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(54) Title: METHOD AND COMPOSITIONS FOR TREATING DECREASED COGNITIVE ABILITY

(57) Abstract: Disclosed are methods and compositions for treating cognitive decline in subjects in need. More specifically, disclosed are methods of administering exogenous adropin to subjects suffering from, or at risk of, cognitive decline including the aged and those suffering from traumatic brain injury. Also disclosed are subjects who would benefit from such treatment and pharmaceutical acceptable compositions comprising adropin, adropin<sup>34-76</sup>, and derivatives or variations thereof.



## **METHOD AND COMPOSITIONS FOR TREATING DECREASED COGNITIVE ABILITY**

### **CROSS REFERENCE TO RELATED APPLICATIONS**

**[0001]** This application claims priority to interational application PCT/US2019/35428, filed June 8, 2019, and U.S. provisional application 62/682,717, filed June 8, 2018, hereby incorporated by reference in their entirety.

### **GOVERNMENT SUPPORT CLAUSE**

**[0002]** The work disclosed herein was supported by award no. R21NS108138. The U.S. Government has certain rights in this invention.

### **FIELD OF THE INVENTION**

**[0003]** The invention relates to methods and compositions for treating reduced cognitive ability. More specifically, the invention relates to using the polypeptide adropin to treat subjects with decreased cognitive ability, due to, by way of example, age related dementia and traumatic brain injury.

### **BACKGROUND**

**[0004]** ‘Dementia’ as a general term describes memory loss and other cognitive deficits severe enough to make normal daily life difficult or impossible. Neurodegenerative conditions such as Alzheimer’s disease (AD), Parkinson’s disease and the degenerative dementias (Lewy body, Frontotemporal) represent a pressing health problem for industrialized nations. ‘Mild Cognitive Impairment’ describes an intermediate stage between cognitive decline that is expected with aging, and the more serious declines observed in dementia. The incidence of these conditions is predicted to increase owing to an aging populace. By 2030, 1 in 5 Americans will be aged 65 years or older. Metabolic disease may also be a predisposing risk factor for neurodegenerative conditions. Type 2 diabetes is a predisposing risk factor for cognitive decline and dementia. Cardiovascular disease, obesity and insulin resistance are also associated with increased risk for neurocognitive decline. The number of people

considered obese or overweight now outnumber those considered to have a healthy body mass index by 2 to 1. The conditions are thus being established for a dramatic rise in the incidence of debilitating neurodegenerative disorders. An increased occurrence of dementias in an aging and increasingly obese population thus represents a clear challenge to the US economy and the health care system. In the absence of new clinical interventions to treat cognitive decline, more than 12 million Americans may suffer from neurodegenerative diseases within 30 years. The search for treatments that delay or prevent cognitive decline is thus an imperative.

**[0005]** Approximately 5.3 million Americans live with permanent disabilities resulting from Traumatic Brain Injury (TBI). Data published by the Department of Defense indicate over 380,000 service members sustained TBI during daily activities, training and deployment between 2001 and 2018. Nationally, the economic costs of TBI-related disabilities are estimated to be ~50 billion per annum. Deficits in participation-level performance of specific cognitive tasks, such as learning and memory, are one of the primary cognitive impairments affecting individuals after a mild TBI. Currently, no FDA-approved preventive or curative interventions are available to improve the long-term suffering of the affected individual and their families. Rehabilitation of patients with moderate TBI involves physiotherapy to improve motor functions and training to assist with cognitive functions (planning, organization). The Inventors describe a use for the synthetic peptide adropin<sup>34-76</sup> for the treatment of TBI, that involves the treatment of these patients with the synthetic peptide in the emergency room, and in the weeks to months following the injury. The delivery of the peptide could be by an intravenous injection route, or by intraperitoneal, subcutaneous or intramuscular injection. Adropin treatment will improve spatial learning, as well as short- and long-term memory. Treatment in the days following injury will reduce the severity of cognitive deficits and improve the quality of life for patients. Improvements in spatial learning and memory would also aid with treatments currently in use, namely physiotherapy and psychiatric care to restore normal behavioral patterns.

**[0006]** Unfortunately, few options are currently available for treating dementias. Health and longevity are maintained, at least in part, by secreted peptides

signaling metabolic condition at a cellular and organismal level. The secreted peptide adropin was identified by one of the inventors as such a signal. The involvement of adropin in metabolic control, was documented, showing that the putative secreted domain (adropin<sup>34-76</sup>) rapidly alters activity of intracellular signaling molecules (e.g., SIRT1, Pgc1 $\alpha$ ) regulating glucose and fatty acid metabolism. While adropin is most abundant in the central nervous system, its functions as a neuropeptide remain unknown. The Inventors have made the surprising discovery that a decline in cognitive performance, whether it be associated with a process such as in aged subjects or with an event, by way of example traumatic brain injured subjects, may be slowed, delayed, attenuated or prevented by sustained adropin activity in the brain. The Inventors describe herein, a method of treating cognitive decline by administering endogenous adropin to a subject in need.

#### SUMMARY OF THE INVENTION

**[0007]** A method of treating cognitive decline in a subject in need, the method comprising administering an effective amount of Adropin, Adropin<sup>34-76</sup>, or variants or derivatives thereof, to the subject parenterally.

**[0008]** A method of treating cognitive decline, whereby the effective amount of Adropin, Adropin<sup>34-76</sup>, is 1000 nmol/kg/day to about 1 nmol/kg/day, preferably about 450 nmol/kg or most preferably about 90 nmol/kg/day.

**[0009]** A method of treating cognitive decline, in a subject wherein the subject is a human subject.

**[0010]** A method of treating cognitive decline, in a subject at risk, including subjects diagnosed with mild cognitive impairment, dementia, hypercholesterolemia, type 2 diabetes, a metabolic dysregulation disorder or advanced age.

**[0011]** A method of treating cognitive declined, wherein a subject in need, or a subject at risk, is of advanced age or a traumatic brain injury.

**[0012]** A method of treating cognitive decline, wherein the effective amount is administered one or more times a day, over a period of 2 weeks or over the remaining life of the subject.

**[0013]** A method of treating cognitive decline, in a subject who has suffered a trinitic brain injury, wherein the effective amount is administered as soon as possible following the brain injury, and then one or more times a day, over a period of 2 days, or 2 weeks, or over the remaining life of the subject.

**[0014]** A method of treating neuroinflammation in a subject in need, the method comprising administering an effective amount of Adropin, Adropin<sup>34-76</sup>, or variants or derivatives thereof, to the subject parenterally.

**[0015]** A method of treating cognitive decline, wherein administering an effective amount consists of administering an oligonucleotide enabled to express an effective amount of Adropin or Adropin<sup>34-76</sup>.

**[0016]** A method of treating cognitive decline, wherein administering an effective amount includes intraperitoneal, subcutaneous, intramuscular, or intravenous injection.

**[0017]** A composition for treating age related dementia or TBI, comprising Adropin, Adropin<sup>34-76</sup>, or variants or derivatives thereof, prepared in a pharmaceutically acceptable aqueous solution.

**[0018]** A composition for treating age related dementia or TBI, comprising Adropin, Adropin<sup>34-76</sup>, or variants or derivatives thereof, prepared in a pharmaceutically acceptable aqueous solution further including but not limited to one or more pharmaceutically acceptable salts, proteins, polypeptides, and preservatives glycerol, liquid polyethylene glycol, and pharmaceutically acceptable oils,

**[0019]** A composition for treating age related dementia or TBI, comprising Adropin, Adropin<sup>34-76</sup>, or variants or derivatives thereof, prepared in a dehydrated or concentrated form, for later use after hydration.

**[0020]** A composition for treating age related dementia or TBI, comprising Adropin, Adropin<sup>34-76</sup>, prepared in pharmaceutically acceptable aqueous solution, whereby the composition is not found in nature.

#### REFERENCE TO COLOR FIGURES

**[0021]** The application file contains at least one figure executed in color. Copies of this patent application publication with color photographs will be provided by the Office upon request and payment of the necessary fee.

#### DESCRIPTION OF THE FIGURES

**[0022]** **Figure 1** Illustrates the relative expression of the ENHO transcript in human tissues (adapted from Mol Cell Proteomics. 2014 Feb13(2):397-406).

**[0023]** **Figure 2** Illustrates the modification of the mouse *Enho* gene with the internal ribosomal entry site (IRES) and causes-recombination (Cre) coding sequence inserted into the 3'-UTR in exon 2. B6.*Enho-Cre* mice were mated with B6.Cg-*Gt(ROSA)26Sor<sup>tm9(CAG-tdTomato)Hze/J</sup>* (Ai9) strain that have a loxP-flanked STOP cassette that prevents transcription of a CAG-promoter-driven variant of the red fluorescent protein (tdTomato). In cells expressing the *Enho* transcript, expression of Cre results in removal of the STOP cassette by site-specific recombination.

**[0024]** **Figure 3** Illustrates (A) Analysis of adropin protein in tissues by western blot. Tissues were homogenized in RIPA buffer; 50 µg of total protein was used in the blot. (B) Schematic of the B6. *Enho-Cre* mouse. An IRES ("internal ribosomal entry site")-Cre inserted in the 3' UTR of the *Enho* gene of C57BL/6J (B6) mice drives Cre-mediated recombination of genomic DNA flanked by LoxP sites. B6.*Enho-Cre* mice were crossed with B6.Cg- *Gt(ROSA)26 Sor<sup>tm9(CAG-tdTomato)Hze/J</sup>* (tdTomato) mice. *Enho*(+ve) cells are identified by activation of tdTomato expression, resulting from Cre-mediated removal of a transcriptional block (TB) 5' of the tdTomato open reading frame; the tdTomato can be visualized directly when exposed to yellow-green light or by immunohistochemistry (IHC).

**[0025] Figure 4** representative photomicrographs showing tdTomato expression in the hippocampus (A-D). Enho-Cre mice crossed with Ai9 reporter strain allow visualization of adropin expressing cells in the hippocampus. (A-C) Codetection of GFAP (A) and tdTomato (B). The merged image (C) suggests that hippocampal astrocytes are devoid of adropin; (D-F) Codetection of NeuN with tdTomato.

**[0026] Figure 5** Illustrates prevention of a decline in adropin protein in the nervous system with aging improves cognition in old B6 mice. (A) Western blot showing adropin protein in 4 and 18 month old AdrTG and age-matched wild type (WT) controls (n=3/group); HSP90 is presented as a loading control. (B-C) Learning and memory of male 18 mo. old AdrTG is significantly improved relative to aged matched WT controls (NOR: novel object recognition test; Acq: memory acquisition in aversive T-maze test; Ret: memory retention in aversive T-maze test) (AdrTG, n=11-14; WT, n=11-14). Significantly different from WT; \*\*  $p<0.01$ ; \*\*\*,  $p<0.001$  (Student's t-test).

**[0027] Figure 6** illustrates reduced expression of inflammatory cytokines in cortical (A) and hippocampal (B) tissue samples from 18 mo. old AdrTG relative to age-matched controls. \*,  $p<0.05$ ; \*\*,  $p<0.01$ .

**[0028] Figure 7** illustrates Western blots indicating increased 4-HNE with aging in male B6 mice (A), that suggest lower 4-HNE in 18-month old adropin transgenic mice (AdrTG) compared to age-matched control mice (B). In panel B, the western blot and quantitated data are shown.

**[0029] Figure 8** Body composition (A-B) and glucose tolerance (C-D) are normal in 18 mo. old AdrTG mice compared to age-matched controls.

**[0030] Figure 9** illustrates reduced JNK phosphorylation (Thr183/Tyr185) in cortical extracts from 18-month old AdrTG mice compared to age-matched controls. Western blot showing phosphorylated JNK (pJNK), total JNK and Actin immunoreactivity (A). Quantitation of pJNK, total JNK (adjusted for actin) and the pJNK/JNK ratio (B).

**[0031] Figure 10** illustrates body weight in 18-month-old male C57BL/6J (B6) mice treated with vehicle or exogenous adropin<sup>34-76</sup>.

**[0032]** **Figure 11** illustrates Novel Object Recognition in 18-month-old male C57BL/6J (B6) mice treated with vehicle or exogenous adropin<sup>34-76</sup>.

**[0033]** **Figure 12** illustrates T-maze Acquisition and T-maze Retention in 18-month-old male C57BL/6J (B6) mice treated with vehicle or exogenous adropin<sup>34-76</sup>.

**[0034]** **Figure 13** illustrates body weight in C57BL/6J (B6), LdLr<sup>-/-</sup>, and ADRTG/LdLr<sup>-/-</sup>, male and female mice.

**[0035]** **Figure 14** illustrates body composition in C57BL/6J (B6), LdLr<sup>-/-</sup>, and ADRTG/LdLr<sup>-/-</sup>, male and female mice.

**[0036]** **Figure 15** illustrates Discrimination index, the percent of total time spent exploring with the novel object, in the Novel Object Recognition test. Controls (C57BL/6J (B6)), LdLr<sup>-/-</sup>, and ADRTG/LdLr<sup>-/-</sup>, transgenic mice.

**[0037]** **Figure 16** illustrates the results of T-maze data from adropin treated and adropin transgenic mice after traumatic brain injury. SHAM are mice handled but not subject to injury. Veh are mice treated with 0.9% saline. Adro are mice treated with adropin<sup>34-76</sup> delivered by ip. injection 1hr and the morning after TBI (150 nmol/kg). (A) Mice used were males, aged 10-14 weeks, purchased from the Jackson laboratory. (B) "TG" are adropin transgenic mice. "WT" are wild-type littermates. All mice were males aged 9-12 months.

**[0038]** **Figure 17** illustrates plasma cytokine levels of mice that were: uninjured, subjected to TBI, and subjected to TBI followed by adropin injections.

**[0039]** **Figure 18** illustrates Hippocampal gene expression. There are increases in the expression of cytokines (significant for IL16, CCL2, IL17) in the hippocampus of TBI-veh mice versus uninjured (sham) controls TBI also appears to result in changes in expression of growth factors (BDNF, VEGF). In most cases, these changes were attenuated by adropin treatment.

#### DETAILED DESCRIPTION OF THE INVENTION

**[0040]** Neurodegenerative diseases and traumatic brain injury (TBI) are pressing health problems for industrialized nations for which few treatments are

currently available. Health and longevity are maintained, at least in part, by secreted peptides signaling metabolic condition at an organismal and cellular level. One of the Inventors has identified the secreted peptide adropin as such a signal in 2008. Full length adropin protein (76 aa) is encoded by the Energy Homeostasis Associated (ENHO) gene which is located on human chromosome 9p13.3. Adropin is a secretory signal peptide. Early analyses of adropin function suggested links with insulin resistance and the control of carbohydrate and lipid metabolism in the periphery. Recent studies suggest a protective role in cerebral ischemia. (Yang et al. (2018) *Aging and Disease*;9 (2): 322-330). The Inventors have reported associations between plasma adropin concentrations and relative intake of fats and carbohydrates, and further demonstrated a metabolic response to exogenously administered adropin<sup>34-76</sup>, when they delivered the putative secretory domain (adropin<sup>34-76</sup>) to mice by intraperitoneal injection. (Stevens, et al., (2016) *Obesity* 24, 1731-1740; St-Onge, et al., (2014) *Obesity* 22,1056---1063). Initial examination of ENHO expression using northern blot suggested abundant expression in mouse brain and liver (Kumar, et al., (2008) *Cell metabolism*, 8, 468-481), whereas recent studies using RNA-seq in humans suggest adropin expression is most abundant in brain (Fig.1). An independent lab reported adropin protein in mice is most abundant in the CNS. (Wong, et al., (2014) *The Journal of biological chemistry* 289, 25976-25986). The Inventors' analysis using a monoclonal antibody confirms abundant adropin immunoreactivity in the CNS relative to peripheral tissues of male C57BL/6J (B6) mice Fig. 3A). Adropin may function primarily as a neuropeptide, with circulating adropin originating predominately from the CNS.

**[0041]** Analysis of *Enho* expression in the mouse brain using in situ hybridization in one of the inventor's laboratories, initially indicated a very broad distribution but were unable to define the cell-types and areas of expression (Kumar, et al., (2008) *Cell metabolism*, 8, 468---481). The Inventors were able to show, using a Cre-inducible reporter mouse, that adropin is expressed in hippocampal neurons. To identify cell-types expressing adropin, the "ENHO-Cre" strain was developed (Fig. 2). Mating ENHO-Cre mice with the tdTomato reporter strain (Madisen, et al, (2010) *Nature Neuroscience* 13, 133-140) allows for visualizing cell-types expressing adropin, mediated by Cre- removal of a LoxP-flanked transcription block (TB) (Fig. 2B, C). These

studies indicate hippocampal pyramidal neurons express adropin (Fig. 4), suggesting a potential role in cognition.

**[0042]** Analysis of mRNA expression indicates that adropin is most abundant in the CNS (Kumar et al., (2008) *Cell metabolism* 8(6):468-81; Wong et al., (2014) *The Journal of biological chemistry* 289(37):25976--86). Immunohistochemistry also indicates high expression of adropin in the CNS (Fig 3A).

**[0043]** The inventors have engineered transgenic mice that over express adropin ("AdrTG") in the nervous system and peripheral tissues. They found that continuous elevated adropin in the CNS protects cognitive function during aging. In addition, reduced JNK phosphorylation and expression of cytokines indicates reduced neuroinflammatory processes. Inflammation is also increasingly considered an important predisposing risk factor for age-related neurodegeneration (Irwin & Vitiello (2019) *Lancet Neurol.* 18(3):296-306; Madhavan et al., *Nat Rev Cardiol.* 15(12):744-756). The transgenic mice demonstrate a delay or prevention in the decline of learning and memory associated with aging. The inventors demonstrated improved cognition in older subjects with continuous elevated adropin compared to age-matched controls, through the use of cognitive assessments: novel object recognition; spatial memory acquisition; and retention, using an aversive T-maze. When the inventors compared C57BL/6J adropin transgenic mice (AdrTG) with littermate controls, they found that while cognitive performance is normal in young (less than 12 month) AdrTG, mice, old (18 month) AdrTG mice exhibit significantly better memory acquisition and retention compared to age-matched controls. In addition, the inventors have also shown that adropin levels may be increased systemically by endogenous administration. When adropin was administered via intraperitoneal injection, the subjects with diet-induced obesity experienced metabolic changes suggesting improvements in glucose control and reduced risk for type 2 diabetes. (Kumar *et al.* 2008; Gao *et al.* 2014, 2015)

**[0044]** The Inventors disclose a method of treating cognitive decline in subjects by increasing levels of adropin or, adropin<sup>34-76</sup> in the subject's brain by way of exogenous administration.

**[0045]** In one embodiment, subjects experiencing or at risk of age-related dementia are treated by intravenous, subcutaneous, or intraperitoneal injection of adropin or adropin<sup>34--76</sup>.

**[0046]** In one embodiment, subjects experiencing or at risk of age-related dementia are treated by intravenous, subcutaneous, or intraperitoneal injection of adropin, adropin<sup>34--76</sup> or variants or derivatives thereof which reduce the likelihood of cognitive decline.

**[0047]** In another embodiment, subjects experiencing or at risk of dementia due to traumatic brain injury are treated by intravenous, subcutaneous, or intraperitoneal injection of adropin or adropin<sup>34--76</sup>.

**[0048]** In another embodiment, subjects experiencing or at risk of dementia due to traumatic brain injury are treated by intravenous, subcutaneous, or intraperitoneal injection of adropin, adropin<sup>34--76</sup>, or variants or derivatives thereof, which reduce the likelihood of cognitive decline.

**[0049]** In another embodiment, subjects experiencing or at risk of neuroinflammation due to traumatic brain injury are treated by intravenous, subcutaneous, or intraperitoneal injection of adropin, adropin<sup>34--76</sup>, or variants or derivatives thereof, which reduce the likelihood of cognitive decline.

**[0050]** In another embodiment, is a method of treating a subject who may have received TBI related injury, by administering intravenous, subcutaneous, or intraperitoneal injection of adropin, adropin<sup>34--76</sup>, or variants or derivatives thereof, to reduce the likelihood of an occurrence of TBI related dementia, within 24 hours of injury.

**[0051]** In another embodiment, is a method of treating a subject who may have received TBI related injury, by administering intravenous, subcutaneous, or intraperitoneal injection of adropin, adropin<sup>34--76</sup>, or variants or derivatives thereof, to reduce the likelihood of an occurrence of TBI related dementia, within 24 hours of injury, and/or one or more subsequent days thereafter.

**[0052]** In another embodiment, subjects experiencing neuroinflammation are treated by intravenous, subcutaneous, or intraperitoneal injection of an effective amount of adropin, adropin<sup>34--76</sup>, or variants or derivatives thereof.

**[0053]** In another embodiment, is an injectable preparation of adropin, adropin<sup>34--76</sup>, or variants or derivatives thereof, and a pharmaceutical acceptable preparation. A pharmaceutical acceptable preparation may also include a preservative, a composition to block non-specific binding of adropin, by way of example a protein such as gelatin or albumin, or a compound, such as a surfactant, by way of example, Tween 20 preferably at 0.1 to 1 percent. The solution may also include compounds to prevent degradation or aggregation of the peptide while staying within physiological acceptable parameters for injection. By way example, the pH may vary from 5.0 to 6.0, 6.0 to 7.0, 7.0, to 8.0, or 9.0 to 10.0. One or more salts may also be included. By way of non-limiting example, a physiological acceptable concentration of salt may be a concentration, plus or minus up to 5, 10, or 15 percent that normally found in the subject. Non-naturally occurring salts may also be included, by way of example, Tris, HCL. Formulations of adropin may be concentrated to some degree or lyophilized for storage and later hydrated before use.

**[0054]** The aqueous solution may further contain various salts or buffers that are well known in the art. Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are, Ringer's solution, or isotonic sodium chloride solution.

**[0055]** Adropin Sequences

**[0056]** Human adropin Amino Acid Sequence (SEQ ID NO.:1):

|                                                        |    |    |    |    |
|--------------------------------------------------------|----|----|----|----|
| 10                                                     | 20 | 30 | 40 | 50 |
| MGAAISQGAL IAIVCNGLVG FLLLLLWVIL CWACHSRSAD VDSLSESSPN |    |    |    |    |

60 70  
SSPGPCPEKA PPPQKPSHEG SYLLQP

**[0057]** Adropin<sup>34-76</sup>, peptide used in the examples for inducing metabolic and cognitive activity (SEQ ID NO.:2):

34 76  
CHSR SAD VDSLSESSPN SSPGPCPEKA PPPQKPSHEG SYLLQP

**[0058]** The sequences disclosed herein represent non-limiting examples. It is anticipated that variations of Adropin and Adropin<sup>34--76</sup>, may also be effective, particularly those with conservative amino acid substitutions. In addition, variants, analogs and derivatives of Adropin polypeptide sequences are anticipated including but not limited to the following list of possible modifications. Chemical design strategies to avoid aggregation, including corruption of hydrophobic patches by means of amino acid substitutions or *N*-methylation of amino acids. Physicochemical properties of adropin may be improved by the introduction of stabilizing  $\alpha$ -helices, introduction of salt bridge formations or lactam bridges. The half-life may be extended through identifying cleavage sites and substitution of amino acids, stabilizing the secondary structure of the molecule, peptide acylation, insertion of albumin-binding elements into the peptides backbone, conjunction to antibody fragments, or polyethylene-glycol (PEG)-ylation.

**[0059]** Sequence identity or percent identity is intended to mean the percentage of same residues between two sequences. In sequence comparisons, the two sequences being compared are aligned using the Clustal method (Higgins et al, Cabios 8:189-191, 1992) of multiple sequence alignment in the Lasergene biocomputing software (DNASTAR, INC, Madison, Wis.). In this method, multiple alignments are carried out in a progressive manner, in which larger and larger alignment groups are assembled using similarity scores calculated from a series of pairwise alignments. Optimal sequence alignments are obtained by finding the maximum alignment score, which is the average of all scores between the separate residues in the alignment, determined from a residue weight table representing the probability of a

given amino acid change occurring in two related proteins over a given evolutionary interval. Penalties for opening and lengthening gaps in the alignment contribute to the score. The default parameters used with this program are as follows: gap penalty for multiple alignment =10; gap length penalty for multiple alignment=10; k-tuple value in pairwise alignment=1; gap penalty in pairwise alignment=3; window value in pairwise alignment=5; diagonals saved in pairwise alignment=5. The residue weight table used for the alignment program is PAM250 (Dayhoff et al., in Atlas of Protein Sequence and Structure, Dayhoff, Ed., NBRF, Washington, Vol. 5, suppl. 3, p. 345, 1978).

**[0060]** Variations in the amino acid sequence will likely be comprised of conservative amino acid substitutions, or substitutions of amino acids outside of function regions. It is anticipated that polypeptides with 85 percent or more, 90 percent or more, 95 percent or more or 98 percent or more identity to Adropin and Adropin<sup>34--76</sup>, may be effective at treating cognitive decline. It is also anticipated that the variants described herein include variants not found in nature.

## Subjects

**[0061]** It is envisioned that subjects selected for treatment would include human subjects, particularly human subjects diagnosed with, dementia, or suffering from cognitive decline or at risk for cognitive decline. Human subjects at risk for cognitive decline, would include, by way of example, the elderly, subjects at risk for, or who have experienced: stroke, Parkinson's Disease, people identified as being "at risk" for early-onset Alzheimer's disease including those with a family history or with risk genes: APOE-e4, amyloid precursor protein, presenilin-1, presenilin-2), traumatic brain injury, chronic poorly-controlled hypertension, type 2 diabetes, sleep apnea, disruption of normal sleeping patterns, elevated biomarkers of systemic and/or cerebral inflammation, hypercholesterolemia, and tests for biomarkers in the blood plasma that are currently under development. Surgical interventions have also been indicated to induce inflammation in the nervous system and dementia. The inventors anticipate administration of endogenous adropin or adropin<sup>34--76</sup> may be used as an effective post-operative prophylactic to reduce risk for dementia in elderly patients.

**[0062]** Subjects suffering from cognitive decline or at risk of cognitive decline due to a TBI would include any subject who has received a TBI, particularly those who have received a TBI recently, by way of example within, 1 to 24 hours, from 24 to 48 hours, or 48 hours or longer. It is expected that adropin therapy would be administered as soon as possible following a TBI event. A traumatic brain injury may include any trauma to the head due to, by way of example, vehicular accidents, falls, or injury to the brain including injury resulting in ischemic or not resulting in ischemic. Subjects would also include those diagnosed with, traumatic brain injury, closed head injury, neurological trauma, neuroinflammation, concussion, head injury, or memory loss. Subjects would include adults, children, adolescents or the elderly,

**[0063]** Adropin may also be administered prophylactically, to a subject at risk. These subjects would include, persons involved in high risk activities, including but not limited to athletes, construction workers, some members of the military, or the like.

**[0064]** Other subjects who may benefit from Adropin treatment include, by way of non-limiting example, laboratory animals, show animals and house hold pets.

## Treatment

**[0065]** Adropin, Adropin<sup>34-76</sup>, or variants, analogs, or derivatives thereof, may be administered to a subject parenterally. By way of non-limiting example, by subcutaneous, intramuscular, intraperitoneal, or intravenous injection. Solutions or suspensions of the peptide may be prepared in pharmaceutically acceptable salts as an aqueous solution. Peptide solutions may also be prepared in glycerol, liquid polyethylene glycols, or mixtures thereof of pharmaceutically acceptable oils. A preservative may be added to the peptide preparation to prevent microbial growth. Preparations may also contain a pharmaceutically acceptable protein, such as gelatin or albumin, to inhibit nonspecific binding of the protein to storage vehicles. By way of example, studies in mice using adropin<sup>34-76</sup> to improve cognition have used 0.1% bovine serum albumin for this purpose.

**[0066]** Using mice, the Inventors have shown that administration of adropin<sup>34-76</sup>, in doses as low as 90 nmol/kg/day, delivered as two 45 nmol/kg injections, can be effective in producing changes in metabolism. Dose as high as 900 nmol/kg/d are well tolerated in mice. While the doses required for a desired clinical effect in humans is expected to vary between patients, the 90 nmol/kg dose administered by subcutaneous, intramuscular, or intravenous injection, either once or twice a day, may represent a preferred treatment protocol. It is anticipated that the treatments would be administered daily, although long-acting formulations involving weekly injections are envisaged. The optimum dose and frequency for a particular subject may be determined by the patient's treating physician, based on the patient's cognitive response. It is anticipated that adropin therapy may or may not improve a subject's cognitive function relative to current baseline, however adropin therapy may result in significant delays in the advancement of the disease. Improvement may manifest as a significant delay or a reduced rate of subsequent cognitive decline.

**[0067]** In one non-limiting embodiment, is a human subject, exhibiting evidence of age-related cognitive decline or dementia, treated with an injection of about 450 nmol/kg of adropin<sup>34-76</sup>, a dose known to affect metabolic control in male B6 mice.

**[0068]** In another non-limiting embodiment, is a human subject having received a traumatic brain injury, the subject is treated with an injection of about 450 nmol/kg of adropin<sup>34-76</sup>, within the first 24 hours, and another treatment about 450 nmol/kg of adropin<sup>34-76</sup>, within the next 24 hours.

**[0069]** Symptoms of dementia can vary greatly between human subjects. At least two of the following core mental functions must be significantly impaired for a diagnosis of dementia to be considered: memory, communication and language, ability to focus and pay attention, reasoning and judgement, visual perception. Medical doctors will diagnose Alzheimer's and other types of dementia based on a careful examination of medical history; physical examination; laboratory tests; characteristic changes in thinking; and assessment of day-to-day function and behavior associated with each type and can determine that a person has dementia with a high level of certainty. Non-limiting symptoms dementia include loss of: memory, communication and language

skills, and an ability to focus and pay attention, reasoning and judgment, as well as visual perception. It is anticipated that treatment would be initiated soon after the diagnosis of dementia, or the subject is determined to be at risk of dementia. The treatment will be or may be repeated daily for the remaining life of the subject. Similar subjects may receive sham treatment. After an appropriate period of time for the particular subject, both groups will be subjected to cognitive testing. It is anticipated that subjects receiving adropin or continuous adropin administration, will perform better on cognitive testing compared to subjects receiving sham treatment.

**[0070]** It is anticipated that adropin treatment would improve performance on cognitive tasks including but not limited to: spatial learning, as well as short- and long-term memory. Treatment in the hours or days following a traumatic brain injury would reduce the severity of cognitive deficits, and improve the quality of life for patients. Improvements in spatial learning and memory could also aid with the currently used treatments, including physiotherapy and psychiatric care to help restore subject's normal behavioral patterns.

**[0071]** Preferred embodiments of the invention are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples.

## EXAMPLES

### Materials and Methods (Examples 1-6)

**[0072]** Using a Cre-inducible reporter model developed by the Inventors (Fig. 3), adropin expression was observed in hippocampal neurons (Fig. 4) typically related to learning and memory (Eichenbaum et al., (2017) Curr Opin Behav Sci. 2017;17, 65-70).

**[0073]** The AdrTG model used for these experiments was developed by the Inventors using a human  $\beta$ -actin promotor to drive overexpression of the adropin open reading frame in a synthetic gene in B6 mice (Kumar, et al., (2008) Cell metabolism, 8, 468---481; Gao, et al., (2014) Diabetes 63, 3242---3252). Increased expression of adropin is evident in all tissues examined, including the nervous system (*Id.*) and data not shown, particularly in tissues where expression of the endogenous gene is low (for e.g., skeletal and cardiac muscle, adipose tissue). An analysis of adropin expression in the hippocampus of male mice housed at SLU suggests a 40% increase in relative expression (WT,  $1.00 \pm 0.02$ ,  $n=6$ ; AdrTG,  $1.42 \pm 0.07$ ,  $n=7$ ;  $p < 0.01$  by Student's t-test).

#### Transgenic Mouse Model

**[0074]** AdrTG were generated and maintained as previously described (Kumar et al., (2008) Cell metabolism.;8(6):468-481; Ghoshal et al., (2018) Molecular metabolism 8:51-64; Gao S, et al., (2014) Diabetes. 2014;63(10):3242-3252) For aging studies, male mice were maintained in group housing (3-4/cage) on standard rodent chow. Fasting glucose and glucose clearance were assessed in mice fasted for 6h. Glucose tolerance tests were performed using 1 mg/kg dextrose administered intraperitoneally (ip.), as previously described. (Kumar, et al., (2008) Cell metabolism, 8, 468---481; Gao, et al., (2014) Diabetes 63, 3242-3252)

**[0075]** For visualization of cells expressing adropin, an IRES-Cre was inserted into the 3'untranslated region (UTR) of the adropin open reading frame in exon 2 (Fig. 1). The targeting vector was constructed using recombineering system. Isogenic DNA containing the Enho locus was retrieved from genomic colony RP23-100C7 of C57Bl/6 BAC genomic library via gap repair. An IRES-Cre-Frt-neo-Frt was inserted into 3' 53 bp downstream of the translational stop codon in exon 2. For gene targeting, 50 $\mu$ g of linearized targeting vector consisting of 3.5kb 5'arm and 7.2 kb 3' arm was electroporated into Bruce4 B6 embryonic stem (ES) cells. Correct homologous recombination in targeted clones was confirmed with Fidelity PCR at the 5' and 3' ends. The fragments produced from Fidelity PCR with these primers were sequenced to further verify the correctness of recombination. Correctly targeted ES cells were injected

into Albino B6 blastocysts; germline transmitting chimeric mice were obtained and mated with Albino B6 mice to generate heterozygous carriers of the *Enh1RES-Cre-Frt-neo-Frt* on the B6 background. The *Frt-neo-Frt* sequence was removed using B6;S<sub>J</sub>L-Tg(ACTFLPe)<sup>9205Dym</sup>/J transgenic mice purchased from the Jackson laboratory. *Enh1RES-Cre* mice were then crossed onto the B6.Cg-Gt(ROSA)<sup>26Sortm9</sup>(CAG-tdTomato)<sup>Hze</sup>/J strain in which a loxP-flanked stop cassette prevents transcription of a red fluorescent protein variant (tdTomato) driven by a CAG promoter (Madisen et al., (2010) *Nature neuroscience.*;13(1):133-140).

**[0076]** Gene expression was assessed using qRT-PCR as previously described. Hippocampal and cortical tissue samples were dissected from fresh brains and snap frozen brains on dry-ice cold isopentane. Total RNA was extracted using Trizol (Invitrogen, Life Technologies) and treated with a DNasefree kit (Ambion, Life Technologies). 800 ng of total RNA were used for cDNA synthesis (Superscript III Reverse transcription kit, Invitrogen). Quantitative PCR was performed in 96-well plates using Taqman gene expression and QuantStudio 7 Detection Systems (Applied Biosystems, Life Technologies); 36B4 was used as the reference gene.

#### Assessment of Cognitive Function.

**[0077]** Memory retention and acquisition were assessed using an aversive T-maze and novel objective recognition, as previously described (Farr et al., (2016) *J Alzheimers Dis.*; 54(4):1339-1348). Briefly, the T-maze consists of a black plastic alley with a start box at one end and two goal boxes at the other. An electrifiable floor of stainless steel rods run throughout the maze to deliver a foot-shock using a scrambled grid floor shocker (Model E13-08, Coulbourn Instruments, Whitehall, PA). Mice are not permitted to explore the maze prior to training. A block of training trials begins when mice are placed in the start box. The guillotine door is raised and a cue buzzer sounded simultaneously (doorbell type, at 55dB); 5 s later a mild aversive foot-shock is applied with an intensity of 0.35mA. The arm of the maze entered on the first trial is designated “incorrect” and the mild foot-shock continued until the mouse enters the other goal box, which in all subsequent trials is designated “correct” for each mouse. Mice are trained until they made one active avoidance, with an inter-trial interval of 30–35 s. The number

of trials to make one active avoidance is the measure of acquisition. Retention is tested 7d later, with training continued until the criterion of making five active avoidances in six consecutive trials is achieved. The number of trials needed to reach this criterion is the measure of retention (Butler et al., (2012) *The Journal of clinical endocrinology and metabolism.*;97(10):3783-3791; Wong et al., (2014) *The Journal of biological chemistry.*; 289(37):25976-25986).

**[0078]** For the novel object recognition task, mice are habituated to an empty apparatus, a 58×66×11-cm white plastic box, for 5min a day for 3 consecutive days prior to entry of objects. On the first day of training, mice are placed in the testing apparatus with two identical objects (A and B). 24h later the animal is reintroduced to the arena but this time one of the original objects is removed and a new object (C). Total time spent exploring each of the two objects is recorded. Anxiety will be assessed using open field and elevated plus maze tests. The experimental apparatus consists of a central platform, two open arms and two equal-sized closed arms opposite to each other. The test consisted of placing a mouse in the central platform facing an enclosed arm and allowing it to freely explore the maze for 5 min. The number of entries into the open and closed arms and the time spent in open arms was measure by an observer blind to treatment

**[0079]** Statistical Analysis. Data were compiled in Microsoft Excel. Statistical analysis requiring analysis of covariance used univariate analysis in SPSS Statistics Version 23 (IBM). Student's t-test was used for comparisons of 2 groups.

#### EXAMPLE 1

**[0080]** The Inventors assessed cognitive performance using a strain of B6 mice over expressing the adropin open reading frame under the control of a human b $\beta$ -actin promotor (AdrTG) (Kumar, et al., (2008) *Cell metabolism*, 8, 468---481; Gao, et al., (2014) *Diabetes* 63, 3242---3252) . Analysis of male adropin transgenic mice (AdrTG), mice aged <1yr, suggested normal performance in tests of declarative memory, and of spatial learning and memory (data not shown). However, at 18 months of age, clear differences in performance were noted (Fig. 3), indicating a

neuroprotective role from age related cognitive decline. Alternatively, there may be a “ceiling effect” in younger mice, with improved cognitive performance only observed when the ability for memory consolidation declines in the control group. Performance in tests of declarative memory, and of spatial learning and memory, were not significantly different in AdrTG and littermate controls aged <12 months (data not shown). However, at 18 months AdrTG exhibited significantly improved cognitive performance (Fig. 5). Adropin signaling in the CNS may thus prevent and/or delay cognitive decline observed with aging of male B6 mice. The transgenic animals created by the inventors demonstrate that if adropin levels in the brain are maintained, cognitive decline may be slowed, delayed or prevented.

## EXAMPLE 2

**[0081]** Continuous exposure to adropin reduces stress in transgenic mice. Adropin transgenic mice show reduced stress indicators compared to wide type. Reduced inflammation (Figure 6) and reduced oxidative stress (Figure 7) are seen in AdrTG transgenic mice. Reduced expression of inflammatory cytokines in cortical (Figure 6A) and hippocampal (Figure 6B) are seen in tissue samples from 18 mo. old AdrTG relative to age-matched controls. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ . In addition, increased 4-HNE is seen with aging in male B6 mice (Figure 7A), but less so in adropin transgenic mice (AdrTG) (Figure 7B) suggesting a relative reduction in oxidative stress in the AdrTG transgenic mice. Body composition (Figure 8A-B) and glucose tolerance (Figure 8C-D) are normal in 18 mo. old AdrTG mice compared to age-matched controls. Reduced SAPK/JNK activity in the nervous system of 18-month old adropin transgenic mice is consistent with reduced neuroinflammation (Figure 9). The stress-activated protein kinase/Jun-amino-terminal kinase SAPK/JNK is potently and preferentially activated by a variety of environmental stresses including UV and gamma radiation, ceramides, inflammatory cytokines, and in some instances, growth factors and GPCR agonists (1-6). As with the other MAPKs, the core signaling unit is composed of a MAPKKK, typically MEKK1-MEKK4, or by one of the mixed lineage kinases (MLKs), which phosphorylate and activate MKK4/7. Upon activation, MKKs phosphorylate and activate the SAPK/JNK kinase (Ichijo, (1999) Oncogene 18, 6087-93.). Stress signals

are delivered to this cascade by small GTPases of the Rho family (Rac, Rho, cdc42) (Kyriakis and Avruch, (2001) *Physiol Rev* 81, 807-69.). Both Rac1 and cdc42 mediate the stimulation of MEKKs and MLKs (*Id.*). Alternatively, MKK4/7 can be activated in a GTPase-independent mechanism via stimulation of a germinal center kinase (GCK) family member (Kyriakis (1999) *J Biol Chem* 274, 5259-62.). There are three SAPK/JNK genes each of which undergoes alternative splicing, resulting in numerous isoforms (Kyriakis and Avruch, (2001) *Physiol Rev* 81, 807-69.). SAPK/JNK, when active as a dimer, can translocate to the nucleus and regulate transcription through its effects on c-Jun, ATF-2, and other transcription factors (Kyriakis and Avruch, (2001) *Physiol Rev* 81, 807-69; Leppä and Bohmann (1999) *Oncogene* 18, 6158-62.).

**[0082]** Total JNK was measured by Western Blot using Recombinant Anti-JNK2 antibody [EP1595Y] (ab76125) from Abcam. Phosphorylation of JNK (an indicator of activation) was measured using Phospho-SAPK/JNK (Thr183/Tyr185) Antibody #9251 (Cell Signaling Technology). The Phospho-SAPK/JNK (Thr183/Tyr185) Antibody detects endogenous levels of p46 and p54 SAPK/JNK dually phosphorylated at threonine 183 (Thr183) and tyrosine 185 (Tyr185). Actin was detected using Cell Signaling 3700s (monoclonal antibody), and is used to estimate loading.

### EXAMPLE 3

**[0083]** To examine the effects of endogenous adropin on a subject's cognitive ability, 34 male C57BL/6J (B6) mice, aged 18 months, were treated with exogenous adropin<sup>34-76</sup>. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME). After acclimation for 1 month, mice were separated into two weight matched groups of 15 mice: Group 1 adropin<sup>34-76</sup> treatment group and Group 2 saline vehicle control.

**[0084]** The mice were administered a single intraperitoneal injection (volume 100 µl) each morning (0900h) for 4 weeks. Group 1 was administered adropin<sup>34-76</sup> at a dose of 90 nmol/kg/day. Group 2 was administered saline. The peptide was purchased from Neoscientific Inc. Behavioral testing began after 2 weeks of injections. Injections continued though testing until 4 weeks.

**[0085]** There was no significant effect of adropin<sup>34-76</sup> treatment on body weight (mean  $\pm$  SD for weight gain in grams for group 1,  $0.7 \pm 3.1$ ; group 2,  $0.6 \pm 2.3$ ,  $p=0.95$  by Student's t-test). One mouse in group 1 died on 08/23/19. (Figure 10)

#### EXAMPLE 4

**[0086]** Adropin treatment improved cognitive performance as measured by the Novel Object Recognition test (Figure 11, Table 1). A non-amnesic subject will spend more time exploring the novel object than the familiar one. When adropin treated and vehicle treated mice were subjected to the Novel Object Recognition test, adropin treated mice spent more time exploring the novel object (Figure 11, Table 1)

**[0087]** Table 1 Novel Object Recognition test 24 Hour delay % Novel Object

| Vehicle | Adropin |
|---------|---------|
| 65.00   | 40.74   |
| 43.48   | 64.58   |
| 46.88   | 62.50   |
| 40.00   | 58.82   |
| 31.48   | 55.56   |
| 44.44   | 77.50   |
| 0.00    | 66.67   |
| 42.50   | 79.10   |
| 60.00   | 51.06   |
| 40.00   | 100.00  |
| 52.94   | 51.61   |
| 33.33   | 46.43   |

|       |       |
|-------|-------|
| 52.63 | 42.86 |
| 71.79 | 53.06 |
| 33.96 | 37.50 |

**[0088]** Statistical analysis of Table 1.

|                                     |                         |
|-------------------------------------|-------------------------|
| Statistical analysis                | Data 1                  |
| Column B                            | Adropin                 |
| vs.                                 | vs.                     |
| Column A                            | Vehicle                 |
| Unpaired t test                     |                         |
| P value                             | 0.0183                  |
| P value summary                     | *                       |
| Significantly different? (P < 0.05) | Yes                     |
| One- or two-tailed P value?         | Two-tailed              |
| t, df                               | t=2.505 df=28           |
| How big is the difference?          |                         |
| Mean $\pm$ SEM of column A          | 43.90 $\pm$ 4.350, n=15 |
| Mean $\pm$ SEM of column B          | 58.88 $\pm$ 4.106, n=15 |
| Difference between means            | 14.99 $\pm$ 5.982       |
| 95% confidence interval             | 2.733 to 27.24          |
| R squared                           | 0.1831                  |
| F test to compare variances         |                         |
| F,DFn, Dfd                          | 1.122, 14, 14           |
| P value                             | 0.8320                  |
| P value summary                     | ns                      |
| Significantly different? (P < 0.05) | No                      |

## Example 5

**[0089]** Adropin treatment also improved performance during the Acquisition T-maze test. Mice prepared with adropin or placebo in Example 3 were subjected to the Acquisition T-maze test. Adropin treated mice performed better (reduced time in maze) than placebo treated mice (Figure 12, Table 2).

**[0090]** Table 2. T- maze acquisition

| Vehicle | Adropin |
|---------|---------|
| 8.      | 7.      |
| 13.     | 6.      |
| 10.     | 7.      |
| 7.      | 8.      |
| 8.      | 8.      |
| 12.     | 7.      |
| 9.      | 8.      |
| 10.     | 9.      |
| 14.     | 6.      |
| 12.     | 9.      |
| 13.     | 7.      |
| 13.     | 6.      |
| 10.     | 8.      |
| 11.     | 7.      |

12.

**[0091]** Statistical analysis of Table 2

|                                     |                             |
|-------------------------------------|-----------------------------|
| Statistical analysis                | T-maze Act                  |
| Column B                            | Adropin                     |
| vs.                                 | vs.                         |
| Column A                            | Vehicle                     |
| Unpaired t test                     |                             |
| P value                             | < 0.0001                    |
| P value summary                     | ****                        |
| Significantly different? (P < 0.05) | Yes                         |
| One- or two-tailed P value?         | Two-tailed                  |
| t, df                               | t=5.464 df=27               |
| How big is the difference?          |                             |
| Mean $\pm$ SEM of column A          | 10.80 $\pm$ 0.5538,<br>n=15 |
| Mean $\pm$ SEM of column B          | 7.357 $\pm$ 0.2695,<br>n=14 |
| Difference between means            | -3.443 $\pm$ 0.6301         |
| 95% confidence interval             | -4.736 to -2.150            |
| R squared                           | 0.5251                      |
| F test to compare variances         |                             |
| F, DFn, Dfd                         | 4.525, 14, 13               |
| P value                             | 0.0099                      |
| P value summary                     | **                          |

Significantly different? ( $P < 0.05$ ) Yes

**[0092]** Adropin treated mice also showed superior performance in the T maize retention. (Figure 12, Table 3) Mice were retested in the maze after 7 days and Adropin treated mice retained learned memories and complete the maze in less time.

**[0093]** Table 3. T-maze Retention

| Vehicle | Adropin |
|---------|---------|
| 9.      | 6.      |
| 9.      |         |
| 10.     | 7.      |
| 11.     | 6.      |
| 7.      | 6.      |
| 9.      | 7.      |
| 9.      | 7.      |
| 11.     | 8.      |
| 9.      | 6.      |
| 9.      | 6.      |
| 9.      | 9.      |
| 9.      | 7.      |
| 10.     | 7.      |
| 7.      | 7.      |
| 10.     | 7.      |

**[0094]** Statically analysis of Table 3

|                                     |                          |
|-------------------------------------|--------------------------|
| Statically analysis                 | T-maze Ret               |
| Column B                            | Adropin                  |
| vs.                                 | vs.                      |
| Column A                            | Vehicle                  |
| Unpaired t test                     |                          |
| P value                             | < 0.0001                 |
| P value summary                     | ****                     |
| Significantly different? (P < 0.05) | Yes                      |
| One- or two-tailed P value?         | Two-tailed               |
| t, df                               | t=6.178 df=27            |
| How big is the difference?          |                          |
| Mean $\pm$ SEM of column A          | 9.200 $\pm$ 0.2960, n=15 |
| Mean $\pm$ SEM of column B          | 6.857 $\pm$ 0.2310, n=14 |
| Difference between means            | -2.343 $\pm$ 0.3792      |
| 95% confidence interval             | -3.121 to -1.565         |
| R squared                           | 0.5857                   |
| F test to compare variances         |                          |
| F,DFn, Dfd                          | 1.759, 14, 13            |
| P value                             | 0.3169                   |
| P value summary                     | ns                       |
| Significantly different? (P < 0.05) | No                       |

## Example 6

**[0095]** Epidemiological studies suggest that the development of hypercholesterolemia in the mid- (as opposed to late-) stages of life associates with increased risk for Alzheimer's disease. Type 2 diabetes is also a major risk factor for dementia and is commonly associated with dyslipidemia. The inventors set out to demonstrate that adropin would be protective under such conditions and to this end, crossbreed transgenic mice to produce mice with severe metabolic dysregulation, which were also continuously exposed to elevated levels of adropin. Low-density lipoprotein receptor deficient (Ldlr<sup>-/-</sup>) mice were used as a model of hypercholesterolemia (see Ishibashi, et. al. J. Clin. Invest., 92 (1993), pp. 883-893). Adropin transgenic mice (AdrTG) were crossed onto the Ldlr<sup>-/-</sup> background (see Ghoshal et. al., Mol Metab. 2018 Feb;8:51-64.). It was found that the adropin transgene did not prevent hypercholesterolemia due to loss of LDLR.

**[0096]** To further enhance the effects of metabolic dysregulation in their transgenic mouse model, the inventors placed these mice on a high fat/high sugar diet (HDF) (Research Diets 12451, 45% kcal/fat, 35% kcal/sucrose, 20% kcal/protein) for 3 months. At the end of this time, spatial learning and memory testing was compared between AdrTG;Ldlr<sup>-/-</sup> mice and Ldlr<sup>-/-</sup> mice. A group of 10 age-matched male C57BL/6J mice maintained on standard low-fat rodent chow were included as controls.

**[0097]** Ldlr<sup>-/-</sup> mice (11 females, 7 males), AdrTG;Ldlr<sup>-/-</sup> mice (6 females, 10 males) and age-matched C57BL/6J mice (n=12) were placed on a high fat diet for 3 months. Note all transgenic mice are on the same C57BL/6J background. Body composition was determined at baseline and after 3 months, after which cognitive function was assessed by Novel Object Recognition and Aversive T-maze testing. All mice gained weight during the study irrespective of sex (Figure 13). Mice fed HFD gained more weight than B6 mice fed chow (13 grams vs. 3 grams). Body composition indicated increases in fat mass (FM) relative to total body weight in animals fed the HFD compared to the control group fed chow. (Figure 14)

**[0098]** Fed blood glucose levels in all animals were high (>350 mg/dL), indicating dysregulation of glucose metabolism. (Table 4)

**[0099]** Table 4. Blood glucose levels in transgenic mice on HFC diets

| Dependent Variable: Blood Glucose |      |            |                         |             |
|-----------------------------------|------|------------|-------------------------|-------------|
| group                             | Mean | Std. Error | 95% Confidence Interval |             |
|                                   |      |            | Lower Bound             | Upper Bound |
| Female, Ldlr-/-                   | 400  | 24         | 353                     | 448         |
| Male, Ldlr-/-                     | 443  | 29         | 384                     | 501         |
| Female, AdrTG;Ldlr-/-             | 374  | 29         | 315                     | 432         |
| Male, AdrTG;Ldlr-/-               | 462  | 25         | 411                     | 513         |
| B6 chow                           | 356  | 21         | 313                     | 399         |

**[00100]** Performance in the novel objective recognition (NOR) test was significantly improved in AdrTG;Ldlr-/- mice relative to Ldlr-/- mice, suggesting improved learning and memory. AdrTG;Ldlr-/- mice spent significantly more time exploring the novel object compared to Ldlr-/- mice. Values are adjusted for sex (Figure 15).

**[00101]** The inventors demonstrated that the adropin transgene improves cognitive function in a mouse model of type 2 diabetes and hypercholesterolemia. Older patients with type 2 diabetes who are at increased risk for dementia, and who may exhibit symptoms of mild cognitive impairment, could therefore benefit from treatment with adropin<sup>34-76</sup> administered by daily injection or any of the treatment methods discussed herein.

#### Materials and Methods for Examples 7-10

**[00102]** The Farr laboratory established procedures for assessing learning and memory function using active avoidance T-maze tasks. In the Examples that follow, researchers involved were “blinded” for the study. At the end of the trials, the key for the genotype was provided for the data analysis. Behavior studies were conducted between 7:30 AM and 11:00 AM. In the Examples that follow, Male C57BL/6J B6 mice subjected

to TBI, exhibit deficits of spatial learning, memory and executive functions or problem solving 1 month after the insult.

**[00103]** TBI PROCEDURE: Mice were exposed to mild closed-head concussive TBI. Mice were anesthetized with isoflurane 2-4% and placed under a device consisting of a Plexiglas tube (inner diameter 13 mm) placed vertically over the animal's head. Adequate anesthesia was determined by the lack of both the corneal reflex and toe pinch reflex. Each mouse received 90 sec Isoflurane anesthesia in the induction chamber. The injury was induced by dropping a 25-gm acrylic weight from 80 cm height down the Plexiglas tube, striking the skull. This weight was chosen based upon literature using this type of device (Tweedie et al. (2016) PLoS One. 11(6):e0156493; Zohar et al. (2011) Acta Neurobiol Exp. 71:36-45). The head was placed on an immobilization sponge board. The sham control mice were anesthetized and placed on the sponge board but will not receive the weight drop. Following Isoflurane anesthesia and the weight drop procedure, mouse respiration, heart rate and righting reflex was monitored to ensure recovery. The righting reflex time was recorded for each mouse. To observe the righting reflex, immediately following the TBI the mouse was placed on its back and the abdomen continuously stroked to provide tactile stimulation. When the mouse moves from a supine to a prone position, i.e., rights itself, the time will be recorded. Mice were housed singly for observation (not more than 30 min), and then will be returned to group housing when fully recovered from anesthesia. Full recovery from anesthesia will be identified by appropriate righting reflex, and normal ambulation and gait. One hour following the weight drop injury four reflexes were checked and recorded: the corneal reflex in both eyes, hindleg extension when held by the tail, startle response from a puff of air, and the ability to walk with a normal gait. Mice with abnormal reflexes or that are unable to ambulate normally 1 hour after TBI were eliminated from study and euthanized by CO<sub>2</sub> asphyxiation.

Assessment of Cognitive Function.

T-MAZE: The T-maze utilized was as described for Examples 1-7.

**[00104]** PUZZLE BOX: The arena consisted of a Plexiglas white box divided by a removable barrier into two compartments: a brightly-lit start zone (58 cm long, 28 cm

wide) and a smaller covered goal zone (15 cm long, 28 cm wide). Introduced into the start zone, mice were trained to move into the goal zone through a narrow underpass (~ 4 cm wide) located under the barrier. Mice underwent a total of nine trials (T1–T9) over 3 consecutive days, with three trials per day, during which they were challenged with obstructions of increasing difficulty placed at the underpass. On day 1 (training), the underpass was be un-blocked, and the barrier will have an open door over the location of the underpass during T1. On T2 and T3 the barrier had no doorway and mice entered via the small underpass. On day 2 (burrowing puzzle), T4 was identical to T2 and T3. On T5 and T6, however, the underpass was filled with sawdust and mice had to dig their way through. On day 3 (plug puzzle), T7 will be a repetition of T5 and T6. However, on T8 and T9, mice were presented with the plug puzzle, with the underpass was obstructed by a cardboard plug that mice had to pull with teeth and paws to enter the goal zone. This sequence allows for the assessment of problem-solving ability (T5 and T8), and learning/short-term memory for species-specific or instrumental responses (T3, T6, and T9; while the repetition on the next day provides a measure of long-term memory (T4 and T7).

**[00105]** ELEVATED PLUS MAZE: To determine if anxiety is involved in learning and memory differences, the inventors tested the mice in the elevated plus maze. The Elevated Plus maze utilized was as described for Examples 1-7. In brief, the test consisted of placing a mouse in the central platform facing an enclosed arm and allowing it to freely explore the maze for 5 min. The test arena was wiped with a damp cloth after each trial. The number of entries into the open and closed arms and the time spent in open arms were recorded by the ANY-maze. Anxiolytic activity was indicated by increase in time spent in closed arms and the number of open arms entries. The percentage of time spent in the open arms was also be calculated as well as the frequency of arm changes.

**[00106]** TREATMENT: Male B6 mice treated with 450 nmol/kg adropin<sup>34--76</sup>, administered by intravenous injection at 1h post-injury and treated again with 450 nmol/kg by intraperitoneal injection at 24h post-injury. Adropin<sup>34--76</sup> treated mice exhibited protection from deficits in spatial learning and memory. In addition, male B6

mice genetically engineered to over-express the full-length adropin sequence also exhibited protection from deficits in spatial learning and memory.

#### Example 7

##### T-Maze Measure of Retention

**[00107]** TBI mice that were administered adropin, were better able to retain memory of the maze than TBI mice treated with saline. In fact, TBI + adropin treated mice were statistically the same as mice that did not receive TBI and were treated with saline. (See Figure 16). Figure 16 (A) illustrates the mean number of trials required for TBI mice to achieve the criterion of the T-maze. TBI mice followed by adropin treatment (TBI+Adro), TBI mice followed by saline treatment (TBI+Vhe), and uninjured mice followed by saline treatment (SHAM+Vhe), were subjected to the T-maze retention assessment as described above. TBI+ adropin treated mice were significantly improved compared to mice that were subjected to TBI followed by saline treatment. In addition, TBI+ adropin treated mice were not significantly different than uninjured vehicle treated control mice (\*\* indicates 99% confidence)

#### Example 8

**[00108]** Transgenic Adropin mice were better able to retain memory following TBI than similarly treated wild type mice as determined by the T-maze retention assessment (see Figure 16). Figure 16 (B) illustrates the mean number of trials required for Wild type (Wt) and Transgenic Adropin mice (Tg) in the T-maze retention maze following TBI. Wild type uninjured mice (WT Sham) and Wild type TBI mice (Wt TBI), along with, transgenic adropin uninjured mice, (Tg Sham), and transgenic adropin TBI mice, (TG TBI), were compared in the T-maze Retention assessment described above. (\*\* indicates 99% confidence) TBI transgenic adropin mice were not significantly different from uninjured transgenic or wild type control mice and were significantly improved compared to TBI wild type mice, in the T-maze retention assessment (see Figure 16).

#### Example 9

**[00109]** A measurement of cytokines in TBI and non-injured adropin treated mice indicates that some cytokines maybe reduced by adropin treatment. The inventors compare cytokine levels in control and adropin treated mice following TBI (see Figure 17). Shown are the plasma cytokine levels of mice that were either: uninjured, subjected to TBI followed by adropin and subjected to TBI followed by saline. The results indicate **a** possible reduction in inflammatory responses to TBI in mice receiving adropin injections.

#### Example 10

**[00110]** The Inventors examined Hippocampal gene expression and found cytokine and growth factor expression were altered by adropin treatment. The expression of cytokines in the hippocampus of TBI mice (TBI+veh) increased compared to uninjured controls (Sham) (significant for IL16, CCL2, IL17). TBI also appears to result in changes in expression of growth factors (BDNF, VEGF). However, if TBI was followed by adropin treatment (TBI+Adr), these changes are attenuated in most cases (see Figure 18).

**[00111]** All publications and patents cited in this specification are hereby incorporated by reference in their entirety. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

## CLAIMS

1. What is claimed is:
2. A method of treating cognitive decline, in a subject in need, the method comprising administering an effective amount of (SEQ ID NO.:2) or (SEQ ID NO.:1, to the subject parenterally.
3. The method of claim 1, wherein the subject in need is selected from the group consisting of a subject suffering from: traumatic brain injury, neurological trauma, neuroinflammation, and concussion.
4. The method of claim 1, wherein the subject is suffering from advanced age.
5. The method of claim 1, wherein the effective amount is 1000 nmol/kg/day to about 1 nmol/kg/day.
6. The method of claim 1 wherein, an effective amount is about 450 nmol/kg/day.
7. The method of claim 1, wherein the effective amount is about 90 nmol/kg/day.
8. The method of claim 1, wherein the subject is a human subject.
9. The method of claim 1, wherein the subject is a post-surgery or post brain injury, with symptoms of neuroinflammation.
10. The method of claim 1, wherein the effective amount is administered one or more times within 24 hours of a traumatic brain injury event.
11. The method of claim 1, wherein the effective amount is administered one or more times within 48 hours of a traumatic brain injury event.
12. The method of claim 1 wherein the effective amount is administered one or more times a day, over a period of 1 or more weeks.
13. The method of claim 1 wherein administering an effective amount consists of administering an oligonucleotide enabled to express (SEQ ID NO.:2) or (SEQ ID NO.:1).

14. The method of claim 1, wherein, parenterally administration is selected from the group consisting of intraperitoneal, subcutaneous, intramuscular, and intravenous injection.
15. The method of claim 1, further comprising a response from the subject wherein the subject's cognitive decline is arrested, reduced or slowed.
16. A method of treating neuroinflammation in a subject in need, the method comprising administering an effective amount of (SEQ ID NO.:2), (SEQ ID NO.:1), to the subject parenterally.
17. A method of treating cognitive decline, in a subject in need, the method comprising administering an effective amount of (SEQ ID NO.:2), (SEQ ID NO.:1), or variants or derivatives thereof, to the subject parenterally, wherein the subject's cognitive decline is arrested, reduced or slowed.
18. The method of claim 16, wherein the variants or derivatives thereof, are polypeptides with conservative amino acid substitutions.
19. The method of claim 16, wherein the variants or derivatives thereof, are polypeptides with 90 percent identity or greater.
20. A composition for treating cognitive decline, comprising (SEQ ID NO.:2), (SEQ ID NO.:1), variants or derivatives thereof, prepared in pharmaceutically acceptable aqueous solution.
21. The composition of claim 19, wherein, ((SEQ ID NO.:2), (SEQ ID NO.:1), variants or derivatives thereof, is selected from the group consisting of (SEQ ID NO.:2) and (SEQ ID NO.:1).
22. The composition of claim 19, further comprising one or more pharmaceutically acceptable compounds selected from the group consisting of, salts, proteins, surfactants, polypeptides, and preservatives.
23. The composition of claim 19, further comprising one or more compounds selected from the group consisting of: glycerol, liquid polyethylene glycol, and pharmaceutically acceptable oils.

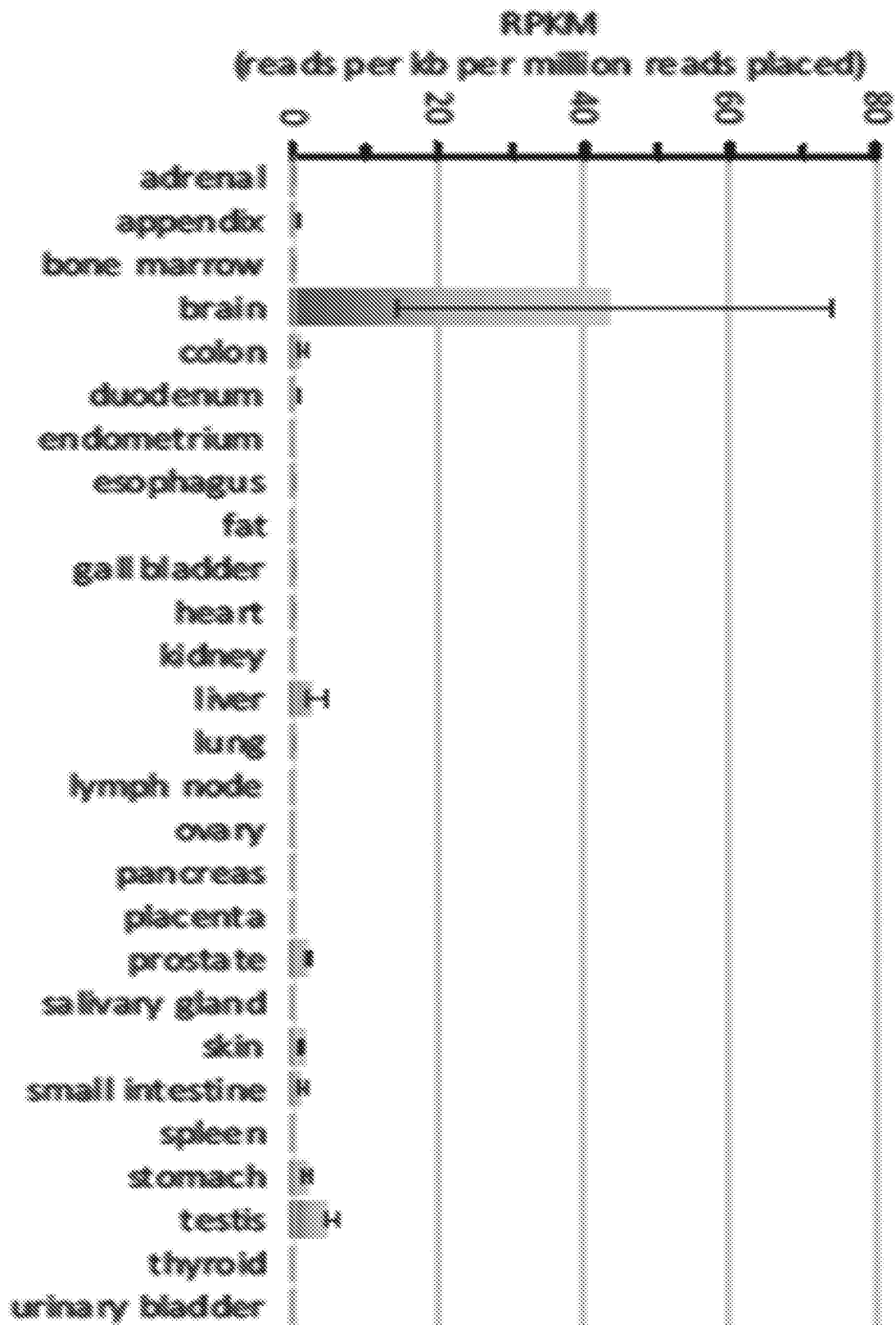


Figure 1

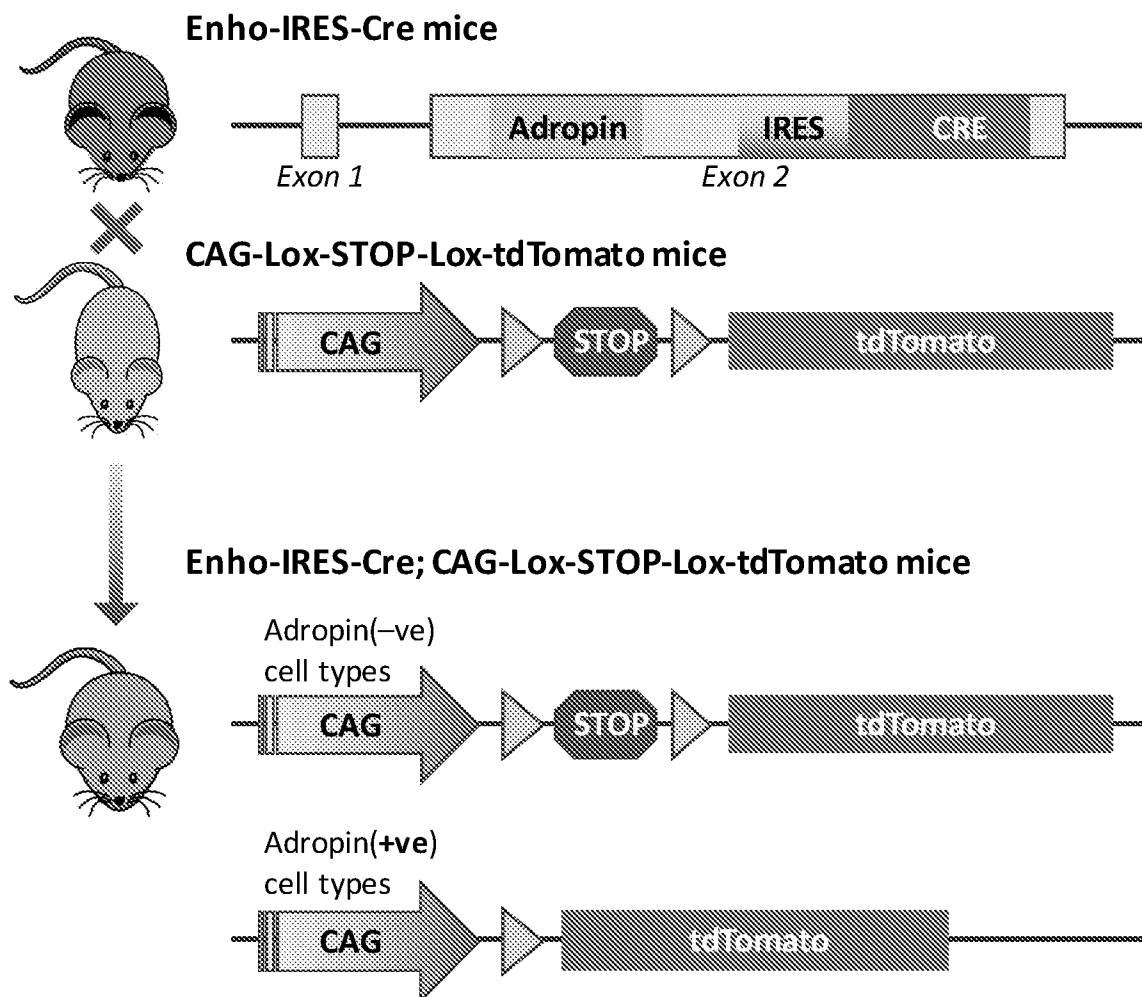


Figure 2

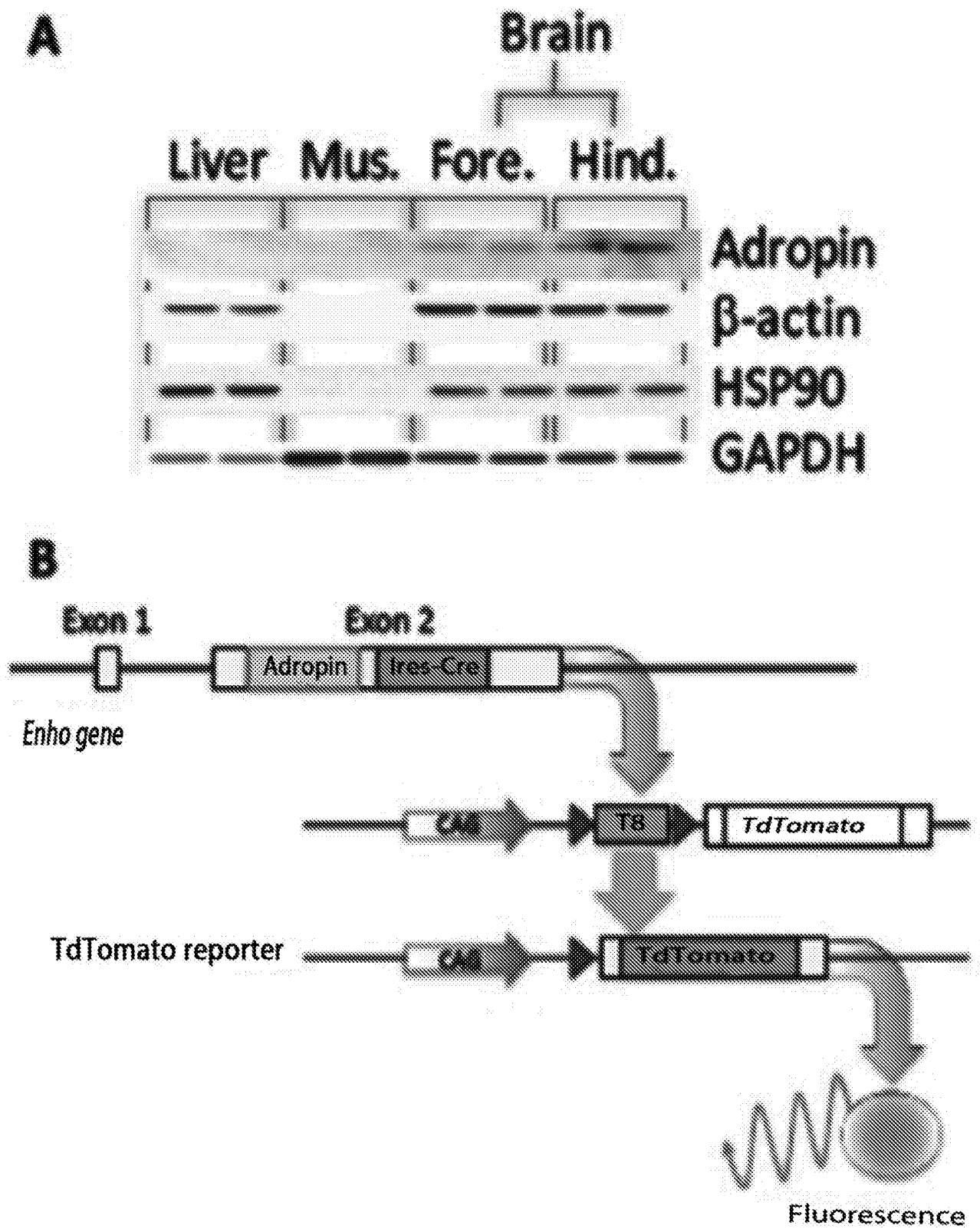


Figure 3

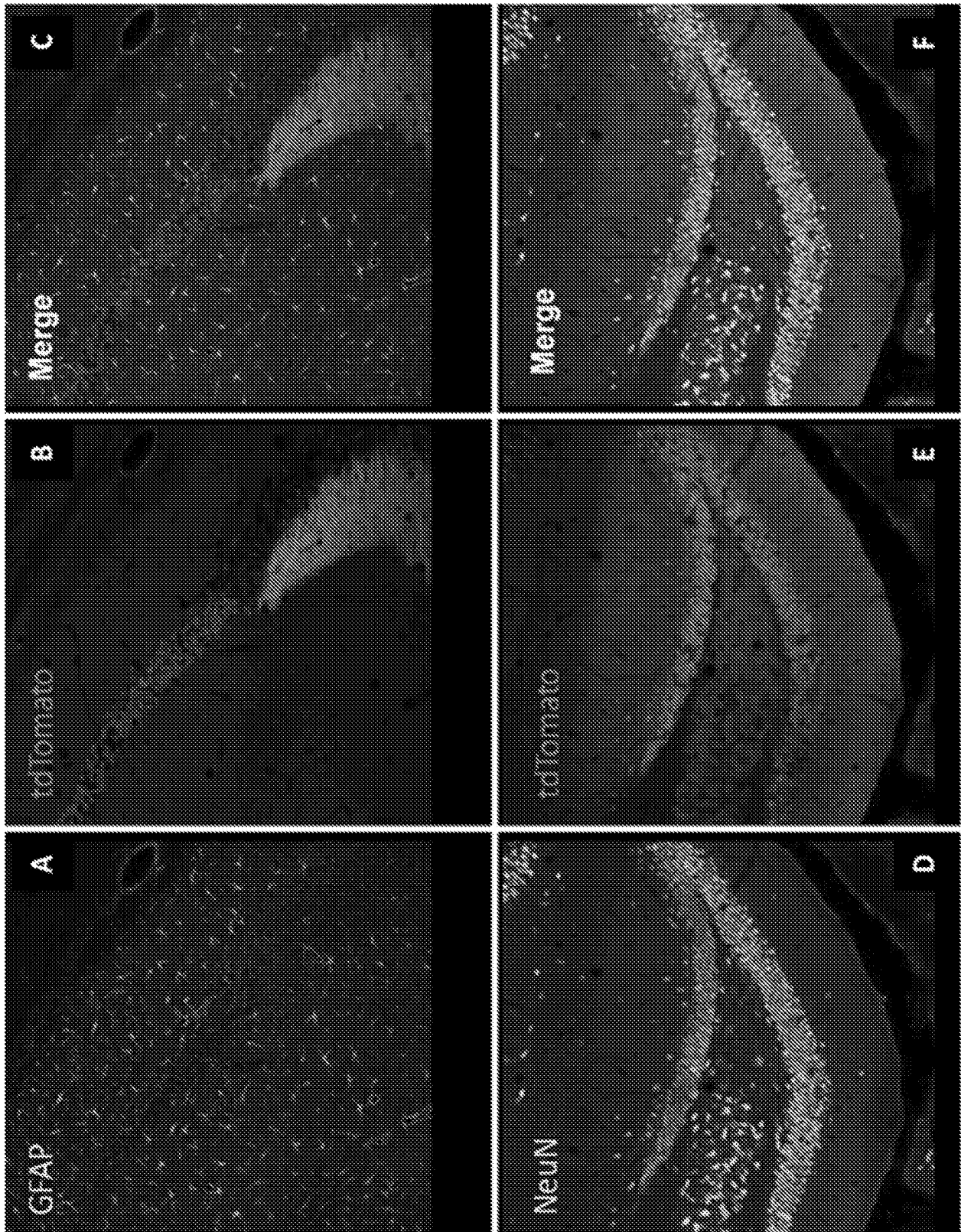


Figure 4

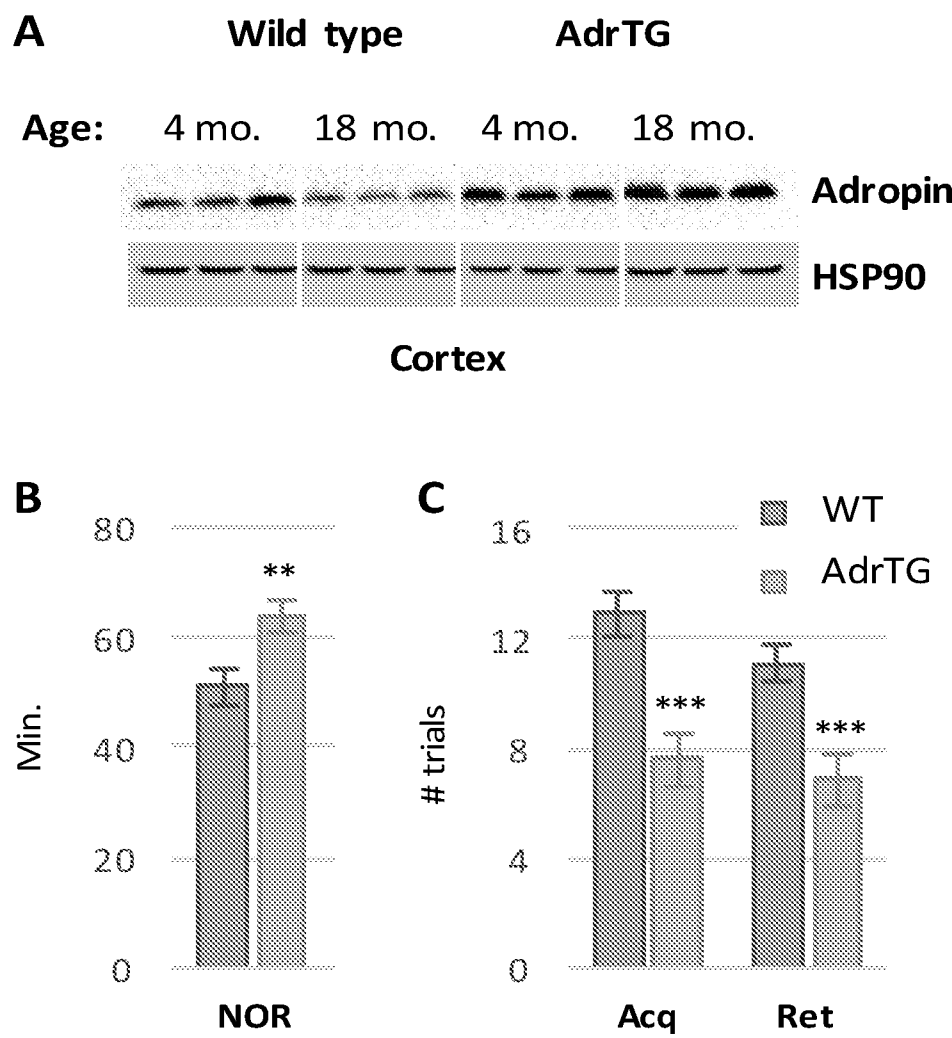


Figure 5

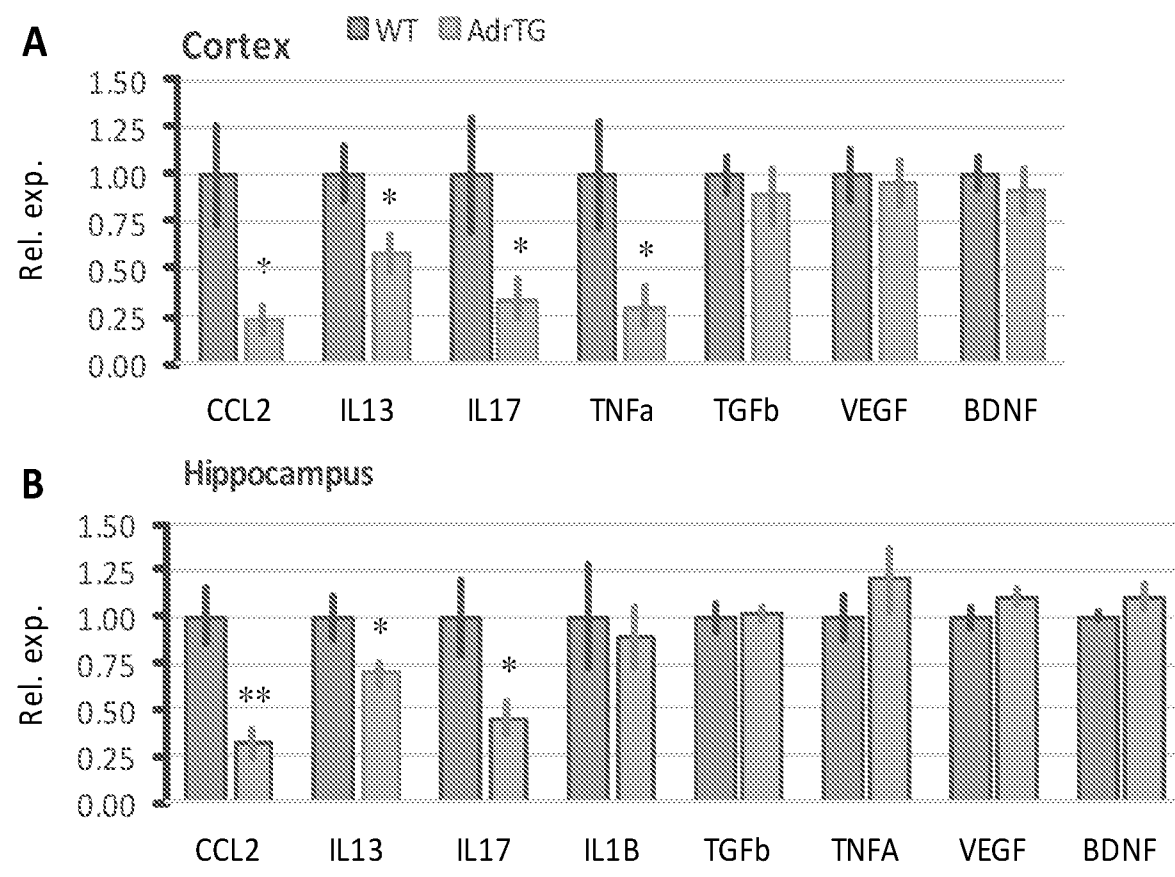


Figure 6

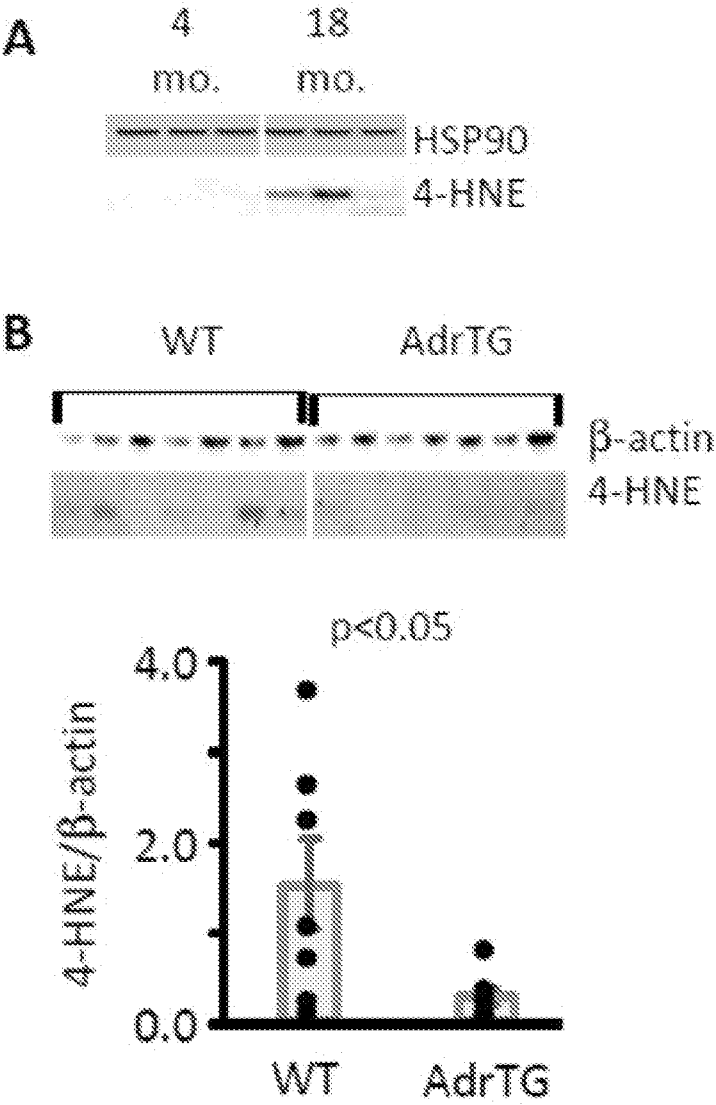
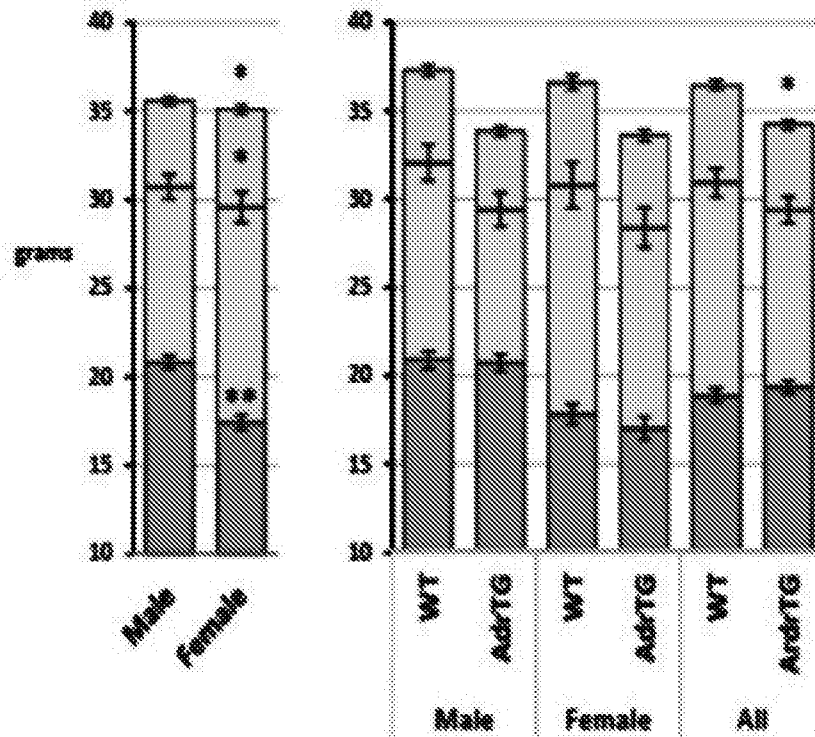
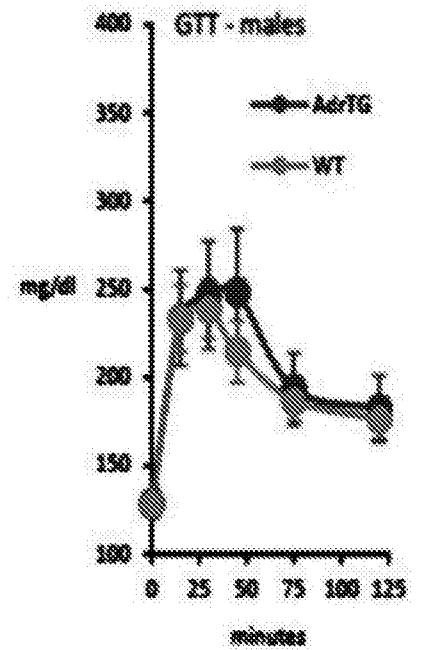


Figure 7

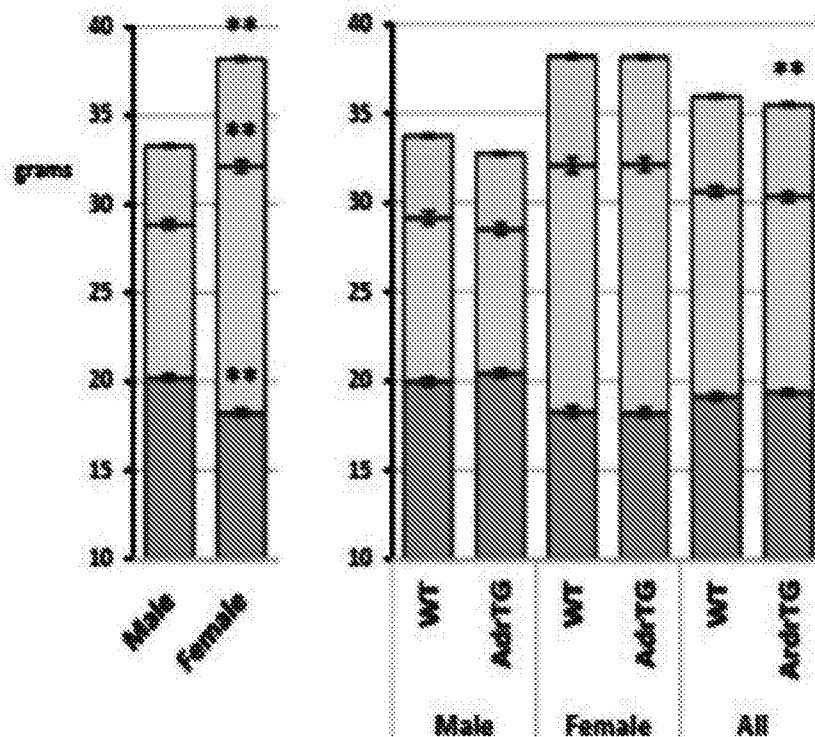
A. Actual values



C.



B. Estimated marginal means adjusted for total body mass



D.

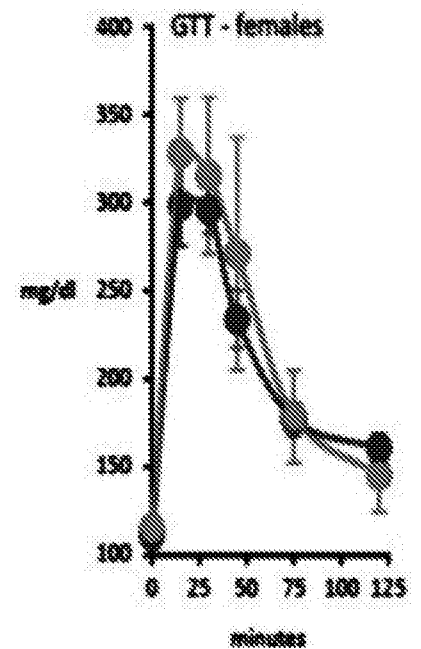


Figure 8

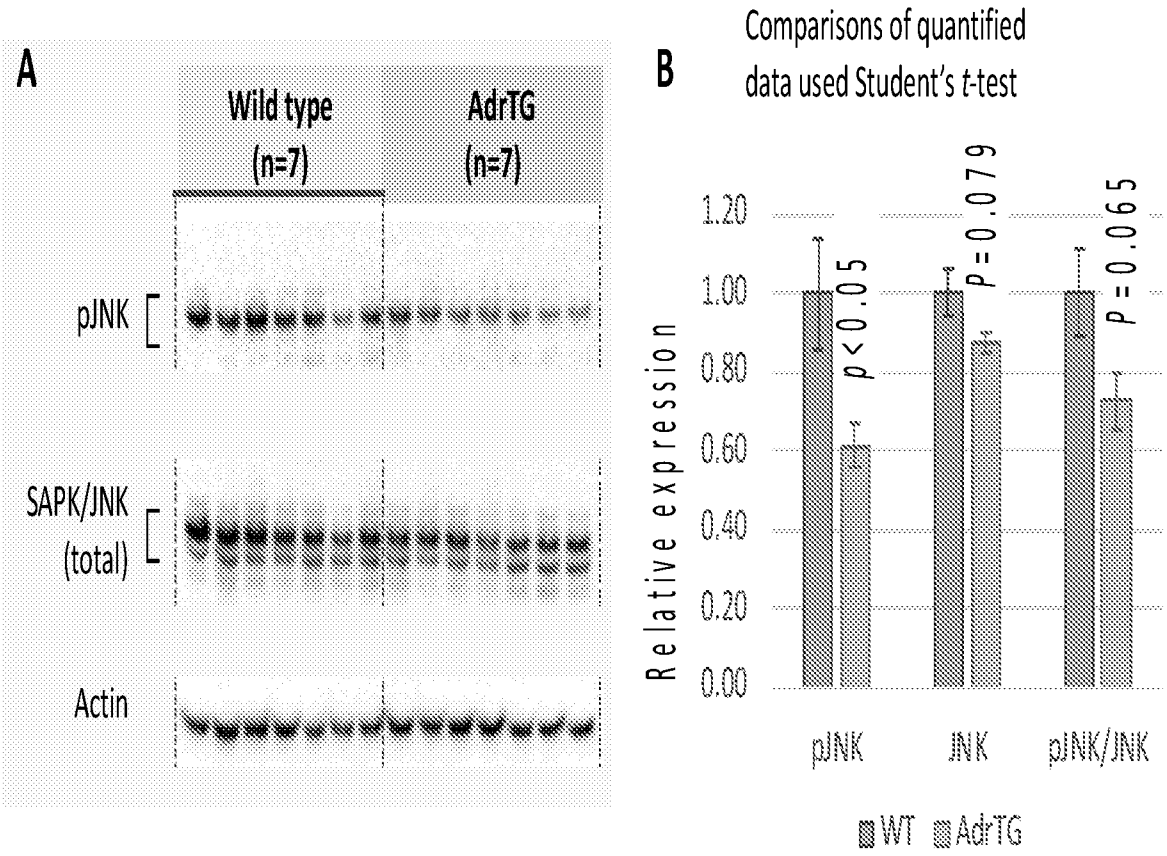


Figure 9

## Body weight (mean, SEM) during treatment

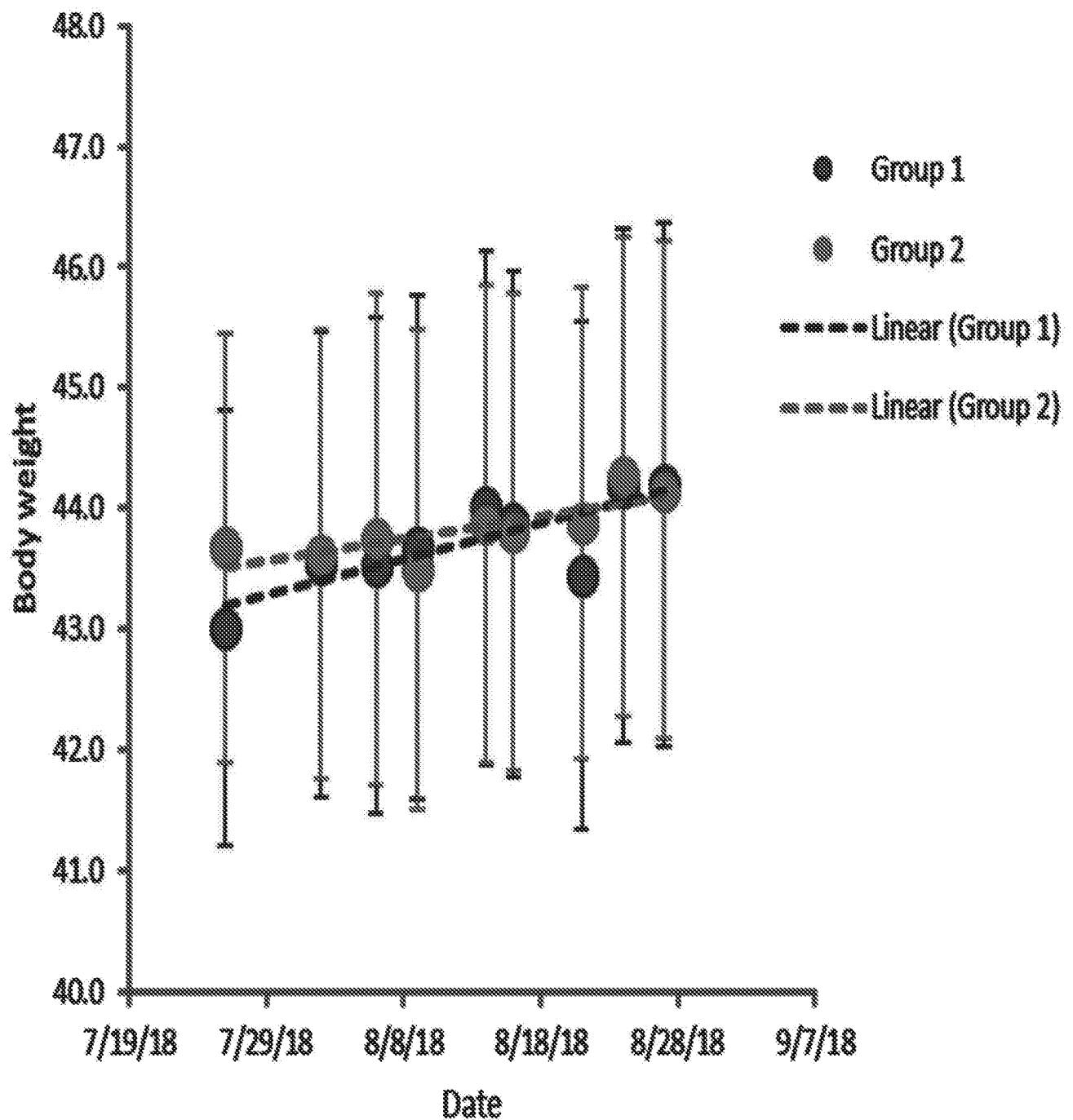
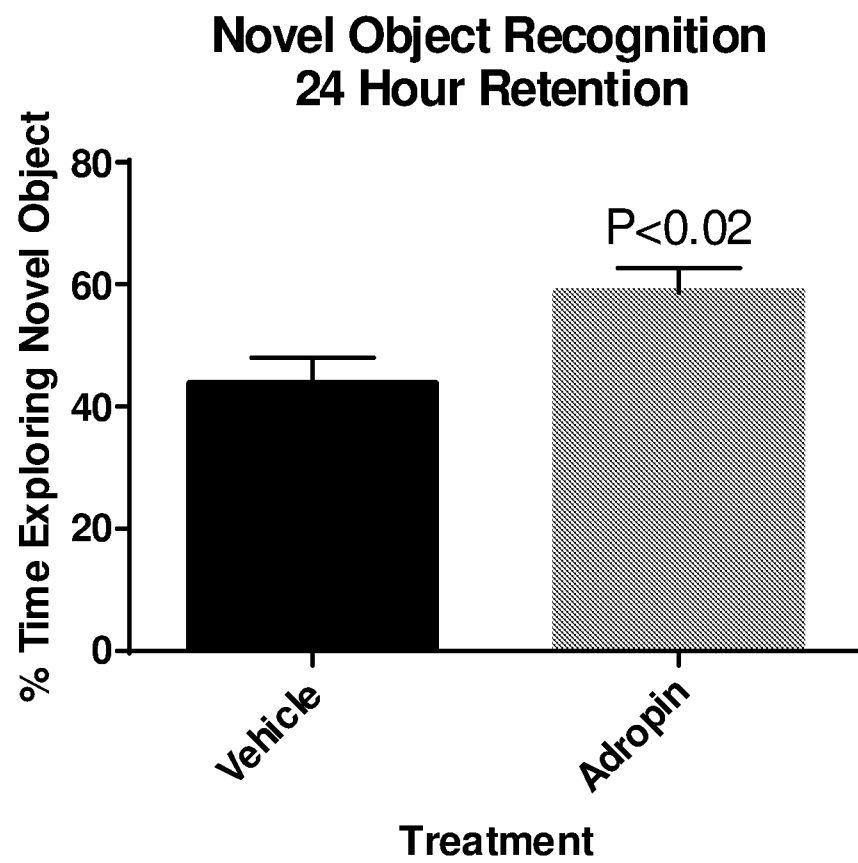


Figure 10

**Figure 11**

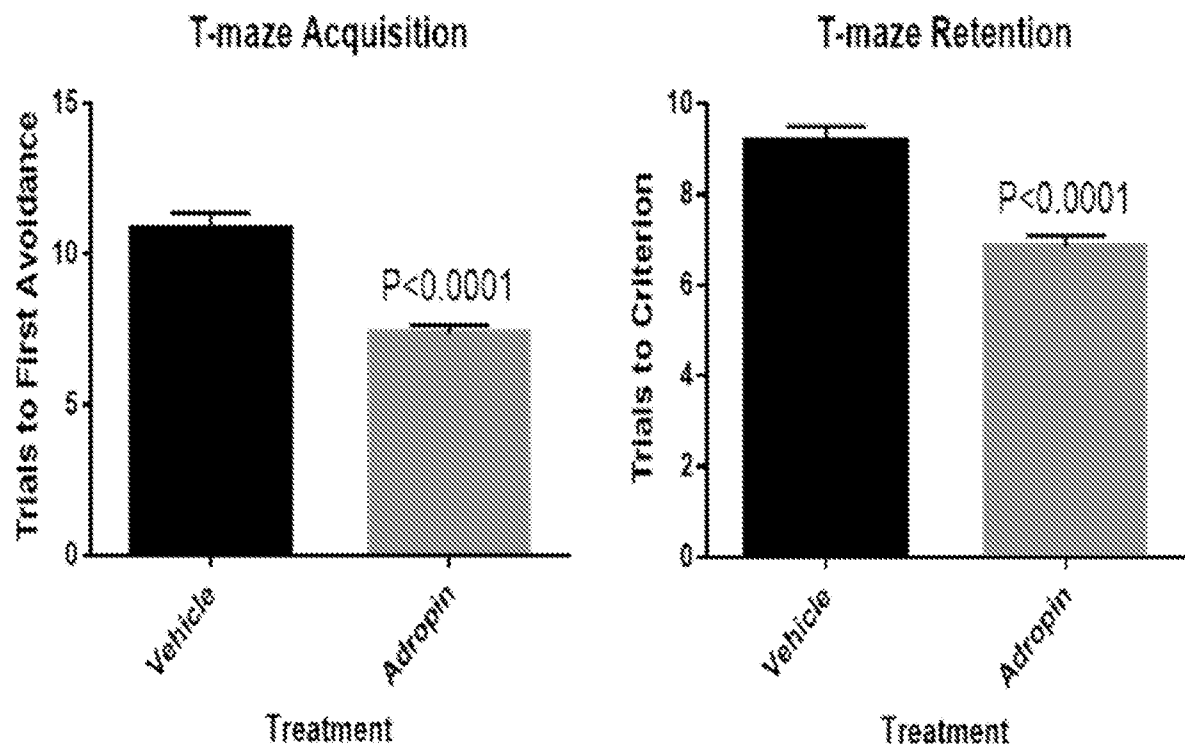
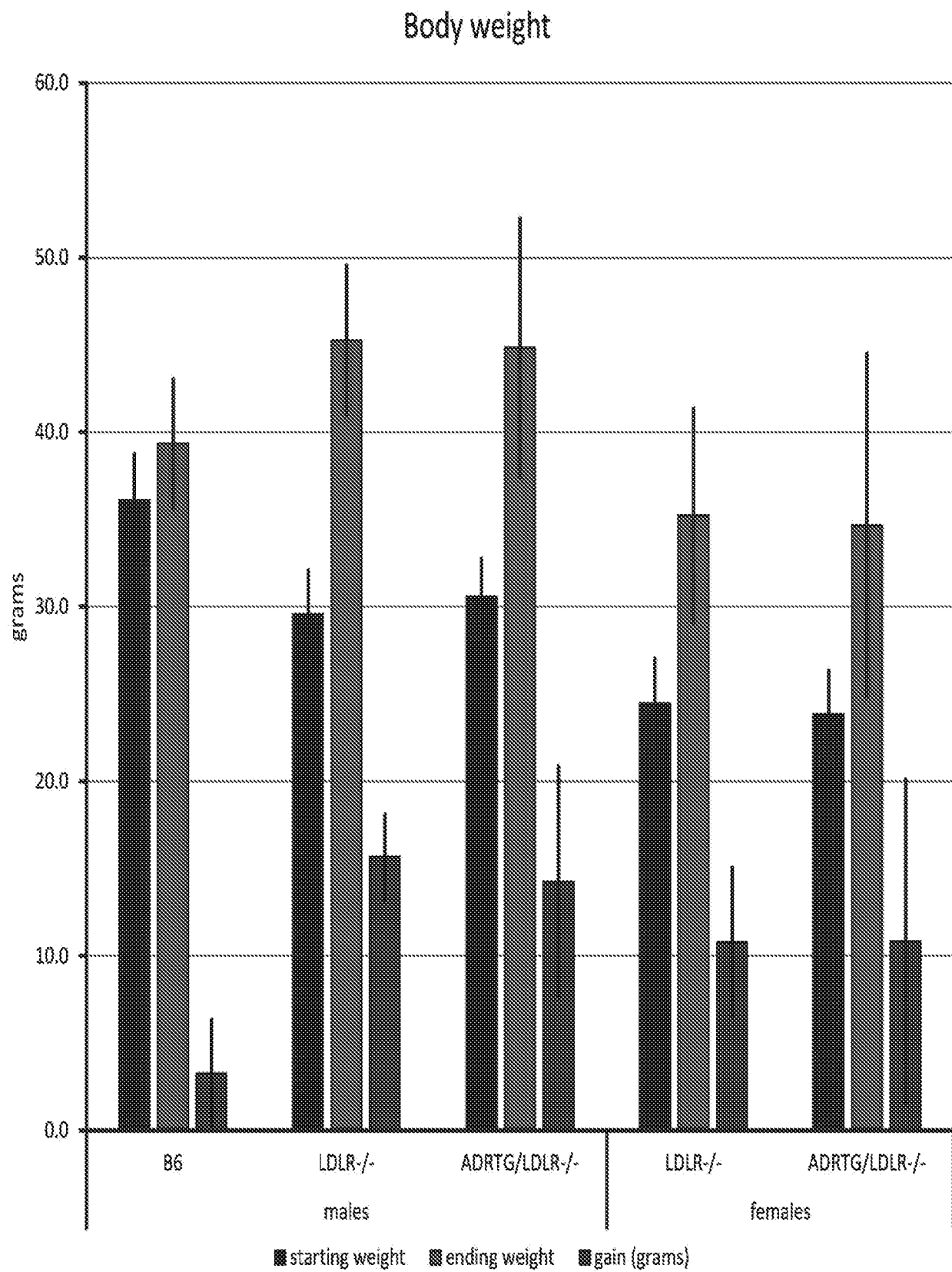


Figure 12

**Figure 13**

## Body composition relative to total body weight

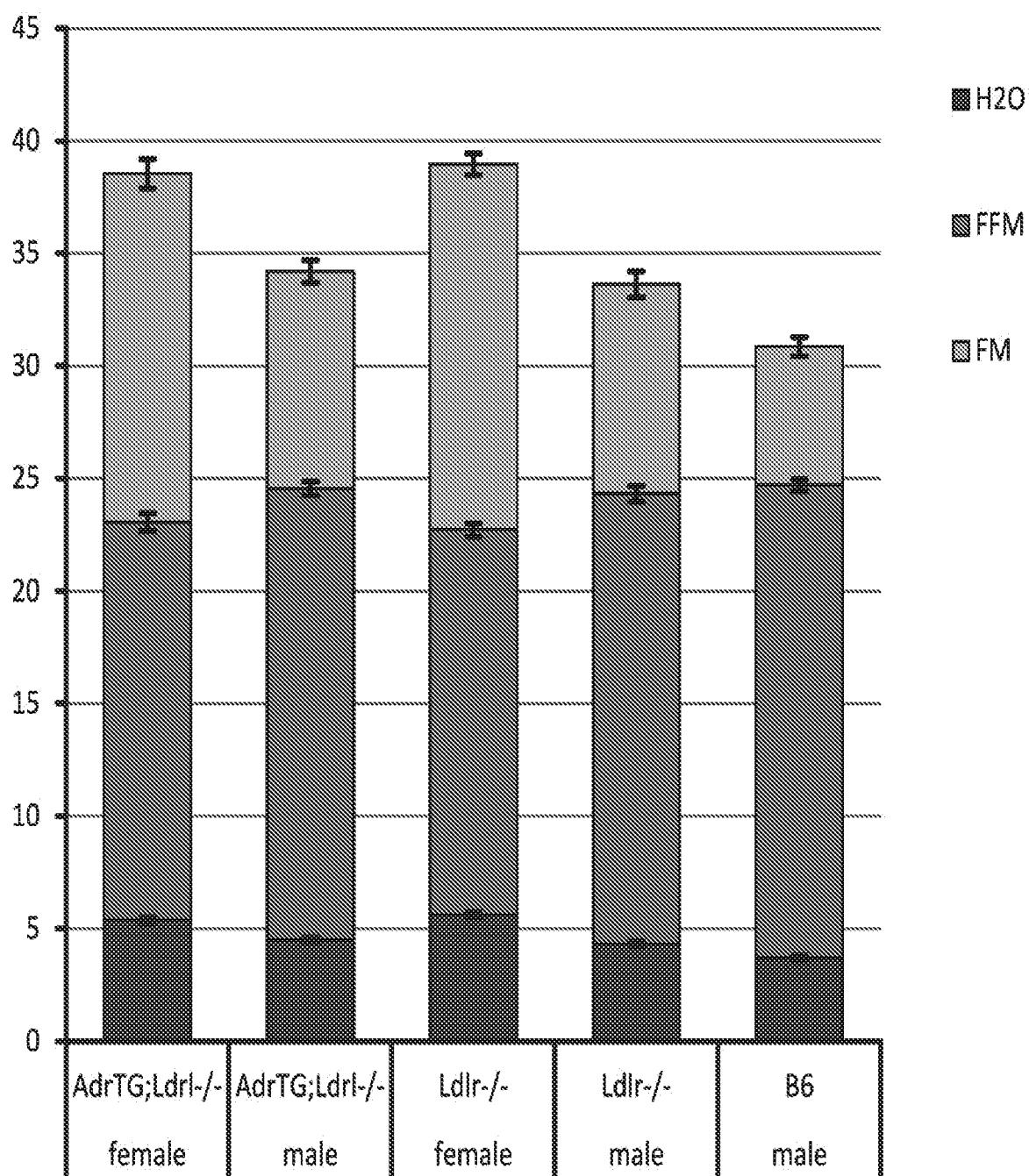


Figure 14

## Discrimination index (deviation from 50%)

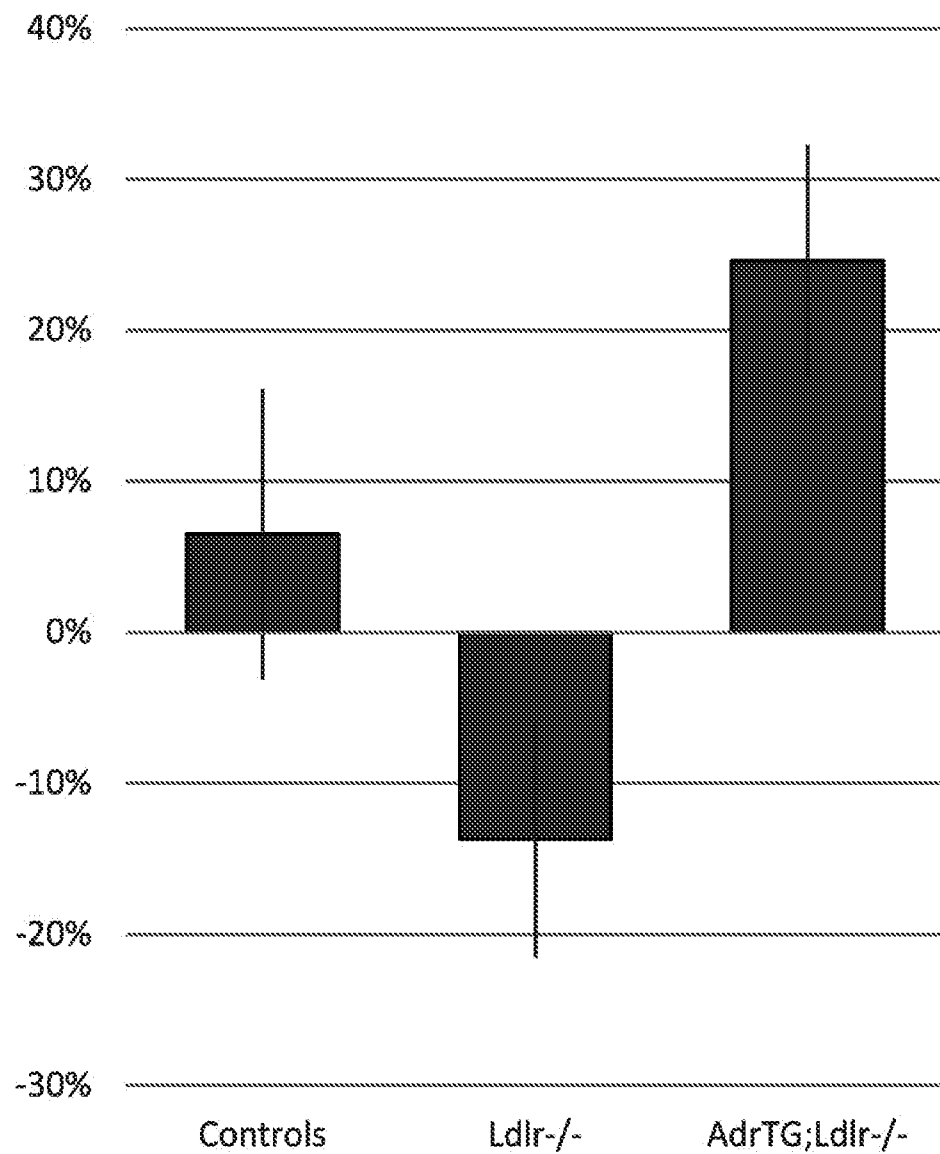


Figure 15

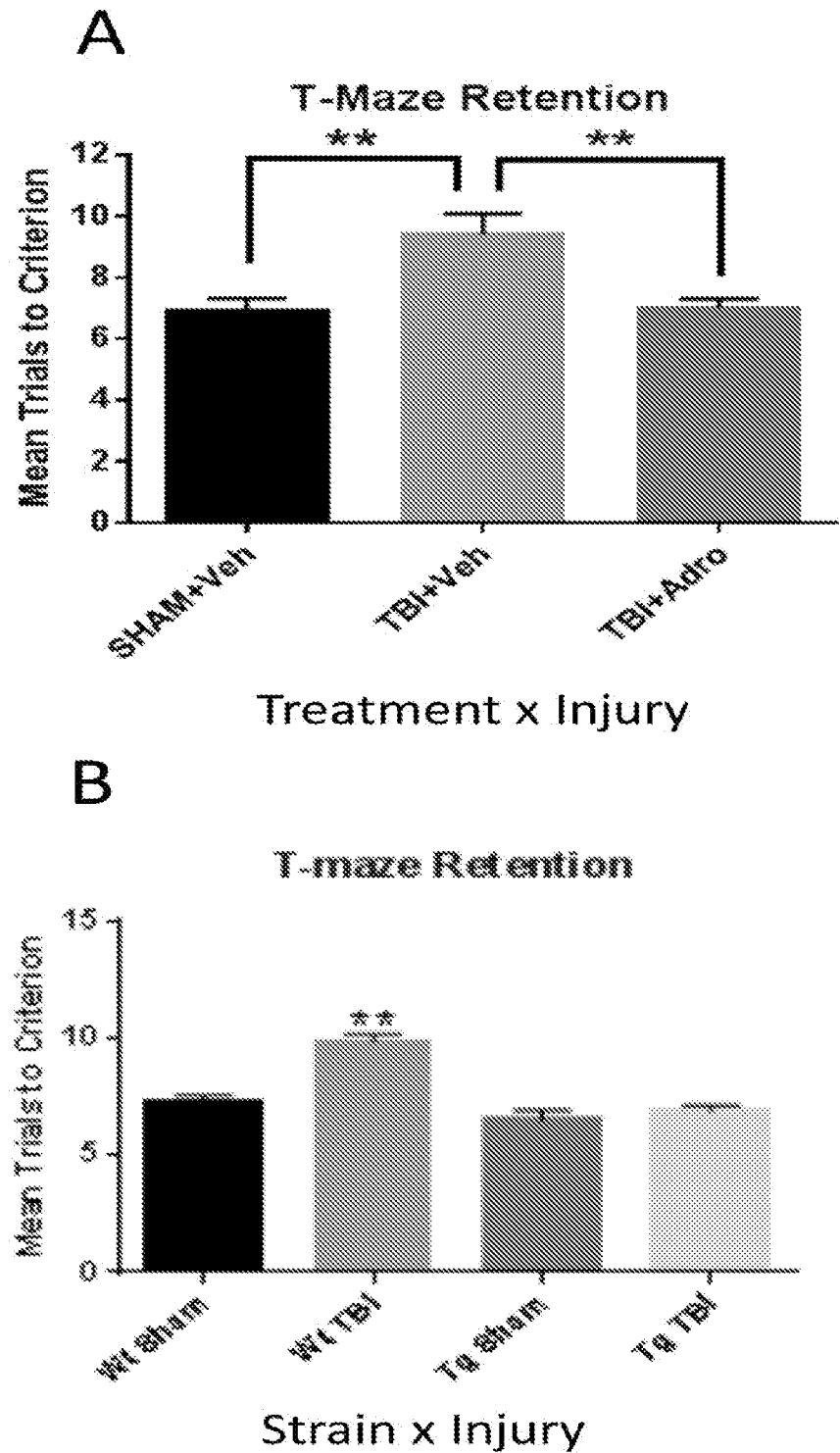


Figure 16

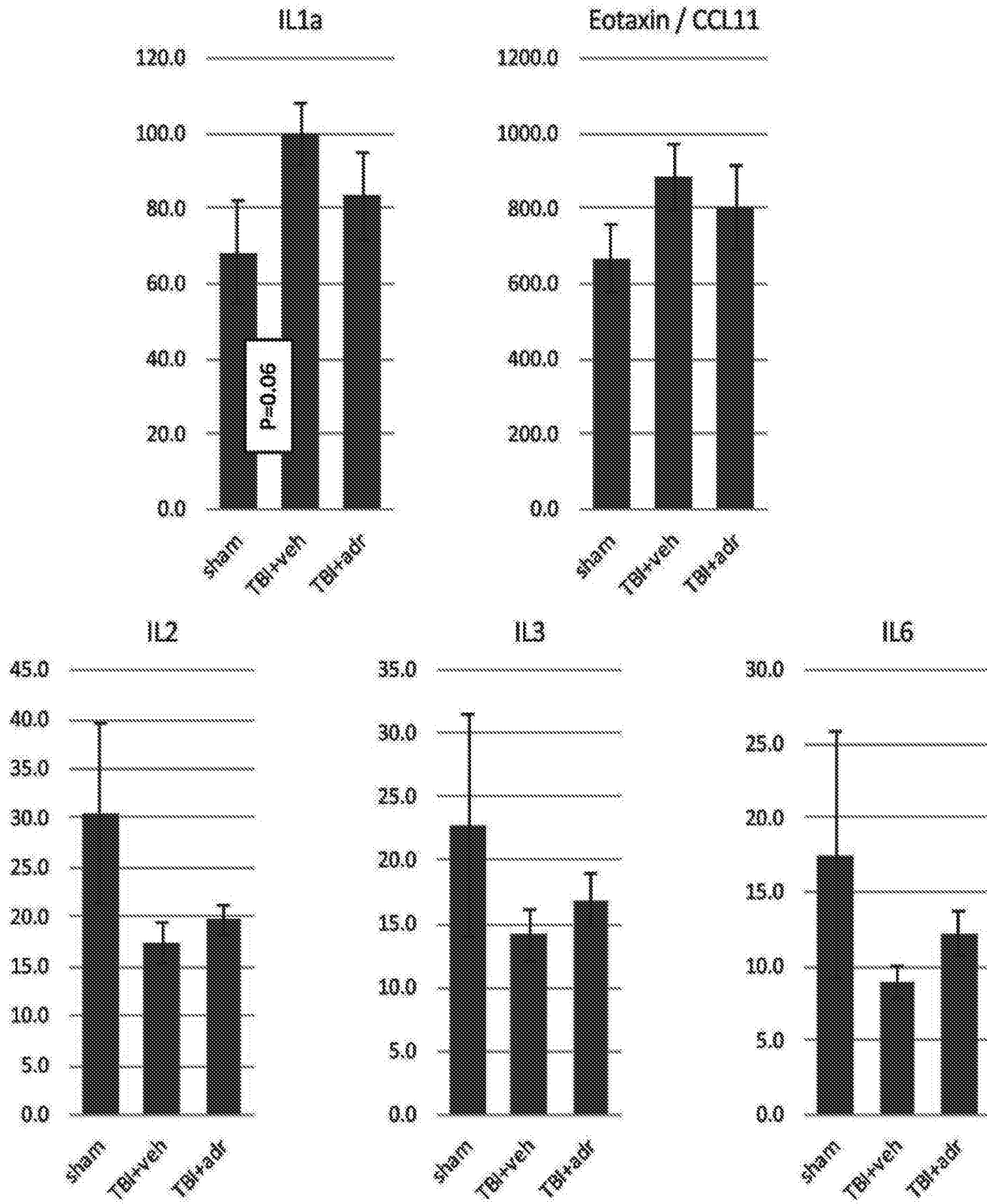


Figure 17

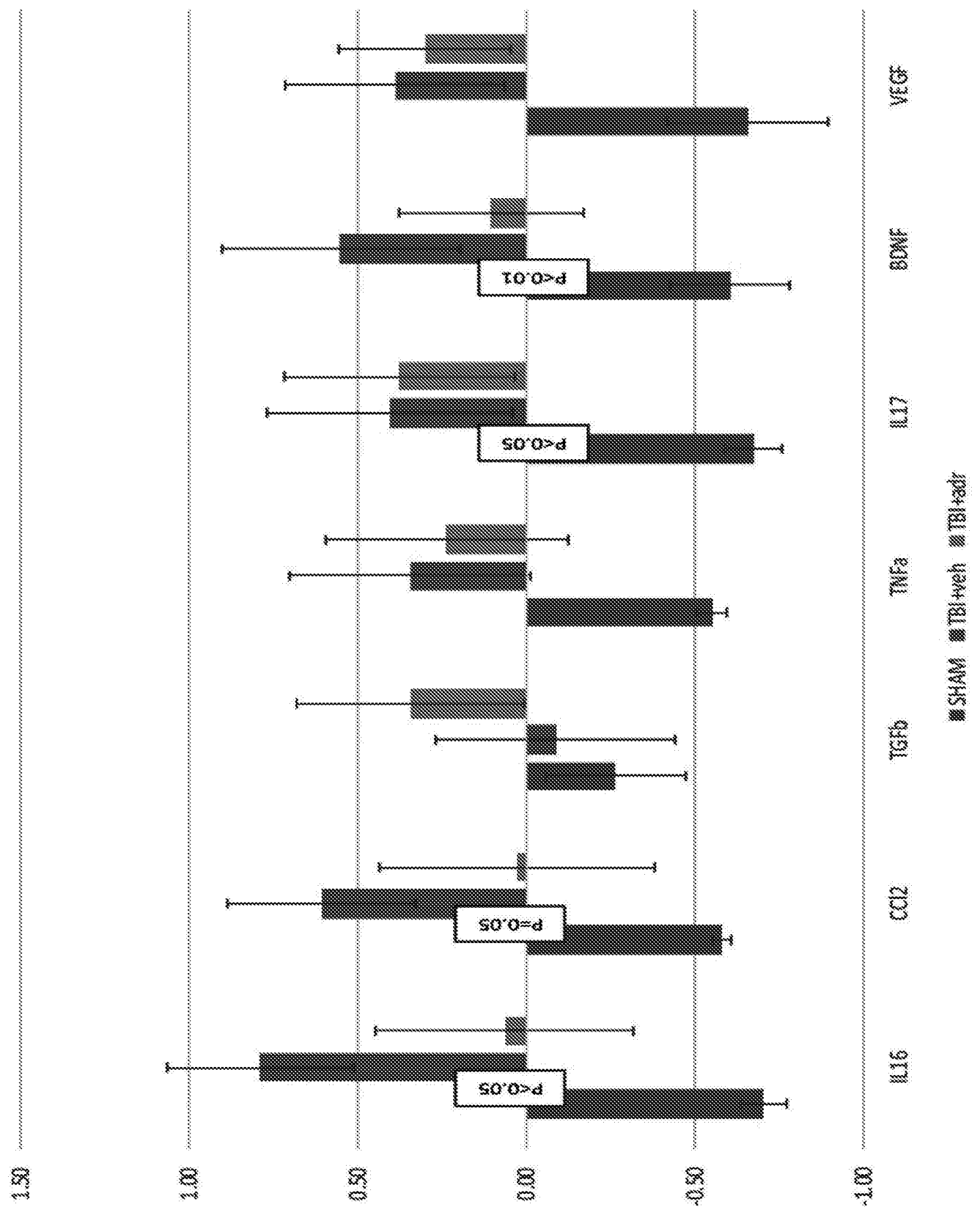


Figure 18

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/60836

## A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/17 (2020.01)

CPC - A61K 38/22, A61K 38/2264, G01N 33/5058, G01N 2800/2821

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                   | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Y         | YU et al. "Adropin preserves the blood-brain barrier through a Notch1/Hes1 pathway after intracerebral hemorrhage in mice" J. Neurochem. 2017, Vol 143, pages 750-760; abstract, pg 750, col 1, para 1, pg 751, col 2, para 3, pg 753, col 1, para 1                                                                                                                                                                 | 2-19                  |
| Y         | US 2009/0253619 A1 (BUTLER et al.) 08 October 2009 (08.10.2009) Claim 1, Claim 14, Claim 19, Claim 38, para [0125], [0136], [0187], [0189]                                                                                                                                                                                                                                                                           | 2-23                  |
| Y         | FARR et al. "Reduced Neuroinflammation and Improved Memory In Old Male C57bL/6J Mice Overexpressing Adropin" Alzheimer's & Dementia, July 2018, Vol 14, No 7, Supplement, Page P736 [online]. [Retrieved 29 January 2019]. Retrieved from the internet: <URL: <a href="https://aandjournal.net/article/S1552-5260(18)31037-9/fulltext">https://aandjournal.net/article/S1552-5260(18)31037-9/fulltext</a> > abstract | 4, 9, 16, 18-23       |

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

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"D" document cited by the applicant in the international application

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

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

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

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

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

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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

29 January 2020

Date of mailing of the international search report

18 FEB 2020

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