations.

Also contemplated are methods of treating a disease that can benefit from treatment with the active ingredients of the article of manufacture of the present invention in a subject in need thereof, the methods comprising administering to the subject a therapeutically effective amount
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of the component active ingredients of the article of manufacture as described herein, thereby treating the disease in the subject.

In specific embodiments, the disease is a disease associated with aging.

In other embodiments, the disease is an inflammatory disease.

5 In still other embodiments, the disease is a neurodegenerative disease or cognitive deficit.

In some embodiments, the disease is Alzheimer's disease.

In other embodiments, the disease is an autistic spectrum disorder and/or intellectual disability.

In some embodiments, the disease is ADNP syndrome.

10 In some embodiments, the disease is selected from the group consisting of stress, anxiety, bi-polar disease, schizophrenia and aggression.

In other embodiments, the disease is selected from the group consisting of high blood pressure, swelling, congestive heart failure, hepatic disease and renal disease.

As used herein, the term "treating" refers to abrogating, substantially inhibiting, slowing
15 or reversing the progression of a pathology (disease, disorder or condition, e.g. autism spectrum disorder, intellectual disability, Alzheimer's disease, ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B
20 syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleeftstra syndrome, and Smith-Magenis syndrome, stress, anxiety, schizophrenia, hypertension, hepatic and renal disease, etc., e.g. a condition which can benefit from treatment with the component active ingredients of the article of manufacture of the invention), substantially ameliorating a symptom of a pathology and/or improving survival
25 rate in a subject diagnosed with the pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology or reduction or regression of a pathology, as further disclosed herein.

As used herein, the term "preventing" refers to keeping a pathology from occurring in a subject that has not yet been diagnosed as having the pathology and/or preventing the
30 manifestation of a symptom associated with the pathology before it occurs.

As used herein, the term "improvement" or "improving" refers to reducing or alleviating the severity, frequency or duration of negative aspects of the subject's disease, condition or disorder, or increasing (frequency, strength or duration of) or producing the positive, beneficial or

desired aspects of the subject's health and/or well-being associated with the disease, condition or disorder. Some non-limiting examples of improvement following treatment with the SIRT1 activator, anti-aging agent, immune modulator agent, ADNF polypeptide or article of manufacture according to the invention is improvement in intellectual and/or cognitive capacity, reduction in autism spectrum behaviors, reduction of blood pressure.

Non-limiting examples of diseases that can be treated according to some embodiments of the invention include inflammatory diseases, neurodegenerative diseases, cognitive deficits, autistic spectrum disorder, mental disorder, cytoskeletal disorder (e.g. Dravet syndrome, Rett syndrome and fragile X syndrome), high blood pressure and swelling (e.g. as a results of heart failure, liver failure, or kidney problems e.g. the nephrotic syndrome), autoimmune disease, allergic disease, infectious disease, graft-rejection disease and cancerous disease.

As used herein, the term "cognitive deficit" encompasses both intellectual disability and cognitive impairment (typically associated with a mental or neurodegenerative disease).

As used herein the term "intellectual disability (ID)", also known as general learning disability or mental retardation (MR), refers to a generalized neurodevelopmental disorder characterized by significantly impaired intellectual and adaptive functioning.

Non-limiting examples of neurodegenerative disease or cognitive deficits include, diseases of central motor systems including degenerative conditions affecting the basal ganglia (Huntington's disease, Wilson's disease, striatonigral degeneration, corticobasal ganglionic degeneration), Tourette's syndrome, Parkinson's disease, progressive supranuclear palsy, progressive bulbar palsy, familial spastic paraplegia, spinomuscular atrophy, ALS and variants thereof, dentatorubral atrophy, olivopontocerebellar atrophy, paraneoplastic cerebellar degeneration, and dopamine toxicity; diseases affecting sensory neurons such as Friedreich's ataxia, diabetes, peripheral neuropathy, retinal neuronal degeneration; diseases of limbic and cortical systems such as cerebral amyloidosis, Pick's atrophy, Retts syndrome; neurodegenerative pathologies involving multiple neuronal systems and/or brainstem including Alzheimer's disease, Parkinson's disease, AIDS-related dementia, Leigh's disease, diffuse Lewy body disease, multiple sclerosis, epilepsy, multiple system atrophy, Guillain-Barre syndrome, lysosomal storage disorders such as lipofuscinosis, late-degenerative stages of Down's syndrome, Alper's disease, vertigo as result of CNS degeneration, ALS, corticobasal degeneration, and progressive supranuclear palsy; pathologies associated with developmental retardation and learning impairments, Down's syndrome, fragile X syndrome, Klinefelter's syndrome, Prader-Willi syndrome, cri du chat syndrome and oxidative stress induced neuronal death; pathologies arising

with aging and chronic alcohol or drug abuse including, for example, (i) with alcoholism, the degeneration of neurons in locus coeruleus, cerebellum, cholinergic basal forebrain, (ii) with aging, degeneration of cerebellar neurons and cortical neurons leading to cognitive and motor impairments, and (iii) with chronic amphetamine abuse, degeneration of basal ganglia neurons leading to motor impairments; pathological changes resulting from focal trauma such as stroke, focal ischemia, vascular insufficiency, hypoxic-ischemic encephalopathy, hyperglycemia, hypoglycemia, closed head trauma, and direct trauma; post traumatic stress disorder (PTSD), pathologies arising as a negative side-effect of therapeutic drugs and treatments (e.g., degeneration of cingulate and entorhinal cortex neurons in response to anticonvulsant doses of antagonists of the NMDA class of glutamate receptor).

Non-limiting examples of autistic spectrum disorders and/or intellectual disability include ADNP syndrome, Dravet syndrome, fragile X syndrome, Down's syndrome, SYNGAP1 syndrome, POGZ syndrome (White-Sutton syndrome), CHD8 syndrome, SCN2A syndrome, ARID1B syndrome, Phelan McDermid syndrome, NRXN1 syndrome, DYRK1A syndrome, GRIN disorder, POGZ (White-Sutton syndrome), FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, CHD2 syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-Magenis syndrome.

According to specific embodiments, the disease is not an autistic spectrum disorder.

According to specific embodiments, the disease is not ADNP syndrome.

Non-limiting examples of mental disorders include mood disorders (e.g., major depression disorder (i.e., unipolar disorder), mania, dysphoria, bipolar disorder, dysthymia, cyclothymia), psychotic disorders (e.g., schizophrenia, schizoaffective disorder, schizophreniform disorder, delusional disorder, brief psychotic disorder, and shared psychotic disorder), personality disorders, post traumatic stress disorder (PTSD) aggression, anxiety disorders (e.g., obsessive-compulsive disorder and attention deficit disorders) as well as other mental disorders such as substance -related disorders, childhood disorders, dementia, adjustment disorder, delirium, multi-infarct dementia, and Tourette's disorder as described in Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, (DSM IV) (see also Benitez-King G. et al, Curr Drug Targets CNS Neurol Disord. 2004 Dec;3(6):515-33. Review). Typically, such disorders have a complex genetic and/or a biochemical component.

Inflammatory diseases - Include, but are not limited to, chronic inflammatory diseases and acute inflammatory diseases.

Inflammatory diseases associated with hypersensitivity

Examples of hypersensitivity include, but are not limited to, Type I hypersensitivity, Type II hypersensitivity, Type III hypersensitivity, Type IV hypersensitivity, immediate hypersensitivity, antibody mediated hypersensitivity, immune complex mediated hypersensitivity, T lymphocyte mediated hypersensitivity and DTH.

Type I or immediate hypersensitivity, such as asthma.

Type II hypersensitivity include, but are not limited to, rheumatoid diseases, rheumatoid autoimmune diseases, rheumatoid arthritis (Krenn V. *et al.*, *Histol Histopathol* 2000 Jul;15 (3):791), spondylitis, ankylosing spondylitis (Jan Voswinkel *et al.*, *Arthritis Res* 2001; 3 (3): 189), systemic diseases, systemic autoimmune diseases, systemic lupus erythematosus (Erikson J. *et al.*, *Immunol Res* 1998;17 (1-2):49), sclerosis, systemic sclerosis (Renaudineau Y. *et al.*, *Clin Diagn Lab Immunol.* 1999 Mar;6 (2):156); Chan OT. *et al.*, *Immunol Rev* 1999 Jun;169:107), glandular diseases, glandular autoimmune diseases, pancreatic autoimmune diseases, diabetes, Type I diabetes (Zimmet P. *Diabetes Res Clin Pract* 1996 Oct;34 Suppl:S125), thyroid diseases, autoimmune thyroid diseases, Graves' disease (Orgiazzi J. *Endocrinol Metab Clin North Am* 2000 Jun;29 (2):339), thyroiditis, spontaneous autoimmune thyroiditis (Braley-Mullen H. and Yu S, *J Immunol* 2000 Dec 15;165 (12):7262), Hashimoto's thyroiditis (Toyoda N. *et al.*, *Nippon Rinsho* 1999 Aug;57 (8):1810), myxedema, idiopathic myxedema (Mitsuma T. *Nippon Rinsho.* 1999 Aug;57 (8):1759); autoimmune reproductive diseases, ovarian diseases, ovarian autoimmunity (Garza KM. *et al.*, *J Reprod Immunol* 1998 Feb;37 (2):87), autoimmune anti-sperm infertility (Diekman AB. *et al.*, *Am J Reprod Immunol.* 2000 Mar;43 (3):134), repeated fetal loss (Tincani A. *et al.*, *Lupus* 1998;7 Suppl 2:S107-9), neurodegenerative diseases, neurological diseases, neurological autoimmune diseases, multiple sclerosis (Cross AH. *et al.*, *J Neuroimmunol* 2001 Jan 1;112 (1-2):1), Alzheimer's disease (Oron L. *et al.*, *J Neural Transm Suppl.* 1997;49:77), myasthenia gravis (Infante AJ. And Kraig E, *Int Rev Immunol* 1999;18 (1-2):83), motor neuropathies (Kornberg AJ. *J Clin Neurosci.* 2000 May;7 (3):191), Guillain-Barre syndrome, neuropathies and autoimmune neuropathies (Kusunoki S. *Am J Med Sci.* 2000 Apr;319 (4):234), myasthenic discases, Lambert-Eaton myasthenic syndrome (Takamori M. *Am J Med Sci.* 2000 Apr;319 (4):204), paraneoplastic neurological diseases, cerebellar atrophy, paraneoplastic cerebellar atrophy, non-paraneoplastic stiff man syndrome, cerebellar atrophies, progressive cerebellar atrophies, encephalitis, Rasmussen's encephalitis, amyotrophic lateral sclerosis, Sydeham chorea, Gilles de la Tourette syndrome, polyendocrinopathies, autoimmune polyendocrinopathies (Antoine JC. and Honnorat J. *Rev Neurol (Paris)* 2000 Jan;156 (1):23);

neuropathies, dysimmune neuropathies (Nobile-Orazio E. *et al.*, Electroencephalogr Clin Neurophysiol Suppl 1999;50:419); neuromyotonia, acquired neuromyotonia, arthrogryposis multiplex congenita (Vincent A. *et al.*, Ann N Y Acad Sci. 1998 May 13;841:482), cardiovascular diseases, cardiovascular autoimmune diseases, atherosclerosis (Matsuura E. *et al.*, Lupus. 1998;7 Suppl 2:S135), myocardial infarction (Vaarala O. Lupus. 1998;7 Suppl 2:S132), thrombosis (Tincani A. *et al.*, Lupus 1998;7 Suppl 2:S107-9), granulomatosis, Wegener's granulomatosis, arteritis, Takayasu's arteritis and Kawasaki syndrome (Praprotnik S. *et al.*, Wien Klin Wochenschr 2000 Aug 25;112 (15-16):660); anti-factor VIII autoimmune disease (Lacroix-Desmazes S. *et al.*, Semin Thromb Hemost.2000;26 (2):157); vasculitises, necrotizing small vessel vasculitises, microscopic polyangiitis, Churg and Strauss syndrome, glomerulonephritis, pauci-immune focal necrotizing glomerulonephritis, crescentic glomerulonephritis (Noel LH. Ann Med Interne (Paris). 2000 May;151 (3):178); antiphospholipid syndrome (Flamholz R. *et al.*, J Clin Apheresis 1999;14 (4):171); heart failure, agonist-like β -adrenoceptor antibodies in heart failure (Wallukat G. *et al.*, Am J Cardiol. 1999 Jun 17;83 (12A):75H), thrombocytopenic purpura (Moccia F. Ann Ital Med Int. 1999 Apr-Jun;14 (2):114); hemolytic anemia, autoimmune hemolytic anemia (Efremov DG. *et al.*, Leuk Lymphoma 1998 Jan;28 (3-4):285), gastrointestinal diseases, autoimmune diseases of the gastrointestinal tract, intestinal diseases, chronic inflammatory intestinal disease (Garcia Herola A. *et al.*, Gastroenterol Hepatol. 2000 Jan;23 (1):16), celiac disease (Landau YE. and Shoenfeld Y. Harefuah 2000 Jan 16;138 (2):122), autoimmune diseases of the musculature, myositis, autoimmune myositis, Sjogren's syndrome (Feist E. *et al.*, Int Arch Allergy Immunol 2000 Sep;123 (1):92); smooth muscle autoimmune disease (Zauli D. *et al.*, Biomed Pharmacother 1999 Jun;53 (5-6):234), hepatic diseases, hepatic autoimmune diseases, autoimmune hepatitis (Manns MP. J Hepatol 2000 Aug;33 (2):326) and primary biliary cirrhosis (Strassburg CP. *et al.*, Eur J Gastroenterol Hepatol. 1999 Jun;11 (6):595).

Type IV or T cell mediated hypersensitivity, include, but are not limited to, rheumatoid diseases, rheumatoid arthritis (Tisch R, McDevitt HO. Proc Natl Acad Sci U S A 1994 Jan 18;91 (2):437), systemic diseases, systemic autoimmune diseases, systemic lupus erythematosus (Datta SK., Lupus 1998;7 (9):591), glandular diseases, glandular autoimmune diseases, pancreatic diseases, pancreatic autoimmune diseases, Type 1 diabetes (Castano L. and Eisenbarth GS. Ann. Rev. Immunol. 8:647); thyroid diseases, autoimmune thyroid diseases, Graves' disease (Sakata S. *et al.*, Mol Cell Endocrinol 1993 Mar;92 (1):77); ovarian diseases (Garza KM. *et al.*, J Reprod Immunol 1998 Feb;37 (2):87), prostatitis, autoimmune prostatitis (Alexander RB. *et al.*, Urology 1997 Dec;50 (6):893), polyglandular syndrome, autoimmune polyglandular syndrome, Type I

autoimmune polyglandular syndrome (Hara T. *et al.*, Blood. 1991 Mar 1;77 (5):1127), neurological diseases, autoimmune neurological diseases, multiple sclerosis, neuritis, optic neuritis (Soderstrom M. *et al.*, J Neurol Neurosurg Psychiatry 1994 May;57 (5):544), myasthenia gravis (Oshima M. *et al.*, Eur J Immunol 1990 Dec;20 (12):2563), stiff-man syndrome (Hiemstra HS. *et al.*, Proc Natl Acad Sci U S A 2001 Mar 27;98 (7):3988), cardiovascular diseases, cardiac autoimmunity in Chagas' disease (Cunha-Neto E. *et al.*, J Clin Invest 1996 Oct 15;98 (8):1709), autoimmune thrombocytopenic purpura (Semple JW. *et al.*, Blood 1996 May 15;87 (10):4245), anti-helper T lymphocyte autoimmunity (Caporossi AP. *et al.*, Viral Immunol 1998;11 (1):9), hemolytic anemia (Sallah S. *et al.*, Ann Hematol 1997 Mar;74 (3):139), hepatic diseases, hepatic autoimmune diseases, hepatitis, chronic active hepatitis (Franco A. *et al.*, Clin Immunol Immunopathol 1990 Mar;54 (3):382), biliary cirrhosis, primary biliary cirrhosis (Jones DE. Clin Sci (Colch) 1996 Nov;91 (5):551), nephric diseases, nephric autoimmune diseases, nephritis, interstitial nephritis (Kelly CJ. J Am Soc Nephrol 1990 Aug;1 (2):140), connective tissue diseases, ear diseases, autoimmune connective tissue diseases, autoimmune ear disease (Yoo TJ. *et al.*, Cell Immunol 1994 Aug;157 (1):249), disease of the inner ear (Gloddek B. *et al.*, Ann N Y Acad Sci 1997 Dec 29;830:266), skin diseases, cutaneous diseases, dermal diseases, bullous skin diseases, pemphigus vulgaris, bullous pemphigoid and pemphigus foliaceus.

Examples of delayed type hypersensitivity include, but are not limited to, contact dermatitis and drug eruption.

Examples of types of T lymphocyte mediating hypersensitivity include, but are not limited to, helper T lymphocytes and cytotoxic T lymphocytes.

Examples of helper T lymphocyte-mediated hypersensitivity include, but are not limited to, T_H1 lymphocyte mediated hypersensitivity and T_H2 lymphocyte mediated hypersensitivity.

Autoimmune diseases

Include, but are not limited to, cardiovascular diseases, rheumatoid diseases, glandular diseases, gastrointestinal diseases, cutaneous diseases, hepatic diseases, neurological diseases, muscular diseases, nephric diseases, diseases related to reproduction, connective tissue diseases and systemic diseases.

Examples of autoimmune cardiovascular diseases include, but are not limited to atherosclerosis (Matsuura E. *et al.*, Lupus. 1998;7 Suppl 2:S135), myocardial infarction (Vaarala O. Lupus. 1998;7 Suppl 2:S132), thrombosis (Tincani A. *et al.*, Lupus 1998;7 Suppl 2:S107-9), Wegener's granulomatosis, Takayasu's arteritis, Kawasaki syndrome (Praprotnik S. *et al.*, Wien Klin Wochenschr 2000 Aug 25;112 (15-16):660), anti-factor VIII autoimmune disease (Lacroix-

Desmazes S. *et al.*, Semin Thromb Hemost.2000;26 (2):157), necrotizing small vessel vasculitis, microscopic polyangiitis, Churg and Strauss syndrome, pauci-immune focal necrotizing and crescentic glomerulonephritis (Noel LH. Ann Med Interne (Paris). 2000 May;151 (3):178), antiphospholipid syndrome (Flamholz R. *et al.*, J Clin Apheresis 1999;14 (4):171), antibody-induced heart failure (Wallukat G. *et al.*, Am J Cardiol. 1999 Jun 17;83 (12A):75H), thrombocytopenic purpura (Moccia F. Ann Ital Med Int. 1999 Apr-Jun;14 (2):114; Semple JW. *et al.*, Blood 1996 May 15;87 (10):4245), autoimmune hemolytic anemia (Efremov DG. *et al.*, Leuk Lymphoma 1998 Jan;28 (3-4):285; Sallah S. *et al.*, Ann Hematol 1997 Mar;74 (3):139), cardiac autoimmunity in Chagas' disease (Cunha-Neto E. *et al.*, J Clin Invest 1996 Oct 15;98 (8):1709) and anti-helper T lymphocyte autoimmunity (Caporossi AP. *et al.*, Viral Immunol 1998;11 (1):9).

Examples of autoimmune rheumatoid diseases include, but are not limited to rheumatoid arthritis (Krenn V. *et al.*, Histol Histopathol 2000 Jul;15 (3):791; Tisch R, McDevitt HO. Proc Natl Acad Sci units S A 1994 Jan 18;91 (2):437) and ankylosing spondylitis (Jan Voswinkel *et al.*, Arthritis Res 2001; 3 (3): 189).

Examples of autoimmune glandular diseases include, but are not limited to, pancreatic disease, Type I diabetes, thyroid disease, Graves' disease, thyroiditis, spontaneous autoimmune thyroiditis, Hashimoto's thyroiditis, idiopathic myxedema, ovarian autoimmunity, autoimmune anti-sperm infertility, autoimmune prostatitis and Type I autoimmune polyglandular syndrome. Diseases include, but are not limited to autoimmune diseases of the pancreas, Type 1 diabetes (Castano L. and Eisenbarth GS. Ann. Rev. Immunol. 8:647; Zimmet P. Diabetes Res Clin Pract 1996 Oct;34 Suppl:S125), autoimmune thyroid diseases, Graves' disease (Orgiazzi J. Endocrinol Metab Clin North Am 2000 Jun;29 (2):339; Sakata S. *et al.*, Mol Cell Endocrinol 1993 Mar;92 (1):77), spontaneous autoimmune thyroiditis (Braley-Mullen H. and Yu S, J Immunol 2000 Dec 15;165 (12):7262), Hashimoto's thyroiditis (Toyoda N. *et al.*, Nippon Rinsho 1999 Aug;57 (8):1810), idiopathic myxedema (Mitsuma T. Nippon Rinsho. 1999 Aug;57 (8):1759), ovarian autoimmunity (Garza KM. *et al.*, J Reprod Immunol 1998 Feb;37 (2):87), autoimmune anti-sperm infertility (Diekman AB. *et al.*, Am J Reprod Immunol. 2000 Mar;43 (3):134), autoimmune prostatitis (Alexander RB. *et al.*, Urology 1997 Dec;50 (6):893) and Type I autoimmune polyglandular syndrome (Hara T. *et al.*, Blood. 1991 Mar 1;77 (5):1127).

Examples of autoimmune gastrointestinal diseases include, but are not limited to, chronic inflammatory intestinal diseases (Garcia Herola A. *et al.*, Gastroenterol Hepatol. 2000 Jan;23 (1):16), celiac disease (Landau YE. and Shoenfeld Y. Harefuah 2000 Jan 16;138 (2):122), colitis, ileitis and Crohn's disease.

Examples of autoimmune cutaneous diseases include, but are not limited to, autoimmune bullous skin diseases, such as, but are not limited to, pemphigus vulgaris, bullous pemphigoid and pemphigus foliaceus.

Examples of autoimmune hepatic diseases include, but are not limited to, hepatitis, autoimmune chronic active hepatitis (Franco A. *et al.*, Clin Immunol Immunopathol 1990 Mar;54 (3):382), primary biliary cirrhosis (Jones DE. Clin Sci (Colch) 1996 Nov;91 (5):551; Strassburg CP. *et al.*, Eur J Gastroenterol Hepatol. 1999 Jun;11 (6):595) and autoimmune hepatitis (Manns MP. J Hepatol 2000 Aug;33 (2):326).

Examples of autoimmune neurological diseases include, but are not limited to, multiple sclerosis (Cross AH. *et al.*, J Neuroimmunol 2001 Jan 1;112 (1-2):1), Alzheimer's disease (Oron L. *et al.*, J Neural Transm Suppl. 1997;49:77), myasthenia gravis (Infante AJ. And Kraig E, Int Rev Immunol 1999;18 (1-2):83; Oshima M. *et al.*, Eur J Immunol 1990 Dec;20 (12):2563), neuropathies, motor neuropathies (Kornberg AJ. J Clin Neurosci. 2000 May;7 (3):191); Guillain-Barre syndrome and autoimmune neuropathies (Kusunoki S. Am J Med Sci. 2000 Apr;319 (4):234), myasthenia, Lambert-Eaton myasthenic syndrome (Takamori M. Am J Med Sci. 2000 Apr;319 (4):204); paraneoplastic neurological diseases, cerebellar atrophy, paraneoplastic cerebellar atrophy and stiff-man syndrome (Hiemstra HS. *et al.*, Proc Natl Acad Sci units S A 2001 Mar 27;98 (7):3988); non-paraneoplastic stiff man syndrome, progressive cerebellar atrophies, encephalitis, Rasmussen's encephalitis, amyotrophic lateral sclerosis, Sydenham chorea, Gilles de la Tourette syndrome and autoimmune polyendocrinopathies (Antoine JC. and Honnorat J. Rev Neurol (Paris) 2000 Jan;156 (1):23); dysimmune neuropathies (Nobile-Orazio E. *et al.*, Electroencephalogr Clin Neurophysiol Suppl 1999;50:419); acquired neuromyotonia, arthrogryposis multiplex congenita (Vincent A. *et al.*, Ann N Y Acad Sci. 1998 May 13;841:482), neuritis, optic neuritis (Soderstrom M. *et al.*, J Neurol Neurosurg Psychiatry 1994 May;57 (5):544) and neurodegenerative diseases.

Examples of autoimmune muscular diseases include, but are not limited to, myositis, autoimmune myositis and primary Sjogren's syndrome (Feist E. *et al.*, Int Arch Allergy Immunol 2000 Sep;123 (1):92) and smooth muscle autoimmune disease (Zauli D. *et al.*, Biomed Pharmacother 1999 Jun;53 (5-6):234).

Examples of autoimmune nephric diseases include, but are not limited to, nephritis and autoimmune interstitial nephritis (Kelly CJ. J Am Soc Nephrol 1990 Aug;1 (2):140).

Examples of autoimmune diseases related to reproduction include, but are not limited to, repeated fetal loss (Tincani A. *et al.*, Lupus 1998;7 Suppl 2:S107-9).

Examples of autoimmune connective tissue diseases include, but are not limited to, ear diseases, autoimmune ear diseases (Yoo TJ. *et al.*, Cell Immunol 1994 Aug;157 (1):249) and autoimmune diseases of the inner ear (Gloddek B. *et al.*, Ann N Y Acad Sci 1997 Dec 29;830:266).

5 Examples of autoimmune systemic diseases include, but are not limited to, systemic lupus erythematosus (Erikson J. *et al.*, Immunol Res 1998;17 (1-2):49) and systemic sclerosis (Renaudineau Y. *et al.*, Clin Diagn Lab Immunol. 1999 Mar;6 (2):156); Chan OT. *et al.*, Immunol Rev 1999 Jun;169:107).

10 According to specific embodiments, the autoimmune disease is selected from the group consisting of multiple sclerosis, myasthenia gravis, Guillan-Barre syndrome (antiphospholipid syndrome), systemic lupus erythematosus, Behcet's syndrome, Sjogrens syndrome, rheumatoid arthritis, Hashimoto's disease/hypothyroiditis, primary biliary cirrhosis, mixed connective tissue disease, chronic active hepatitis, Graves' disease/hyperthyroiditis, scleroderma, chronic idiopathic thrombocytopenic purpura, diabetic neuropathy and septic shock (see, e.g., Schneider
15 A. et al, J Biol Chem. 279:55833-9 (2004)).

Infectious diseases

Examples of infectious diseases include, but are not limited to, chronic infectious diseases, subacute infectious diseases, acute infectious diseases, viral diseases, bacterial diseases, protozoan diseases, parasitic diseases, fungal diseases, mycoplasma diseases and prion diseases.

Graft rejection diseases

20 Examples of diseases associated with transplantation of a graft include, but are not limited to, graft rejection, chronic graft rejection, subacute graft rejection, hyperacute graft rejection, acute graft rejection and graft versus host disease.

Allergic diseases

25 Examples of allergic diseases include, but are not limited to, asthma, hives, urticaria, pollen allergy, dust mite allergy, venom allergy, cosmetics allergy, latex allergy, chemical allergy, drug allergy, insect bite allergy, animal dander allergy, stinging plant allergy, poison ivy allergy and food allergy.

Cancerous diseases

30 Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma, and leukemia. Particular examples of cancerous diseases but are not limited to: Myeloid leukemia such as Chronic myelogenous leukemia. Acute myelogenous leukemia with maturation. Acute promyelocytic leukemia, Acute nonlymphocytic leukemia with increased basophils, Acute

monocytic leukemia. Acute myelomonocytic leukemia with eosinophilia; Malignant lymphoma, such as Birkitt's Non-Hodgkin's; Lymphocytic leukemia, such as Acute lymphoblastic leukemia. Chronic lymphocytic leukemia; Myeloproliferative diseases, such as Solid tumors Benign Meningioma, Mixed tumors of salivary gland, Colonic adenomas; Adenocarcinomas, such as Small cell lung cancer, Kidney, Uterus, Prostate, Bladder, Ovary, Colon, Sarcomas, Liposarcoma, myxoid, Synovial sarcoma, Rhabdomyosarcoma (alveolar), Extraskeletal myxoid chondrosarcoma, Ewing's tumor; other include Testicular and ovarian dysgerminoma, Retinoblastoma, Wilms' tumor, Neuroblastoma, Malignant melanoma, Mesothelioma, breast, skin, prostate, and ovarian.

According to some aspects of the invention the disease or condition is a disease associated with SHANK3.

As used herein the term "SHANK3" [also known as SH3 and multiple ankyrin repeat domains 3 and proline-rich synapse-associated protein 2 (ProSAP2)] refers to the expression product e.g. RNA or protein of the SHANK3 gene (Gene ID 85358). The gene encodes a protein that contains 5 interaction domains or motifs including the ankyrin repeats domain (ANK), a src 3 domain (SH3), a proline-rich domain, a PDZ domain and a sterile α motif domain (SAM).

According to specific embodiments, the SHANK3 is the human SHANK3, such as provided in the following Accession Nos. NM_001080420, NM_001372044, NP_277052.

As used herein the term "disease associated with SHANK3" refers to a disease associated with SHANK3 malfunction (e.g. due to a mutation) for onset and/or progression. A non-limiting example of such a disease is Phelan McDermid syndrome.

The present inventors have uncovered sex-related differences in some, but not all aspects of the effects of the polypeptides and active agents disclosed herein. Thus, in some embodiments, the subject is a male. In other embodiments, the subject is a female.

In some embodiments, treatment with methods, compositions or active components of the articles of manufacture of the invention can be of greater efficacy in a specific age group, for example, younger subjects where neurodevelopmental processes are affected, and, conversely, older subjects where neurodegenerative or aging processes are affected. Thus, in some embodiments, the subject is under 18 years old, namely 0-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17, but less than 18 years old. In other embodiments, the subject is over 60 years old, namely in the range of 61-65, 64-70, 69-75, 74-80, 79-85, 84-90, 89-95, 94-100 and greater than 100 years old. It will be appreciated that the ranges of age include all of the intervening ages.

As used herein, the term “cognitive deficit” encompasses both intellectual disability and cognitive impairment (typically associated with a mental or neurodegenerative disease).

As used herein the term “intellectual disability (ID)”, also known as general learning disability or mental retardation (MR), refers to a generalized neurodevelopmental disorder characterized by significantly impaired intellectual and adaptive functioning.

As used herein, the term "activity-dependent neuroprotective factor (ADNF)" refers to ADNF III (also known as ADNP) and/or ADNF I.

As used herein, the term “ADNF polypeptide” refers to the amino acid sequence of human ADNF III and/or ADNF I, or a functional homolog thereof, having at least one of the activities of ADNF III or ADNF I, as further described hereinbelow. According to specific embodiments, the phrase "ADNF polypeptide" refers to a mixture of an ADNF III polypeptide and an ADNF I polypeptide.

As use herein, the phrase “a functional homolog” refers to a fragment, a naturally occurring or synthetically/recombinantly produced homolog, a non-human homolog, an allelic or polymorphic variant, an amino acid sequence comprising conservative and non-conservative amino acid substitutions deletions or additions, an analog, a lipophilic variant and/or a chemically modified variant, which maintains at least one of the activities of the full length protein, e.g. neurotrophic/neuroprotective activity, binding EB1 and/or EB3, binding an SH3 domain, as further described hereinbelow.

As used herein, the term “polypeptide”, “peptide” or “amino acid sequence”, which are interchangeably used herein, encompasses native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

Peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated amide bonds (-N(CH₃)-CO-), ester bonds (-C(=O)-O-), ketomethylene bonds (-CO-CH₂-), sulfinylmethylene bonds (-S(=O)-CH₂-), α -aza bonds (-NH-N(R)-CO-), wherein R is

any alkyl (e.g., methyl), amine bonds (-CH₂-NH-), sulfide bonds (-CH₂-S-), ethylene bonds (-CH₂-CH₂-), hydroxyethylene bonds (-CH(OH)-CH₂-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), fluorinated olefinic double bonds (-CF=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH₂-CO-), wherein R is the "normal" side chain, naturally present on the carbon atom.

These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) bonds at the same time.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted by non-natural aromatic amino acids such as 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), naphthylalanine, ring-methylated derivatives of Phe, halogenated derivatives of Phe or O-methyl-Tyr.

The polypeptides of some embodiments of the invention may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

The term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally in vivo, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-amino adipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

According to specific embodiments, the polypeptide comprises at least one D-amino acid.

According to specific embodiments, the polypeptide comprises at least two, at least three, at least 4, at least 5, at least 6, at least 8 D-amino acids.

According to specific embodiments, all the polypeptide amino acids are D-amino acids.

Tables 1 and 2 below list naturally occurring amino acids (Table 2), and non-conventional or modified amino acids (e.g., synthetic, Table 3) which can be used with some embodiments of the invention.

Table 1

<i>Amino Acid</i>	<i>Three-Letter Abbreviation</i>	<i>One-letter Symbol</i>
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E

Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Lcu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

Table 2

<i>Non-conventional amino acid</i>	<i>Code</i>	<i>Non-conventional amino acid</i>	<i>Code</i>
ornithine	Orn	hydroxyproline	Hyp
α -aminobutyric acid	Abu	aminonorbornyl-carboxylate	Norb
D-alanine	Dala	aminocyclopropane-carboxylate	Cpro
D-arginine	Darg	N-(3-guanidinopropyl)glycine	Narg
D-asparagine	Dasn	N-(carbamylmethyl)glycine	Nasn
D-aspartic acid	Dasp	N-(carboxymethyl)glycine	Nasp
D-cysteine	Dcys	N-(thiomethyl)glycine	Ncys
D-glutamine	Dgln	N-(2-carbamylethyl)glycine	Ngln
D-glutamic acid	Dglu	N-(2-carboxyethyl)glycine	Nglu
D-histidine	Dhis	N-(imidazolethyl)glycine	Nhis
D-isoleucine	Dile	N-(1-methylpropyl)glycine	Nile
D-leucine	Dleu	N-(2-methylpropyl)glycine	Nleu
D-lysine	Dlys	N-(4-aminobutyl)glycine	Nlys
D-methionine	Dmet	N-(2-methylthioethyl)glycine	Nmet
D-ornithine	Dorn	N-(3-aminopropyl)glycine	Norn
D-phenylalanine	Dphe	N-benzylglycine	Nphe
D-proline	Dpro	N-(hydroxymethyl)glycine	Nser
D-serine	Dscr	N-(1-hydroxyethyl)glycine	Nthr
D-threonine	Dthr	N-(3-indolylethyl) glycine	Nhtrp
D-tryptophan	Dtrp	N-(<i>p</i> -hydroxyphenyl)glycine	Ntyr
D-tyrosine	Dtyr	N-(1-methylethyl)glycine	Nval
D-valine	Dval	N-methylglycine	Nmgly
D-N-methylalanine	Dnmala	L-N-methylalanine	Nmala
D-N-methylarginine	Dnmarg	L-N-methylarginine	Nmarg
D-N-methylasparagine	Dnmasn	L-N-methylasparagine	Nmasn
D-N-methylasparatate	Dnmasp	L-N-methylaspartic acid	Nmasp
D-N-methylcysteine	Dnmcys	L-N-methylcysteine	Nmcys
D-N-methylglutamine	Dnmglu	L-N-methylglutamine	Nmglu
D-N-methylglutamate	Dnmglu	L-N-methylglutamic acid	Nmglu
D-N-methylhistidine	Dnmhis	L-N-methylhistidine	Nmhis
D-N-methylisoleucine	Dnmile	L-N-methylisoleucine	Nmile

D-N-methylleucine	Dnmleu	L-N-methylleucine	Nmleu
D-N-methyllysine	Dnmlys	L-N-methyllysine	Nmlys
D-N-methylmethionine	Dnmmt	L-N-methylmethionine	Nmmt
D-N-methylornithine	Dnmorn	L-N-methylornithine	Nmorn
D-N-methylphenylalanine	Dnmphe	L-N-methylphenylalanine	Nmphe
D-N-methylproline	Dnmpro	L-N-methylproline	Nmpro
D-N-methylserine	Dnmser	L-N-methylserine	Nmser
D-N-methylthreonine	Dnmthr	L-N-methylthreonine	Nmthr
D-N-methyltryptophan	Dnmtrp	L-N-methyltryptophan	Nmtrp
D-N-methyltyrosine	Dnmtyr	L-N-methyltyrosine	Nmtyr
D-N-methylvaline	Dnmval	L-N-methylvaline	Nmval
L-norleucine	Nle	L-N-methylnorleucine	Nnmle
L-norvaline	Nva	L-N-methylnorvaline	Nnmva
L-ethylglycine	Etg	L-N-methyl-ethylglycine	Nmetg
L-t-butylglycine	Tbug	L-N-methyl-t-butylglycine	Nmtbug
L-homophenylalanine	Hphe	L-N-methyl-homophenylalanine	Nmhphe
α -naphthylalanine	Anap	N-methyl- α -naphthylalanine	Nmanap
penicillamine	Pen	N-methylpenicillamine	Nmpen
γ -aminobutyric acid	Gabu	N-methyl- γ -aminobutyrate	Nmgabu
cyclohexylalanine	Chexa	N-methyl-cyclohexylalanine	Nmchexa
cyclopentylalanine	Cpen	N-methyl-cyclopentylalanine	Nmcpen
α -amino- α -methylbutyrate	Aabu	N-methyl- α -amino- α -methylbutyrate	Nmaabu
α -aminoisobutyric acid	Aib	N-methyl- α -aminoisobutyrate	Nmaib
D- α -methylarginine	Dmarg	L- α -methylarginine	Marg
D- α -methylasparagine	Dmasn	L- α -methylasparagine	Masn
D- α -methylaspartate	Dmasp	L- α -methylaspartate	Masp
D- α -methyleysteine	Dmcys	L- α -methyleysteine	Mcys
D- α -methylglutamine	Dmgln	L- α -methylglutamine	Mgln
D- α -methyl glutamic acid	Dmglu	L- α -methylglutamate	Mglu
D- α -methylhistidine	Dmhis	L- α -methylhistidine	Mhis
D- α -methylisoleucine	Dmile	L- α -methylisoleucine	Mile
D- α -methylleucine	Dmleu	L- α -methylleucine	Mleu
D- α -methyllysine	Dmlys	L- α -methyllysine	Mlys
D- α -methylmethionine	Dmmt	L- α -methylmethionine	Mmt
D- α -methylornithine	Dmorn	L- α -methylornithine	Morn
D- α -methylphenylalanine	Dmphe	L- α -methylphenylalanine	Mphe
D- α -methylproline	Dmpro	L- α -methylproline	Mpro
D- α -methylserine	Dmser	L- α -methylserine	Mser
D- α -methylthreonine	Dmthr	L- α -methylthreonine	Mthr
D- α -methyltryptophan	Dmtrp	L- α -methyltryptophan	Mtrp
D- α -methyltyrosine	Dmtyr	L- α -methyltyrosine	Mtyr
D- α -methylvaline	Dmval	L- α -methylvaline	Mval
N-cyclobutylglycine	Ncbut	L- α -methylnorvaline	Mnva
N-cycloheptylglycine	Nchep	L- α -methyl-ethylglycine	Metg
N-cyclohexylglycine	Nchex	L- α -methyl- <i>t</i> -butylglycine	Mtbug
N-cyclodecylglycine	Ncdod	L- α -methyl-homophenylalanine	Mhphe
N-cyclododecylglycine	Ncdod	α -methyl- α -naphthylalanine	Manap

N-cyclooctylglycine	Ncoct	α -methylpenicillamine	Mpen
N-cyclopropylglycine	Ncpro	α -methyl- γ -aminobutyrate	Mgab
N-cycloundecylglycine	Ncund	α -methyl-cyclohexylalanine	Mchexa
N-(2-aminoethyl)glycine	Naeg	α -methyl-cyclopentylalanine	Mcpen
N-(2,2-diphenylethyl)glycine	Nbhm	N-(N-(2,2-diphenylethyl) carbamylmethyl-glycine	Nnbhm
N-(3,3-diphenylpropyl)glycine	Nbhc	N-(N-(3,3-diphenylpropyl) carbamylmethyl-glycine	Nnbhc
1-carboxy-1-(2,2-diphenyl ethylamino)cyclopropane	Nmbc	1,2,3,4-tetrahydroisoquinoline- 3-carboxylic acid	Tic
phosphoserine	pSer	phosphothreonine	pThr
phosphotyrosine	pTyr	O-methyl-tyrosine	
2-aminoadipic acid		hydroxylysine	

The amino acids of the polypeptides of some embodiments of the present invention may be substituted either conservatively or non-conservatively.

The term "conservative substitution" as used herein, refers to the replacement of an amino acid present in the native sequence in the peptide with a naturally or non-naturally occurring amino or a peptidomimetics having similar steric properties. Where the side-chain of the native amino acid to be replaced is either polar or hydrophobic, the conservative substitution should be with a naturally occurring amino acid, a non-naturally occurring amino acid or with a peptidomimetic moiety which is also polar or hydrophobic (in addition to having the same steric properties as the side-chain of the replaced amino acid).

As naturally occurring amino acids are typically grouped according to their properties, conservative substitutions by naturally occurring amino acids can be easily determined bearing in mind the fact that in accordance with the invention replacement of charged amino acids by sterically similar non-charged amino acids are considered as conservative substitutions.

For producing conservative substitutions by non-naturally occurring amino acids it is also possible to use amino acid analogs (synthetic amino acids) well known in the art. A peptidomimetic of the naturally occurring amino acid is well documented in the literature known to the skilled practitioner.

When affecting conservative substitutions the substituting amino acid should have the same or a similar functional group in the side chain as the original amino acid.

Conservative substitution tables providing functionally similar amino acids are well known in the art. Guidance concerning which amino acid changes are likely to be phenotypically silent can also be found in Bowie et al., 1990, Science 247: 1306 1310.

The phrase "non-conservative substitutions" as used herein refers to replacement of the amino acid as present in the parent sequence by another naturally or non-naturally occurring

amino acid, having different electrochemical and/or steric properties. Thus, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the native amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted. Examples of non-conservative substitutions of this type include the substitution of phenylalanine or cyclohexylmethyl glycine for alanine, isoleucine for glycine, or $\text{-NH-CH[(-CH}_2\text{)}_5\text{-COOH]-CO-}$ for aspartic acid. Those non-conservative substitutions which fall under the scope of the present invention are those which still constitute a peptide having neuroprotective properties.

The polypeptides of some embodiments of the invention are preferably utilized in a linear form, although it will be appreciated that in cases where cyclicization does not severely interfere with peptide characteristics, cyclic forms of the peptide can also be utilized.

Since according to specific embodiments, the present polypeptides are utilized in therapeutics which require the peptides to be in soluble form, the polypeptides of some embodiments of the invention include one or more non-natural or natural polar amino acids, including but not limited to serine and threonine which are capable of increasing peptide solubility due to their hydroxyl-containing side chain.

According to specific embodiments, the polypeptide is less than 100, less than 50, less than 20 or less than 10 amino acids in length.

According to specific embodiments, the polypeptide is 4-100, 4-50, 4-40, 4-20, 4-15, 4-10, 4-8 or 8 amino acids in length, each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the polypeptide is at least 4, at least 5, at least 6, at least 7, at least 8 amino acids in length.

According to specific embodiments, the polypeptide is attached, directly or through a spacer or a linker, to a cell penetrating and/or stabilizing moiety. Such moieties are well known in the art and are further described in details hereinbelow.

According to specific embodiments, the N and/or C termini of the polypeptides of some embodiments of the present invention may be protected by functional groups (i.e. end-capping moieties). Examples of such functional groups can be found, for example, in Green *et al.*, "Protective Groups in Organic Chemistry", (Wiley, 2^{sup}.nd ed. 1991), Harrison *et al.*, "Compendium of Synthetic Organic Methods", Vols. 1-8 (John Wiley and Sons, 1971-1996); and Green and Wuts, "Protecting Groups in Organic Synthesis", John Wiley and Sons, Chapters 5 and 7, 1991, the teachings of which are incorporated herein by reference. Preferred protecting groups

are those that increase stability of the polypeptide and/or facilitate transport of the compound attached thereto into a cell, for example, by reducing the hydrophilicity and increasing the lipophilicity of the compounds.

According to specific embodiments, the end-capping comprises an N terminus end-capping.

Representative examples of N-terminus end-capping moieties include, but are not limited to, formyl, acetyl (also denoted herein as "Ac"), stearyl, trifluoroacetyl, benzyl, benzyloxycarbonyl (also denoted herein as "Cbz"), tert-butoxycarbonyl (also denoted herein as "Boc"), trimethylsilyl (also denoted "TMS"), 2-trimethylsilyl-ethanesulfonyl (also denoted "SES"), trityl and substituted trityl groups, allyloxycarbonyl, 9-fluorenylmethyloxycarbonyl (also denoted herein as "Fmoc"), and nitro-veratryloxycarbonyl ("NVOC").

According to specific embodiments, the N terminus end-capping comprises an Acetyl.

According to specific embodiments, the N terminus end-capping comprises a stearyl (see e.g. Gozes I, et al. Proc Natl Acad Sci U S A. 1996 Jan 9; 93(1): 427-32).

According to specific embodiments, the end-capping comprises a C terminus end-capping.

Representative examples of C-terminus end-capping moieties are typically moieties that lead to acylation of the carboxy group at the C-terminus and include, but are not limited to, benzyl and trityl ethers as well as alkyl ethers, tetrahydropyranyl ethers, trialkylsilyl ethers, allyl ethers, monomethoxytrityl and dimethoxytrityl. Alternatively the -COOH group of the C-terminus end-capping may be modified to an amide group.

According to specific embodiments, the C terminus end-capping comprises an Amide.

Other end-capping modifications of peptides include replacement of the amine and/or carboxyl with a different moiety, such as hydroxyl, thiol, halide, alkyl, aryl, alkoxy, aryloxy and the like.

According to other specific embodiments of the invention, the polypeptide is attached to a non-proteinaceous moiety.

According to specific embodiments, the polypeptide and the attached non-proteinaceous moiety are covalently attached, directly or through a spacer or a linker.

The phrase "non-proteinaceous moiety" as used herein refers to a molecule not including peptide bonded amino acids that is attached to the above-described polypeptide. According to a specific embodiment the non-proteinaceous is a non-toxic moiety. Exemplary non-proteinaceous moieties which may be used according to the present teachings include, but are not limited to a

drug, a chemical, a small molecule, a polynucleotide, a detectable moiety, polyethylene glycol (PEG), Polyvinyl pyrrolidone (PVP), poly(styrene comaleic anhydride) (SMA), and divinyl ether and maleic anhydride copolymer (DIVEMA). According to specific embodiments of the invention, the non-proteinaceous moiety comprises polyethylene glycol (PEG).

Such a molecule is highly stable (resistant to in-vivo proteolytic activity probably due to steric hindrance conferred by the non-proteinaceous moiety) and may be produced using common solid phase synthesis methods which are inexpensive and highly efficient, as further described hereinbelow. However, it will be appreciated that recombinant techniques may still be used, whereby the recombinant peptide product is subjected to in-vitro modification (e.g., PEGylation as further described hereinbelow).

Bioconjugation of the peptide amino acid sequence with PEG (*i.e.*, PEGylation) can be effected using PEG derivatives such as N-hydroxysuccinimide (NHS) esters of PEG carboxylic acids, monomethoxyPEG₂-NHS, succinimidyl ester of carboxymethylated PEG (SCM-PEG), benzotriazole carbonate derivatives of PEG, glycidyl ethers of PEG, PEG p-nitrophenyl carbonates (PEG-NPC, such as methoxy PEG-NPC), PEG aldehydes, PEG-orthopyridyl-disulfide, carbonyldimazol-activated PEGs, PEG-thiol, PEG-maleimide. Such PEG derivatives are commercially available at various molecular weights [See, e.g., Catalog, Polyethylene Glycol and Derivatives, 2000 (Shearwater Polymers, Inc., Huntsville, Ala.)]. If desired, many of the above derivatives are available in a monofunctional monomethoxyPEG (mPEG) form. In general, the PEG added to the peptide of some embodiments of the present invention should range from a molecular weight (MW) of several hundred Daltons to about 100 kDa (e.g., between 3-30 kDa). Larger MW PEG may be used, but may result in some loss of yield of PEGylated polypeptides. The purity of larger PEG molecules should be also watched, as it may be difficult to obtain larger MW PEG of purity as high as that obtainable for lower MW PEG. It is preferable to use PEG of at least 85 % purity, and more preferably of at least 90 % purity, 95 % purity, or higher. PEGylation of molecules is further discussed in, e.g., Hermanson, Bioconjugate Techniques, Academic Press San Diego, Calif. (1996), at Chapter 15 and in Zalipsky et al., "Succinimidyl Carbonates of Polyethylene Glycol," in Dunn and Ottenbrite, eds., Polymeric Drugs and Drug Delivery Systems, American Chemical Society, Washington, D.C. (1991).

Conveniently, PEG can be attached to a chosen position in the peptide by site-specific mutagenesis as long as the activity of the conjugate is retained. A target for PEGylation could be any Cysteine residue at the N-terminus or the C-terminus of the peptide sequence. Additionally

or alternatively, other Cysteine residues can be added to the peptide amino acid sequence (e.g., at the N-terminus or the C-terminus) to thereby serve as a target for PEGylation. Computational analysis may be effected to select a preferred position for mutagenesis without compromising the activity.

5 Various conjugation chemistries of activated PEG such as PEG-maleimide, PEG-vinylsulfone (VS), PEG-acrylate (AC), PEG-orthopyridyl disulfide can be employed. Methods of preparing activated PEG molecules are known in the arts. For example, PEG-VS can be prepared under argon by reacting a dichloromethane (DCM) solution of the PEG-OH with NaH and then with di-vinylsulfone (molar ratios: OH 1: NaH 5: divinyl sulfone 50, at 0.2 gram
10 PEG/mL DCM). PEG-AC is made under argon by reacting a DCM solution of the PEG-OH with acryloyl chloride and triethylamine (molar ratios: OH 1: acryloyl chloride 1.5: triethylamine 2, at 0.2 gram PEG/mL DCM). Such chemical groups can be attached to linearized, 2-arm, 4-arm, or 8-arm PEG molecules.

Resultant conjugated molecules (e.g., PEGylated or PVP-conjugated peptide) are
15 separated, purified and qualified using e.g., high-performance liquid chromatography (HPLC) as well as biological assays.

The polypeptides and compositions of matter of the present invention may be attached (either covalently or non-covalently) to a penetrating moiety.

According to other specific embodiments, the polypeptide is not attached to a
20 heterologous penetrating moiety. Thus, for Example, the ADNF polypeptide NAP (SEQ ID NO: 2) is bioavailable by endocytosis (see e.g. Ivashko-Pachima Y, Gozes I.J Mol Neurosci. 2020 Jul;70(7):993-998), thus being a cell penetrating peptide by itself.

As used herein the phrase "penetrating moiety" refers to an agent which enhances translocation of any of the attached polypeptide or composition of matter comprising same
25 across a cell membrane.

According to one embodiment, the penetrating moiety is a peptide and is attached to the polypeptide (either directly or non-directly) via a peptide bond.

Typically, peptide penetrating moieties have an amino acid composition containing either a high relative abundance of positively charged amino acids such as lysine or arginine, or have
30 sequences that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids.

By way of non-limiting example, cell penetrating peptide (CPP) sequences may be used in order to enhance intracellular penetration; however, the disclosure is not so limited, and any suitable penetrating agent may be used, as known by those of skill in the art.

Cell-Penetrating Peptides (CPPs) are short peptides (<40 amino acids), with the ability to gain access to the interior of almost any cell. They are highly cationic and usually rich in arginine and lysine amino acids. They have the exceptional property of carrying into the cells a wide variety of covalently and noncovalently conjugated cargoes such as proteins, oligonucleotides, and even 200 nm liposomes. Therefore, according to additional exemplary embodiment CPPs can be used to transport the ADNP polypeptide to the interior of cells.

TAT (transcription activator from HIV-1), pAntp (also named penetratin, *Drosophila* antennapedia homeodomain transcription factor) and VP22 (from Herpes Simplex virus) are non-limiting examples of CPPs that can enter cells in a non-toxic and efficient manner and may be suitable for use with some embodiments of the invention. Protocols for producing CPPs-cargos conjugates and for infecting cells with such conjugates can be found, for example L Theodore et al. [The Journal of Neuroscience, (1995) 15(11): 7158-7167], Fawell S, et al. [Proc Natl Acad Sci USA, (1994) 91:664-668], and Jing Bian et al. [Circulation Research. (2007) 100: 1626-1633].

According to another exemplary embodiment the polypeptide may be incorporated into a particulated delivery vehicle, e.g., a liposome, or a nano- or microparticle by any of the known methods in the art [for example, Liposome Technology, Vol. II, Incorporation of Drugs, Proteins, and Genetic Material, CRC Press; Monkkonen, J. *et al.*, 1994, J. Drug Target, 2:299-308; Monkkonen, J. *et al.*, 1993, Calcif. Tissue Int., 53:139-145; Lasic D D., Liposomes Technology Inc., Elsevier, 1993, 63-105. (chapter 3); Winterhalter M, Lasic D D, Chem Phys Lipids, 1993 September;64(1-3):35-43].

Liposomes include any synthetic (i.e., not naturally occurring) structure composed of lipid bilayers, which enclose a volume. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. Liposomes can be of different sizes, may contain a low or a high pH and may be of different charge.

The polypeptides of some embodiments of the invention may be synthesized by any techniques that are known to those skilled in the art of peptide synthesis, such as, but not limited to, solid phase and recombinant techniques.

For solid phase peptide synthesis, a summary of the many techniques may be found in J. M. Stewart and J. D. Young, *Solid Phase Peptide Synthesis*, W. H. Freeman Co. (San Francisco), 1963 and J. Meienhofer, *Hormonal Proteins and Peptides*, vol. 2, p. 46, Academic Press (New York), 1973. For classical solution synthesis see G. Schroder and K. Lupke, *The Peptides*, vol. 1, Academic Press (New York), 1965.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then either be attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially or concurrently, to afford the final peptide compound. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide and so forth. Further description of peptide synthesis is disclosed in U.S. Pat. No. 6,472,505.

Large scale peptide synthesis is described by Andersson *Biopolymers* 2000;55(3):227-50. Specific embodiments of the present invention contemplate the use of a combined treatment/prophylaxis comprising the polypeptide and a therapeutic agent other than the polypeptides disclosed herein.

Hence, according to specific embodiments, the polypeptides disclosed herein may be provided to the individual with additional active agents to achieve an improved therapeutic or preventive effect as compared to treatment with each agent by itself. Thus, the polypeptide can be administered alone or with other established or experimental therapeutic regimen to treat or prevent diseases associated with evoked potential and/or speech impairment, autism spectrum disorder and intellectual disability, Alzheimer's disease, autism spectrum disorder, neurodegenerative disease, cognitive deficit, mental disorder and cytoskeletal disorder as detailed herein. In such therapy, measures (e.g., dosing and selection of the complementary

agent) are taken to minimize or eliminate adverse side effects which may be associated with combination therapies.

Non-limiting examples of ADNF polypeptides that can be used with specific embodiments of the invention are described in detail in e.g. International Patent Application Publication Nos. WO1992/018140, WO9611948, WO 98/35042, WO 0027875, WO 00/53217, WO01/12654, WO 01/92333, WO 2004/080957, WO 2006/099739, WO2007/096859, 2008/08448, WO 2011/021186, WO/2009/026687, WO2010/075635, 2011/083461, WO 2011/099011, WO2013/171595, WO 2017/130190, WO 2004/060309, WO2003022226 and U.S. Patent Nos. US5767240, US6174862, US6613740 and US8586548; herein each incorporated by reference in their entirety; and further hereinbelow.

According to specific embodiments, ADNF is ADNF III.

“ADNF III”, also known as ADNP (activity- dependent neuroprotective protein), refers to the polypeptide encoded by the *ADNP* gene (Gene ID 23394). According to specific embodiments, ADNF III is human ADNF III. Full length human ADNF III (ADNP) has a predicted molecular weight of 123,562.8 Da (>1000 amino acid residues) and a theoretical pi of about 6.97. The human ADNF III gene is localized to chromosome 20q13.13-13.2, a region associated with cognitive function. Exemplary full-length amino acid and nucleic acid sequences of ADNF III can be found in WO 98/35042, WO 00/27875, US Patent Nos. 6,613,740 and 6,649,411. According to specific embodiments, ADNF III amino acid sequence comprises SEQ ID NO: 1.

The ADNF III polypeptide described herein possesses at least one of the activities of the full length ADNF III e.g. neurotrophic/neuroprotective activity as measured with in vitro cortical neuron culture assays, binding EB1 and/or EB3, binding an SH3 domain.

Assays for testing neurotrophic/neuroprotective activity are well known in the art and include, but not limited to, in vitro cortical neuron culture assays described by, e.g., Hill et al, Brain Res. 603:222-233 (1993); Brenneman & Gozes, J. Clin. Invest. 97:2299-2307 (1996), Gozes et al, Proc. Natl. Acad. Sci USA 93, 427-432 (1996).

Assays for testing binding are well known in the art and include, but not limited, to flow cytometry, BiaCore, bio-layer interferometry Blitz® assay, HPLC.

Non-limiting examples of ADNF III polypeptides that can be used with specific embodiments of the invention are provided in Table 3 hereinbelow.

According to specific embodiments, the ADNF III polypeptide comprises an amino acid sequence having 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 % identity or homology to any of SEQ ID NO: 1-22.

As used herein, “identity” or “sequence identity” refers to global identity, *i.e.*, an identity over the entire amino acid or nucleic acid sequences disclosed herein and not over portions thereof.

Sequence identity or homology can be determined using any protein or nucleic acid sequence alignment algorithm such as Blast, ClustalW, and MUSCLE.

According to specific embodiments, ADNF is ADNF I.

“ADNF I” refers to the activity dependent neurotrophic factor described in Gozes I, Brenneman DE. J Mol Neurosci. 1996 Winter;7(4):235-44; Brenneman DE, Gozes I. J Clin Invest. 1996 May 15;97(10):2299-307 and Brenneman DE, et al. J Pharmacol Exp Ther. 1998 May;285(2):619-27, the contents of each are incorporated herein by reference in their entirety. According to specific embodiments, ADNF I is human ADNF I. Full length human ADNF I has a predicted molecular weight of about 14,000 Da with a pI of 8.3 ± 0.25 . According to specific embodiments, ADNF I amino acid sequence comprises any of SEQ ID NO: 24 or 45.

The ADNF I polypeptide described herein possesses at least one of the activities of the full length ADNF I e.g. neurotrophic/neuroprotective activity as measured with in vitro cortical neuron culture assays, binding EB1 and EB3.

Non-limiting examples of ADNF I polypeptides that can be used with specific embodiments of the invention are provided in Table 3 hereinbelow.

According to specific embodiments, the ADNF I polypeptide comprises an amino acid sequence having 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 % identity or homology to any of SEQ ID NO: 24-48.

Table 3: list of possible ADNF polypeptides that can be used with specific embodiments of the invention.

SEQ ID NO:	ADNF III polypeptide		SEQ ID NO:	ADNF I polypeptide	
	Single letter aa code	Three letters aa code		Single letter aa code	Three letters aa code
1	Full length		24	SALLRSIPA	Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
2	NAPVSIPQ	Asn-Ala-Pro-Val-Ser-Ile-Pro-	25	VAGGGSALLRSIPA	Val-Ala-Gly-Gly-Gly-Ser-Ala-Leu-

		Gln			Leu-Arg-Ser-Ile-Pro-Ala
3	SVRLGLGGN APVSIPQQS	Ser-Val-Arg-Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser	26	VEEGIVLGGGS ALLRSIPA	Val-Glu-Glu-Gly-Ile-Val-Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
4	LGLGGNAPVS IPQQS	Leu-Gly-Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser	27	VLGGGCALLR CIPA	Val-Leu-Gly-Gly-Gly-Cys-Ala-Leu-Leu-Arg-Cys-Ile-Pro-Ala
5	LGGNAPVSIP QQS	Leu-Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln-Gln-Ser	28	ALLRSIPA	Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
6	GGNAPVSIPQ	Gly-Gly-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln	29	GSALLRSIPA	Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
7	Ac- NAPVSKIPQ	Ac-Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln	30	SALLRS	Ser-Ala-Leu-Leu-Arg-Ser
8	NAPVSKIPQ	Asn-Ala-Pro-Val-Ser-Lys-Ile-Pro-Gln	31	GSALLRSIPA	Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
9	NAPBSAIPQ	Asn-Ala-Pro-Asx-Ser-Ala-Ile-Pro-Gln	32	VAGGGSALLRS I	Val-Ala-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile
10	NAPVSRIPQ	Asn-Ala-Pro-Val-Ser-Arg-Ile-Pro-Gln	33	VLGGGSALLR	Val-Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg
11	NAPVTRIPQ	Asn-Ala-Pro-Val-Thr-Arg-Ile-Pro-Gln	34	VLGGGSALL	Val-Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu
12	NAPVAAAAQ	Asn-Ala-Pro-Val-Ala-Ala-Ala-Ala-Gln	35	VAGGGSAL	Val-Ala-Gly-Gly-Gly-Ser-Ala-Leu
13	All d-amino acid NAPVSIPQ	All d-amino acid Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln	36	All d-amino acid SALLRSIPA	All d-amino acid Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
14	AAPVSIPQ	Ala-Ala-Pro-Val-Ser-Ile-Pro-Gln	37	AALLRSIPA	Ala-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
15	NAAVSIPQ	Asn-Ala-Ala-Val-Ser-Ile-Pro-Gln	38	SAALRSIPA	Ser-Ala-Ala-Leu-Arg-Ser-Ile-Pro-Ala
16	NAPASIPQ	Asn-Ala-Pro-Ala-Ser-Ile-Pro-Gln	39	SALARSIPA	Ser-Ala-Leu-Ala-Arg-Ser-Ile-Pro-Ala
17	NAPVAIPQ	Asn-Ala-Pro-Val-Ala-Ile-Pro-Gln	40	SALLASIPA	Ser-Ala-Leu-Leu-Ala-Ser-Ile-Pro-Ala

18	NAPVSAPQ	Asn-Ala-Pro-Val-Ser-Ala-Pro-Gln	41	SALLRAIPA	Ser-Ala-Leu-Leu-Arg-Ala-Ile-Pro-Ala
19	NAPVSIAQ	Asn-Ala-Pro-Val-Ser-Ile-Ala-Gln	42	SALLRSAPA	Ser-Ala-Leu-Leu-Arg-Ser-Ala-Pro-Ala
20	NAPVSIPA	Asn-Ala-Pro-Val-Ser-Ile-Pro-Ala	43	SALLRSIAA	Ser-Ala-Leu-Leu-Arg-Ser-Ile-Ala-Ala
21	SKIP	Ser-Lys-Ile-Pro	44	SALLRSIPAPAG ASRLLLLTGEID I.P	Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala-Pro-Ala-Gly-Ala-Ser-Arg-Leu-Leu-Leu-Leu-Thr-Gly-Glu-Ile-Asp-Leu-Pro
22	Ac-SKIP-NH2	Ac-Ser-Lys-Ile-Pro-NH2	45	VLGGGSALLRS IPA	Val-Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
			46	LGGGSALLRSIP A	Leu-Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
			47	GGGSALLRSIP A	Gly-Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala
			48	GGGSALLRSIPA	Gly-Gly-Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala

In further aspects, the polypeptide comprises an active core site comprising the amino acid sequence of NAPVSIPQ (SEQ ID NO: 2) or SALLRSIPA (SEQ ID NO:24), or conservatively modified variants (e.g., deletion, addition, or substitutions of one or more amino acids) or chemically modified variants thereof, that have neurotrophic/neuroprotective activity as measured with in vitro cortical neuron culture assays as described. An ADNF polypeptide can be derived from an ADNF I polypeptide, an ADNF III polypeptide, their alleles, polymorphic variants, analogs, interspecies homolog, any subsequences thereof or lipophilic variants that exhibit neuroprotective/neurotrophic action on, e.g., neurons originating in the central nervous system either in vitro or in vivo. An ADNF-related neuroprotective peptide can range from as short as four to eight amino acids and can have, e.g., between 8-20, 8-50, 10-100, or about 200, 500, or more amino acids. One non-limiting example of a variant ADNP-related neuroprotective peptide is a 4-amino acid peptide of SKIP (SEQ ID NO: 21), see Amram et al. Sexual Divergence

in Microtubule Function: The Novel Intranasal Microtubule Targeting SKIP Normalizes Axonal Transport and Enhances Memory. *Mol Psychiatry*, 2016; 21:1467-76. Further examples include, but are not limited to all D-amino acid derivatives of NAPVSIPQ (SEQ ID NO: 13) and SALLRSIPA (SEQ ID NO:36).

Thus, according to further aspects of the invention, the polypeptide has the formula $(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$ (SEQ ID NO: 49), in which R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and x and y are independently selected and are equal to zero or one. In further embodiments, the core amino acid sequence “Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln” of SEQ ID NO: 49 (“Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln” is identical to SEQ ID NO: 2) is replaced by an analogue of SEQ ID NO: 2.

In further aspects of the invention, the polypeptide has the formula $(R^1)_x$ -Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala- $(R^2)_y$ (SEQ ID NO: 50), in which R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and x and y are independently selected and are equal to zero or one. In further embodiments, the amino acid sequence “Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala” of SEQ ID NO: 50 (“Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala” is identical to SEQ ID NO: 24) is replaced by an analogue of SEQ ID NO: 24.

In some embodiments, the ADNF polypeptide is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-22. In other embodiments, the ADNF polypeptide is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-20. In still further embodiments, the ADNF polypeptide is a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

The polypeptides and/or therapeutic agents described herein can be provided to the subject *per se*, or as part of a pharmaceutical composition where it is mixed with a pharmaceutically acceptable carrier.

The agents, polypeptides and ingredients of some embodiments of the invention which are described hereinabove may be included in an article of manufacture, preferably along with appropriate instructions for use and labels indicating FDA approval for use, for example, in treatment of the indications described herein.

5 Such an article of manufacture can include, for example, at least one container including at least one of the above-described polypeptides and ingredients (e.g., cannabinoids, ketamine, cytokine, etc.) packed in another container. The article of manufacture may also include appropriate buffers and preservatives for improving the shelf-life of the article of manufacture.

10 As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

 Herein the term "active ingredient" refers to the polypeptide or therapeutic agent accountable for the biological effect.

15 Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

20 Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

25 Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

30 Suitable routes of administration may, for example, include oral, sublingual, topical, intra-dermal, rectal, transmucosal (including eye drops), especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous, transdermal (by pressure) and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, intrapulmonary or intraocular injections.

 According to specific embodiments, the active ingredients are provided in a systemic manner.

According to specific embodiments, the route of administration is intranasal or intrapulmonary administration.

According to specific embodiments, the polypeptide is formulated for nasal administration as described in WO16/073,199, the contents of which are fully incorporated
5 herein by reference.

According to other specific embodiments, the route of administration is into the skin. Methods of administering an active agent into a skin are known in the art and include, for example, intradermal injections, gels, liquid sprays, devices and patches which comprise the active agent and which are applied on the outer surface of the skin.

10 According to some embodiments of the invention, administration of the active agent into the skin of the subject is performed topically (on the skin).

According to some embodiments of the invention, administration of the active agent into the skin of the subject is performed non-invasively, e.g., using a gel, a liquid spray or a patch (e.g. reservoir type patch and matrix type patch), or device comprising the active ingredient,
15 which are applied onto the skin of the subject.

It should be noted that in order to increase delivery of the active agent into the skin, the active agent can be formulated with various vehicles designed to increase delivery to the epidermis or the dermis layers. Such vehicles include, but are not limited to liposomes, dendrimers, noisome, transfersome, microemulsion and solid lipid nanoparticles.

20 According to some embodiments of the invention, administering is performed by an intradermal injection.

Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intrahippocampal (IH), intracranial (IC), intracerebral injection, intracerebroventricular injection (ICV) or infusion or intrathecal administration); molecular
25 manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol
30 carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size

limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer, or saline or slow-release solutions. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired,

disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

According to specific embodiments, the pharmaceutical composition is formulated for oral administration.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

According to specific embodiments, the pharmaceutical composition is formulated for inhalation (e.g. intranasal or intrapulmonary).

For administration by inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Pharmaceutical composition suitable for the use of some embodiments of the invention for intranasal administration are conveniently delivered as detailed in US Patent No. 10,912,819, I. Gozes, Inventor, the contents of which are fully incorporated herein in their entirety.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Slow release formulations may also be used in preparation of the pharmaceutical composition for parenteral administration.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of some embodiments of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

The pharmaceutical composition of some embodiments of the invention may also be formulated for sustained-release to provide elevated serum half-life. Such sustained release systems are well known to those of skill in the art and include e.g. microcapsules and nanoparticles. According to specific embodiments, the ProLease biodegradable microsphere delivery system for proteins and peptides (Tracy, 1998, Biotechnol. Prog. 14, 108; Johnson et al., 1996, Nature Med. 2, 795; Herbert et al., 1998, Pharmaceut. Res. 15, 357) a dry powder composed of biodegradable polymeric microspheres containing the protein in a polymer matrix that can be compounded as a dry formulation with or without other agents.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of

a disorder (e.g., ARDS, infectious diseases e.g. Corona virus infection) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

For complete toxicity assessment of the ADNP polypeptide of SEQ ID NO: 2 see Gozes I. Front Neurol. 2020 Nov 24; 11: 608444, the contents of which are fully incorporated herein by reference.

Dosage amount and interval may be adjusted individually to provide that the levels of the active ingredient are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

The doses determined in a mouse model can be converted for the treatment other species such as human and other animals diagnosed with the disease. Conversion Table approved by the FDA is shown in Reagan-Shaw S., et al., FASEB J. 22:659-661 (2007).

The human equivalent dose is calculated as follows: $HED \text{ (mg/kg)} = \text{Animal dose (mg/kg)} \times (\text{Animal } K_m / \text{human } K_m)$.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved. It will be appreciated that treatment will typically be an extended, and in most cases chronic course of

treatment, as the target patient population comprises genetically impaired individuals, whose neurodegenerative conditions require ongoing attention.

According to specific embodiments, the polypeptide is administered once or twice a day.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

According to some embodiments of the invention, the polypeptide is provided at an amount ranging from 0.0001 mg/kg to 1,000 mg/kg including any intermediate subranges and values therebetween, e.g. 0.001 mg/kg, 0.1 mg/kg, 1 mg/kg, 5 mg/kg, 15 mg/kg, 50 mg/kg or 500 mg/kg per dose. According to specific embodiments, the polypeptide is provided in an amount ranging from 0.05 – 0.1 mg/kg e.g. 0.08 mg/kg. In more specific embodiments, the polypeptide is provided in an amount ranging from 0.01 mg/kg to 2mg/kg body weight, including any intermediate subranges and values therebetween, e.g. 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 mg/70 kg subject. In yet further embodiments, the polypeptide is provided in an amount ranging from 1 to 40 mg/70 kg subject, in particular 5, 15 or 30 mg/70 kg subject.

According to specific embodiments, the polypeptide is provided in an amount ranging from 0.1 – 1 mg/kg e.g. 0.4 mg/kg given e.g. subcutaneously.

According to specific embodiments, the polypeptide is provided in an amount ranging from 0.05 – 0.5 mg/kg e.g. 0.2 mg/kg (15 mg to a 70 kg subject) or 0.07 mg/kg (5 mg to a 70 kg subject) e.g. intranasally.

Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in

an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

As used herein the term “about” refers to $\pm 10\%$.

The terms “comprises”, “comprising”, “includes”, “including”, “having” and their
5 conjugates mean “including but not limited to”.

The term “consisting of” means “including and limited to”.

The term “consisting essentially of” means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed
10 composition, method or structure.

As used herein, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a compound” or “at least one compound” may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in
15 a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically
20 disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first
25 indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term “method” refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques
30 and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

When reference is made to particular sequence listings, such reference is to be understood to also encompass sequences that substantially correspond to its complementary sequence as including minor sequence variations, resulting from, e.g., sequencing errors, cloning errors, or other alterations resulting in base substitution, base deletion or base addition, provided that the frequency of such variations is less than 1 in 50 nucleotides, alternatively, less than 1 in 100 nucleotides, alternatively, less than 1 in 200 nucleotides, alternatively, less than 1 in 500 nucleotides, alternatively, less than 1 in 1000 nucleotides, alternatively, less than 1 in 5,000 nucleotides, alternatively, less than 1 in 10,000 nucleotides.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non-limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome

Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. L., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

MATERIALS AND METHODS

Cell cultures - Human neuroblastoma SH-SY5Y cells differentiated to neuron-like cells (10 μ M retinoic acid)¹⁵, human embryonic kidney 293 (HEK293) cell line and induced pluripotent stem cells (iPSCs)³⁷ that were differentiated to neural cells were subjected to immunohistochemistry and/or coimmunoprecipitation.

Docking SIRT1 through its SXIP motif to the EB1 homodimer –

Available structures:

1. SIRT1: PDB ID 4IG9³⁸ X-ray structure of the open state of SIRT1. Chain A was used.
2. EB1 homodimer: created using 3TQ7³⁹ and 3GJO⁴⁰ disclosed in ¹².

Docking methods:

1. Patchdock⁴¹ was used to dock SIRT1 to EB1 homodimer using the known binding sites (residues 452-456 in SIRT1 and 219, 220 and 223 in EB1).
2. Firedock^{42,43} was used to refine the Patchdock results, using the full refinement protocol.
3. Rosetta Dock⁴⁴ was used to further refine and score the best 7 docking poses from Firedock,

Co-immunoprecipitation assay - Proteins were extracted from differentiated human neuroblastoma SH-SY5Y cells using lysis buffer (Pierce, Rockford, IL) and subjected to Co-IP analysis according to the manufacture protocol, as previously described^{13, 25}. Briefly, 10µg of antibodies of interest (EB1, ab53358 EB3, ab99287 Abcam, Cambridge, UK) were cross-linked to the 30µl of A/G PLUS-agarose beads (provided by the Co-IP kit). 1 mg of protein extracts were incubated overnight on end-over-end shaker (ROTAMIX RM1, Riga, Latvia), 4 °C. To enhance interaction with EB1 proteins, 3mg NAP (SEQ ID NO: 2) was added as described in^{13,25}. The Immunoprecipitated materials were subsequently investigated by western blot analysis using primary antibodies as follows: mouse monoclonal SIRT1 antibody (1:1000, ab110304, Cambridge, UK), mouse monoclonal ADNP F9 (1:200, SC-376674, Santa Cruz, CA), rat monoclonal EB1 (1:500), goat polyclonal EB3 (1:2500) and rabbit monoclonal Tau (1:2500, ab32057, Abcam). Next, proteins were visualized SuperSignal Chemiluminescent Substrates (Thermo Scientific, Rockford, IL), using horseradish peroxidase-conjugated goat anti mouse secondary antibody (1:5000, Jackson, Hamburg, Germany), goat anti rat (1:5000, Jackson) donkey anti goat (1:5000, Jackson), and goat anti rabbit (1:5000, Abcam).

RNA-sequencing single cell analysis - The NCBI website was examined for datasets derived from human Single cell transcriptomes of developmental human brain.

1) Single cell RNA sequencing data from human cortex specimen (48 samples from postconceptional weeks 5.85 to 37, PRJNA295469⁴⁵) was analyzed using the UCSC Cell Browser ([www\(dot\)cells\(dot\)ucsc\(dot\)edu/?ds=cortex-dev](http://www(dot)cells(dot)ucsc(dot)edu/?ds=cortex-dev)). 2D plots of single cells were visualized by the t-SNE algorithm, and *ADNP* and *SIRT1* expressing cells were marked in black circles.

2) Single cell RNA sequencing data (GSE36552⁴⁶) from 124 cells from human embryonic stem cells and human preimplantation embryos. Single cell expression levels of *ADNP*, *SIRT1*, *MAPRE1* and *MAPRE3* were analyzed with Single Cell Expression Atlas⁴⁷, t-SNE plots were generated with perplexity=50.

Tissue RNA expression analysis - *ADNP*, *SIRT1*, *MAPRE1*, and *MAPRE3* total bulk RNA expression levels in human tissues were analyzed using the Genotype-Tissue Expression

(GTEx)⁴⁸ data. The data expression levels were visualized with t-SNE using the UCSC Cell Browser ([www\(dot\)cells\(dot\)ucsc\(dot\)edu/?ds=gtx8](http://www.cellsc.ucsc.edu/?ds=gtx8)).

Animals - All procedures involving animals have been approved by the Animal Care and Use Committee of Tel Aviv University and the Israeli Ministry of Health. The *Adnp*^{+/-} mice, on a mixed C57BL and 129/Sv background, a valid model for the ADNP syndrome^{11,12}, were previously described^{1,16,49}. For continuous breeding, an ICR outbred mouse line was used^{12,49}. Animals were housed in a 12-h light/12-h dark cycle with free access to rodent chow and water. Genotyping was performed by Transnetyx (Memphis, TN).

Histone modification screening - Histone proteins were extracted from the hippocampi of 3 and 5-month-old male and female *Adnp*^{1/-} and *Adnp*^{+/+} mice with Histone Extraction Kit (ab113476, Abcam) according to manufacture protocol. Histone proteins were measured with Bradford Protein Assay (Cat # 500-0006, BIO-RAD). Histone H3 Modification Multiplex Assay Kit (ab185910, Abcam) was used to screening and quantify Histone H3 modification in *Adnp* mice. 500 ng of total histone proteins were used per assay according to the protocol booklet. The absorbance was measured with a microplate reader at 450 nm wavelength with a reference wavelength of 655 nm.

Motif analysis - Factorbook⁵⁰ database was used for the analysis binding sites from ENCODE chip-seq datasets⁵¹. ADNP (eGFP-ADNP tagged K562 cell line, ENCSR440VKE), HDAC2 (ENCSR000AQQ), SMARCA4 (ENCSR000EHO) AND YY1 (ENCSR000BKU) in K562 cells. Chip-seq data binding peaks were analyzed to generate enrichment of motif sequences. Average histone modification profiles were generated for ADNP chip-seq binding site using Factorbook⁵⁰ database. The histone peaks are separated for peaks that proximal (<1 kb) to the transcription start site (TSS) and distal to TSS, each is 2 kb around the position with most sequence reads⁵⁰.

Chip-seq data mining - Cistrome DB⁵² was used for Chip-seq data mining and visualized with WashU Epigenome Browser³². Chip-seq binding peaks of H3K79me2 (GSM733653), ADNP (eGFP-ADNP tagged K562 cell line, GSE105573), HDAC2 (GSM1003447), YY1(GSM803470, GSM803446), and SMARCA4 (GSE91946) from Erythroblast (cell line K562) was downloaded and further visualized with WashU Epigenome Browser.

Correlation matrix analysis - Human tissue RNA expression levels were extracted from RNA-seq dataset (BioProject: PRJEB4337) from 27 normal tissue samples from 95 human individuals⁵³. The NCBI Gene Expression Omnibus (GEO) GEO website was screened for datasets derived from human AD postmortem brain tissues or blood from live patients and

controls. AD postmortem brain data set (GSE5281)⁵³ from entorhinal cortex, hippocampus, medial temporal gyrus, posterior cingulate, superior frontal gyrus and primary visual cortex including samples from 74 controls and 87 patients. The second cohort analyzed was a blood dataset (GSE63060)⁵⁴ including samples from 105 controls and 146 AD patients. Additionally, RNA-seq data from the BrainSpan atlas⁵¹ of postmortem brain structures across human brain development was assessed.

The RNA-seq dataset (GSE72664)¹² of *Adnp*^{+/-} and *Adnp*^{+/+} mice, mice hippocampus (N=23), was used for Pearson correlations of RNA expression levels of *Sirt1* and *Adnp*. The expression levels were downloaded, and Pearson correlation coefficient was calculated using R software. The correlation matrix was generated with the Corrplot package; the stars represent the significance level *** P<0.001, ** P<0 .01, * P<0 .05.

Additional details can be found in Hadar A, Kapitansky O, et al. Mol Psychiatry. 2021 Nov;26(11):6550-6561, the contents of which are incorporated herein in their entirety by reference.

EXAMPLE 1

ADNP and SIRT1 share microtubule end binding proteins (EB1) motif and interact with EB1/EB3 at the single cell and biochemical level

STRING protein interaction analysis ([www\(string-db\(dot\)org/\)](http://www.string-db.org/)) showed the complexity of ADNP interactions and SIRT1 network proteins (data not shown). However, this current knowledge on potential ADNP-SIRT1 interactions does not imply of any major crossroads. Regardless, TP53 appeared in the SIRT1 network and as was shown before, TP53 is regulated by ADNP⁴, suggesting common pathways.

Therefore, the present inventors sought out to discover whether there are potential physical interaction points between ADNP and SIRT1, perhaps through other protein mediators. For this purpose, the Eukaryotic Linear Motif (ELM) prediction analysis⁵⁵ was used (data not shown), revealing multiple binding motifs of the WD repeat-containing protein 5 (WDR5) that mediates the assembly of histone modification complexes (Figure 1A). As a scaffold protein, WDR5 contributes to histone modification. By recruitment of the core histone methylation and acetylation complexes, WDR5 plays a key role in H3K4 methylation/demethylation⁵⁶ and in H4 acetylation on several lysine residues⁵⁷.

Following, the present inventors showed LC3 interacting sites on both ADNP as SIRT1, as well as eukaryotic initiation factor 4E interaction sites⁴⁹ (Figure 1A). Additionally, the SIRT1-

EB binding domain was shown, with SSIP on SIRT1 position 453-456 (Figure 1A), as well as the known ADNP SIP motifs.

In silico docking for the SKIP¹² and for the NAPVSIPQ motifs from ADNP⁵⁸, was previously shown. Looking at SIRT1-EB1 interactions - 88 docking poses were obtained from Patchdock, and refined in Firedock. Then, the 7 best docking poses were further refined using the Rosetta Dock high resolution application. 1000 decoys were calculated for each docking pose. The best pose was selected by the total score of the complex (Figure 1B).

Given the shared interacting proteins for ADNP and SIRT1, the physical interaction was further addressed experimentally, at the cellular level by immunocytochemistry and at the protein biochemical level by co-immunoprecipitation. Specifically, HEK293T cells were immune-stained with antibodies recognizing ADNP or SIRT1, and cell nuclei counter-stained/visualized with DAPI. The nuclear co-localization of the two proteins is represented in yellow and quantified on a graph (Figure 1C). The biological significance of cellular co-localization was extended by staining human iPSC-derived neural cells³⁷ (Figure 1D), revealing nuclear and cytoplasmic co-existence.

Following, co-immunoprecipitation with proteins extracted from the neuronal-differentiated human neuroblastoma SH-SY5Y, in which ADNP is mostly cytoplasmic⁹, was performed. The results (Figure 1E) showed specific co-immunoprecipitation of ADNP and SIRT1 in the presence of either EB1 or EB3 antibodies. Co-immunoprecipitation with EB1 was also performed with extracts from human iPSC-derived neural progenitor cells, in the absence (Figure 1F) and in the presence of the ADNP snippet NAP enhancing ADNP-EB-Tau interaction¹³ (Figure 1G) (with Tau known to directly bind SIRT1²³). The results did not reveal SIRT1 in the EB1 antibody eluted fractions (Figure 1F, upper panels, E1-E3). Furthermore, the loading was saturated and EB1-like immunoreactivity was detected also in the flow through (Figure 1F, FT, lower panel). The FT fraction was then reacted with the immobilized EB1 antibodies including 3mg NAP (SEQ ID NO: 2). Electrophoresis and immunoblotting then identified a faint SIRT1-like band at the expected position for the SIRT1 antibodies and a potential Tau band (Figure 1G, arrow), further strongly identified by Tau antibodies (lower panel).

The co-localization question was further extended to the developing human cortex utilizing bioinformatics tools and publicly available single cell RNA-seq libraries⁴⁵, revealing 16.8% *ADNP* and *SIRT1* co-expressing cells (Figure 2A). Looking at the cellular expression in multiple human tissues, a high similarity in the patterns of *ADNP* and *SIRT1* expression was

discovered (Figure 2B). Interestingly, looking at EB1 and EB3 (mRNA, *MAPRE1* and *MAPRE3*) expression (data not shown), *MAPRE1*, but not *MAPRE3* mimicked the *ADNP/SIRT1* patterns of expression. This was also reproducible in stem cells (Figure 2C) and during development (data not shown).

Additional details can be found in Hadar A, Kapitanisky O, et al. Mol Psychiatry. 2021 Nov;26(11):6550-6561, the contents of which are incorporated herein in their entirety by reference.

EXAMPLE 2

ADNP and SIRT1 are co-regulated at the transcriptional level and both control specific histone H3 modifications

The SWI/SNF complex including ADNP⁵ constitutes a major part of the chromatin remodeling complexes³³. SIRT1 is implicated in chromatin remodeling linked to oocyte aging, displaying changes in histone methylation⁵⁹ Histone H3 methylations have also been associated with ADNP, with HP1 (binding to ADNP)² found to recruit ADNP to H3K9me3 marked pericentromeric heterochromatin for silencing of major satellite repeats⁶. Furthermore, loss of ADNP led to increased ratio of H3K4me3/H3K27me3 at key primitive endoderm gene promoters in embryonic stem cells⁸.

Subsequently, a comprehensive approach was used to look at Adnp-regulated histone modification in the 5-month-old female hippocampus, comparing *Adnp*^{+/+} to *Adnp*^{+/-} mice, which show marked dysregulation of gene expression at the RNA-seq level¹². While no *Adnp* gene dosage effect was seen at the level of total histone H3 methylation (Figure 3A), specific methylation sites were affected. For example, H3K79me2 (dotted red line box) was identified as markedly reduced due to Adnp deficiency. To substantiate these results, the present inventors further looked at Chip-seq binding peaks on the promoter/control regions (data not shown) of ADNP, SIRT1 and MAPRE1 (co-localized in the same cells, Figures 1C-G and 2A-C) of H3K79me2 (GSM733653), ADNP (GSE105573), regulating its own expression^{2,60}, HDAC2 (GSM1003447) and YY1 (GSM803470, GSM803446) interacting with SIRT1^{61,62}, and the ADNP-interacting⁵ BRG1 (SMARCA4) (GSE91946) in the erythroblast cell line K562 (with BRG1, ADNP⁶³ as well as SIRT⁶¹ regulating erythrocyte maturation, and globin expression). WashU Epigenome Browser view identified adjoining histone modification peaks of the major ADNP-related modification, most notably in activating and transcriptional histone marks (Figure 3B box). Figure 3B further shows highly reproducible promoter engagement of all the tested

proteins, suggesting co-interaction and co-regulation at the transcriptional level. Mechanistically, exploring the promoter regions identified shared motifs among ADNP, YY1, BRG1 (SMARCA4) and HDAC2, with HDAC2 showing the highest similarity to ADNP (Figure 3C). In a further mechanistic exploration, key histone-interacting proteins were selected suggesting a novel transcriptional regulating/chromatin modifying complex (Figure 4A). Additional studies also analyzed the sex and age-dependent histone H3 methylation, showing different and potential hotspot patterns of methylation, suggesting a complex regulation (data not shown).

Additional details can be found in Hadar A, Kapitanisky O, et al. Mol Psychiatry. 2021 Nov;26(11):6550-6561, the contents of which are incorporated herein in their entirety by reference.

EXAMPLE 3

Correlation of expression and developmental co-regulation, showing a marked dysregulation in Alzheimer's disease postmortem brains

As co-localization and interaction of ADNP, SIRT1 and MAPRE1 (EB1) was shown (Figures 1A-G and 2A-C), it was of further interest to investigate expression correlations of *ADNP* and *SIRT1*, as well as related gene products. Figure 4B shows a correlation matrix plot which depicts RNA-seq expression levels of 27 normal tissues from 95 human individuals (data from BioProject: PRJEB4337⁵³), indicating a very high correlation of *SIRT1* and *ADNP*. Figure 4C extends this analysis to selected RNA species coding for the ADNP-SIRT1 interacting proteins in the normal brain. High correlations were indicated, except for *HAT1*, *RBBP7*, *HDAC1* and *MAPRE3*, with most of the transcripts except *MAPRE3* and *NMNAT1* showing positive correlations.

To corroborate the correlation results, two additional experimental paradigms were employed: 1) assessing *Adnp*^{+/-} vs. *Adnp*^{+/+} mice¹¹ and 2) using additional publicly available human datasets. The mouse results are depicted in Figure 4B (insert, RNA-seq). Further analysis of males and females separately, revealed extensive correlation in five-months-old females (data not shown, $r=1$, $p=0.005$) and not in males or one-month-old mice. Corroboration by qRT-PCR, with double the number of mice at one month of age indicated correlation in males (data not shown $r=0.669$, $p=0.0343$), implicating a possible age and sex-dependency.

For the human, assorted AD and ASD blood samples showed minor differences between AD and ASD data and respective control data (data not shown). Lastly, a public dataset GSE5281⁶⁴ was assessed and a correlation matrix plot of postmortem brain gene expression

levels of grouped AD patients and controls was depicted (Figure 5). Positive correlations are shown in a blue scale and negative correlations are shown in a red scale. A dramatic difference was observed, with complete loss of correlation in the AD brain (Figure 5, comparing different brain areas identified highly significant difference in the primary visual cortex and superior frontal gyrus, associated with visuospatial cognition⁶⁵). In Parkinson's disease brains compared to controls, the correlation was maintained except for the most affected substantia nigra (data not shown).

Figure 6 summarizes the findings presented in Examples 1-3 hereinabove, modelling multiple non-limiting SIRT1-ADNP interactions at the chromatin and the cytoplasmic level.

Additional details can be found in Hadar A, Kapitansky O, et al. Mol Psychiatry. 2021 Nov;26(11):6550-6561, the contents of which are incorporated herein in their entirety by reference.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

It is the intent of the applicant(s) that all publications, patents and patent applications referred to in this specification are to be incorporated in their entirety by reference into the specification, as if each individual publication, patent or patent application was specifically and individually noted when referenced that it is to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting. In addition, any priority document(s) of this application is/are hereby incorporated herein by reference in its/their entirety.

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WHAT IS CLAIMED IS:

1. An article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and a SIRT1 activator.
2. The article of manufacture of claim 1, wherein said polypeptide and said SIRT1 activator are provided in a co-formulation.
3. The article of manufacture of claims 1, wherein said polypeptide and said SIRT1 activator are provided in separate formulations.
4. A method of treating a disease selected from the group consisting of ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a SIRT1 activator, thereby treating the disease in the subject.
5. The article of manufacture of any one of claims 1-3 or the method of claim 4, wherein said SIRT1 activator is a small molecule.
6. The article of manufacture or the method of claim 5, wherein said SIRT1 activator is selected from the group consisting of Resveratrol, Quercetin, Butein, Beberine, Curcumin, Fisetin, Honokiol, YK 3-237, SRT1720, SRT1460, SRT2183, STAC-5, STAC-9, STAC-10, BML-278 and Piceatannol, or an analog or derivative thereof.
7. The article of manufacture or the method of claim 5, wherein said SIRT1 activator is Resveratrol or an analog or derivative thereof.

8. The article of manufacture of any one of claims 1-3 or the method of claim 4, wherein said SIRT1 activator is NAD⁺ or an analog or derivative thereof, or nicotinamide riboside (NR).

9. An article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and an anti-aging agent, wherein said anti-aging agent is not an anti-oxidant.

10. The article of manufacture of claim 9, wherein said polypeptide and said agent are provided in a co-formulation.

11. The article of manufacture of claims 9, wherein said polypeptide and said agent are provided in separate formulations.

12. A method of treating a disease selected from the group consisting of ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an anti-aging agent, thereby treating the disease in the subject.

13. The method of claim 12, wherein said anti-aging agent is not an anti-oxidant.

14. The article of manufacture of any one of claims 9-11 or the method of any one of claims 12-13, wherein said anti-aging agent is a SIRT1 activator.

15. The article of manufacture of any one of claims 9-11 or the method of any one of claims 12-13, wherein said anti-aging agent is selected from the group consisting of rapamycin,

metformin, melatonin, carnosine, nicotinamide mononucleotide, delta-sleep-inducing-peptide and small molecule Klotho enhancer, or an analog or derivative thereof.

16. The article of manufacture of any one of claims 9-11 or the method of any one of claims 12-13, wherein said anti-aging agent comprises a calorie restriction diet.

17. An article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and an immune-modulator agent selected from the group consisting of chemokine receptor modulator, immune-check point modulator and a cytokine, wherein said cytokine is not IFN β .

18. The article of manufacture of claim 17, wherein said polypeptide and said agent are provided in a co-formulation.

19. The article of manufacture of claims 17, wherein said polypeptide and said agent are provided in separate formulations.

20. A method of treating a disease selected from the group consisting of ADNP syndrome, Dravet syndrome, fragile X syndrome, SYNGAP1-related intellectual disability, Phelan McDermid syndrome, GRIN disorder, CHD8-related disorder, DYRK1A syndrome, POGZ syndrome, FOXP1 syndrome, SLC5A1-related disorder, Coffin-Siris syndrome, ARID1B-related syndrome, KMT5B syndrome, PTEN autism syndrome, Rett syndrome, Okihiro syndrome plus developmental delay, Angelman syndrome, Noonan syndrome, Kleefstra syndrome, and Smith-Magenis syndrome in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an immune-modulator agent selected from the group consisting of chemokine receptor modulator, immune-check point modulator and a cytokine, thereby treating the disease in the subject.

21. The method of claim 20, wherein said cytokine is not IFN β .

22. The article of manufacture of any one of claims 17-19 or the method of any one of claims 20-21, wherein said chemokine receptor is selected from CCR5 and CXCR4.

23. The article of manufacture or the method of any one of claims 17-22, wherein said modulator is an inhibitor.

24. The article of manufacture or the method of any one of claims 17-23, wherein said chemokine receptor modulator is selected from the group consisting of maraviroc, leronlimab, aplaviroc, vicriviroc, plerixafor, mavorixafor, BL-8040 and TG-0054, or an analog or derivative thereof.

25. The article of manufacture or the method of any one of claims 17-23, wherein said chemokine receptor modulator is selected from the group consisting of maraviroc and plerixafor, or an analog or derivative thereof.

26. The article of manufacture or the method of any one of claims 17-25, wherein said cytokine is selected from the group consisting of IL-6, IL-10 and TNF α .

27. An article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and bumetanide or an analog or derivative thereof.

28. The article of manufacture of claim 27, wherein said polypeptide and said bumetanide or an analog or derivative thereof are provided in a co-formulation.

29. The article of manufacture of claims 27, wherein said polypeptide and said bumetanide or an analog or derivative thereof are provided in separate formulations.

30. An article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and a cannabinoid.

31. The article of manufacture of claim 30, wherein said polypeptide and said cannabinoid are provided in a co-formulation.

32. The article of manufacture of claim 30, wherein said polypeptide and said cannabinoid are provided in separate formulations.

33. The article of manufacture of any one of claims 30-32, wherein said cannabinoid is selected from the group consisting of THC and CBD.

34. An article of manufacture comprising as active ingredients an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay; and ketamine or an analog or derivative thereof.

35. The article of manufacture of claim 34, wherein said polypeptide and ketamine or an analog or derivative are provided in a co-formulation.

36. The article of manufacture of claims 34, wherein said polypeptide and said ketamine or an analog or derivative are provided in separate formulations.

37. The method of any one of claims 4-8, 12-16 and 20-26, further comprising administering to said subject a therapeutically effective amount of an ADNF polypeptide, wherein said ADNF polypeptide has a neurotrophic/neuroprotective activity in an in vitro cortical neuron culture assay.

38. The article of manufacture or the method of any one of claims 1-3, 5-9, 14-19 and 22-37, wherein said ADNF polypeptide is capable of binding EB1 and or EB3.

39. The article of manufacture or the method of any one of claims 1-3, 5-9, 14-19 and 22-38, wherein said ADNF polypeptide is an ADNF III polypeptide.

40. The article of manufacture or the method of claim 39, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-22.

41. The article of manufacture or the method of claim 39, wherein said polypeptide comprises SEQ ID NO: 2.

42. The article of manufacture or the method of claim 39, wherein said polypeptide has the formula $(R^1)_x$ -Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln- $(R^2)_y$ (SEQ ID NO: 49), or an analogue thereof, in which R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and x and y are independently selected and are equal to zero or one.

43. The article of manufacture or the method of any one of claims 1-3, 5-9, 14-19 and 22-38, wherein said ADNF polypeptide is an ADNF I polypeptide.

44. The article of manufacture or the method of claim 43, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24-48.

45. The article of manufacture or the method of claim 43, wherein said polypeptide comprises SEQ ID NO: 24.

46. The article of manufacture or the method of claim 43, wherein said polypeptide has the formula $(R^1)_x$ -Ser-Ala-Leu-Leu-Arg-Ser-Ile-Pro-Ala- $(R^2)_y$ (SEQ ID NO: 50), or an analogue thereof, in which R^1 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; R^2 is an amino acid sequence comprising from 1 to about 40 amino acids wherein each amino acid is independently selected from the group consisting of naturally occurring amino acids and amino acid analogs; and x and y are independently selected and are equal to zero or one.

47. The article of manufacture or the method of any one of claims 1-3, 5-9, 14-19 and 22-46, wherein said polypeptide comprises at least one D-amino acid.

48. The article of manufacture or the method of any one of claims 1-3, 5-9, 14-19 and 22-38 and 22-47, wherein said polypeptide is less than 50 amino acids in length.

49. The article of manufacture or the method of any one of claims 1-3, 5-9, 14-19 and 22-47, wherein said polypeptide is less than 20 amino acids in length.

50. The article of manufacture or the method of any one of claims 1-3, 5-9, 14-19 and 22-49, wherein said polypeptide is attached to a cell penetrating or stabilizing moiety.

51. The method of any one of claims 4-8, 12-16 and 20-26 and 37, wherein said subject is a female.

52. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said subject is a male.

53. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said subject is under 18 years old.

54. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said subject is over 60 years old.

55. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said disease is associated with aging.

56. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said disease is an inflammatory disease.

57. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said disease is a neurodegenerative disease or cognitive deficit.

58. The method of claim 57, wherein said disease is mild cognitive impairment, Parkinson's disease, amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy (PSP) and Alzheimer's disease.

59. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said disease is an autistic spectrum disorder and/or intellectual disability.

60. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said disease is an ADNP syndrome.

61. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said disease is selected from the group consisting of stress, anxiety, bi-polar disease, schizophrenia and aggression.

62. The method of any one of claims 4-8, 12-16, 20-26 and 37, wherein said disease is selected from the group consisting of high blood pressure, swelling, congestive heart failure, hepatic disease and renal disease.

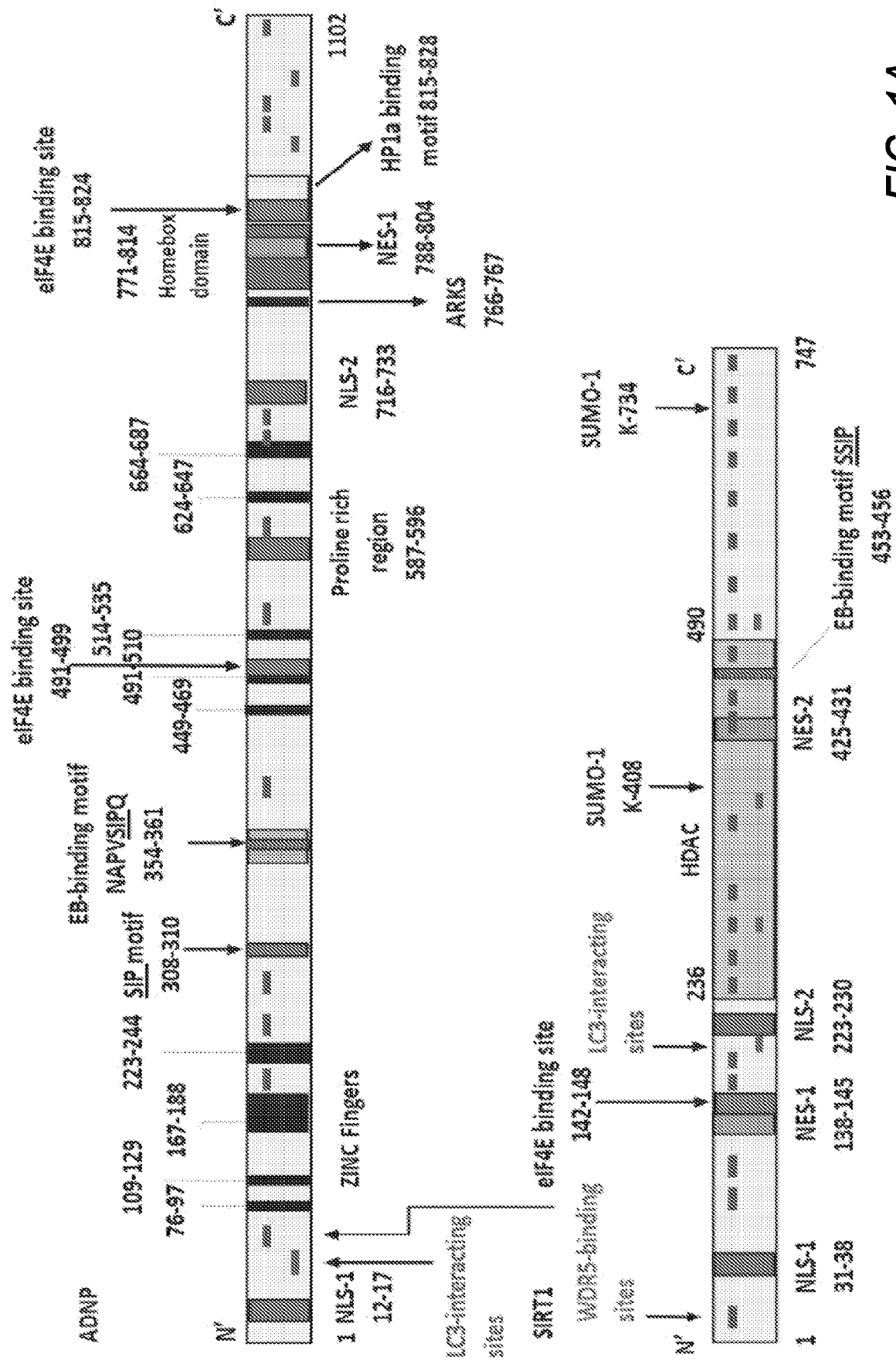


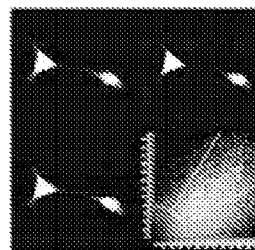
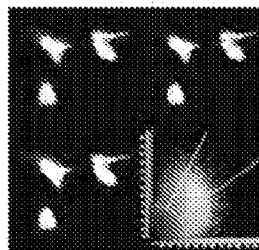
FIG. 1A

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FIG. 1B

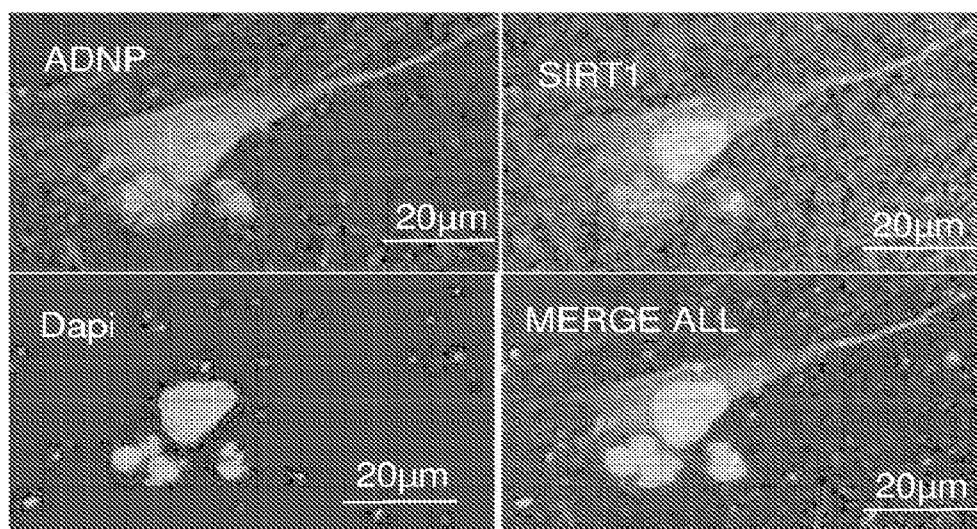
Colocalization



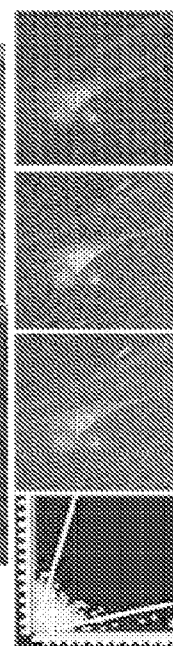
HEK293T

FIG. 1C

FIG. 1D



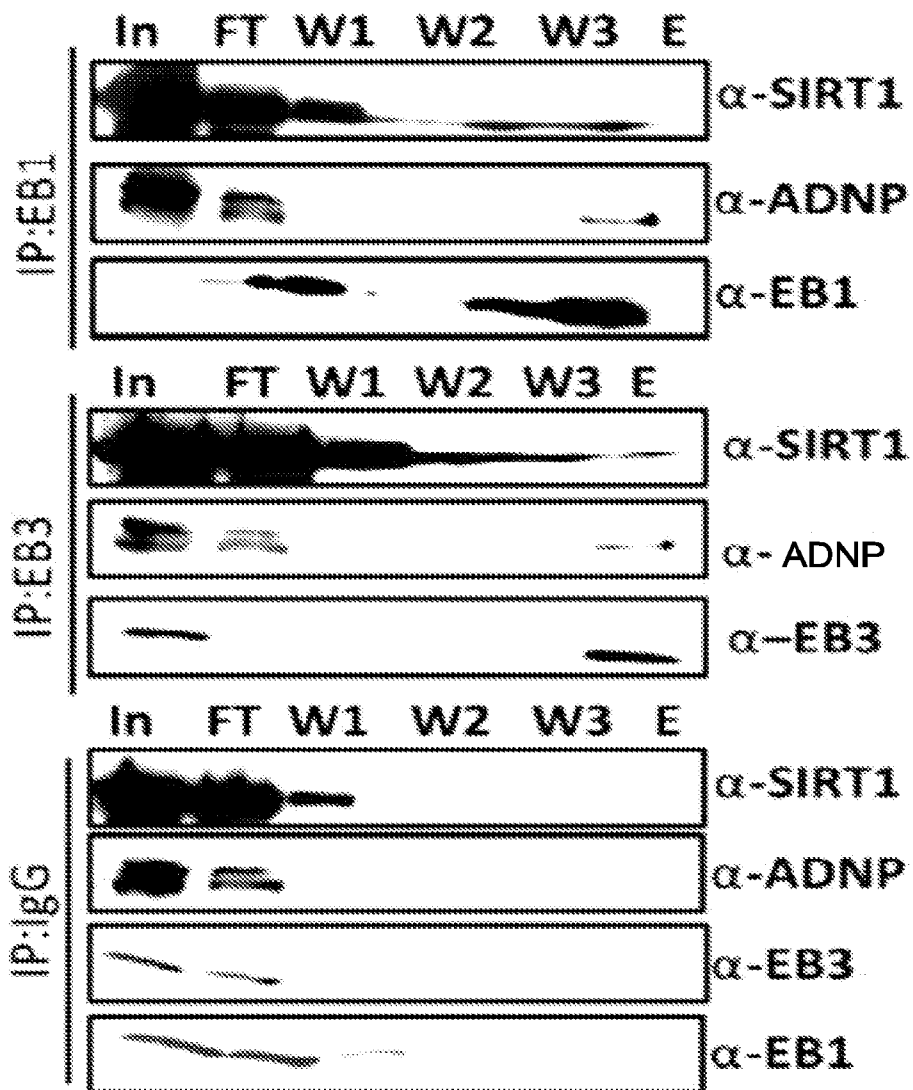
Colocalization



Human iPSC-derived neural cells

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SH-SY5Y

**FIG. 1E**

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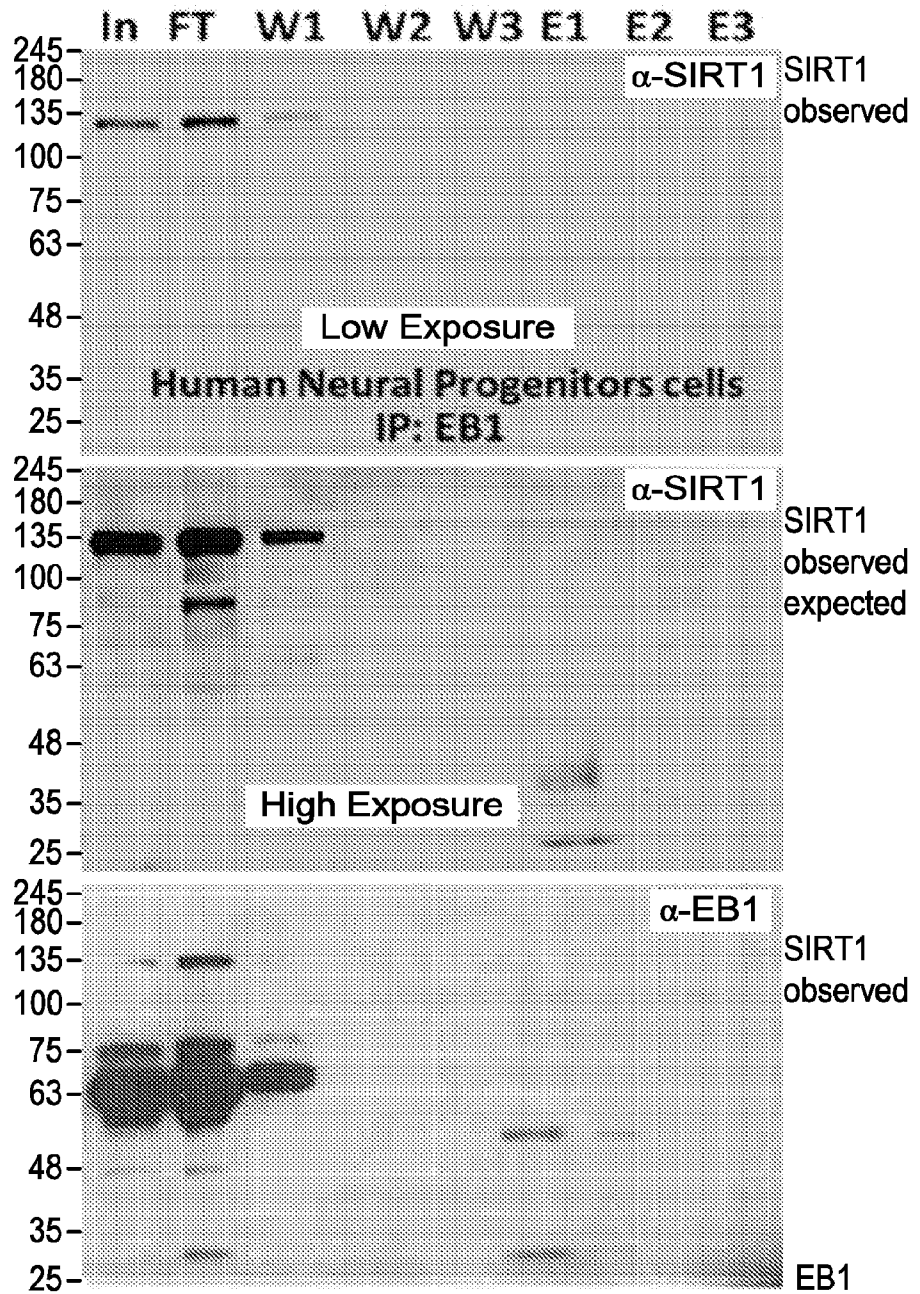


FIG. 1F

FIG. 1G

+ NAP 16h



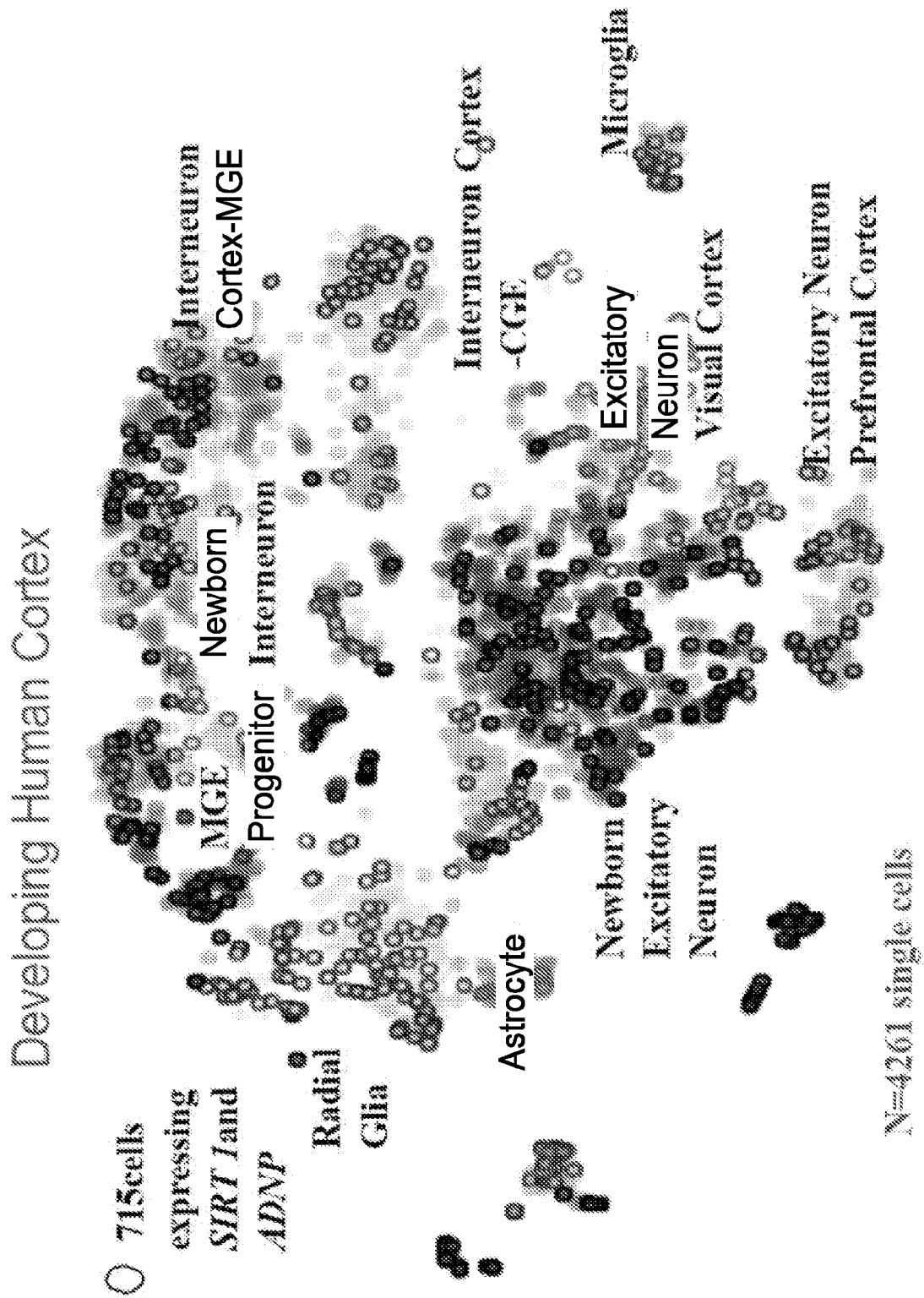


FIG. 2A

ADNP

All Tissues

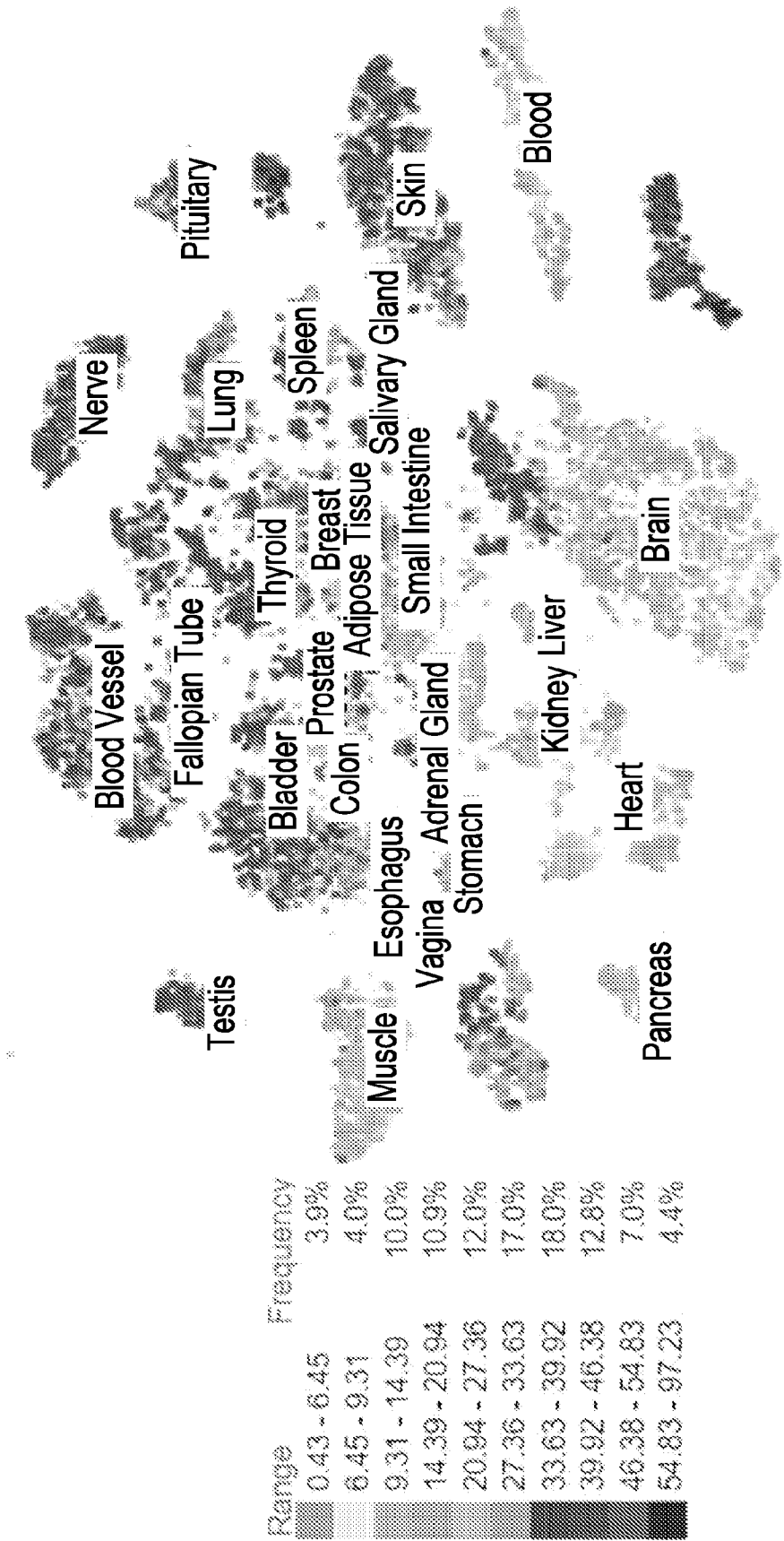


FIG. 2B

SIRT1

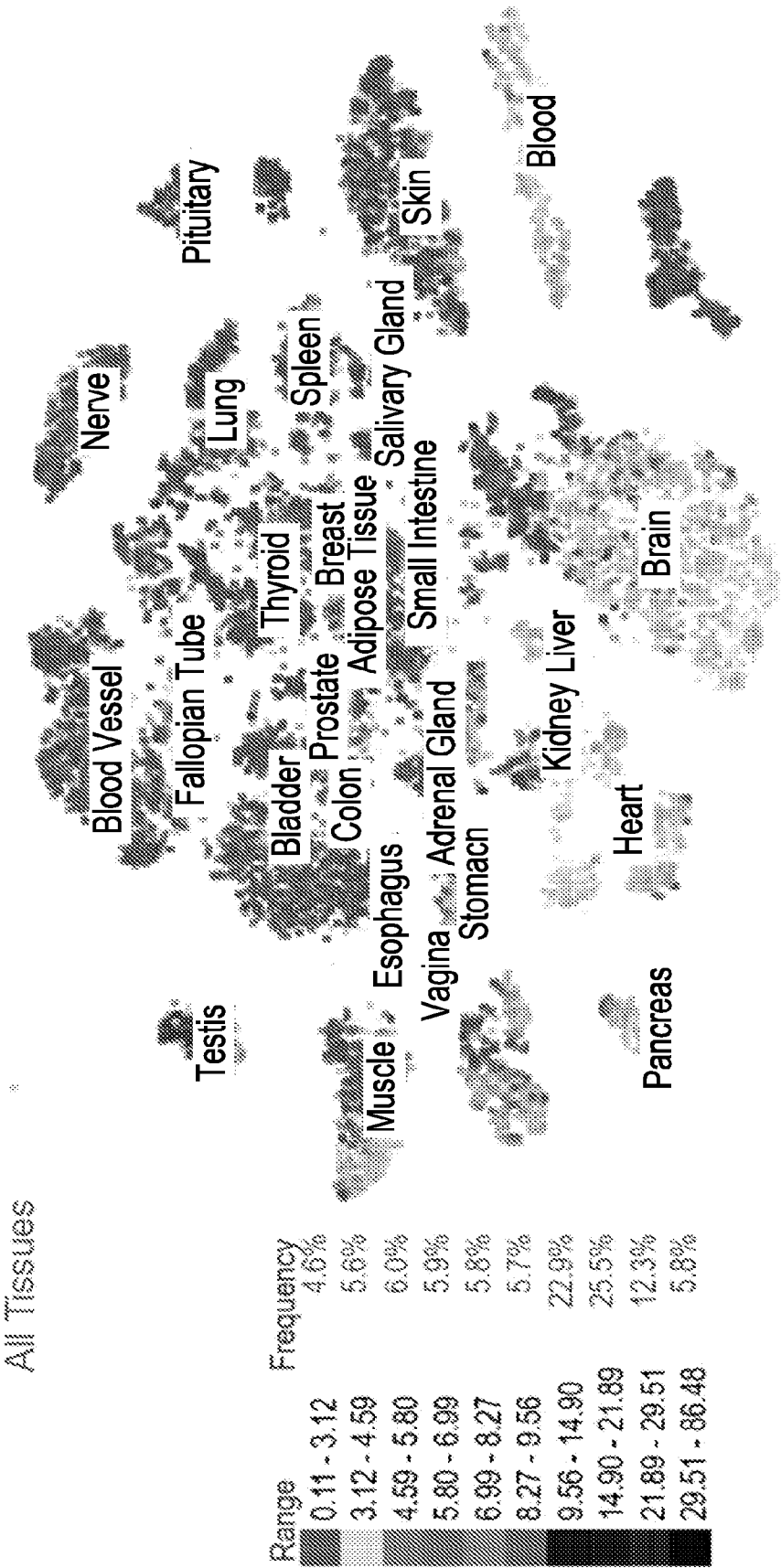


FIG. 2B (continued)

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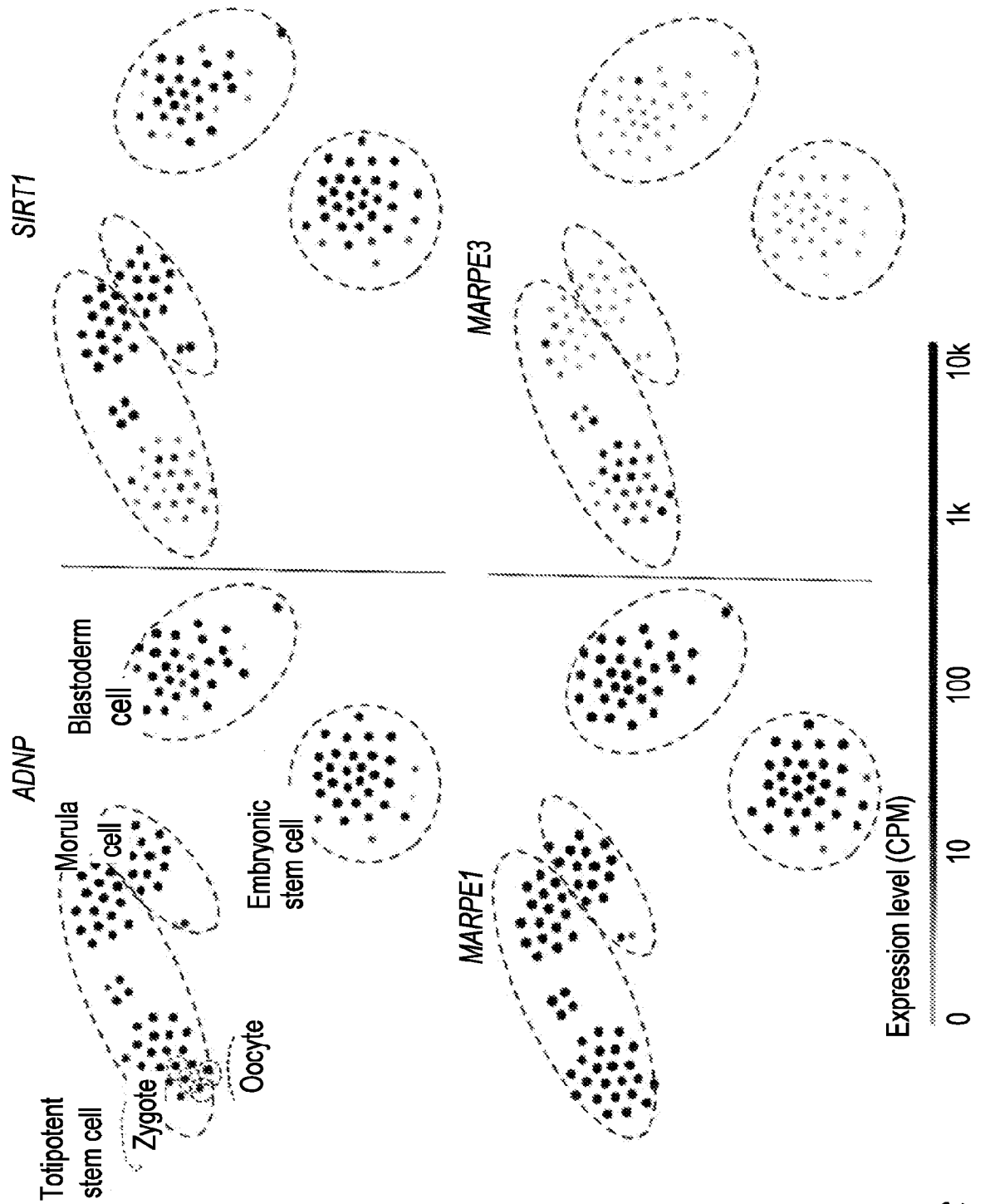


FIG. 2C

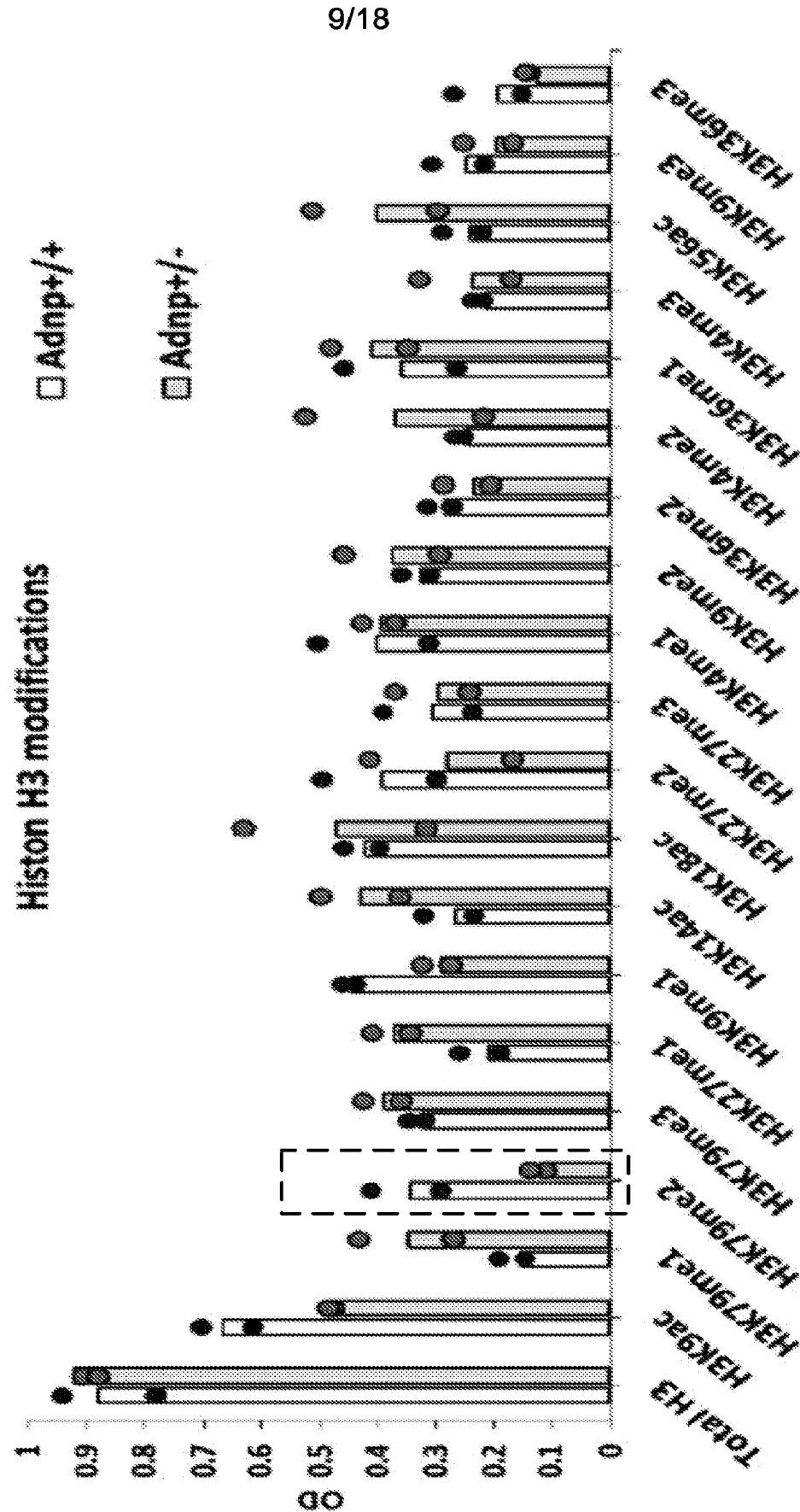


FIG. 3A

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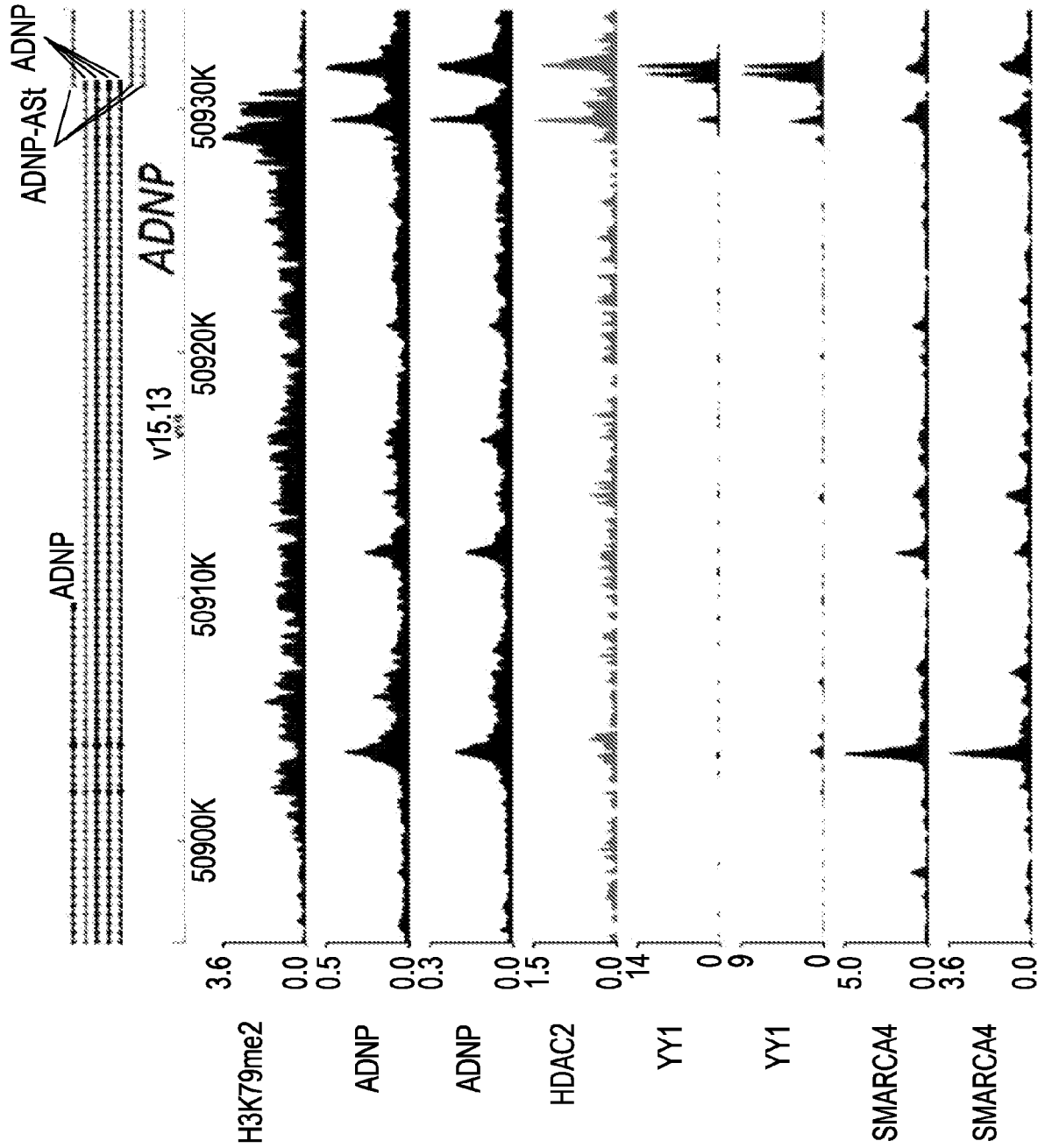


FIG. 3B

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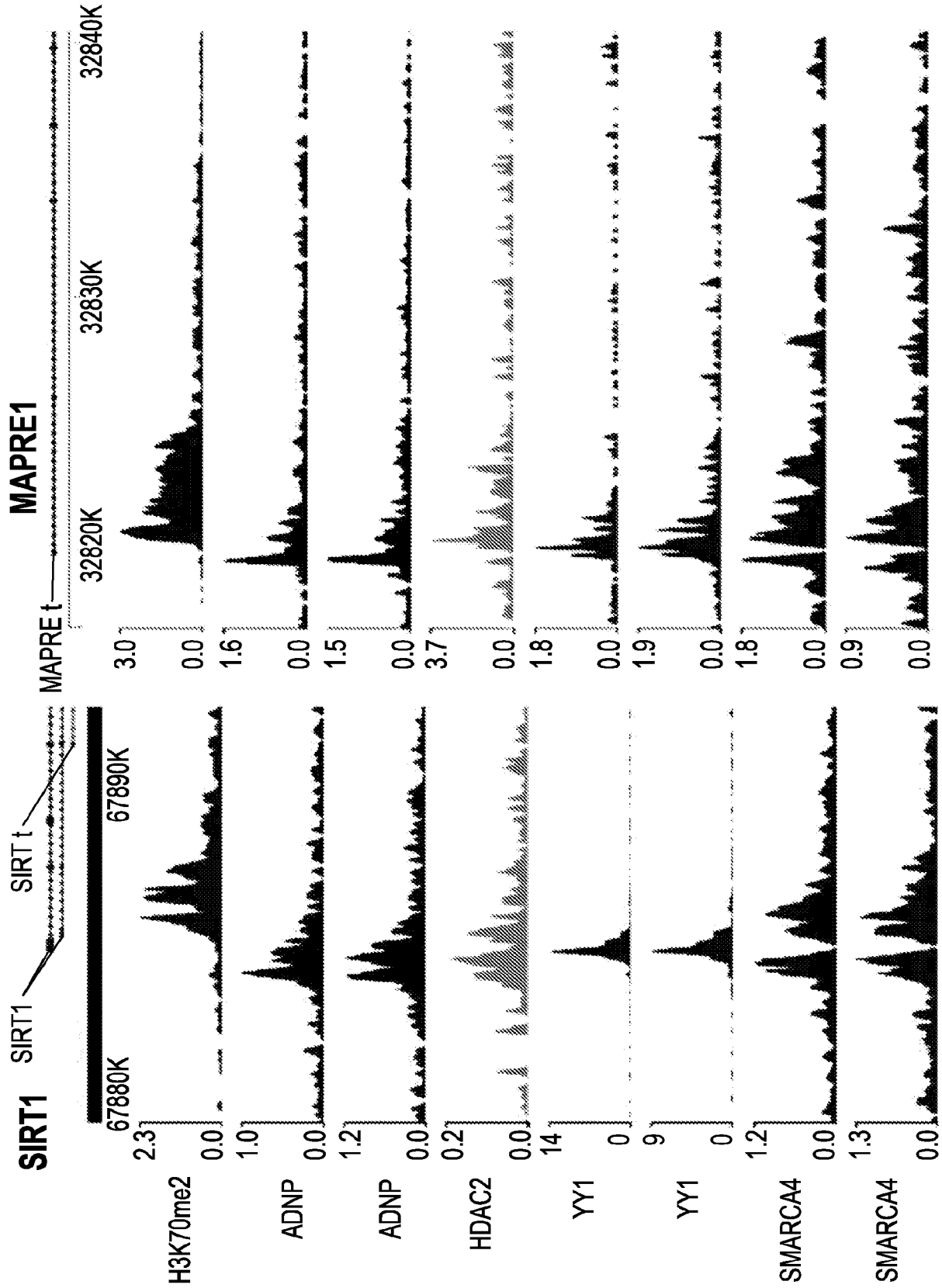


FIG. 3B (continued)

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Histone modification adjoining ADNP peaks

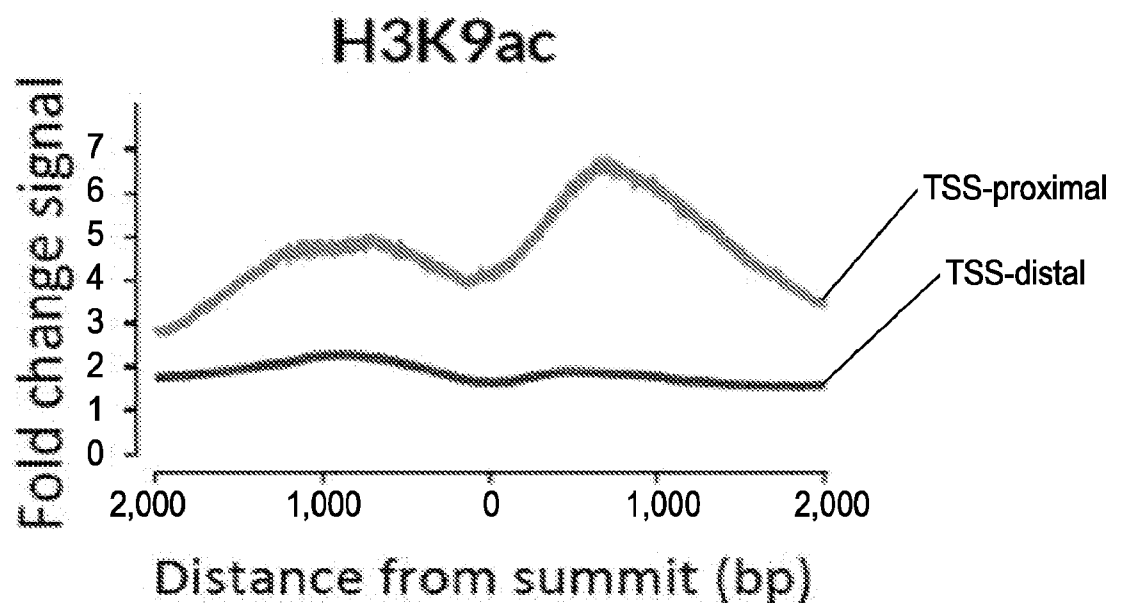
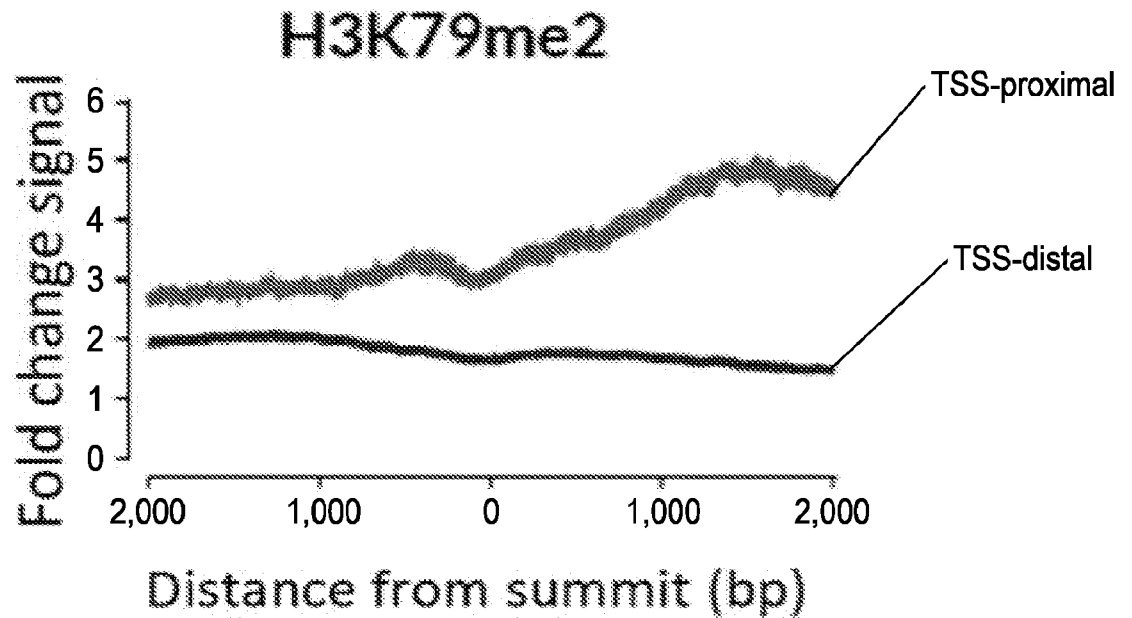


FIG. 3B (continued)

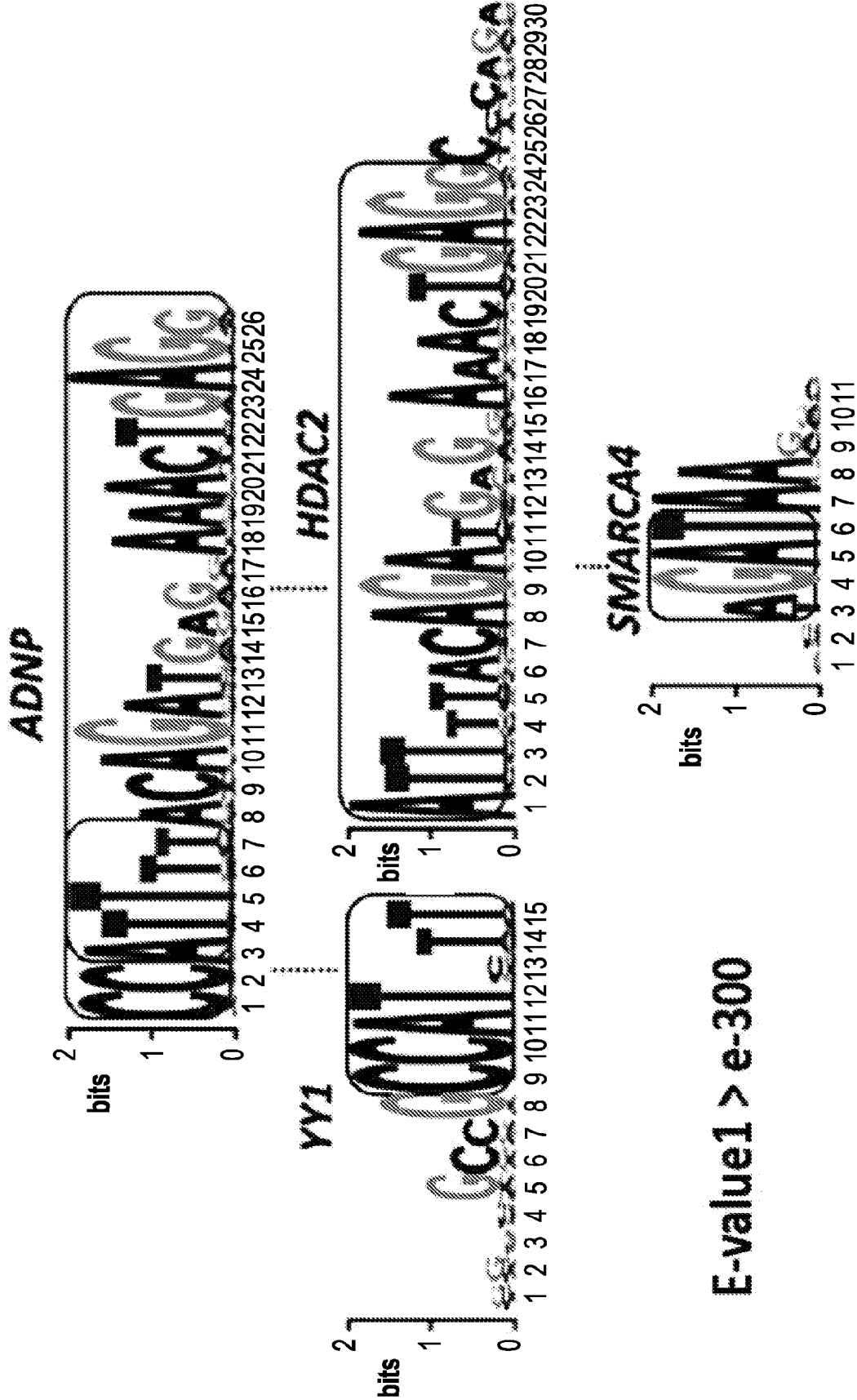
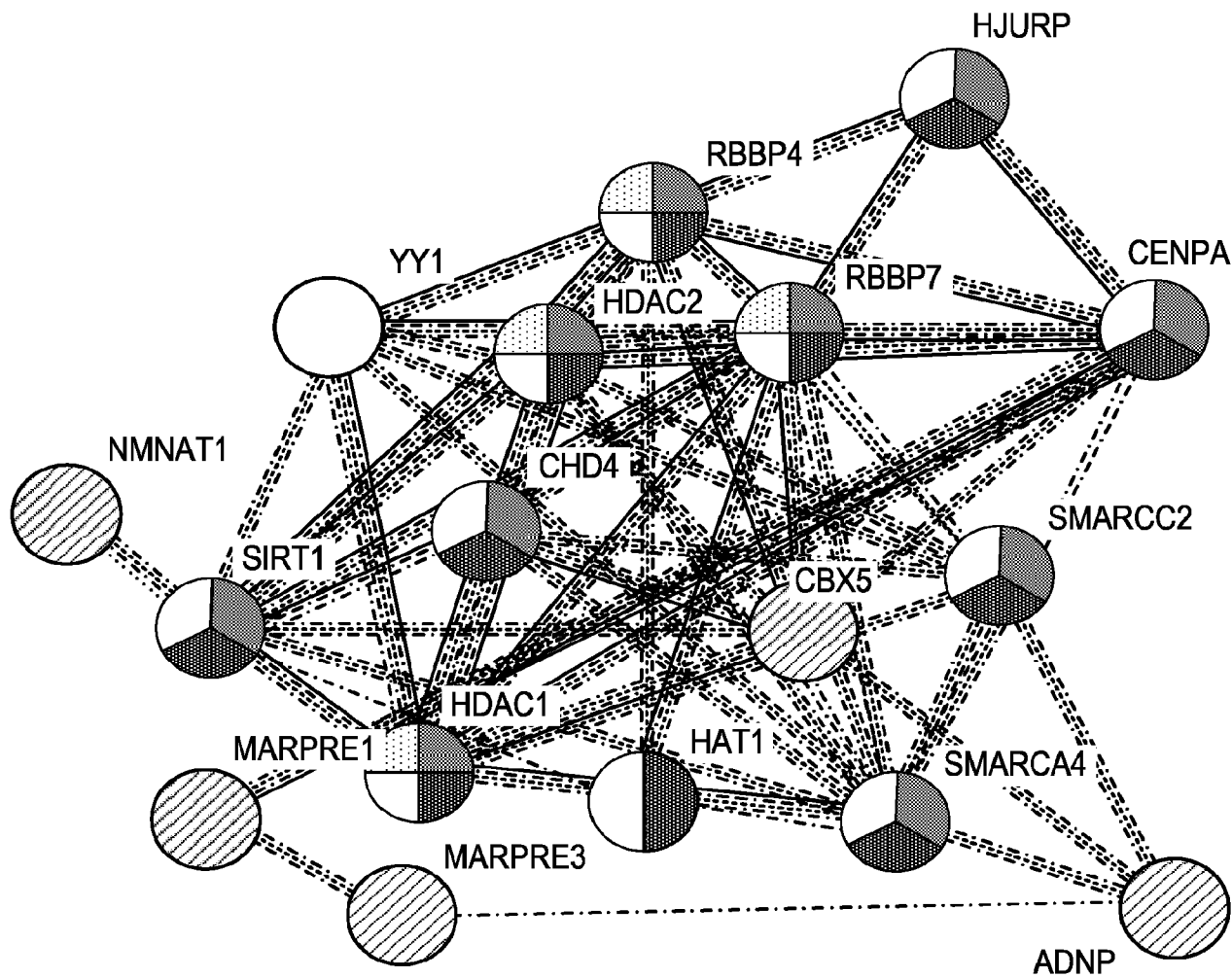


FIG. 3C



Biological Processing (GO)

<i>GO-term</i>	<i>description</i>	<i>count in gene set</i>	<i>false discovery rate</i>	
GO:0006338	chromatin remodeling	8 of 156	5.18e-10	●
GO:0006325	chromatin organization	11 of 683	5.82e-10	●
GO:0051276	chromosome organization	12 of 999	6.09e-10	○
GO:0016575	histone deacetylation	6 of 57	2.91e-09	●
GO:0034728	nucleosome organization	7 of 167	9.74e-09	◐

FIG. 4A

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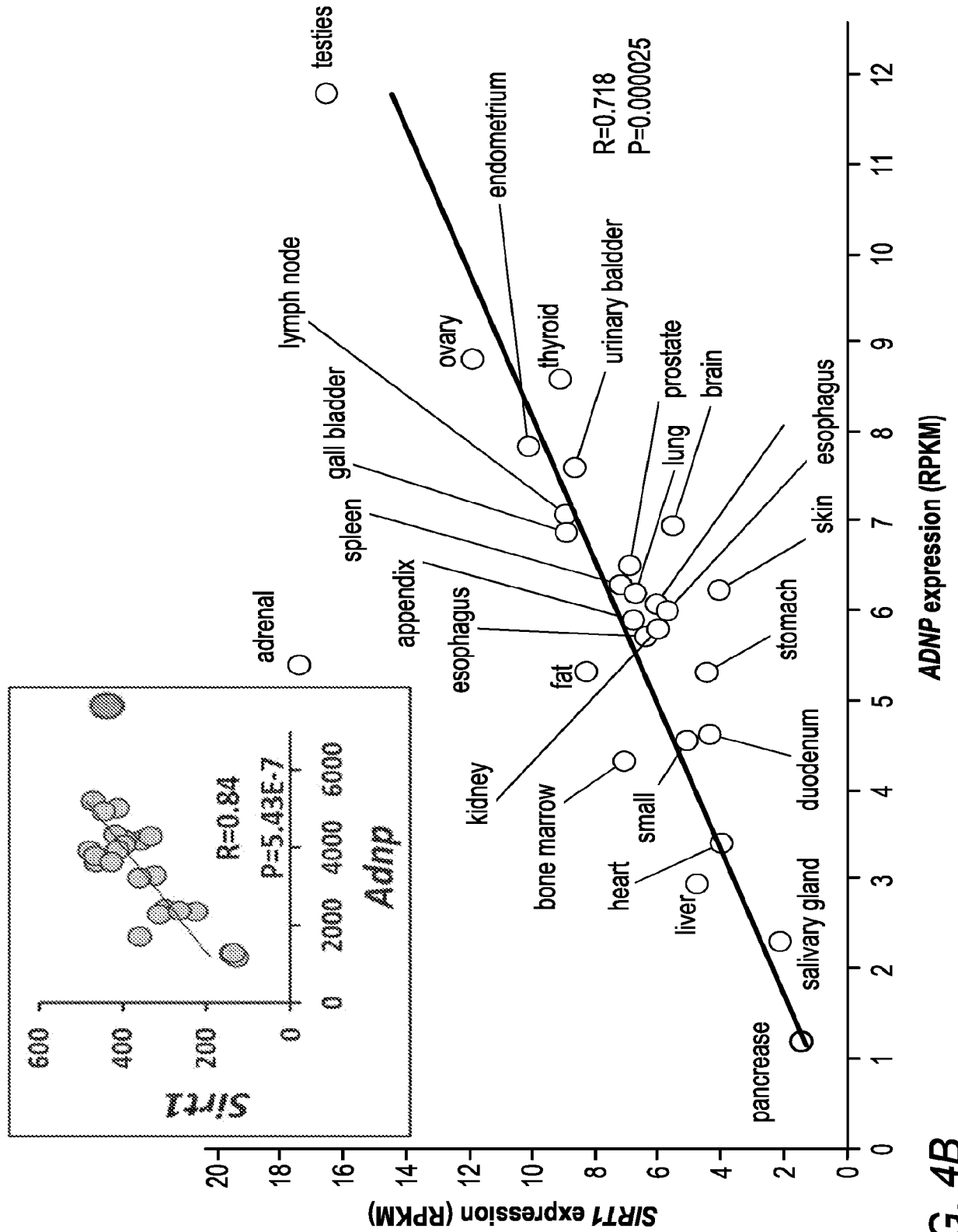


FIG. 4B

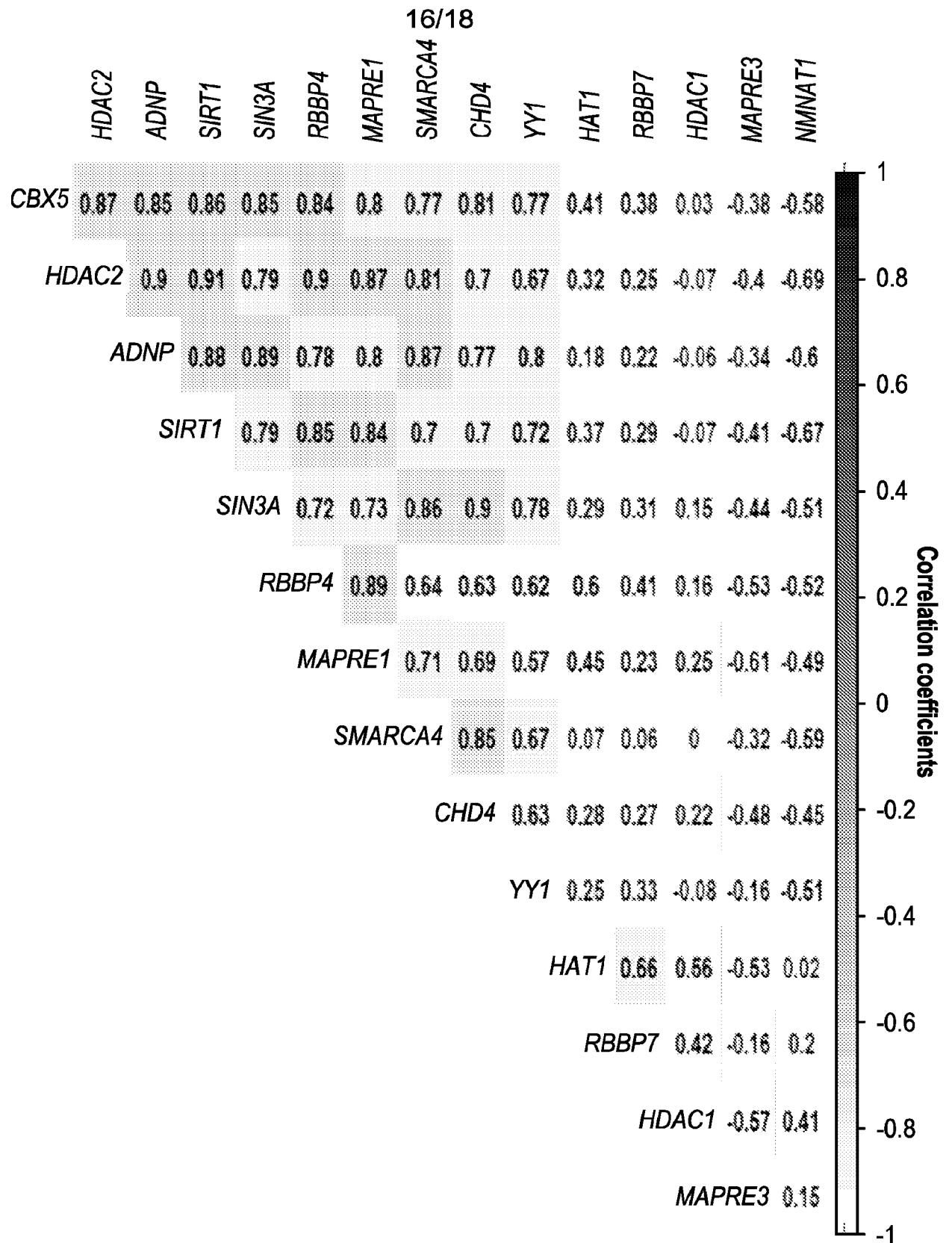
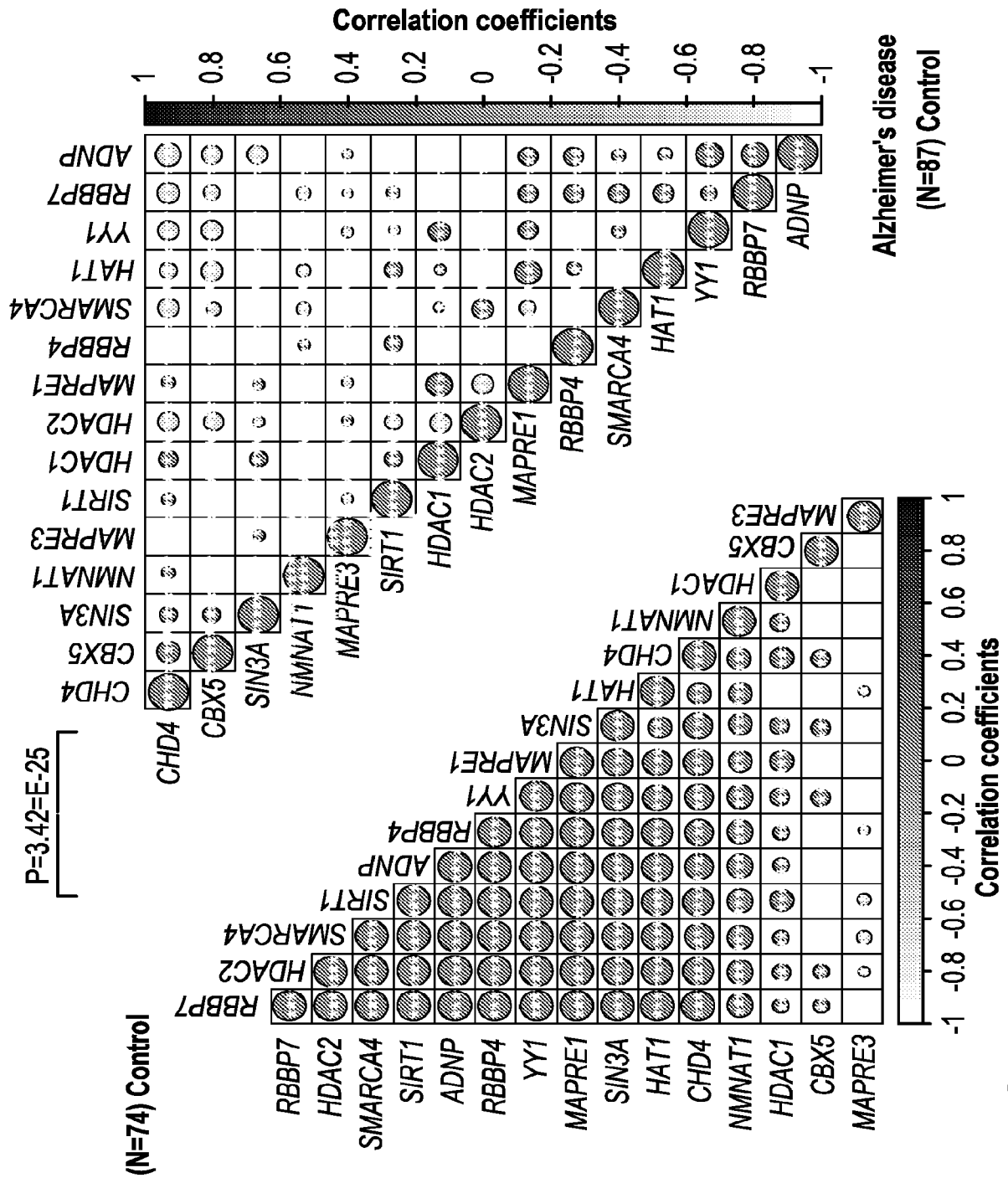


FIG. 4C



Sig. level ***p<0.001, **p<0.01, *p<0.05

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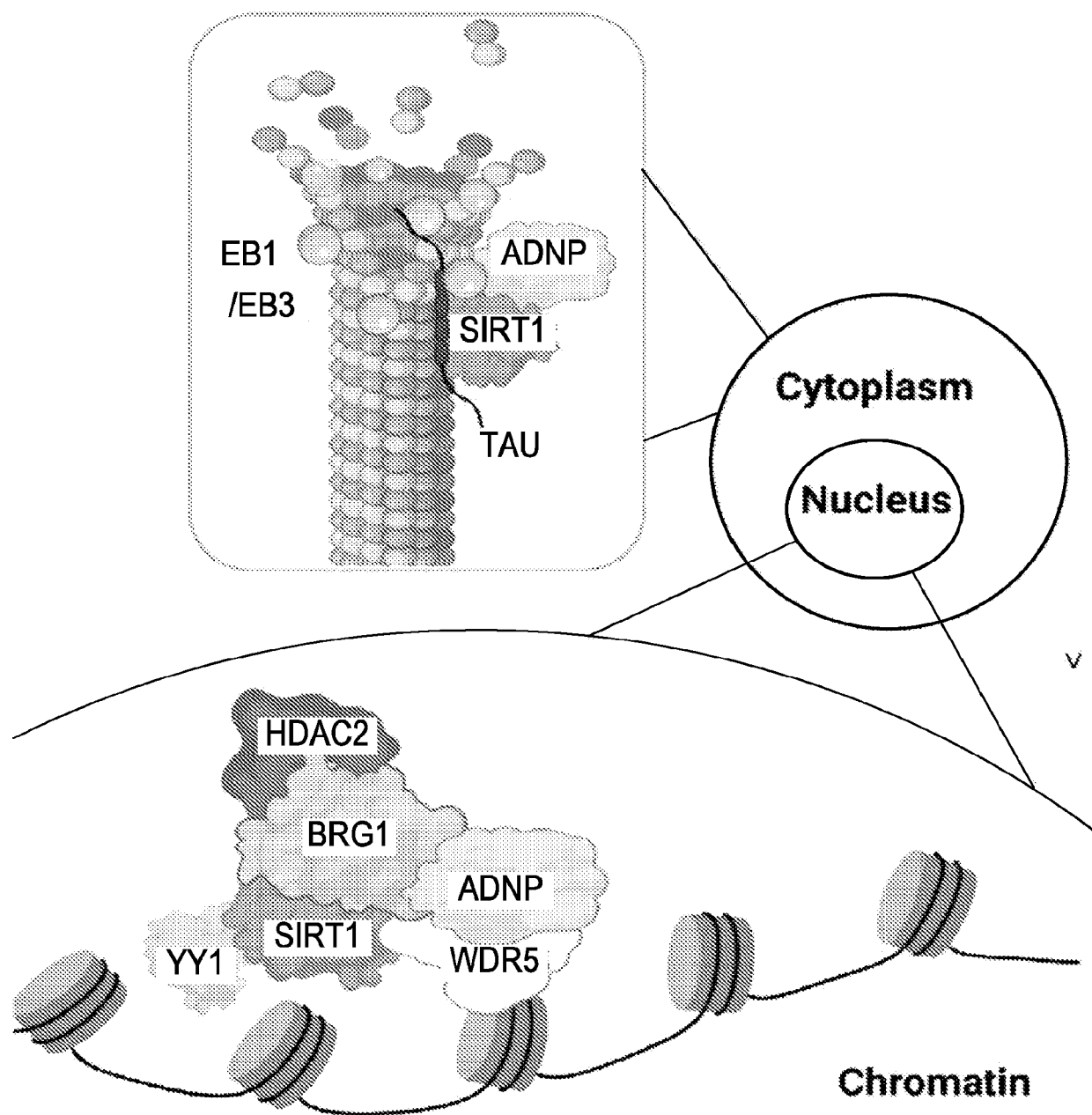
**FIG. 6**



FIG. 1B