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(54) **Title:** FIBROBLAST GROWTH FACTOR 21 (FGF21) GENE THERAPY

(57) **Abstract:** Described herein is a gene construct comprising a nucleotide sequence encoding a fibroblast growth factor 21 (FGF21), for use in the treatment and/or prevention of a metabolic disorder, wherein the therapy involves expression of the gene construct in the central nervous system (CNS).

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Fibroblast growth factor 21 (FGF21) gene therapy

Background

5 The prevalence of diabetes is growing at an alarming rate and is a major health problem worldwide. Obesity is strongly associated with insulin resistance and type 2 diabetes (T2D) (Moller, D. E., and Flier, J. S., 1991. N. Engl. J. Med. 325:938-948). Both T2D and obesity increase the risk of mortality (Peeters, A. et al., 2003. Ann. Intern. Med. 138:24-32) and also increase the risk of highly morbid chronic diseases, including cardiovascular disease, hypertension and certain types of cancers (Haslam, D. W. et al., 2005, 10 Lancet. 366, 1197–1209; Roberts, D. L. et al., 2010, Annu. Rev. Med. 61, 301–316). Insulin resistance and obesity-associated diseases are subsequently linked to reduced life expectancy and poor quality of life.

It is now well-accepted that during obesity there is a chronic, low-grade, inflammation in peripheral tissues, such as adipose tissue, liver, or skeletal muscle that may be responsible for metabolic 15 dysfunction, including the development of insulin resistance (Valdearcos, M. et al., 2015, Annu. Rev. Physiol. 77, 131–160; Hotamisligil, G. S. et al., 2017, Nature. 542, 177–185). Recently, a growing body of literature has demonstrated that obesity and insulin resistance are also associated with inflammation in the brain (Guillemot-Legris, O. et al., 2017, Trends Neurosci. 40, 237–253; Beilharz, J.E. et al., 2016, Behav. Brain Res. 306, 1–7). Moreover, obesity and insulin resistance are not only linked to 20 neuroinflammation but also with deficits in cognitive function in animal models and humans (Guillemot-Legris, O. et al., 2017, Trends Neurosci. 40, 237–253).

Fibroblast growth factor 21 (FGF21), a growth factor predominantly secreted by the liver, but also by adipose tissue and pancreas (Muise, E. S. et al., 2008. Mol. Pharmacol. 74:403-412), has been shown 25 to increase brown adipose tissue (BAT) growth and expression of thermogenic genes in BAT and white adipose tissue (WAT), stimulating energy expenditure (Coskun, T. et al., 2008. Endocrinology 149:6018-6027; Fisher, F. M. et al., 2012. Genes Dev. 26:271-281; Kharitonenkov, A. et al., 2005. J. Clin. Invest 115:1627-1635; Konishi, M. et al., 2000. J. Biol. Chem. 275:12119-12122; Tomlinson, E. et al., 2002. Endocrinology 143:1741-1747; Xu, J. et al., 2009. Diabetes 58:250-259).

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Native FGF21 protein exhibits poor pharmacokinetic characteristics. It has a short half-life, and it is susceptible to *in vivo* proteolytic degradation and *in vitro* aggregation (Huang, J. et al., 2013. *J Pharmacol Exp Ther.* 346(2):270-80; So, W. Y. and Leung, P.S. 2016. *Med Res Rev.* 36(4):672-704; Zhang, J. and Li, Y. 2015. *Front Endocrinol (Lausanne).* 6:168). Various engineering approaches have been developed 35 to extend the half-life and to improve the stability and solubility of FGF21. Currently, two engineered FGF21 mimetics (LY2405319 and PF-05231023) are being tested in humans. Nevertheless, those FGF21 mimetics require multiple administrations, which poses a significant burden to the patients. Moreover, engineered FGF21 mimetics/analogs may exhibit a higher risk of immunogenicity than native FGF21, e.g. patients treated with LY2405319 developed injection site reactions, anti-drug antibodies and 40 a serious hypersensitivity reaction (Gaich, G. et al., 2013. *Cell Metab.* 18(3):333-40). Thus, the long-term

and effective expression provided by a single administration of the vectors of the invention represents a significant advantage over other therapies.

Given the importance that neuroinflammation seems to play in the cognitive decline and whole-body energy and glucose metabolism observed in diabetes and obesity, new therapeutic approaches addressing the inflammation of the central nervous system (CNS) may be of compelling importance. Recent studies have shown that FGF21 peripheral metabolic effects may indeed be mediated by FGF21 signalling in the CNS, particularly in the hypothalamus, which is the major site of the brain regulating whole-body energy metabolism (D. A. Sarruf *et al.*, *Diabetes.* **59**, 1817–1824 (2010); A. L. Bookout *et al.*, *Nat. Med.* **19**, 1147–1152 (2013); B. M. Owen *et al.*, *Cell Metab.* **20**, 670–677 (2014); N. Douris *et al.*, *Endocrinology*. **156**, 2470–2481 (2015).

Field

Aspects herein pertain to the medical field, comprising gene therapy compositions for use in the treatment of a metabolic disorder in mammals, particularly in human beings.

Summary

In a first aspect, there is provided a gene construct comprising a nucleotide sequence encoding a fibroblast growth factor 21 (FGF21), for use in therapy, wherein the therapy involves expression of the gene construct in the central nervous system (CNS), preferably in the brain, more preferably in the hypothalamus. In some embodiments, there is provided a gene construct comprising a nucleotide sequence encoding a fibroblast growth factor 21 (FGF21), for use in the treatment of a metabolic disorder, wherein the therapy involves expression of the gene construct in the central nervous system (CNS), preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

Preferably, the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous promoter. In a preferred embodiment, the ubiquitous promoter is selected from the group consisting of a CAG promoter and a CMV promoter, preferably wherein the ubiquitous promoter is a CAG promoter. Preferably, the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous promoter and at least one target sequence of a microRNA expressed in a tissue where the expression of FGF21 is wanted to be prevented.

Preferably, the at least one target sequence of a microRNA is selected from those target sequences that bind to microRNAs expressed in the heart and/or the liver of a mammal.

More preferably, the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous promoter and at least one target sequence of a microRNA expressed in the liver and at least one target sequence of a microRNA expressed in the heart.

Preferably, a target sequence of a microRNA expressed in the heart is selected from SEQ ID NO's: 13 and 21-25 and a target sequence of a microRNA expressed in the liver is selected from SEQ ID NO's: 12 and 14-20.

More preferably, the gene construct comprises a target sequence of microRNA-122a and a target sequence of microRNA-1.

Preferably, the ubiquitous promoter is selected from the group consisting of a CAG promoter and a CMV promoter, preferably wherein the ubiquitous promoter is the CAG promoter.

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Preferably, the nucleotide sequence encoding FGF21 is selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising an amino acid sequence that has at least 60% sequence identity with the amino acid sequence of SEQ ID NO: 1, 2 or 3;

(b) a nucleotide sequence that has at least 60% sequence identity with the nucleotide
10 sequence of SEQ ID NO: 4, 5, 6, 7, 8, 9, 10 or 11; and

(c) a nucleotide sequence the sequence of which differs from the sequence of a nucleotide sequence of (b) due to the degeneracy of the genetic code.

In a second aspect, there is provided an expression vector comprising a gene construct as described
15 in the first aspect, for use in therapy, wherein the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the hypothalamus. In some embodiments, there is provided an expression vector comprising a gene construct as described in the first aspect, for use in the treatment of a metabolic disorder, wherein the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the
20 hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus Preferably, the expression vector is a viral vector.

Preferably, the expression vector is selected from the group consisting of adenoviral vectors, adeno-associated viral vectors, retroviral vectors, and lentiviral vectors, preferably wherein the expression vector is an adeno-associated viral vector.

25 Preferably, the expression vector is an adeno-associated viral vector of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, rh10, rh8, Cb4, rh74, DJ, 2/5, 2/1, 1/2 or Anc80, more preferably wherein the expression vector is an adeno-associated viral vector of serotype 1, 2 or 9.

In a third aspect, there is provided a pharmaceutical composition comprising a gene construct as
30 described in the first aspect and/or an expression vector as described in the second aspect, together with one or more pharmaceutically acceptable ingredients, for use in therapy, wherein the therapy involves expression of the gene construct in the CNS and/or the brain. In some embodiments, there is provided a pharmaceutical composition comprising a gene construct as described in the first aspect and/or an expression vector as described in the second aspect, together with one or more
35 pharmaceutically acceptable ingredients, for use in the treatment of a metabolic disorder, wherein the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

40 In a fourth aspect, there is provided a gene construct for use as described in the first aspect and/or an expression vector for use as described in the second aspect and/or a pharmaceutical composition

for use as described in the third aspect, wherein the gene construct and/or expression vector and/or pharmaceutical composition is administered by intra-CSF administration.

In a fifth aspect, there is provided a gene construct for use as described in the first aspect and/or an expression vector for use as described in the second aspect and/or a pharmaceutical composition for use as described in the third aspect, for use in the treatment and/or prevention of a metabolic disorder, preferably wherein the metabolic disorder is a diabetes and/or obesity.

Description

The present inventors have developed an improved gene therapy strategy based on FGF21 gene therapy directed to the central nervous system (CNS) to counteract obesity and/or diabetes. Particularly, as elaborated in the experimental part, the present inventors have found the following unexpected advantages of brain-directed FGF21 gene therapy:

- The gene constructs and vectors as described herein can obtain a robust and wide-spread overexpression in the brain (Examples 1, 2, 3 and 4)
- The gene constructs and vectors as described herein cause decreased adipocyte size, decreased fat accumulation in brown adipocytes, increased thermogenesis, reduced circulating triglycerides and free fatty acids, healthier pancreas (increase number of islet, amelioration of beta cell mass) and reduced systemic inflammation (reduction or pro-inflammatory cytokines such as F4/80, IL-6, TNFalpha) (Example 1.1).
- In a widely used mouse model of obesity and diabetes, expression of FGF21 in the brain led to a clear reduction in weight gain, adiposity and liver weight as well as complete normalization of fed glycemia (Example 1), improved insulin resistance, improved glucose tolerance and decreased gluconeogenesis (Example 4)
- In a widely used mouse model of senescence with age-related brain pathologies, expression of FGF21 in the brain led to a clear reduction in weight gain and liver weight (Example 2).
- In both mouse models, inflammation of the hypothalamus is reduced (Examples 1, 2).

Accordingly, the aspects and embodiments of the present invention as described herein solve at least some of the problems and needs as discussed herein.

<u>Gene construct</u>

In a first aspect, there is provided a gene construct comprising a nucleotide sequence encoding a fibroblast growth factor 21 (FGF21).

A “gene construct” as described herein has its customary and ordinary meaning as understood by one of skill in the art in view of this disclosure. A “gene construct” can also be called "expression cassette" or "expression construct" and refers to a gene or a group of genes, including a gene that encodes a protein of interest, which is operatively linked to a promoter that controls its expression. The part of this application entitled “general information” comprises more detail as to a “gene construct”. "Operatively linked" as used herein is further described in the part of this application entitled "general

information".

In some embodiments, a gene construct as described herein is suitable for expression in a mammal. As used herein, “suitable for expression in a mammal” may mean that the gene construct includes one or more regulatory sequences, selected on the basis of the mammalian host cells to be used for expression, that is operatively linked to the nucleotide sequence to be expressed. Preferably, said mammalian host cells to be used for expression are human, murine or canine cells.

In some embodiments, a gene construct as described herein is for use in therapy. In a preferred embodiment, a gene construct as described herein is for use in the treatment and/or prevention of a metabolic disorder. In a preferred embodiment, the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the hypothalamus. In some embodiments, expression of the gene construct in the brain may mean expression of the gene construct in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, preferably the hypothalamus. Accordingly, expression of the gene construct in the brain may mean expression of the gene construct in at least one or at least two or at least three or all brain regions selected from the group consisting of the hypothalamus, the cortex, the hippocampus, the cerebellum and the olfactory bulb. In a preferred embodiment, the therapy involves expression of the gene construct in the hypothalamus. In some embodiments, expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb may mean specific expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb. In an embodiment, expression does not involve expression in the liver, pancreas, adipose tissue, skeletal muscle and/or heart. In some embodiments, expression does not involve expression in at least one, at least two, at least three, at least four or all organs selected from the group consisting of the liver, pancreas, adipose tissue, skeletal muscle and heart. A description of CNS- and/or brain- and/or hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific expression has been provided under the section entitled “general information”.

Expression may be assessed as described under the section entitled “general information”. A description of “CNS”, “brain” and “hypothalamus” has been provided under the section entitled “general information”.

In some embodiments, a gene construct as described herein is for use in therapy, wherein the gene construct is administered by intra-CSF (cerebrospinal fluid) administration (via cisterna magna, intrathecal or intraventricular delivery), intraparenchymal administration or intranasal administration. A preferred administration is intra-CSF administration.

"Intra-CSF administration", “intranasal administration”, “intraparenchymal administration” “intra-cisterna magna administration”, “intrathecal administration” and “intraventricular administration”, as used herein, are described in the part of this application entitled "general information".

In some embodiments, the gene construct as described herein comprises a nucleotide sequence encoding an FGF21 to be expressed in the CNS, preferably in the brain, more preferably in the

hypothalamus. In some embodiments, the gene construct as described herein is suitable for expression in the CNS, preferably in the brain, more preferably in the hypothalamus. In some embodiments, expression of the gene construct in the brain may mean expression of the gene construct in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb. Accordingly, expression of the gene construct in the brain may mean expression of the gene construct in at least one or at least two or at least three or all brain regions selected from the group consisting of the hypothalamus, the cortex, the hippocampus, the cerebellum and the olfactory bulb. Expression in the hypothalamus is most preferred. Expression may be assessed as described under the section entitled “general information”.

In the context of embodiments of the invention, an FGF21 to be expressed in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb; and a gene construct suitable for expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, refer to the preferential or predominant (at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 60% higher, at least 70% higher, at least 80% higher, at least 90% higher, at least 100% higher, at least 150% higher, at least 200% higher or more) expression of FGF21 in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb as compared to other organs or tissues. Other organs or tissues may be the liver, pancreas, adipose tissue, skeletal muscle, heart, kidney, colon, hematopoietic tissue, lung, ovary, spleen, stomach, testis and others. Preferably, other organs are the liver and/or the heart. In an embodiment, expression is not detectable in the liver, pancreas, adipose tissue, skeletal muscle and/or heart. In some embodiments, expression is not detectable in at least one, at least two, at least three, at least four or all organs selected from the group consisting of the liver, pancreas, adipose tissue, skeletal muscle, heart, kidney, colon, hematopoietic tissue, lung, ovary, spleen, stomach and testis. Expression may be assessed as described under the section entitled “general information”.

A nucleotide sequence encoding an FGF21 present in a gene construct according to the invention may be derived from any FGF21 gene or FGF21 coding sequence, preferably an FGF21 gene or FGF21 coding sequence from human, mouse or dog; or a mutated FGF21 gene or FGF21 coding sequence, preferably from human, mouse or dog; or a codon optimized FGF21 gene or FGF21 coding sequence, preferably from human, mouse or dog.

Accordingly, in some embodiments, a preferred nucleotide sequence encoding an FGF21 encodes a polypeptide comprising an amino acid sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 similarity with SEQ ID NO: 1, 2 or 3. SEQ ID NO: 1 represents an amino acid sequence of human FGF21. SEQ ID NO: 2 represents an amino acid sequence of murine FGF21. SEQ ID NO: 3

represents an amino acid sequence of canine FGF21. In some embodiments, a nucleotide sequence encoding an FGF21 present in a gene construct according to the invention has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 with any sequence selected from the group consisting of SEQ ID NO's: 4, 5, 6, 7, 8, 9, 10 or 11.

A description of “identity” or “sequence identity” and “similarity” or “sequence similarity” has been provided under the section entitled “general information”.

In some embodiments, a nucleotide sequence encoding a human FGF21 present in a gene construct according to the invention has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 with SEQ ID NO: 4, 5, 6 or 7. SEQ ID NO: 4 is a nucleotide sequence encoding human FGF21. SEQ ID NO: 5 is a codon optimized nucleotide sequence encoding human FGF21, variant 1. SEQ ID NO: 6 is a codon optimized nucleotide sequence encoding human FGF21, variant 2. SEQ ID NO: 7 is a codon optimized nucleotide sequence encoding human FGF21, variant 3. Variant 1, variant 2 and variant 3 encode for the same human FGF21 protein and were obtained by different algorithms of codon optimization. A description of “codon optimization” has been provided under the section entitled “general information”.

In some embodiments, a nucleotide sequence encoding mouse FGF21 present in a gene construct according to the invention has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 with SEQ ID NO: 8 or 9. SEQ ID NO: 8 is a nucleotide sequence encoding mouse FGF21. SEQ ID NO: 9 is a codon optimized nucleotide sequence encoding mouse FGF21.

In some embodiments, a nucleotide sequence encoding canine FGF21 present in a gene construct according to the invention has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 with SEQ ID NO:

10 or 11. SEQ ID NO: 10 is a nucleotide sequence encoding canine FGF21. SEQ ID NO: 11 is a codon optimized nucleotide sequence encoding canine FGF21.

In some embodiments, there is provided a gene construct as described herein, wherein the nucleotide sequence encoding an FGF21 is selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising an amino acid sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity or similarity with the amino acid sequence of SEQ ID NO: 1, 2 or 3.

(b) a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with the nucleotide sequence of SEQ ID NO: 4, 5, 6, 7, 8, 9, 10 or 11.

(c) a nucleotide sequence the sequence of which differs from the sequence of a nucleotide sequence of (b) due to the degeneracy of the genetic code.

In a preferred embodiment, a nucleotide sequence encoding an FGF21 is a codon-optimized nucleotide sequence, preferably a codon-optimized human sequence, preferably selected from the sequences of SEQ ID NO: 5, 6 and 7.

An FGF21 encoded by the nucleotide sequences described herein exerts at least a detectable level of an activity of an FGF21 as known to a person of skill in the art. An activity of an FGF21 can be to exhibit an anti-obesity and/or an anti-diabetes effect as described in more detail later herein. An activity of an FGF21 can also be to increase insulin sensitivity. This activity could be assessed by methods known to a person of skill in the art, for example by using an insulin tolerance test or a glucose tolerance test.

In some embodiments, the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous promoter. A preferred ubiquitous promoter is selected from the CMV promoter and the CAG promoter, preferably the CAG promoter. In some embodiments, the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous promoter and at least one target sequence of a microRNA expressed in a tissue where the expression of FGF21 is wanted to be prevented.

A description of “ubiquitous promoter”, “operably linked” and “microRNA” has been provided under the section entitled “general information”. A “target sequence of a microRNA expressed in a tissue” or

"target sequence binding to a microRNA expressed in a tissue" or "binding site of a microRNA expressed in a tissue" as used herein refers to a nucleotide sequence which is complementary or partially complementary to at least a portion of a microRNA expressed in said tissue, as described elsewhere herein.

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In some embodiments, the at least one target sequence of a microRNA is selected from those target sequences that bind to microRNAs expressed in heart and/or liver of a mammal.

In some embodiments, the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous 10 promoter and at least one target sequence of a microRNA expressed in the liver and at least one target sequence of a microRNA expressed in the heart.

A "target sequence of a microRNA expressed in the liver" or "target sequence binding to a microRNA expressed in the liver" or "binding site of a microRNA expressed in the liver" as used herein refers to a nucleotide sequence which is complementary or partially complementary to at least a portion of a 15 microRNA expressed in the liver. Similarly, a "target sequence of a microRNA expressed in the heart" or "target sequence binding to a microRNA expressed in the heart" or "binding site of a microRNA expressed in the heart" as used herein refers to a nucleotide sequence which is complementary or partially complementary to at least a portion of a microRNA expressed in the heart.

A portion of a microRNA expressed in the liver or a portion of a microRNA expressed in the heart, 20 as described herein, means a nucleotide sequence of at least four, at least five, at least six or at least seven consecutive nucleotides of said microRNA. The binding site sequence can have perfect complementarity to at least a portion of an expressed microRNA, meaning that the sequences are a perfect match without any mismatch occurring. Alternatively, the binding site sequence can be partially complementary to at least a portion of an expressed microRNA, meaning that one mismatch in four, 25 five, six or seven consecutive nucleotides may occur. Partially complementary binding sites preferably contain perfect or near perfect complementarity to the seed region of the microRNA, meaning that no mismatch (perfect complementarity) or one mismatch per four, five, six or seven consecutive nucleotides (near perfect complementarity) may occur between the seed region of the microRNA and its binding site. The seed region of the microRNA consists of the 5' region of the microRNA from about nucleotide 30 2 to about nucleotide 8 of the microRNA. The portion as described herein is preferably the seed region of said microRNA. Degradation of the messenger RNA (mRNA) containing the target sequence for a microRNA expressed in the liver or a microRNA expressed in the heart may be through the RNA interference pathway or via direct translational control (inhibition) of the mRNA. This invention is in no way limited by the pathway ultimately utilized by the miRNA in inhibiting expression of the transgene or 35 encoded protein.

In the context of the invention, a target sequence that binds to microRNAs expressed in the liver may be replaced by a nucleotide sequence comprising a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, 40 at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 12 or 14-20.

In a preferred embodiment, the target sequence of a microRNA expressed in the liver may be replaced by a nucleotide sequence comprising a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 12. In a further embodiment, at least one copy of a target sequence of a microRNA expressed in the liver, as described in SEQ ID NO: 12 or 14-20, is present in the gene construct of the invention. In a further embodiment, two, three, four, five, six, seven or eight copies of a target sequence of a microRNA expressed in the liver, as described in SEQ ID NO: 12 or 14-20, are present in the gene construct of the invention. In a preferred embodiment, one, two, three, four, five, six, seven or eight copies of the sequence miRT-122a (SEQ ID NO: 12) are present in the gene construct of the invention. A preferred number of copies of a target sequence of a microRNA expressed in the liver is four.

A target sequence of a microRNA expressed in the liver as used herein exerts at least a detectable level of activity of a target sequence of a microRNA expressed in the liver as known to a person of skill in the art. An activity of a target sequence of a microRNA expressed in the liver is to bind to its cognate microRNA expressed in the liver and, when operatively linked to a transgene, to mediate detargeting of transgene expression in the liver. This activity may be assessed by measuring the levels of transgene expression in the liver on the level of the mRNA or the protein by standard assays known to a person of skill in the art, such as qPCR, Western blot analysis or ELISA.

In the context of the invention, a target sequence of a microRNA expressed in the heart may be replaced by a nucleotide sequence comprising a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 13 or 21-25.

In a preferred embodiment, the target sequence of a microRNA expressed in the heart may be replaced by a nucleotide sequence comprising a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 13. In a further embodiment, at least one copy of a target sequence of a microRNA expressed in the heart, as described in SEQ ID NO: 13 or 21-25, is present in the gene construct of the invention. In a further embodiment, two, three, four, five, six, seven or eight copies of a target sequence of a microRNA expressed in the heart, as described in SEQ ID NO: 13 or 21-25, are present in the gene construct of the invention. In a preferred embodiment, one, two, three, four, five, six, seven or eight copies of a nucleotide sequence encoding miRT-1 (SEQ ID NO: 13), are present in the gene construct of the invention. A preferred number of copies of a target sequence of a microRNA expressed in the heart is four.

A target sequence of a microRNA expressed in the heart as used herein exerts at least a detectable level of activity of a target sequence of a microRNA expressed in the heart as known to a person of skill in the art. An activity of a target sequence of a microRNA expressed in the heart is to bind to its cognate microRNA expressed in the heart and, when operatively linked to a transgene, to mediate detargeting of transgene expression in the heart. This activity may be assessed by measuring the levels of transgene expression in the heart on the level of the mRNA or the protein by standard assays known to a person of skill in the art, such as qPCR, Western blot analysis or ELISA.

In some embodiments, at least one copy of a target sequence of a microRNA expressed in the liver, as described in SEQ ID NO: 12 or 14-20, and at least one copy of a target sequence of a microRNA expressed in the heart, as described in SEQ ID NO: 13 or 21-25, are present in the gene construct of the invention. In a further embodiment, two, three, four, five, six, seven or eight copies of a target sequence of a microRNA expressed in the liver, as described in SEQ ID NO: 12 or 14-20, and two, three, four, five, six, seven or eight copies of a target sequence of a microRNA expressed in the heart, as described in SEQ ID NO: 13 or 21-25, are present in the gene construct of the invention. In a further embodiment one, two, three, four, five, six, seven or eight copies of a nucleotide sequence encoding miRT-122a (SEQ ID NO: 12) and one, two, three, four, five, six, seven or eight copies nucleotide sequence encoding miRT-1 (SEQ ID NO: 13) are combined in the gene construct of the invention. In a further embodiment, four copies of a nucleotide sequence encoding miRT-122a (SEQ ID NO: 12) and four copies of nucleotide sequence encoding miRT-1 (SEQ ID NO: 13) are combined in the gene construct of the invention.

In some embodiments there is provided a gene construct as described above, wherein the target sequence of a microRNA expressed in the liver and the target sequence of a microRNA expressed in the heart is selected from a group consisting of sequences SEQ ID NO: 12 to 25 and/or combinations thereof. In some embodiments there is provided a gene construct as described above, wherein the target sequence of a microRNA expressed in the heart is selected from SEQ ID NO's: 13 and 21-25 and a target sequence of a microRNA expressed in the liver is selected from SEQ ID NO's: 12 and 14-20. In some embodiments there is provided a gene construct as described above, wherein the gene construct comprises a target sequence of microRNA-122a and a target sequence of microRNA-1.

In some embodiments, a ubiquitous promoter as described herein is selected from the group consisting of a CAG promoter, a CMV promoter, a mini-CMV promoter, a β-actin promoter, a rous-

sarcoma-virus (RSV) promoter, an elongation factor 1 alpha (EF1α) promoter, an early growth response factor-1 (Egr-1) promoter, an Eukaryotic Initiation Factor 4A (elF4A) promoter, a ferritin heavy chain-encoding gene (FerH) promoter, a ferritin heavy light-encoding gene (FerL) promoter, a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter, a GRP78 promoter, a GRP94 promoter, a heat-shock protein 70 (hsp70) promoter, an ubiquitin B promoter, a SV40 promoter, a Beta-Kinesin promoter, a ROSA26 promoter and a PGK-1 promoter.

In a preferred embodiment, the ubiquitous promoter is a CAG promoter. CAG promoters are demonstrated in the examples to be suitable for use in a gene construct according to the invention. In some embodiments, a CAG promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 27.

Another preferred ubiquitous promoter is a cytomegalovirus (CMV) promoter. In some embodiments, a CMV promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 28. Preferably said CMV promoter is used together with an intronic sequence. In some embodiments, an intronic sequence comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 26.

Another preferred ubiquitous promoter is a mini-CMV promoter. In some embodiments, a mini-CMV promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 36.

Another preferred ubiquitous promoter is an EF1α promoter. In some embodiments, an EF1α promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 37.

Another preferred ubiquitous promoter is an RSV promoter. In some embodiments, an RSV promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 38.

In some embodiments, the nucleotide sequence encoding FGF21 is operably linked to a tissue-specific promoter. In a preferred embodiment, a tissue-specific promoter is a CNS-specific promoter, more preferably a brain-specific promoter, most preferably a hypothalamus-specific promoter.

A description of “tissue-specific promoter” has been provided under the section entitled “general information”.

In some embodiments, a CNS-specific promoter as described herein is selected from the group consisting of a Synapsin 1 promoter, a Neuron-specific enolase (NSE) promoter, a Calcium/calmodulin-dependent protein kinase II (CaMKII) promoter, a tyrosine hydroxylase (TH) promoter, a Forkhead Box A2 (FOXA2) promoter, an alpha-internexin (INA) promoter, a Nestin (NES) promoter, a Glial fibrillary acidic protein (GFAP) promoter, an Aldehyde Dehydrogenase 1 Family Member L1 (ALDH1L1) promoter, a myelin-associated oligodendrocyte basic protein (MOBP) promoter, a Homeobox Protein 9 (HB9) promoter and a Myelin basic protein (MBP) promoter.

In some embodiments, a brain-specific promoter as described herein is selected from the group consisting of a Synapsin 1 promoter, a Neuron-specific enolase (NSE) promoter, a Calcium/calmodulin-dependent protein kinase II (CaMKII) promoter, a tyrosine hydroxylase (TH) promoter, a Forkhead Box A2 (FOXA2) promoter, an alpha-internexin (INA) promoter, a Nestin (NES) promoter, a Glial fibrillary acidic protein (GFAP) promoter, an Aldehyde Dehydrogenase 1 Family Member L1 (ALDH1L1) promoter, a myelin-associated oligodendrocyte basic protein (MOBP) promoter and a Myelin basic protein (MBP) promoter.

In some embodiments, a hypothalamus-specific promoter may be a Gonadotropin-releasing hormone (GnRH) promoter.

In a preferred embodiment, the CNS- and/or brain-specific promoter is a synapsin 1 promoter. In some embodiments, a synapsin 1 promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 39.

Another preferred CNS- and/or brain-specific promoter is a calcium/calmodulin-dependent protein kinase II (CaMKII) promoter. In some embodiments, a calcium/calmodulin-dependent protein kinase II (CaMKII) promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 40.

Another preferred CNS- and/or brain-specific promoter is a Glial fibrillary acidic protein (GFAP) promoter. In some embodiments, a Glial fibrillary acidic protein (GFAP) promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 41.

Another preferred CNS- and/or brain-specific promoter is a Nestin promoter. In some embodiments, a Nestin promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 42.

Another preferred CNS-specific promoter is a Homeobox Protein 9 (HB9) promoter. In some embodiments, a Homeobox Protein 9 (HB9) promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 43.

Another preferred CNS- and/or brain-specific promoter is a tyrosine hydroxylase (TH) promoter. In some embodiments, a tyrosine hydroxylase (TH) promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 44.

Another preferred CNS- and/or brain-specific promoter is a Myelin basic protein (MBP) promoter. In some embodiments, a Myelin basic protein (MBP) promoter comprises, consists essentially of, or consists of a nucleotide sequence that has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% sequence identity with SEQ ID NO: 45.

In some embodiments, CNS-, brain- and/or hypothalamus-specific promoters as described herein direct expression of said nucleotide sequence in at least one cell of the CNS and/or brain and/or hypothalamus. Preferably, said promoter directs expression in at least 10%, 20%, 30%, 40%, 40%, 60%, 70%, 80%, 90%, or 100% of cells of the CNS and/or the brain and/or the hypothalamus. A CNS- and/or brain-specific promoter, as used herein, also encompasses promoters directing expression in a specific region or cellular subset of the CNS and/or brain. Accordingly, CNS- and/or brain specific promoters as described herein may also direct expression in at least 10%, 20%, 30%, 40%, 40%, 60%, 70%, 80%, 90%, or 100% of cells of the hippocampus, the cerebellum, the cortex, the hypothalamus and/or the olfactory bulb. Expression may be assessed as described under the section entitled “general information”.

A promoter as used herein (especially when the promoter sequence is described as having a minimal identity percentage with a given SEQ ID NO) should exert at least an activity of a promoter as known to a person of skill in the art. Preferably a promoter described as having a minimal identity percentage with a given SEQ ID NO should control transcription of the nucleotide sequence to which it is operably linked (i.e. at least a nucleotide sequence encoding a FGF21) as assessed in an assay known to a person of skill in the art. For example, such assay could involve measuring expression of the transgene. Expression may be assessed as described under the section entitled “general information”.

Additional sequences may be present in the gene construct of the invention. Exemplary additional sequences suitable herein include inverted terminal repeats (ITRs), an SV40 polyadenylation signal (SEQ ID NO: 32), a rabbit β-globin polyadenylation signal (SEQ ID NO: 33), a CMV enhancer sequence (SEQ ID NO: 29). Within the context of the invention, “ITRs” is intended to encompass one 5’ITR and one 3’ITR, each being derived from the genome of an AAV. Preferred ITRs are from AAV2 and are represented by SEQ ID NO: 30 (5’ ITR) and SEQ ID NO: 31 (3’ ITR). Within the context of the invention, it is encompassed to use the CMV enhancer sequence (SEQ ID NO: 29) and the CMV promoter sequence (SEQ ID NO: 28) as two separate sequences or as a single sequence (SEQ ID NO: 34). Each of these additional sequences may be present in a gene construct according to the invention.

Optionally, additional nucleotide sequences may be operably linked to the nucleotide sequence(s) encoding an FGF21, such as nucleotide sequences encoding signal sequences, nuclear localization signals, expression enhancers, and the like.

In some embodiments, there is provided a gene construct comprising a nucleotide sequence encoding FGF21, optionally wherein the gene construct does not comprise a target sequence of a microRNA expressed in a tissue where the expression of FGF21 is wanted to be prevented.

Expression vector

Gene constructs described herein can be placed in expression vectors. Thus, in another aspect there is provided an expression vector comprising a gene construct as described in any of the preceding embodiments. A description of “expression vector” has been provided under the section entitled “general information”.

In some embodiments, an expression vector as described herein is for use in therapy. In a preferred embodiment, an expression vector as described herein is for use in the treatment and/or prevention of a metabolic disorder. In a preferred embodiment, the therapy involves expression of the gene construct comprised in the expression vector in the CNS, preferably in the brain, more preferably in the hypothalamus. In some embodiments, expression of the gene construct in the brain may mean expression of the gene construct in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, preferably the hypothalamus. Accordingly, expression of the gene construct in the brain may mean expression of the gene construct in at least one or at least two or at least three or all brain regions selected from the group consisting of the hypothalamus, the cortex, the hippocampus, the cerebellum and the olfactory bulb. In a preferred embodiment, the therapy involves expression of the gene construct in the hypothalamus. In some embodiments, expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb may mean specific expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb. In an embodiment, expression does not involve expression in the liver, pancreas, adipose tissue, skeletal muscle and/or heart. In some embodiments, expression does not involve expression in at least one, at least two, at least three, at least four or all organs selected from the group consisting of the liver, pancreas, adipose tissue, skeletal muscle and heart. A description of CNS- and/or brain- and/or

hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific expression has been provided under the section entitled “general information”.

Expression may be assessed as described under the section entitled “general information”. A description of “CNS”, “brain” and “hypothalamus” has been provided under the section entitled “general information”.

In some embodiments, an expression vector as described herein is for use in therapy, wherein the expression vector is administered by intra-CSF (cerebrospinal fluid) administration (via cisterna magna, intrathecal or intraventricular delivery), intraparenchymal or by intranasal administration. A preferred administration is intra-CSF administration.

"Intra-CSF administration", “intranasal administration”, “intraparenchymal administration” “intra-cisterna magna administration”, “intrathecal administration” and “intraventricular administration”, as used herein, are described in the part of this application entitled "general information".

In some embodiments, the expression vector is a viral expression vector. A description of “viral expression vector” has been provided under the section entitled “general information”.

A viral vector may be a viral vector selected from the group consisting of adenoviral vectors, adeno-associated viral vectors, retroviral vectors and lentiviral vectors. An adenoviral vector is also known as an adenovirus derived vector, an adeno-associated viral vector is also known as an adeno-associated virus derived vector, a retroviral vector is also known as a retrovirus derived vector and a lentiviral vector is also known as a lentivirus derived vector. A preferred viral vector is an adeno-associated viral vector. A description of “adeno-associated viral vector” has been provided under the section entitled “general information”.

In some embodiments, the vector is an adeno-associated vector or adeno-associated viral vector or an adeno-associated virus derived vector (AAV) selected from the group consisting of AAV of serotype 1 (AAV1), AAV of serotype 2 (AAV2), AAV of serotype 3 (AAV3), AAV of serotype 4 (AAV4), AAV of serotype 5 (AAV5), AAV of serotype 6 (AAV6), AAV of serotype 7 (AAV7), AAV of serotype 8 (AAV8), AAV of serotype 9 (AAV9), AAV of serotype rh10 (AAVrh10), AAV of serotype rh8 (AAVrh8), AAV of serotype Cb4 (AAVCb4), AAV of serotype rh74 (AAVrh74), AAV of serotype DJ (AAVDJ), AAV of serotype 2/5 (AAV2/5), AAV of serotype 2/1 (AAV2/1), AAV of serotype 1/2 (AAV1/2), AAV of serotype Anc80 (AAVAnc80).

In a preferred embodiment, the vector is an AAV of serotype 1, 2 or 9 (AAV1, AAV2, or AAV9). These AAV serotypes are demonstrated in the examples to be suitable for use as an expression vector according to the invention.

In a preferred embodiment, the expression vector is an AAV1 or AAV2 or AAV9, preferably an AAV9, and comprises a gene construct comprising a nucleotide sequence encoding FGF21. More preferably such gene construct comprises a CAG promoter comprising, consisting essentially of, or consisting of a nucleotide sequence that has at least 60% with SEQ ID NO:27. More preferably, such gene construct comprises at least one target sequence of a microRNA expressed in a tissue where the expression of FGF21 is wanted to be prevented as described herein.

In another preferred embodiment, the expression vector is an AAV1 and comprises a gene construct comprising a nucleotide sequence encoding FGF21, optionally wherein the gene construct does not comprise a target sequence of a microRNA. In an embodiment, the gene construct does not comprise a target sequence of a miRNA, which is expressed in a tissue where the expression of FGF21 is wanted to be prevented. More preferably such gene construct comprises a CAG promoter comprising, consisting essentially of, or consisting of a nucleotide sequence that has at least 60% with SEQ ID NO:27.

Composition

In a further aspect there is provided a composition comprising a gene construct as described above and/or a viral vector as described above, together with one or more pharmaceutically acceptable ingredients.

Such composition may be called a gene therapy composition. Preferably, the composition is a pharmaceutical composition.

As used herein, “pharmaceutically acceptable ingredients” include pharmaceutically acceptable carriers, fillers, preservatives, solubilizers, vehicles, diluents and/or excipients. Accordingly, the one or more pharmaceutically acceptable ingredients may be selected from the group consisting of pharmaceutically acceptable carriers, fillers, preservatives, solubilizers, vehicles, diluents and/or excipients. Such pharmaceutically acceptable carriers, fillers, preservatives, solubilizers, vehicles, diluents and/or excipients may for instance be found in Remington: The Science and Practice of Pharmacy, 22nd edition. Pharmaceutical Press (2013).

In some embodiments, a composition as described herein is for use in therapy. In a preferred embodiment, a composition as described herein is for use in the treatment and/or prevention of a metabolic disorder. In a preferred embodiment, the therapy involves expression of the gene construct comprised in the composition in the CNS, preferably in the brain, more preferably in the hypothalamus. In some embodiments, expression of the gene construct in the brain may mean expression of the gene construct in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, preferably the hypothalamus. Accordingly, expression of the gene construct in the brain may mean expression of the gene construct in at least one or at least two or at least three or all brain regions selected from the group consisting of the hypothalamus, the cortex, the hippocampus, the cerebellum and the olfactory bulb. In a preferred embodiment, the therapy involves expression of the gene construct in the hypothalamus. In some embodiments, expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb may mean specific expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb. In an embodiment, expression does not involve expression in the liver, pancreas, adipose tissue, skeletal muscle and/or heart. In some embodiments, expression does not involve expression in at least one, at least two, at least three, at least four or all organs selected from the group consisting of the liver, pancreas, adipose tissue, skeletal muscle and heart. A description of CNS- and/or brain- and/or

hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific expression has been provided under the section entitled “general information”.

Expression may be assessed as described under the section entitled “general information”. A description of “CNS”, “brain” and “hypothalamus” has been provided under the section entitled “general information”.

In some embodiments, a composition as described herein is for use in therapy, wherein the composition is administered by intra-CSF (cerebrospinal fluid) administration (via cisterna magna, intrathecal or intraventricular delivery), intraparenchymal or by intranasal administration. A preferred administration is intra-CSF administration.

"Intra-CSF administration", “intranasal administration”, “intraparenchymal administration” “intra-cisterna magna administration”, “intrathecal administration” and “intraventricular administration”, as used herein, are described in the part of this application entitled "general information".

A further compound may be present in a composition of the invention. Said compound may help in delivery of the composition. Suitable compounds in this context are: compounds capable of forming complexes, nanoparticles, micelles and/or liposomes that deliver each constituent as described herein, complexed or trapped in a vesicle or liposome through a cell membrane. Many of these compounds are known in the art. Suitable compounds comprise polyethylenimine (PEI), or similar cationic polymers, including polypropyleneimine or polyethylenimine copolymers (PECs) and derivatives; synthetic amphiphiles (SAINT-18); lipofectin™, DOTAP. A person of skill in the art will know which type of formulation is the most appropriate for a composition as described herein.

Method and use

In a further aspect, there is provided a gene construct as described herein, for use in therapy, wherein the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

Further provided is an expression vector as described herein, for use in therapy, wherein the therapy involves expression of the gene construct comprised in the expression vector in the CNS , preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

Further provided is a pharmaceutical composition as described herein, for use in therapy, wherein the therapy involves expression of the gene construct comprised in the pharmaceutical composition in the CNS , preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

In some embodiments, a gene construct as described herein, and/or expression vector as described herein and/or a pharmaceutical composition as described herein is for use in the treatment and/or prevention of a metabolic disorder, preferably obesity and/or diabetes, wherein the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the

hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

In a further aspect there is provided a method of treatment, comprising administering a gene construct, an expression vector or a pharmaceutical composition as described herein, wherein the method involves the expression of a gene construct as described herein in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

In some embodiments, administering a gene construct, an expression vector or a pharmaceutical composition means administering to a subject in need thereof a therapeutically effective amount of a gene construct, an expression vector or a pharmaceutical composition.

In some embodiments there is provided a method of treatment, comprising administering a gene construct, an expression vector or a pharmaceutical composition as described herein, wherein the method is for treating and/or preventing a metabolic disorder and involves the expression of a gene construct as described herein in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

In a further aspect there is provided a use of a gene construct, an expression vector or a pharmaceutical composition as described herein, for the manufacture of a medicament, wherein said medicament involves the expression of a gene construct as described herein in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

In some embodiments there is provided a use of a gene construct, an expression vector or a pharmaceutical composition as described herein, for the manufacture of a medicament, wherein said medicament is for the treatment and/or prevention of a metabolic disorder and involves the expression of a gene construct as described herein in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

In a further aspect there is provided a use of a gene construct, an expression vector or a pharmaceutical composition as described herein, for medical treatment, wherein said medical treatment involves the expression of a gene construct as described herein in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

In some embodiments there is provided a use of a gene construct, an expression vector or a pharmaceutical composition as described herein, for medical treatment, wherein said medical treatment is for the treatment and/or prevention of a metabolic disorder and involves the expression of a gene construct as described herein in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, “involving the expression of a gene construct” may be replaced by “causing the expression of a gene construct” or “inducing the expression of a gene construct”.

Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, the therapy and/or treatment and/or medicament may involve expression of the gene construct in the CNS, preferably the brain, more preferably the hypothalamus. In some embodiments, expression of the gene construct in the brain may mean expression of the gene construct in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, preferably in the hypothalamus. Accordingly, expression of the gene construct in the brain may mean expression of the gene construct in at least one or at least two or at least three or all brain regions selected from the group consisting of the hypothalamus, the cortex, the hippocampus, the cerebellum and the olfactory bulb. In a preferred embodiment, the therapy involves expression of the gene construct in the hypothalamus. In some embodiments, expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb may mean specific expression in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb. In an embodiment, expression does not involve expression in the liver, pancreas, adipose tissue, skeletal muscle and/or heart. In some embodiments, expression does not involve expression in at least one, at least two, at least three, at least four or all organs selected from the group consisting of the liver, pancreas, adipose tissue, skeletal muscle and heart. A description of CNS- and/or brain- and/or hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific expression has been provided under the section entitled “general information”.

Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, a gene construct and/or an expression vector and/or a pharmaceutical composition may be administered by intra-CSF (cerebrospinal fluid) administration (via cisterna magna, intrathecal or intraventricular delivery).

Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, a gene construct and/or an expression vector and/or a pharmaceutical composition may be administered by intraparenchymal administration.

Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, a gene construct and/or an expression vector and/or a pharmaceutical composition may be administered by intranasal administration.

"Intra-CSF administration", “intranasal administration”, “intraparenchymal administration” “intra-cisterna magna administration”, “intrathecal administration” and “intraventricular administration”, as used herein, are described in the part of this application entitled "general information".

Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, the therapy and/or treatment and/or medicament may be for use in the treatment and/or prevention of a metabolic disorder, preferably obesity and/or diabetes. Complications of a metabolic disorder may also be encompassed.

5 Metabolic disorders may include metabolic syndrome, diabetes, obesity, obesity-related comorbidities, diabetes-related comorbidities, hyperglycaemia, insulin resistance, glucose intolerance, hepatic steatosis, alcoholic liver diseases (ALD), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), coronary heart disease (CHD), hyperlipidemia, atherosclerosis, endocrinopathies, osteosarcopenic obesity syndrome (OSO), diabetic nephropathy, chronic kidney 10 disease (CKD), cardiac hypertrophy, diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, arthritis, sepsis, ocular neovascularization, neurodegeneration, dementia, and may also include depression, adenoma, carcinoma.

Diabetes may include prediabetes, hyperglycaemia, Type 1 diabetes, Type 2 diabetes, maturity-onset diabetes of the young (MODY), monogenic diabetes, neonatal diabetes, gestational diabetes, 15 brittle diabetes, idiopathic diabetes, drug- or chemical-induced diabetes, Stiff-man syndrome, lipoatrophic diabetes, latent autoimmune diabetes in adults (LADA).

Obesity may include overweight, central/upper body obesity, peripheral/lower body obesity, morbid obesity, osteosarcopenic obesity syndrome (OSO), pediatric obesity, Mendelian (monogenic) syndromic obesity, Mendelian non-syndromic obesity, polygenic obesity.

20 Preferred metabolic disorders are obesity and/or a diabetes.

In a preferred embodiment, a treatment or a therapy or a use or the administration of a medicament as described herein does not have to be repeated. In some embodiments, a treatment or a therapy or a use or the administration of a medicament as described herein may be repeated each year or each 2, 25 3, 4, 5, 6, 7, 8, 9 or 10, including intervals between any two of the listed values, years.

The subject treated may be a higher mammal, such as a cat, a rodent, (preferably mice, rats, gerbils and guinea pigs, and more preferably mice and rats), a dog, or a human being.

30 Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, a gene construct and/or an expression vector and/or a pharmaceutical composition as described herein preferably exhibits an anti-diabetes effect and/or an anti-obesity effect.

An anti-diabetes effect may be reached when glucose disposal in blood is increased and/or when 35 glucose tolerance is improved and/or when insulin sensitivity is increased. This could be assessed using techniques known to a person of skill in the art such as measurement of glycaemia, insulinemia and/or performance of an insulin tolerance test and/or of a glucose tolerance test, for example as done in the experimental part. In this context, “increase” (respectively “improvement”) means at least a detectable increase (respectively a detectable improvement) using an assay known to a person of skill in the art. 40 The increase may be an increase of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100% using assays such

as the measurement of glycaemia, insulinemia and/or performance of an insulin tolerance test and/or of a glucose tolerance test.

An anti-obesity effect may be reached when body weight, body weight gain and/or body fat percentage is decreased. An anti-obesity effect may also be reached when body mass index (BMI), waist circumference, waist-to-hip ratio (WHR) and/or waist-to-height ratio (WHtR) is decreased. An anti-obesity effect may also be reached when weight of tissues, such as the liver, is decreased. This could be assessed using techniques known to a person of skill in the art, for example as done in the experimental part. In this context, “decrease” (respectively “improvement”) means at least a detectable decrease (respectively a detectable improvement) using an assay known to a person of skill in the art, such as assays as carried out in the experimental part. Anti-obesity effects include both prevention of obesity and reversion of obesity.

An anti-diabetes effect and/or an anti-obesity effect may also be observed when the progression of a typical symptom (e.g. insulitis, beta cell loss, decrease of beta cellmass, increase of body weight) has been slowed down as assessed by a physician. A decrease of a typical symptom may mean a slowdown in progression of symptom development or a complete disappearance of symptoms. Symptoms, and thus also a decrease in symptoms, can be assessed using a variety of methods, to a large extent the same methods as used in diagnosis of diabetes and/or obesity, including clinical examination and routine laboratory tests. Such methods include both macroscopic and microscopic methods, as well as molecular methods, radiographic methods such as X-rays, biochemical methods, immunohistochemical methods and others. Beta cell loss and/or decrease of beta cell mass may be assessed using immunohistochemical methods, preferably as carried out in the experimental part.

An anti-diabetes effect and/or an anti-obesity effect may also be observed when a reduced systemic inflammation is assessed (reduction of pro-inflammatory cytokines such as F4/80, IL-6, TNFalpha). In this context, “decrease” means at least a detectable decrease using an assay known to a person of skill in the art. The decrease may be a decrease of at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100% using assays such as the measurement of a pro-inflammatory cytokine such as F4/80, IL-6 and/or TNF alpha using techniques known to the skilled person, preferably those used in the experimental part (i.e. RTPCR).

Within the context of gene constructs for use, expression vectors for use, pharmaceutical compositions for use, methods and uses according to the invention, a gene construct and/or an expression vector and/or a pharmaceutical composition as described herein preferably alleviates one or more symptom(s) of a metabolic disorder, such as a diabetes and/or obesity, in an individual, in a cell, tissue or organ of said individual or alleviates one or more characteristic(s) or symptom(s) of a cell, tissue or organ of said individual.

A gene construct and/or an expression vector and/or a pharmaceutical composition as described herein is preferably able to alleviate a symptom or a characteristic of a patient or of a cell, tissue or organ of said patient if after at least one week, one month, six months, one year or more of treatment using a gene construct and/or an expression vector and/or a composition of the invention, said symptom or characteristic has decreased (e.g. is no longer detectable or has slowed down), as described herein.

A gene construct and/or an expression vector and/or a pharmaceutical composition as described herein may be suitable for administration to a cell, tissue and/or an organ *in vivo* of individuals affected by or at risk of developing a metabolic disorder, such as a diabetes and/or obesity, and may be administered *in vivo*, *ex vivo* or *in vitro*. Said gene construct and/or expression vector and/or pharmaceutical composition may be directly or indirectly administered to a cell, tissue and/or an organ *in vivo* of an individual affected by or at risk of developing a metabolic disorder, such as a diabetes and/or obesity, and may be administered directly or indirectly *in vivo*, *ex vivo* or *in vitro*.

An administration mode may be intravenous, intramuscular, intrathecal, intraventricular, intraperitoneal, via inhalation, intranasal, intra-ocular and/or intraparenchymal administration. Preferred administration modes are intranasal, intraparenchymal and intra-CSF (via cisterna magna, intrathecal or intraventricular delivery) administration. Intra-CSF administration is most preferred.

A viral expression construct and/or a viral vector and/or a nucleic acid molecule and/or a composition of the invention may be directly or indirectly administered using suitable means known in the art. Improvements in means for providing an individual or a cell, tissue, organ of said individual with a viral expression construct and/or a viral vector and/or a nucleic acid molecule and/or a composition of the invention are anticipated, considering the progress that has already thus far been achieved. Such future improvements may of course be incorporated to achieve the mentioned effect of the invention. A viral expression construct and/or a viral vector and/or a nucleic acid molecule and/or a composition can be delivered as is to an individual, a cell, tissue or organ of said individual. Depending on the disease or condition, a cell, tissue or organ of said individual may be as earlier described herein. When administering a viral expression construct and/or a viral vector and/or a nucleic acid molecule and/or a composition of the invention, it is preferred that such viral expression construct and/or vector and/or nucleic acid and/or composition is dissolved in a solution that is compatible with the delivery method.

As encompassed herein, a therapeutically effective dose of a viral expression construct, vector, nucleic acid molecule and/or composition as mentioned above is preferably administered in a single and unique dose hence avoiding repeated periodical administration.

General information

Unless stated otherwise, all technical and scientific terms used herein have the same meaning as customarily and ordinarily understood by a person of ordinary skill in the art to which this invention belongs, and read in view of this disclosure.

Sequence identity/similarity

In the context of the invention, a nucleic acid molecule such as a nucleic acid molecule encoding an FGF21 is represented by a nucleic acid or nucleotide sequence which encodes a protein fragment or a polypeptide or a peptide or a derived peptide. In the context of the invention, an FGF21 protein fragment or a polypeptide or a peptide or a derived peptide as Fibroblast growth factor 21 (FGF21) is represented by an amino acid sequence.

It is to be understood that each nucleic acid molecule or protein fragment or polypeptide or peptide or derived peptide or construct as identified herein by a given sequence identity number (SEQ ID NO) is not limited to this specific sequence as disclosed. Each coding sequence as identified herein encodes a given protein fragment or polypeptide or peptide or derived peptide or construct or is itself a protein fragment or polypeptide or construct or peptide or derived peptide. Throughout this application, each time one refers to a specific nucleotide sequence SEQ ID NO (take SEQ ID NO: X as example) encoding a given protein fragment or polypeptide or peptide or derived peptide, one may replace it by:

i. a nucleotide sequence comprising a nucleotide sequence that has at least 60% sequence identity with SEQ ID NO: X;

ii. a nucleotide sequence the sequence of which differs from the sequence of a nucleic acid molecule of (i) due to the degeneracy of the genetic code; or,

iii. a nucleotide sequence that encodes an amino acid sequence that has at least 60% amino acid identity or similarity with an amino acid sequence encoded by a nucleotide sequence SEQ ID NO: X.

Throughout this application, each time one refers to a specific amino acid sequence SEQ ID NO (take SEQ ID NO: Y as example), one may replace it by: a polypeptide comprising an amino acid sequence that has at least 60% sequence identity or similarity with amino acid sequence SEQ ID NO: Y.

Each nucleotide sequence or amino acid sequence described herein by virtue of its identity or similarity percentage (at least 60%) with a given nucleotide sequence or amino acid sequence respectively has in a further preferred embodiment an identity or a similarity of at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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% with the given nucleotide or amino acid sequence, respectively.

Each non-coding nucleotide sequence (i.e. of a promoter or of another regulatory region) could be replaced by a nucleotide sequence comprising a nucleotide sequence that has at least 60% sequence identity or similarity with a specific nucleotide sequence SEQ ID NO (take SEQ ID NO: A as example). A preferred nucleotide sequence has at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, 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 with SEQ ID NO: A. In a preferred embodiment, such non-coding nucleotide sequence such as a promoter exhibits or exerts at least an activity of such a non-coding nucleotide sequence such as an activity of a promoter as known to a person of skill in the art.

The terms “homology”, “sequence identity”, “identity” and the like are used interchangeably herein. Sequence identity is herein described as a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. "Similarity" or “sequence similarity” between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in Bioinformatics and the Cell: Modern Computational Approaches in Genomics, Proteomics and transcriptomics, Xia X., Springer International Publishing, New York, 2018; and Bioinformatics: Sequence and Genome Analysis, Mount D., Cold Spring Harbor Laboratory Press, New York, 2004.

Sequence identity or similarity can be calculated based on the full length of two given SEQ ID NO’s or on part thereof. In some embodiments, part thereof means at least 50%, 60%, 70%, 80%, 90%, or 100% of both SEQ ID NO. In a preferred embodiment, sequence identity or similarity is determined by comparing the whole length of the sequences as identified herein. Unless otherwise indicated herein, identity or similarity with a given SEQ ID NO means identity or similarity based on the full length of said sequence (*i.e.* over its whole length or as a whole). In the art, "identity" also refers to the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences.

Sequence identity or similarity can be determined by alignment of two peptide or two nucleotide sequences using global or local alignment algorithms, depending on the length of the two sequences. Sequences of similar lengths are preferably aligned using a global alignment algorithms (e.g. Needleman-Wunsch) which aligns the sequences optimally over the entire length, while sequences of substantially different lengths are preferably aligned using a local alignment algorithm (e.g. Smith-Waterman). Sequences may then be referred to as "substantially identical” or “essentially similar” when they (when optimally aligned by for example the program EMBOSS needle or EMBOSS water using default parameters) share at least a certain minimal percentage of sequence identity or similarity (as described below).

A global alignment is suitably used to determine sequence identity or similarity when the two sequences have similar lengths. When sequences have a substantially different overall length, local alignments, such as those using the Smith-Waterman algorithm, are preferred. EMBOSS needle uses the Needleman-Wunsch global alignment algorithm to align two sequences over their entire length (full length), maximizing the number of matches and minimizing the number of gaps. EMBOSS water uses the Smith-Waterman local alignment algorithm. Generally, the EMBOSS needle and EMBOSS water default parameters are used, with a gap open penalty = 10 (nucleotide sequences) / 10 (proteins) and gap extension penalty = 0.5 (nucleotide sequences) / 0.5 (proteins). For nucleotide sequences the default scoring matrix used is DNAfull and for proteins the default scoring matrix is Blosum62 (Henikoff & Henikoff, 1992, PNAS 89, 915-919).

Alternatively percentage similarity or identity may be determined by searching against public databases, using algorithms such as FASTA, BLAST, etc. Thus, the nucleic acid and protein sequences of some embodiments of the present invention can further be used as a “query sequence” to perform a search against public databases to, for example, identify other family members or related sequences.

Such searches can be performed using the BLASTn and BLASTx programs (version 2.0) of Altschul, *et al*. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to oxidoreductase nucleic acid molecules of the invention. BLAST protein searches can be performed with the BLASTx
5 program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Res. 25(17): 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., BLASTx and BLASTn) can be used. See the homepage of the National Center for Biotechnology Information
10 accessible on the world wide web at www.ncbi.nlm.nih.gov/.

Optionally, in determining the degree of amino acid similarity, a person of skill in the art may also take into account so-called conservative amino acid substitutions.

As used herein, “conservative” amino acid substitutions refer to the interchangeability of residues having similar side chains. Examples of classes of amino acid residues for conservative substitutions
15 are given in the Tables below.

Acidic Residues	Asp (D) and Glu (E)
Basic Residues	Lys (K), Arg (R), and His (H)
Hydrophilic Uncharged Residues	Ser (S), Thr (T), Asn (N), and Gln (Q)
Aliphatic Uncharged Residues	Gly (G), Ala (A), Val (V), Leu (L), and Ile (I)
Non-polar Uncharged Residues	Cys (C), Met (M), and Pro (P)
Aromatic Residues	Phe (F), Tyr (Y), and Trp (W)

Alternative conservative amino acid residue substitution classes :

1	A	S	T
2	D	E	
3	N	Q	
4	R	K	
5	I	L	M
6	F	Y	W

20 Alternative physical and functional classifications of amino acid residues:

Alcohol group-containing residues	S and T
Aliphatic residues	I, L, V, and M
Cycloalkenyl-associated residues	F, H, W, and Y
Hydrophobic residues	A, C, F, G, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E

Polar residues	C, D, E, H, K, N, Q, R, S, and T
Positively charged residues	H, K, and R
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P and T
Flexible residues	Q, T, K, S, G, P, D, E, and R

For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of 5 amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Substitutional variants of the amino acid sequence disclosed herein 10 are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. Preferably, the amino acid change is conservative. Preferred conservative substitutions for each of the naturally occurring amino acids are as follows: Ala to Ser; Arg to Lys; Asn to Gln or His; Asp to Glu; Cys to Ser or Ala; Gln to Asn; Glu to Asp; Gly to Pro; His to Asn or Gln; Ile to Leu or Val; Leu to Ile or Val; Lys to Arg; Gln or Glu; Met to Leu or Ile; Phe to Met, Leu or Tyr; Ser to Thr; 15 Thr to Ser; Trp to Tyr; Tyr to Trp or Phe; and, Val to Ile or Leu.

Gene or coding sequence

The term "gene" means a DNA fragment comprising a region (transcribed region), which is transcribed into an RNA molecule (e.g. an mRNA) in a cell, operably linked to suitable regulatory regions 20 (e.g. a promoter). A gene will usually comprise several operably linked fragments, such as a promoter, a 5' leader sequence, a coding region and a 3'-nontranslated sequence (3'-end) e.g. comprising a polyadenylation- and/or transcription termination site. A chimeric or recombinant gene (such as a FGF21 gene) is a gene not normally found in nature, such as a gene in which for example the promoter is not associated in nature with part or all of the transcribed DNA region. "Expression of a gene" refers to the 25 process wherein a DNA region which is operably linked to appropriate regulatory regions, particularly a promoter, is transcribed into an RNA, which is biologically active, i.e. which is capable of being translated into a biologically active protein or peptide.

A "transgene" is herein described as a gene or a coding sequence or a nucleic acid molecule (*i.e.* a molecule encoding a FGF21) that has been newly introduced into a cell, *i.e.* a gene that may be present 30 but may normally not be expressed or expressed at an insufficient level in a cell. In this context, “insufficient” means that although said FGF21 is expressed in a cell, a condition and/or disease as described herein could still be developed. In this case, the invention allows the over-expression of a FGF21. The transgene may comprise sequences that are native to the cell, sequences that naturally do not occur in the cell and it may comprise combinations of both. A transgene may contain sequences 35 coding for a FGF21 and/or additional proteins as earlier identified herein that may be operably linked to

appropriate regulatory sequences for expression of the sequences coding for a FGF21 in the cell. Preferably, the transgene is not integrated into the host cell's genome.

Promoter

As used herein, the term "promoter" or "transcription regulatory sequence" refers to a nucleic acid fragment that functions to control the transcription of one or more coding sequences, and is located upstream with respect to the direction of transcription of the transcription initiation site of the coding sequence, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter. A "constitutive" promoter is a promoter that is active in most tissues under most physiological and developmental conditions. An "inducible" promoter is a promoter that is physiologically or developmentally regulated, e.g. by the application of a chemical inducer.

A “ubiquitous promoter” is active in substantially all tissues, organs and cells of an organism.

A "organ-specific" or “tissue-specific” promoter is a promoter that is active in a specific type of organ or tissue, respectively. Organ-specific and tissue-specific promoters regulate expression of one or more genes (or coding sequence) primarily in one organ or tissue, but can allow detectable level (“leaky”) expression in other organs or tissues as well. Leaky expression in other organs or tissues means at least one-fold, at least two-fold, at least three-fold, at least four-fold or at least five-fold lower, but still detectable expression as compared to the organ-specific or tissue-specific expression, as evaluated on the level of the mRNA or the protein by standard assays known to a person of skill in the art (e.g. qPCR, Western blot analysis, ELISA). The maximum number of organs or tissues where leaky expression may be detected is five, six, seven or eight.

A “CNS- or brain- or hypothalamus-specific promoter” is a promoter that is capable of initiating transcription in the CNS and/or brain and/or hypothalamus, whilst still allowing for any leaky expression in other (maximum five, six, seven or eight) organs and parts of the body. Transcription in the CNS and/or brain and/or hypothalamus can be detected in relevant areas, such as the hypothalamus, cortex, hippocampus, cerebellum and olfactory bulb, and cells, such as neurons and/or glial cells.

In the context of the invention, CNS- and/or brain- and/or hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific promoters may be promoters that are capable of driving the preferential or predominant (at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 60% higher, at least 70% higher, at least 80% higher, at least 90% higher, at least 100% higher, at least 150% higher, at least 200% higher or more) expression of FGF21 in the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb as compared to other organs or tissues. Other organs or tissues may be the liver, pancreas, adipose tissue, skeletal muscle, heart, kidney, colon, hematopoietic tissue, lung, ovary, spleen, stomach, testis and others. Preferably, other organs are the liver and the heart. Expression may be assessed as described elsewhere under the section entitled “general information”.

Throughout the application, where CNS- and/or brain- and/or hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific is mentioned in the context of expression, cell-type specific expression of the cell type(s) making up the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb is also envisaged, respectively.

Operably linked

As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a transcription regulatory sequence is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein encoding regions, contiguous and in reading frame. Linking can be accomplished by ligation at convenient restriction sites or at adapters or linkers inserted in lieu thereof, or by gene synthesis.

microRNA

As used herein, “microRNA” or “miRNA” or “miR” has its customary and ordinary meaning as understood by one of skill in the art in view of this disclosure. A microRNA is a small non-coding RNA molecule found in plants, animals and some viruses, that may function in RNA silencing and post-transcriptional regulation of gene expression. A target sequence of a microRNA may be denoted as “miRT”. For example, a target sequence of microRNA-1 or miRNA-1 or miR-1 may be denoted as miRT-1.

Proteins and amino acids

The terms "protein" or "polypeptide" or “amino acid sequence” are used interchangeably and refer to molecules consisting of a chain of amino acids, without reference to a specific mode of action, size, 3-dimensional structure or origin. In amino acid sequences as described herein, amino acids or “residues” are denoted by three-letter symbols. These three-letter symbols as well as the corresponding one-letter symbols are well known to a person of skill in the art and have the following meaning: A (Ala) is alanine, C (Cys) is cysteine, D (Asp) is aspartic acid, E (Glu) is glutamic acid, F (Phe) is phenylalanine, G (Gly) is glycine, H (His) is histidine, I (Ile) is isoleucine, K (Lys) is lysine, L (Leu) is leucine, M (Met) is methionine, N (Asn) is asparagine, P (Pro) is proline, Q (Gln) is glutamine, R (Arg) is arginine, S (Ser) is serine, T (Thr) is threonine, V (Val) is valine, W (Trp) is tryptophan, Y (Tyr) is tyrosine. A residue may be any proteinogenic amino acid, but also any non-proteinogenic amino acid such as D-amino acids and modified amino acids formed by post-translational modifications, and also any non-natural amino acid.

CNS and brain

As used herein, “central nervous system” or “CNS” refers to the part of the nervous system that comprises the brain and the spinal cord, to which sensory impulses are transmitted and from which motor impulses pass out, and which coordinates the activity of the entire nervous system.

As used herein, “brain” refers to the central organ of the nervous system and consists of the cerebrum, the brainstem and the cerebellum. It controls most of the activities of the body, processing, integrating, and coordinating the information it receives from the sense organs, and making decisions as to the instructions sent to the rest of the body.

5 In particular, as used herein, ‘hypothalamus” refers to a region of the forebrain below the thalamus which coordinates both the autonomic nervous system and the activity of the pituitary, controlling body temperature, thirst, hunger, and other homeostatic systems, and involved in sleep and emotional activity.

Gene constructs

10 Gene constructs as described herein could be prepared using any cloning and/or recombinant DNA techniques, as known to a person of skill in the art, in which a nucleotide sequence encoding said FGF21 is expressed in a suitable cell, *e.g.* cultured cells or cells of a multicellular organism, such as described in Ausubel *et al.*, "Current Protocols in Molecular Biology", Greene Publishing and Wiley-Interscience, New York (1987) and in Sambrook and Russell (2001, *supra*); both of which are incorporated herein by 15 reference in their entirety. Also see, Kunkel (1985) Proc. Natl. Acad. Sci. 82:488 (describing site directed mutagenesis) and Roberts *et al.* (1987) Nature 328:731-734 or Wells, J.A., *et al.* (1985) Gene 34: 315 (describing cassette mutagenesis).

Expression vectors

20 The phrase "expression vector" or "vector" generally refers to a nucleotide sequence that is capable of effecting expression of a gene or a coding sequence in a host compatible with such sequences. An expression vector carries a genome that is able to stabilize and remain episomal in a cell. Within the context of the invention, a cell may mean to encompass a cell used to make the construct or a cell wherein the construct will be administered. Alternatively, a vector is capable of integrating into a cell's 25 genome, for example through homologous recombination or otherwise.

These expression vectors typically include at least suitable promoter sequences and optionally, transcription termination signals. An additional factor necessary or helpful in effecting expression can also be used as described herein. A nucleic acid or DNA or nucleotide sequence encoding a FGF21 is incorporated into a DNA construct capable of introduction into and expression in an *in vitro* cell culture. 30 Specifically, a DNA construct is suitable for replication in a prokaryotic host, such as bacteria, *e.g.*, *E. coli*, or can be introduced into a cultured mammalian, plant, insect, (*e.g.*, Sf9), yeast, fungi or other eukaryotic cell lines.

A DNA construct prepared for introduction into a particular host may include a replication system recognized by the host, an intended DNA segment encoding a desired polypeptide, and transcriptional 35 and translational initiation and termination regulatory sequences operably linked to the polypeptide-encoding segment. The term “operably linked” has already been described herein. For example, a promoter or enhancer is operably linked to a coding sequence if it stimulates the transcription of the sequence. DNA for a signal sequence is operably linked to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of a polypeptide. Generally, a DNA sequence that is 40 operably linked are contiguous, and, in the case of a signal sequence, both contiguous and in reading frame. However, enhancers need not be contiguous with a coding sequence whose transcription they

control. Linking is accomplished by ligation at convenient restriction sites or at adapters or linkers inserted in lieu thereof, or by gene synthesis.

The selection of an appropriate promoter sequence generally depends upon the host cell selected for the expression of a DNA segment. Examples of suitable promoter sequences include prokaryotic, and eukaryotic promoters well known in the art (see, *e.g.* Sambrook and Russell, 2001, *supra*). A transcriptional regulatory sequence typically includes a heterologous enhancer or promoter that is recognised by the host. The selection of an appropriate promoter depends upon the host, but promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters are known and available (see, *e.g.* Sambrook and Russell, 2001, *supra*). An expression vector includes the replication system and transcriptional and translational regulatory sequences together with the insertion site for the polypeptide encoding segment. In most cases, the replication system is only functional in the cell that is used to make the vector (bacterial cell as *E. Coli*). Most plasmids and vectors do not replicate in the cells infected with the vector. Examples of workable combinations of cell lines and expression vectors are described in Sambrook and Russell (2001, *supra*) and in Metzger *et al.* (1988) Nature <u>334</u>: 31-36. For example, suitable expression vectors can be expressed in, yeast, *e.g. S.cerevisiae*, *e.g.*, insect cells, *e.g.,* Sf9 cells, mammalian cells, *e.g.*, CHO cells and bacterial cells, *e.g.*, *E. coli*. A cell may thus be a prokaryotic or eukaryotic host cell. A cell may be a cell that is suitable for culture in liquid or on solid media.

Alternatively, a host cell is a cell that is part of a multicellular organism such as a transgenic plant or animal.

The selection of an appropriate promoter sequence generally depends upon the host cell selected for the expression of a DNA segment. Examples of suitable promoter sequences include prokaryotic, and eukaryotic promoters well known in the art (see, *e.g.* Sambrook and Russell, 2001, *supra*). A transcriptional regulatory sequence typically includes a heterologous enhancer or promoter that is recognised by the host. The selection of an appropriate promoter depends upon the host, but promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters are known and available (see, *e.g.* Sambrook and Russell, 2001, *supra*). An expression vector includes the replication system and transcriptional and translational regulatory sequences together with the insertion site for the polypeptide encoding segment. In most cases, the replication system is only functional in the cell that is used to make the vector (bacterial cell as *E. Coli*). Most plasmids and vectors do not replicate in the cells infected with the vector. Examples of workable combinations of cell lines and expression vectors are described in Sambrook and Russell (2001, *supra*) and in Metzger *et al.* (1988) Nature <u>334</u>: 31-36. For example, suitable expression vectors can be expressed in yeast, *e.g. S.cerevisiae*, *e.g.*, insect cells, *e.g.,* Sf9 cells, mammalian cells, *e.g.*, CHO cells and bacterial cells, *e.g.*, *E. coli*. A cell may thus be a prokaryotic or eukaryotic host cell. A cell may be a cell that is suitable for culture in liquid or on solid media.

Alternatively, a host cell is a cell that is part of a multicellular organism such as a transgenic plant or animal.

Viral vector

A viral vector or a viral expression vector a viral gene therapy vector is a vector that comprises a gene construct as described herein.

A viral vector or a viral gene therapy vector is a vector that is suitable for gene therapy. Vectors that are suitable for gene therapy are described in Anderson 1998, Nature 392: 25-30; Walther and Stein, 2000, Drugs 60: 249-71; Kay *et al.*, 2001, Nat. Med. 7: 33-40; Russell, 2000, J. Gen. Virol. 81: 2573-604; Amado and Chen, 1999, Science 285: 674-6; Federico, 1999, Curr. Opin. Biotechnol.10: 448-53; Vigna and Naldini, 2000, J. Gene Med. 2: 308-16; Marin *et al.*, 1997, Mol. Med. Today 3: 396-403; Peng and Russell, 1999, Curr. Opin. Biotechnol. 10: 454-7; Sommerfelt, 1999, J. Gen. Virol. 80: 3049-64; Reiser, 2000, Gene Ther. 7: 910-3; and references cited therein.

A particularly suitable gene therapy vector includes an adenoviral and adeno-associated virus (AAV) vector. These vectors infect a wide number of dividing and non-dividing cell types including synovial cells and liver cells. The episomal nature of the adenoviral and AAV vectors after cell entry makes these vectors suited for therapeutic applications, (Russell, 2000, J. Gen. Virol. 81: 2573-2604; Goncalves, 2005, Virol J. 2(1):43) as indicated above. AAV vectors are even more preferred since they are known to result in very stable long-term expression of transgene expression (up to 9 years in dog (Niemeyer et al, Blood. 2009 Jan 22;113(4):797-806) and ~ 10 years in human (Buchlis, G. et al., Blood. 2012 Mar 29;119(13):3038-41). Preferred adenoviral vectors are modified to reduce the host response as reviewed by Russell (2000, supra). Method for gene therapy using AAV vectors are described by Wang et al., 2005, J Gene Med. March 9 (Epub ahead of print), Mandel et al., 2004, Curr Opin Mol Ther. 6(5):482-90, and Martin et al., 2004, Eye 18(11):1049-55, Nathwani et al, N Engl J Med. 2011 Dec 22;365(25):2357-65, Apparailly et al, Hum Gene Ther. 2005 Apr;16(4):426-34.

Another suitable gene therapy vector includes a retroviral vector. A preferred retroviral vector for application in the present invention is a lentiviral based expression construct. Lentiviral vectors have the ability to infect and to stably integrate into the genome of dividing and non-dividing cells (Amado and Chen, 1999 Science 285: 674-6). Methods for the construction and use of lentiviral based expression constructs are described in U.S. Patent No.'s 6,165,782, 6,207,455, 6,218,181, 6,277,633 and 6,323,031 and in Federico (1999, Curr Opin Biotechnol 10: 448-53) and Vigna *et al.* (2000, J Gene Med 2000; 2: 308-16).

Other suitable gene therapy vectors include an adenovirus vector, a herpes virus vector, a polyoma virus vector or a vaccinia virus vector.

Adeno-associated virus vector (AAV vector)

The terms "adeno associated virus", "AAV virus", "AAV virion", "AAV viral particle" and "AAV particle", used as synonyms herein, refer to a viral particle composed of at least one capsid protein of AAV (preferably composed of all capsid protein of a particular AAV serotype) and an encapsulated polynucleotide of the AAV genome. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide different from a wild-type AAV genome, such as a transgene to be delivered to a mammalian cell) flanked by AAV inverted terminal repeats, then they are typically known as a "AAV vector particle" or "AAV viral vector" or "AAV vector". AAV refers to a virus that belongs to the genus Dependovirus family Parvoviridae. The AAV genome is approximately 4.7 Kb in length and it consists

of single strand deoxyribonucleic acid (ssDNA) that can be positive or negative detected. The invention also encompasses the use of double stranded AAV also called dsAAV or scAAV. The genome includes inverted terminal repeats (ITR) at both ends of the DNA strand, and two open reading frames (ORFs): rep and cap. The frame rep is made of four overlapping genes that encode proteins Rep necessary for AAV lifecycle. The frame cap contains nucleotide sequences overlapping with capsid proteins: VP1, VP2 and VP3, which interact to form a capsid of icosahedral symmetry (see Carter and Samulski ., 2000, and Gao et al, 2004).

A preferred viral vector or a preferred gene therapy vector is an AAV vector. An AAV vector as used herein preferably comprises a recombinant AAV vector (rAAV vector). A "rAAV vector" as used herein refers to a recombinant vector comprising part of an AAV genome encapsidated in a protein shell of capsid protein derived from an AAV serotype as explained herein. Part of an AAV genome may contain the inverted terminal repeats (ITR) derived from an adeno-associated virus serotype, such as AAV1, AAV2, AAV3, AAV4, AAV5 and others. Preferred ITRs are those of AAV2 which are represented by sequences comprising, consisting essentially of, or consisting of SEQ ID NO: 30 (5' ITR) and SEQ ID NO: 31 (3' ITR). The invention also preferably encompasses the use of a sequence having at least 80% (or 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 with SEQ ID NO: 30 as 5' ITR and a sequence having at least 80% (or 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 with SEQ ID NO: 31 as 3' ITR.

Protein shell comprised of capsid protein may be derived from any AAV serotype. A protein shell may also be named a capsid protein shell. rAAV vector may have one or preferably all wild type AAV genes deleted, but may still comprise functional ITR nucleic acid sequences. Functional ITR sequences are necessary for the replication, rescue and packaging of AAV virions. The ITR sequences may be wild type sequences or may have at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% or 100% sequence identity with wild type sequences or may be altered by for example by insertion, mutation, deletion or substitution of nucleotides, as long as they remain functional. In this context, functionality refers to the ability to direct packaging of the genome into the capsid shell and then allow for expression in the host cell to be infected or target cell. In the context of the present invention a capsid protein shell may be of a different serotype than the rAAV vector genome ITR.

A nucleic acid molecule represented by a nucleic acid sequence of choice is preferably inserted between the rAAV genome or ITR sequences as identified above, for example an expression construct comprising an expression regulatory element operably linked to a coding sequence and a 3' termination sequence. Said nucleic acid molecule may also be called a transgene.

"AAV helper functions" generally refers to the corresponding AAV functions required for rAAV replication and packaging supplied to the rAAV vector *in trans*. AAV helper functions complement the AAV functions which are missing in the rAAV vector, but they lack AAV ITRs (which are provided by the rAAV vector genome). AAV helper functions include the two major ORFs of AAV, namely the *rep* coding region and the *cap* coding region or functional substantially identical sequences thereof. Rep and Cap

regions are well known in the art, see *e.g.* Chiorini *et al.* (1999, J. of Virology, Vol 73(2): 1309-1319) or US 5,139,941, incorporated herein by reference. The AAV helper functions can be supplied on an AAV helper construct. Introduction of the helper construct into the host cell can occur *e.g.* by transformation, transfection, or transduction prior to or concurrently with the introduction of the rAAV genome present in the rAAV vector as identified herein. The AAV helper constructs of the invention may thus be chosen such that they produce the desired combination of serotypes for the rAAV vector's capsid protein shell on the one hand and for the rAAV genome present in said rAAV vector replication and packaging on the other hand.

"AAV helper virus" provides additional functions required for AAV replication and packaging. Suitable AAV helper viruses include adenoviruses, herpes simplex viruses (such as HSV types 1 and 2) and vaccinia viruses. The additional functions provided by the helper virus can also be introduced into the host cell via plasmids, as described in US 6,531,456 incorporated herein by reference.

"Transduction" refers to the delivery of a FGF21 into a recipient host cell by a viral vector. For example, transduction of a target cell by a rAAV vector of the invention leads to transfer of the rAAV genome contained in that vector into the transduced cell. "Host cell" or "target cell" refers to the cell into which the DNA delivery takes place, such as the muscle cells of a subject. AAV vectors are able to transduce both dividing and non-dividing cells.

Production of an AAV vector

The production of recombinant AAV (rAAV) for vectorizing transgenes have been described previously. *See* Ayuso E, *et al.*, Curr. Gene Ther. 2010; 10:423-436, Okada T, *et al.*, Hum. Gene Ther. 2009; 20:1013-1021, Zhang H, *et al.*, Hum. Gene Ther. 2009; 20:922-929, and Virag T, *et al.*, Hum. Gene Ther. 2009; 20:807-817. These protocols can be used or adapted to generate the AAV of the invention. In one embodiment, the producer cell line is transfected transiently with the polynucleotide of the invention (comprising the expression cassette flanked by ITRs) and with construct(s) that encodes rep and cap proteins and provides helper functions. In another embodiment, the cell line supplies stably the helper functions and is transfected transiently with the polynucleotide of the invention (comprising the expression cassette flanked by ITRs) and with construct(s) that encodes rep and cap proteins. In another embodiment, the cell line supplies stably the rep and cap proteins and the helper functions and is transiently transfected with the polynucleotide of the invention. In another embodiment, the cell line supplies stably the rep and cap proteins and is transfected transiently with the polynucleotide of the invention and a polynucleotide encoding the helper functions. In yet another embodiment, the cell line supplies stably the polynucleotide of the invention, the rep and cap proteins and the helper functions. Methods of making and using these and other AAV production systems have been described in the art. *See* Muzyczka N, *et al.*, US 5,139,941, Zhou X, *et al.*, US 5,741,683, Samulski R, *et al.*, US 6,057,152, Samulski R, *et al.*, US 6,204,059, Samulski R, *et al.*, US 6,268,213, Rabinowitz J, *et al.*, US 6,491,907, Zolotukhin S, *et al.*, US 6,660,514, Shenk T, *et al.*, US 6,951,753, Snyder R, *et al.*, US 7,094,604, Rabinowitz J, *et al.*, US 7,172,893, Monahan P, *et al.*, US 7,201,898, Samulski R, *et al.*, US 7,229,823, and Ferrari F, *et al.*, US 7,439,065.

The rAAV genome present in a rAAV vector comprises at least the nucleotide sequences of the inverted terminal repeat regions (ITRs) of one of the AAV serotypes (preferably the ones of serotype

AAV2 as disclosed earlier herein), or nucleotide sequences substantially identical thereto or nucleotide sequences having at least 60% identity thereto, and nucleotide sequence encoding a FGF21 (under control of a suitable regulatory element) inserted between the two ITRs. A vector genome requires the use of flanking 5' and a 3' ITR sequences to allow for efficient packaging of the vector genome into the rAAV capsid.

The complete genome of several AAV serotypes and corresponding ITR has been sequenced (Chiorini *et al.* 1999, J. of Virology Vol. 73, No.2, p1309-1319). They can be either cloned or made by chemical synthesis as known in the art, using for example an oligonucleotide synthesizer as supplied *e.g.* by Applied Biosystems Inc. (Fosters, CA, USA) or by standard molecular biology techniques. The ITRs can be cloned from the AAV viral genome or excised from a vector comprising the AAV ITRs. The ITR nucleotide sequences can be either ligated at either end to the nucleotide sequence encoding one or more therapeutic proteins using standard molecular biology techniques, or the AAV sequence between the ITRs can be replaced with the desired nucleotide sequence.

Preferably, the rAAV genome as present in a rAAV vector does not comprise any nucleotide sequences encoding viral proteins, such as the *rep* (replication) or *cap* (capsid) genes of AAV. This rAAV genome may further comprise a marker or reporter gene, such as a gene for example encoding an antibiotic resistance gene, a fluorescent protein (*e.g. gfp*) or a gene encoding a chemically, enzymatically or otherwise detectable and/or selectable product (*e.g. lacZ, aph, etc.*) known in the art.

The rAAV genome as present in said rAAV vector further comprises a promoter sequence operably linked to the nucleotide sequence encoding a FGF21.

A suitable 3' untranslated sequence may also be operably linked to the nucleotide sequence encoding a FGF21. Suitable 3' untranslated regions may be those naturally associated with the nucleotide sequence or may be derived from different genes, such as for example the SV40 polyadenylation signal (SEQ ID NO: 32) and the rabbit β-globin polyadenylation signal (SEQ ID NO: 33).

Expression

Expression may be assessed by any method known to a person of skill in the art. For example, expression may be assessed by measuring the levels of transgene expression in the liver on the level of the mRNA or the protein by standard assays known to a person of skill in the art, such as qPCR, Western blot analysis or ELISA.

Expression may be assessed at any time after administration of the gene construct, expression vector or composition as described herein. In some embodiments herein, expression may be assessed after 1 week, 2 weeks, 3 weeks, 4, weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9, weeks, 10 weeks, 11 weeks, 12 weeks, 14 weeks, 16 weeks, 18 weeks, 20 weeks, 22 weeks, 24 weeks, 28 weeks, 32 weeks, 36 weeks, 40 weeks, or more.

In the context of the invention, CNS- and/or brain- and/or hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific expression refers to the preferential or predominant (at least 10% higher, at least 20% higher, at least 30% higher, at least 40% higher, at least 50% higher, at least 60% higher, at least 70% higher, at least 80% higher, at least 90% higher, at least 100% higher, at least 150% higher, at least 200% higher or more) expression of FGF21 in the CNS

and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb as compared to other organs or tissues. Other organs or tissues may be the liver, pancreas, adipose tissue, skeletal muscle, heart, kidney, colon, hematopoietic tissue, lung, ovary, spleen, stomach, testis and others. Preferably, other organs are the liver and/or the heart. In an embodiment, expression is not detectable in the liver, pancreas, adipose tissue, skeletal muscle and/or heart. In some embodiments, expression is not detectable in at least one, at least two, at least three, at least four or all organs selected from the group consisting of the liver, pancreas, adipose tissue, skeletal muscle, heart, kidney, colon, hematopoietic tissue, lung, ovary, spleen, stomach and testis. Expression may be assessed as described above.

Throughout the application, where CNS- and/or brain- and/or hypothalamus and/or cortex- and/or hippocampus- and/or cerebellum- and/or olfactory bulb-specific is mentioned in the context of expression, cell-type specific expression of the cell type(s) making up the CNS and/or the brain and/or the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb is also envisaged, respectively.

Administration

As used herein, “intra-CSF administration” means direct administration into the CSF, located in the subarachnoid space between the arachnoid and pia mater layers of the meninges surrounding the brain. Intra-CSF administration can be performed via intra-cisterna magna, intraventricular or intrathecal administration. As used herein, “intra-cisterna magna administration” means administration into the cisterna magna, an opening of the subarachnoid space located between the cerebellum and the dorsal surface of the medulla oblongata. As used herein, “intraventricular administration” means administration into the either of both lateral ventricles of the brain As used herein, “intrathecal administration” involves the direct administration into the CSF within the intrathecal space of the spinal column. As used herein, “intraparenchymal administration” means local administration directly into any region of the brain parenchyma. As used herein, “intranasal administration” means administration by way of the nasal structures.

Codon optimization

“Codon optimization”, as used herein, refers to the processes employed to modify an existing coding sequence, or to design a coding sequence, for example, to improve translation in an expression host cell or organism of a transcript RNA molecule transcribed from the coding sequence, or to improve transcription of a coding sequence. Codon optimization includes, but is not limited to, processes including selecting codons for the coding sequence to suit the codon preference of the expression host organism. For example, to suit the codon preference of mammalians, preferably of murine, canine or human expression hosts. Codon optimization also eliminates elements that potentially impact negatively RNA stability and/or translation (e. g. termination sequences, TATA boxes, splice sites, ribosomal entry sites, repetitive and/or GC rich sequences and RNA secondary structures or instability motifs).). In some embodiments, codon-optimized sequences show at least 3%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more increase in transcription, RNA stability and/or translation.

In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, the verb “to consist” may be replaced by “to consist essentially of” meaning that a peptide or peptidomimetic, a culture medium, or a composition as described herein may comprise additional component(s) than the ones specifically identified, said additional component(s) not altering the unique characteristic of the invention. In addition, the verb “to consist” may be replaced by “to consist essentially of” meaning that a method as described herein may comprise additional step(s) than the ones specifically identified, said additional step(s) not altering the unique characteristic of the invention.

Reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

As used herein, "at least" a particular value means that particular value or more. For example, "at least 2" is understood to be the same as "2 or more" i.e., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, ..., etc.

Individual numerical values are stated as approximations as though the values were preceded by the word “about” or “approximately.” Similarly, the numerical values in the various ranges specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word “about” or “approximately.” As used herein, the terms “about” and “approximately” when referring to a numerical value shall have their plain and ordinary meanings to a person of ordinary skill in the art to which the disclosed subject matter is most closely related or the art relevant to the range or element at issue. The amount of broadening from the strict numerical boundary depends upon many factors. For example, some of the factors which may be considered include the criticality of the element and/or the effect a given amount of variation will have on the performance of the claimed subject matter, as well as other considerations known to those of skill in the art. In the absence of any contrary consideration, the word “about” or “approximately” when used in association with a numerical value (e.g. about 10) preferably means that the value may be the given value (of 10) more or less 1% of the value.

As used herein, the term "and/or" indicates that one or more of the stated cases may occur, alone or in combination with at least one of the stated cases, up to with all of the stated cases.

Each embodiment as identified herein may be combined together unless otherwise indicated.

All patent applications, patents, and printed publications cited herein are incorporated herein by reference in the entireties, except for any definitions, subject matter disclaimers or disavowals, and except to the extent that the incorporated material is inconsistent with the express disclosure herein, in which case the language in this disclosure controls.

A person of skill in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

5 The present invention is further described by the following examples which should not be construed as limiting the scope of the invention.

Description of the figures

10 **Figure 1. Expression of *moFGF21* in the brain of db/db mice.** The expression levels of the murine codon-optimized FGF21 (*moFgf21*) coding sequence were measured by RTqPCR in Hypothalamus, Cortex, Hippocampus and Cerebellum of db/db mice, and normalized with *Rplp0* values. Analyses were performed 12 weeks after intra-CSF administration of $5x10^{10}$ vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. ND, non-detected.

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Figure 2. Decreased body and tissue weight of db/db mice after treatment with AAV9-FGF21 vectors. (A) Body weight evolution. Body weight was measured weekly after the AAV administration. **(B)** Body weight gain. Body weight gain was calculated as percentage of the increased weight divided by the body weight at the time of AAV administration. **(C)** Weight of iWAT, eWAT, mWAT, BAT and liver

20 of non-treated and AAV9-FGF21-treated db/db. Analyses were performed 12 weeks after intra-CSF administration of $5x10^{10}$ vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ vs non-treated mice. iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue; mWAT, mesenteric white adipose tissue; BAT, interscapular brown adipose tissue; L, liver.

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Figure 3. Reversal of diabetes in db/db mice by intra-CSF administration of AAV9-FGF21 vectors. Evolution of fed blood glucose levels of non-treated and AAV9-CAG-moFGF21-dmiRT-treated db/db mice after intra-CSF vector administration. Results are expressed as the mean ± SEM, n=9 animals/group. ***$p<0.001$ vs non-treated mice.

30

Figure 4. Reduction of brain inflammation in db/db mice treated with AAV9-FGF21 vectors. Expression levels of astrocyte markers (*Gfap* and *S100b*), microglia markers (*Aif1*) and inflammatory molecules (*Nfkb*, *Il1b* and *Il6*) were measured by RTqPCR in Hypothalamus of db/db mice, and normalized with *Rplp0* values. Analyses were performed 12 weeks after intra-CSF administration of

35 $5x10^{10}$ vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. *$p <0.05$ vs non-treated mice. *Gfap*, glial fibrillary acidic protein; *S100b*, calcium-binding protein B; *Aif1*, allograft inflammatory factor 1; *Nfkb*, nuclear factor kappa B; *Il1b*, interleukin 1 beta; *Il6*, Interleukin 6.

40 **Figure 5. Expression of moFGF21 in the brain of SAMP8 mice.** The expression levels of the murine codon-optimized FGF21 (moFGF21) coding sequence were measured by RTqPCR in

Hypothalamus, Cortex, Hippocampus and Cerebellum of SAMP8 mice, and normalized with *Rplp0* values. Analyses were performed 14 weeks after intra-CSF administration of $5x10^{10}$ vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. ND, non-detected.

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Figure 6. Decreased body and tissue weight of SAMP8 mice after treatment with AAV9-FGF21 vectors. (A) Body weight evolution. Body weight was measured weekly after the AAV administration. **(B)** Body weight gain. Body weight gain was calculated as the percentage of increased weight divided by the body weight at the time of AAV administration. **(C)** Weight of iWAT, eWAT, mWAT, BAT and liver
10 of non-treated and AAV9-FGF21-treated SAMP8. Analyses were performed 14 weeks after intra-CSF administration of $5x10^{10}$ vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. * p<0.05, ** p<0.01 and *** p<0.001 vs non-treated mice. iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue; mWAT, mesenteric white adipose tissue; BAT, interscapular brown adipose tissue, L, liver.

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Figure 7. Reduction of brain inflammation in SAMP8 mice treated with AAV9-FGF21. Expression levels of astrocyte markers (*Gfap* and *S100b*), microglia marker (*Aif1*) and inflammatory molecules (*Nfkb, Il1b* and *Il6*) were measured by RTqPCR in Hypothalamus of SAMP8 mice, and normalized with *Rplp0* values. Analyses were performed 14 weeks after intra-CSF administration of
20 $5x10^{10}$ vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. **p<0.01 vs non-treated mice. *Gfap*, glial fibrillary acidic protein; *S100b*, calcium-binding protein B; *Aif1*, allograft inflammatory factor 1; *Nfkb*, nuclear factor kappa B; *Il1b*, interleukin 1 beta; *Il6*, Interleukin 6.

25 **Figure 8. Expression of moFGF21 in the brain after intra-CSF administration of AAV1-FGF21, AAV2-FGF21 and AAV9-FGF21 vectors.** The expression levels of the murine codon-optimized FGF21 (moFGF21) coding sequence were measured by RTqPCR in Hypothalamus, Cortex, Hippocampus and Cerebellum of wild-type mice 3 weeks after intra-CSF administration of $5x10^{10}$ vg/mouse of AAV1-CAG-moFGF21-dmiRT, AAV2-CAG-moFGF21-dmiRT or AAV9-CAG-moFGF21-dmiRT vectors. Results
30 were normalized with *Rplp0* values and are expressed as the mean ± SEM, n=5 animals/group. ND, non-detected.

Figure 9. FGF21 protein levels in the brain. FGF21 protein content was determined by ELISA in brain homogenates of wild-type mice 3 weeks after administration of $5x10^{10}$ vg/mouse of AAV1-CAG-
35 moFGF21-dmiRT, AAV2-CAG-moFGF21-dmiRT or AAV9-CAG-moFGF21-dmiRT vectors. Results were normalized by total protein levels and are expressed as the mean ± SEM, n=5 animals/group. ND, non-detected.

Figure 10. Reduction of adiposity and increased thermogenesis after treatment with AAV9-
40 **FGF21 vectors**. Representative images of sections stained with hematoxylin and eosin of **(A)** eWAT and **(B)** BAT of AAV9-FGF21-treated and non-treated db/db mice. Original magnification x200 **(C)**

Expression levels of thermogenic markers (*Ucp1* and *Cidea*) were measured by RTqPCR in BAT of db/db mice, and normalized with *Rplp0* values. Analyses were performed 12 weeks after intra-CSF administration of 5x10^{10} vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. ***p<0.001 vs non-treated mice. *Ucp1,* uncoupling protein 1;
5 *Cidea*, cell death-inducing DNA fragmentation factor, alpha subunit-like effector A; eWAT, epididymal white adipose tissue; BAT, brown adipose tissue.

Figure 11. Decreased hepatic triglyceride content in AAV9-FGF21-treated mice. (A) Hepatic triglyceride content. **(B)** Serum triglycerides and **(C)** serum FFA levels. Analyses were performed 12
10 weeks after intra-CSF administration of the vectors. Results are expressed as the mean ± SEM, n=9 animals/group. *p<0.05 vs non-treated mice. FFA, free fatty acids.

Figure 12. **Amelioration of β-cell mass in FGF21-treated db/db mice. (A)** Number of islets and **(B)** β-cell mass was calculated in non-treated and AAV9-FGF21-treated db/db mice after
15 immunohistochemical analysis of pancreas sections stained with anti-insulin antibody. Results are expressed as the mean ± SEM, n=3 animals/group. *p<0.05 vs non-treated mice.

Figure 13. Reduced inflammation in adipose tissue and liver of db/db mice after treatment with AAV9-FGF21 vectors. (A) Representative images of MAC-2 immunohistochemistry of the eWAT
20 from non-treated and AAV9-FGF21-treated db/db mice (n=6 animals/group) **(B)** Expression levels of the inflammatory marker *F4/80* was measured by RTqPCR in eWAT of db/db mice, and normalized with *Rplp0* values. Analyses were performed 12 weeks after intra-CSF administration of 5x10^{10} vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. **(C-D)** Expression levels of the inflammatory markers *F4/80*, *Il6* and *Tnfa* were measured by RTqPCR in BAT (C) and liver (D) of db/db mice, and normalized with *Rplp0*
25 values. Analyses were performed 12 weeks after intra-CSF administration of 5x10^{10} vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. Results are expressed as the mean ± SEM, n=9 animals/group. * p<0.05, ** p<0.01 and ***p<0.001 ***p<0.001 vs non-treated mice. *F4/80,* adhesion G protein-coupled receptor E1; *Il6*, interleukin 6; *Tnfa*, tumor necrosis factor alpha; eWAT, epididymal white adipose tissue; BAT, brown adipose tissue. MAC-2, lectin, galactose binding, soluble 3; Arrows indicate MAC-2
30 signalling.

Figure 14. Expression of FGF21 in the brain of AAV1-FGF21-treated db/db mice. The expression levels of the murine codon-optimized FGF21 (*moFgf21*) coding sequence were measured by RTqPCR in Hypothalamus, Cortex, Hippocampus, Cerebellum and Olfactory Bulb of db/db mice, and
35 normalized with *Rplp0* values. Analyses were performed 16 weeks after intra-CSF administration of 5x10^{10} vg/mouse of AAV1-CAG-moFGF21 vectors. Results are expressed as the mean ± SEM, n=7 animals/group. ND, non-detected.

Figure 15. Decreased body weight of db/db mice after treatment with AAV1-CAG-FGF21
40 **vectors.** Body weight was measured weekly after the AAV administration in non-treated db/+ (lean), non-treated db/db and AAV1-CAG-FGF21-treated db/db mice. Results are expressed as the mean ±

SEM, n=7 animals/group. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ vs db/+ mice. $^{\$\$\$}$ $p<0.001$ vs db/db non-treated mice.

Figure 16. Reversal of diabetes in AAV1-FGF21-treated db/db. (A) Evolution of fed blood glucose levels of lean (db/+), non-treated and AAV9-CAG-moFGF21-dmiRT-treated db/db mice after intra-CSF vector administration. **(B)** Fasted blood glucose levels were measured 11 weeks after AAV1-CAG-FGF21 vector administration. Results are expressed as the mean ± SEM, n=7 animals/group. ** $p<0.01$ and ***$p<0.001$ vs db/+ mice. $^{\$\$\$}$ $p<0.001$ vs db/db non-treated mice.

Figure 17. Increased insulin sensitivity in AAV1-FGF21-treated db/db mice. Intraperitoneal insulin tolerance test. Lean (db/+), non-treated and AAV9-CAG-moFGF21-dmiRT-treated db/db mice were given an intraperitoneal injection of 0.75 U insulin/kg body weight and blood glucose levels were measured at the indicated time points. The test was performed 14 weeks post-AAV administration. Results are expressed as the mean ± SEM, n=7 animals/group. * $p<0.05$, ** $p<0.01$ and ***$p<0.001$ vs db/+ mice. $^{\$}$ $p<0.05$ and $^{\$\$}$ $p<0.01$ vs db/db non-treated mice.

Figure 18. Treatment with AAV1-CAG-FGF21 improves glucose tolerance. Glucose tolerance was studied 11 weeks after AAV administration in non-treated db/+ (lean), non-treated db/db and AAV1-CAG-FGF21-treated db/db mice after an intraperitoneal injection of glucose (1g/kg body weight). Results are expressed as the mean ± SEM, n=7 animals/group. *$p<0.05$ and *** $p<0.001$ vs db/+ mice. $^{\$\$\$}$ $p<0.001$ vs db/db non-treated mice.

Figure 19. Decreased gluconeogenesis in db/db mice after AAV1-FGF21 administration. A pyruvate tolerance test was performed in lean (db/+), non-treated and AAV9-CAG-moFGF21-dmiRT-treated db/db mice. All groups were given an intraperitoneal injection of pyruvate (1g/kg body weight) and blood glucose levels were measured at the indicated time points. The test was performed 12 weeks post-AAV administration. Results are expressed as the mean ± SEM, n=7 animals/group. ***$p<0.001$ vs non-treated mice. $^{\$\$\$}$ $p<0.001$ vs db/db non-treated mice.

Examples

To study the effects of FGF21 in the brain when overexpressed in this organ by using AAV vectors. Three different experiments have been performed:

- Treatment of db/db mice with AAV9-CAG-moFGF21-dmiRT. Dose used: $5x10^{10}$ vg/mouse (Example 1).
- Treatment of SAMP8 mice with AAV9-CAG-moFGF21-dmiRT. Dose used: $5x10^{10}$ vg/mouse (Example 2).
- Treatment of db/db mice with AAV1-CAG-moFGF21. Dose used: $5x10^{10}$ vg/mouse (Example 4).

Moreover, we also examined brain transduction efficiency by AAV1-FGF21, AAV2-FGF21 and AAV9-FGF21 vectors after intra-CSF administration of wild-type mice (Example 3).

dmiRT refers to 4 copies of the miRT-122a and 4 copies of the miRT-1 sequences.

The CAG-moFGF21-dmiRT gene construct sequence is comprised in the sequence of SEQ ID NO: 35. The CAG-moFGF21 gene construct sequence is comprised in the sequence of SEQ ID NO: 46.

5

General procedures to the Examples

Subject characteristics

Male BKS.Cg-+*Lepr*^{db}/+*Lepr*^{db} OlaHsd (db/db), BKS.Cg-m+/+*Lepr*^{db}/OlaHsd (db/+, lean)
10 SAMP8/TaHsd (SAMP8) and C57Bl/6J (wild-type) mice were used. Mice were fed *ad libitum* with a standard diet (2018S Teklad Global Diets®, Harlan Labs., Inc., Madison, WI, US and kept under a light-dark cycle of 12 h (lights on at 8:00 a.m.) and stable temperature (22°C ± 2). For tissue sampling, mice were anesthetized by means of inhalational anesthetic isoflurane (IsoFlo®, Abbott Laboratories, Abbott Park, IL, US) and decapitated. Tissues of interest were excised and kept at -80°C until analysis. All
15 experimental procedures were approved by the Ethics Committee for Animal and Human Experimentation of the Universitat Autònoma de Barcelona.

Recombinant AAV vectors

Single-stranded AAV vectors of serotype 1, 2 and 9 were produced by triple transfection of HEK293
20 cells according to standard methods (Ayuso, E. et al., 2010. Curr Gene Ther. 10(6):423-36). Cells were cultured in 10 roller bottles (850 cm^2, flat; Corning™, Sigma-Aldrich Co., Saint Louis, MO, US) in DMEM 10% FBS to 80% confluence and co-transfected by calcium phosphate method with a plasmid carrying the expression cassette flanked by the AAV2 ITRs (SEQ ID NO: 35), a helper plasmid carrying the AAV2 *rep* gene and the AAV of serotype 1, 2 or 9 *cap* gene, respectively, and a plasmid carrying the
25 adenovirus helper functions. The transgene used was the murine codon-optimized FGF21 coding-sequence (SEQ ID NO: 9) driven by the early enhancer/chicken beta actin (CAG) promoter (SEQ ID NO: 27). In examples 1, 2 and 3 the transgene also contained the addition of four tandem repeats of the miRT-122a sequence (5'CAAACACCATTGTCACACTCCA3', SEQ ID NO: 12) and four tandems repeats of the miRT-1 sequence (5'TTACATACTTCTTTACATTCCA3', SEQ ID NO: 13) cloned in the 3'
30 untranslated region of the expression cassette. In example 4, the cassette was not carrying the miRT-122a and miRT-1; AAVs were purified with an optimized method based on a polyethylene glycol precipitation step and two consecutive cesium chloride (CsCl) gradients. This second-generation CsCl-based protocol reduced empty AAV capsids and DNA and protein impurities dramatically (Ayuso, E. et al., 2010. *Curr Gene Ther.* 10(6):423-36). Purified AAV vectors were dialyzed against PBS, filtered and
35 stored at -80ºC. Titers of viral genomes were determined by quantitative PCR following the protocol described for the AAV2 reference standard material using linearized plasmid DNA as standard curve (Lock M, *et al.*, Hum. Gene Ther. 2010; 21:1273-1285). The vectors were constructed according to molecular biology techniques well known in the art.

40

In vivo intra-CSF administration of AAV vectors

Mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and the skin of the posterior part of the head, from behind the ears to approximately between the scapulas, was shaved and rinsed with ethanol. Mice were held in prone position, with the head at a slightly downward inclination. A 2-mm rostro-caudal incision was made to introduce a Hamilton syringe at an angle of 45–55° into the cisterna magna, between the occiput and the C1-vertebra and 5 µl of vector dilution was administered. Given that the CNS is the main target compartment for vector delivery, mice were dosed with the same number of vector genomes/mouse irrespective of body weight ($5x10^{10}$ vg/mice).

Immunohistochemical and morphometric analysis

Tissues were fixed for 24 h in formalin (Panreac Química), embedded in paraffin, and sectioned. Tissue samples were stained with hematoxylin-eosin and images were taken with the Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) connected to a videocamera with a monitor with an image analysis software (analySIS 3.0; Soft Imaging System, Center Valley, PA, EEUU).

Immunohistochemistry

Tissues were fixed for 12-24 h in 10% formalin, embedded in paraffin and sectioned. For immunohistochemical detection, sections were deparaffinised and incubated overnight at 4ºC with rat anti-MAC2 (1:50; CL8942AP; Cedarlane) and guinea pig anti-insulin (1:100; I-8510; Sigma-Aldrich). Biotinylated rabbit anti-rat (1:300; E0467; Dako) and rabbit anti-guinea pig coupled to peroxidase (1:300; P0141; Dako) were used as secondary antibodies. The ABC peroxidase kit (Pierce) was used for immunodetection, and sections were counterstained in Mayer's hematoxylin. The percentage of β-cell area in the pancreas was analyzed in two insulin-stained sections 200 µm apart, by dividing the area of all insulin positive cells in one section by the total pancreas area of that section. β-cell mass was calculated by multiplying pancreas weight by percentage of β-cell area, as previously described (Casellas et al,2006).

RNA analysis

Total RNA was obtained from hypothalamus, cortex, hippocampus, cerebellum and olfactory bulb using Tripure isolation reagent (Roche Diagnostics Corp., Indianapolis, IN, US) and from white adipose tissue, brown adipose tissue and liver using Qiazol lysis reagent (Qiagen NV, Venlo, NL), and RNeasy Mini Kit or RNeasy Micro Kit for hippocampus samples (Qiagen NV, Venlo, NL). In order to eliminate the residual viral genomes, total RNA was treated with DNAseI (Qiagen NV, Venlo, NL). For RT-PCR analysis, 1 µg of RNA samples was reverse-transcribed using Transcriptor First Strand cDNA Synthesis Kit (04379012001, Roche, California, USA). Real-time quantitative PCR was performed in a SmartCyclerII® (Cepheid, Sunnyvale, USA) using TB Green Premix Ex TaqII (Takara Bio Europe, France). Data was normalized with Rplp0 values and analyzed as previously described (Pfaffl, M., Nucleic Acids Res. 2001; 29(9):e45).

An overview of the primers used is shown below:

moFgf21-Fw: 5'-CCTAACCAGGACGCCACAAG-3' (SEQ ID NO: 47)

moFgf21-Rv: 5'-GTTCCACCATGCTCAGAGGG -3' (SEQ ID NO: 48)
Gfap-Fw: 5'-ACAGACTTTCTCCAACCTCCAG-3' (SEQ ID NO: 49)
Gfap-Rv: 5'-CCTTCTGACACGGATTTGGT-3' (SEQ ID NO: 50)
S100b-Fw: 5'-AACAACGAGCTCTCTCACTTCC-3' (SEQ ID NO: 51)
5 *S100b-Rv*: 5'-CGTCTCCATCACTTTGTCCA-3' (SEQ ID NO: 52)
Aif1-Fw: 5'-TGAGCCAAAGCAGGGATTTG-3' (SEQ ID NO: 53)
Aif1-Rv: 5'-TCAAGTTTGGACGGCAGATC-3' (SEQ ID NO: 54)
Nfkb-Fw: 5'-GACCACTGCTCAGGTCCACT-3' (SEQ ID NO: 55)
Nfkb-Rv: 5'-TGTCACTATCCCGGAGTTCA-3' (SEQ ID NO: 56)
10 *Il1b-Fw*: 5'-ATGAAGGGCTGCTTCCAAAC-3' (SEQ ID NO: 57)
Il1b-Rv: 5'-ATGTGCTGCTGCGAGATTTG-3' (SEQ ID NO: 58)
Il6-Fw: 5'-TCGCTCAGGGTCACAAGAAA-3' (SEQ ID NO: 59)
Il6-Rv: 5'-CATCAGAGGCAAGGAGGAAAAC-3' (SEQ ID NO: 60)
Ucp1-Fw: 5'-GGCCTCTACGACTCAGTCCA-3' (SEQ ID NO: 61)
15 *Ucp1-Rv*: 5'-TAAGCCGGCTGAGATCTTGT-3' (SEQ ID NO: 62)
Cidea-Fw: 5'-AAACCATGACCGAAGTAGCC-3' (SEQ ID NO: 63)
Cidea-Rv: 5'-AGGCCAGTTGTGATGACTAAGAC-3' (SEQ ID NO: 64)
Tnfa-Fw: 5'-CGGCATGGATCTCAAAGACAAC-3' (SEQ ID NO: 65)
Tnfa-Rv: 5'-AGATAGCAAATCGGCTGACG-3' (SEQ ID NO: 66)
20 *F4/80-Fw:* 5'-CTTTGGCTATGGGCTTCCAGTC-3' (SEQ ID NO: 67)
F4/80-Rv: 5'-GCAAGGAGGACAGAGTTTATC-3' (SEQ ID NO: 68)
Rplp0-Fw: 5'-ACTGGTCTAGGACCCGAGAA-3' (SEQ ID NO: 69)
Rplp0-Fw: 5'-TCCCACCTTGTCTCCAGTCT-3' (SEQ ID NO: 70)

25 *Hormone and metabolite assays*

Blood glucose levels were measured with a Glucometer Elite™ analyzer (Bayer, Leverkusen, Germany). Brain levels of FGF21 protein were determined by quantitative sandwich enzyme immunoassay Mouse/Rat FGF-21 ELISA kit (MF2100, R&Dsystems, Abingdon, UK), and normalized by total protein content measured with Bradford reagent (Bio-Rad Protein Assay, Bio-Rad, Germany) in 30 whole brain homogenates. To extract lipids from liver, frozen samples of approximately 100 mg were weighted and homogenized in chloroform:methanol (2:1), as described by Carr *et al*. Hepatic triglycerides and serum triglycerides were quantified spectrophotometrically using an enzymatic assay kit (Horiba-ABX, Montpellier, France). Serum free fatty acids were measured by the acyl-CoA synthase and acyl-CoA oxidaxe methods (Wako Chemicals GmbH, Neuss, Germany). All biochemical parameters 35 were determined using Pentra 400 Analyzer (Horiba-ABX).

Insulin tolerance test

For insulin tolerance test, insulin (0.75 IU/kg body wt; Humulin Regular; Eli Lilly, Indianapolis, IN) was injected intraperitoneally into awake fed mice. Glucose concentration was determined in blood 40 samples obtained from the tail vein at the indicated time points after the insulin injection.

Glucose tolerance test

Awake mice were fasted overnight (16 h) and administered with an intraperitoneal injection of glucose (1 g/kg body weight). Glycemia was measured in tail vein blood samples at the indicated time points.

Pyruvate tolerance test

Awake mice were fasted overnight (16 h) and administered with an intraperitoneal injection of pyruvate (1 g/kg body weight). Glycemia was measured in tail vein blood samples at the indicated time points.

Example 1. Reversion of obesity and diabetes by intra-CSF administration of AAV9-CAG-moFGF21-dmirT vectors in db/db mice

We evaluated the anti-diabetogenic and anti-obesogenic therapeutic potential of the AAV-mediated genetic engineering of the brain with FGF21 in 7-week-old db/db male mice, which have defective leptin signalling and are a widely used genetic model of obesity and diabetes. To this end, db/db mice were administered locally intra-cerebrospinal fluid (CSF), through the cisterna magna, with $5x10^{10}$ vg/mouse of AAV9 vectors encoding a murine codon-optimized FGF21 coding sequence under the control of the CAG ubiquitous promoter which included target sites of the liver-specific miR-122a and the heart-specific miR-1 (AAV9-CAG-moFGF21-dmiRT). As control, non-treated db/db animals were used.

Intra-CSF administration of AAV9-CAG-moFGF21-dmiRT vectors mediated widespread overexpression of FGF21 in the brain, as evidenced by the increased expression levels of the factor in different areas of the brain such as hypothalamus, cortex, hippocampus and cerebellum, 12 weeks after AAV administration (FIG 1).

While non-treated db/db mice continued to gain weight during the 12-week follow-up period (~50% weight gain), there was a clear reduction of weight gain in the cohort treated with FGF21-encoding vectors (~20% weight gain) (FIGs 2A and 2B). In agreement, animals treated with AAV9-CAG-moFGF21-dmiRT vectors showed decreased adiposity and 60% reduction of the weight of the liver (FIG 2C). Noticeably, db/db mice in which FGF21 gene transfer was targeted to the brain also showed complete normalization of fed glycemia, demonstrating counteraction of diabetes in these animals (FIG 3).

Obesity is associated with brain inflammation (O. Guillemot-Legris, G. G. Muccioli, *Trends Neurosci.* 40, 237–253 (2017). Inflammation in this organ was analyzed through the expression of astrocyte markers *Gfap* and *S100b*, the microglia marker *Aif1* and pro-inflammatory molecules, such as *Nfkb*, *Il1b* and *Il6*. Db/db mice treated intra-CSF with AAV9-CAG-moFGF21-dmiRT vectors showed decreased expression of *Gfap*, *S100b*, *Aif1*, *Nfkb*, *Il1b* and *Il6* in the hypothalamus (FIG 4).

Example 1.1

Histological analysis of white adipose tissue by hematoxylin-eosin staining revealed decreased white adipocyte size in eWAT (FIG 10A). In BAT, the histological analysis showed lower lipid accumulation and more multiloculated brown adipocytes (FIG 10B). According to these results, the expression levels of *Ucp1* and *Cidea* were highly increased in BAT of FGF21-treated mice (FIG 10C), suggesting increased thermogenesis after the AAV-FGF21 CNS administration. Hepatic triglyceride content was decreased in AAV9-FGF21-treated db/db mice (FIG 11A). In parallel circulating levels of triglycerides and serum free fatty acids were also decreased in these mice (FIGs 11B and 11C). Immunohistochemical analysis of the pancreas revealed increased number of islets (FIG 12A) and amelioration of β-cell mass (FIG 12B) in db/db mice after the treatment with AAV9-FGF21 vectors.

Obesity and diabetes are associated with systemic inflammation. In white adipose tissue, immunohistochemical analysis against the MAC-2 proinflammatory marker indicated decreased macrophages infiltration in AAV9-FGF21-treated mice (FIG 13A), and this was associated with a decrease in *F4/80* mRNA expression levels (FIG 13B). In brown adipose tissue and in liver, the expression levels of the proinflammatory cytokines *F4/80, Il6,* and *Tnfalpha* were also decreased in FGF21-treated animals (FIGs 13C and 13D, respectively), indicating decreased systemic inflammation after FGF21 gene therapy.

<u>Example 2. Decreased body weight gain by intra-CSF administration of AAV9-CAG-moFGF21-dmirT vectors in SAMP8 mice</u>

Seven-week-old senescence-accelerated mouse-prone 8 (SAMP8) male mice, which is a widely used mouse model of senescence with age-related brain pathologies, were administered locally intra-CSF, through the cisterna magna, with $5x10^{10}$ vg/mouse of AAV9-CAG-moFGF21-dmiRT vectors. As control, non-treated SAMP8 animals were used.

Similar to the observations made in db/db mice, intra-CSF administration of AAV9-CAG-moFGF21-dmiRT vectors mediated robust overexpression of FGF21 in the hypothalamus, cortex, hippocampus and cerebellum of SAMP8 mice (FIG 5), 14 weeks after AAV administration. FGF21-treated mice showed lower body weight gain than the non-treated cohort (FIGs 6A and 6B), which was parallel to a decrease in the weight of the liver (FIG 6C). In addition, expression of the pro-inflammatory cytokines *Il1b* and *Il6* was decreased in the hypothalamus of SAMP8 mice overexpressing FGF21 in the brain (FIG 7).

<u>Example 3. Brain transduction after intra-CSF administration of AAV1-CAG-moFGF21-dmirT, AAV2-CAG-moFGF21-dmirT and AAV9-CAG-moFGF21-dmirT vectors.</u>

To examine whether several AAV serotypes were able to transduce the brain efficiently after direct-CSF administration through the cisterna magna, wild-type mice were treated with $5x10^{10}$vg/mice of AAV1, AAV2 and AAV9 vectors encoding a murine codon-optimized FGF21 coding sequence under the control of the CAG ubiquitous promoter which included target sites of the liver-specific miR-122a and the heart-specific miR-1 (AAV1-CAG-moFGF21-dmiRT, AAV2-CAG-moFGF21-dmiRT and AAV9-CAG-moFGF21-dmiRT, respectively). As control, non-treated wild-type mice were used.

Three weeks after intra-CSF administration of the AAV vectors, brain samples were obtained and RT-PCR analysis showed increased *moFGF21* expression in different brain areas, such as hypothalamus, cortex, hippocampus and cerebellum (FIG 8). Moreover, *moFGF21* overexpression resulted in increased FGF21 protein content in the whole brain (FIG 9).

5

Example 4. Reversion of obesity and diabetes by intra-CSF administration of AAV1-CAG-moFGF21 vectors in db/db mice.

The anti-diabetogenic and anti-obesogenic therapeutic potential of the AAV-mediated genetic engineering of the brain with FGF21 gene therapy, was also evaluated in 7-week-old db/db male mice
10 administered locally intra-cerebrospinal fluid (CSF), through the cisterna magna, with $5x10^{10}$ vg/mouse of AAV1 vectors encoding a murine codon-optimized FGF21 coding sequence under the control of the CAG ubiquitous promoter (AAV1-CAG-moFGF21). As controls, non-treated db/db and non-treated db/+ (lean) mice were used.

15 Intra-CSF administration of AAV1-CAG-moFGF21 vectors mediated widespread overexpression of FGF21 in the brain, as evidenced by the increased expression levels of the factor in different areas of the brain such as hypothalamus, cortex, hippocampus, cerebellum and olfactory bulb, 16 weeks after AAV administration (FIG 14).

20 While non-treated db/db mice continued to gain weight during the 14-week follow-up period, the body weight of the cohort treated with AAV1-FGF21-encoding vectors was not increased (FIG 15). Noticeably, db/db mice in which FGF21 gene transfer was targeted to the brain also showed complete normalization of fed and fasted glycemia (FIGs 16A and 16B), demonstrating counteraction of diabetes in these animals.

25

An insulin tolerance test showed that insulin resistance was improved in db/db after the treatment with AAV1-FGF21 viral vectors (FIG 17) and an intraperitoneal glucose tolerance test on overnight-starved mice showed that db/db mice treated with AAV1-CAG-moFGF21 were more glucose tolerant than db/db non-treated mice (FIG 18). As an indicator of hepatic gluconeogenesis, an intraperitoneal
30 pyruvate tolerance test was performed. After the pyruvate challenge, blood glucose levels rose to 600 mg/dl in db/db non-treated mice and remained elevated during the test, whereas glucose levels of FGF21 db/db-treated mice and lean mice treated rose to a maximum of 150 mg/dl, thus indicating decreased gluconeogenesis after AAV1-CAG-FGF21 treatment (FIG 19).

35

Sequences

SEQ ID NO:	Description of the sequence
1	Amino acid sequence of *homo sapiens* FGF21
2	Amino acid sequence of *mus musculus* FGF21
3	Amino acid sequence of *canis lupus familiaris* FGF21
4	Nucleotide sequence of *homo sapiens* FGF21
5	Codon optimized nucleotide sequence of *homo sapiens* FGF21 – variant 1
6	Codon optimized nucleotide sequence of *homo sapiens* FGF21 – variant 2
7	Codon optimized nucleotide sequence of *homo sapiens* FGF21 – variant 3
8	Nucleotide sequence of *mus musculus* FGF21
9	Codon optimized nucleotide sequence of *mus musculus* FGF21
10	Nucleotide sequence of *canis lupus familiaris* FGF21
11	Codon optimized nucleotide sequence of *canis lupus familiaris* FGF21
12	Nucleotide sequence encoding miRT-122a
13	Nucleotide sequence encoding miRT-1
14	Nucleotide sequence encoding miRT-152
15	Nucleotide sequence encoding miRT-199a-5p
16	Nucleotide sequence encoding miRT-199a-3p
17	Nucleotide sequence encoding miRT-215
18	Nucleotide sequence encoding miRT-192
19	Nucleotide sequence encoding miRT-148a
20	Nucleotide sequence encoding miRT-194
21	Nucleotide sequence encoding miRT-133a
22	Nucleotide sequence encoding miRT-206
23	Nucleotide sequence encoding miRT-208-5p
24	Nucleotide sequence encoding miRT-208a-3p
25	Nucleotide sequence encoding miRT-499-5p
26	Nucleotide sequence of chimeric intron composed of introns from human β-globin and immunoglobulin heavy chain genes
27	Nucleotide sequence of CAG promoter
28	Nucleotide sequence of CMV promoter
29	Nucleotide sequence of CMV enhancer
30	Truncated AAV2 5' ITR
31	Truncated AAV2 3' ITR
32	SV40 polyadenylation signal
33	Rabbit β-globin polyadenylation signal
34	CMV promoter and CMV enhancer sequence
35	pAAV-CAG-moFGF21-dmiRT
36	mini-CMV promoter
37	EF1α promoter
38	RSV promoter
39	Synapsin 1 promoter
40	Calcium/calmodulin-dependent protein kinase II (CaMKII) promoter
41	Glial fibrillary acidic protein (GFAP) promoter
42	Nestin promoter
43	Homeobox Protein 9 (HB9) promoter
44	Tyrosine hydroxylase (TH) promoter

45	Myelin basic protein (MBP) promoter
46	pAAV-CAG-moFGF21
47-70	RT-qPCR primers

Amino acid sequence of *homo sapiens* FGF21 (SEQ ID NO: 1)
MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLYTDDAQQTEAHLEIREDG
TVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRPDGALYGSLHFDPEACSFRELLLEDGYNVY
5 QSEAHGLPLHLPGNKSPHRDPAPRGPARFLPLPGLPPALPEPPGILAPQPPDVGSSDPLSMVGPSQG
RSPSYAS
Nucleotide sequence of *homo sapiens* FGF21 (SEQ ID NO: 4)
ATGGACTCGGACGAGACCGGGTTCGAGCACTCAGGACTGTGGGTTTCTGTGCTGGCTGGTCT
TCTGCTGGGAGCCTGCCAGGCACACCCCATCCCTGACTCCAGTCCTCTCCTGCAATTCGGGGGC
10 CAAGTCCGGCAGCGGTACCTCTACACAGATGATGCCCAGCAGACAGAAGCCCACCTGGAGATCA
GGGAGGATGGGACGGTGGGGGGCGCTGCTGACCAGAGCCCCGAAAGTCTCCTGCAGCTGAAA
GCCTTGAAGCCGGGAGTTATTCAAATCTTGGGAGTCAAGACATCCAGGTTCCTGTGCCAGCGGC
CAGATGGGGCCCTGTATGGATCGCTCCACTTTGACCCTGAGGCCTGCAGCTTCCGGGAGCTGCT
TCTTGAGGACGGATACAATGTTTACCAGTCCGAAGCCCACGGCCTCCCGCTGCACCTGCCAGGG
15 AACAAGTCCCCACACCGGGACCCTGCACCCCGAGGACCAGCTCGCTTCCTGCCACTACCAGGC
CTGCCCCCCGCACTCCCGGAGCCACCCGGAATCCTGGCCCCCCAGCCCCCCGATGTGGGCTCC
TCGGACCCTCTGAGCATGGTGGGACCTTCCCAGGGCCGAAGCCCCAGCTACGCTTCCTGA
Codon optimized nucleotide sequence of *homo sapiens* FGF21 – variant 1 (SEQ ID NO: 5)
ATGGATTCTGATGAGACAGGCTTCGAGCACAGCGGCCTGTGGGTTTCAGTTCTGGCTGGACT
20 GCTGCTGGGAGCCTGTCAGGCACACCCTATTCCAGATAGCAGCCCTCTGCTGCAGTTCGGCGGA
CAAGTGCGGCAGAGATACCTGTACACCGACGACGCCCAGCAGACAGAAGCCCACCTGGAAATCA
GAGAGGATGGCACAGTTGGCGGAGCCGCCGATCAGTCTCCTGAATCTCTGCTCCAGCTGAAGGC
CCTGAAGCCTGGCGTGATCCAGATCCTGGGCGTGAAAACCAGCCGGTTCCTGTGCCAAAGACCT
GACGGCGCCCTGTATGGCAGCCTGCACTTTGATCCTGAGGCCTGCAGCTTCAGAGAGCTGCTGC
25 TTGAGGACGGCTACAACGTGTACCAGTCTGAGGCCCATGGCCTGCCTCTGCATCTGCCTGGAAA
CAAGAGCCCTCACAGAGATCCCGCTCCTAGAGGCCCTGCCAGATTTCTGCCTCTTCCTGGATTG
CCTCCTGCTCTGCCAGAGCCTCCTGGAATTCTGGCTCCTCAGCCTCCTGATGTGGGCAGCTCTG
ATCCTCTGAGCATGGTCGGACCTAGCCAGGGCAGATCTCCTAGCTACGCCTCTTGA
Codon optimized nucleotide sequence of *homo sapiens* FGF21 – variant 2 (SEQ ID NO: 6)
30 ATGGACAGCGATGAAACCGGGTTCGAGCACAGCGGTCTGTGGGTGTCCGTGCTGGCCGGAC
TGCTCCTGGGAGCCTGTCAGGCGCACCCCATCCCTGACTCCTCGCCGCTGCTGCAATTCGGCG
GACAAGTCCGCCAGAGATACCTGTACACCGACGACGCCCAGCAGACCGAAGCCCACCTGGAAAT
TCGGGAGGACGGGACTGTGGGAGGCGCTGCAGATCAGTCACCCGAGTCCCTCCTCCAACTGAA
GGCCTTGAAGCCCGGCGTGATTCAGATCCTGGGCGTGAAAACTTCCCGCTTCCTTTGCCAACGG
35 CCGGATGGAGCTCTGTACGGATCCCTGCACTTCGACCCCGAAGCCTGCTCATTCCGCGAGCTGC
TCCTTGAGGACGGCTATAACGTGTACCAGTCTGAGGCCCATGGACTCCCCCTGCATCTGCCCGG
CAACAAGTCCCCTCACCGGGATCCTGCCCCAAGAGGCCCAGCTCGGTTTCTGCCTCTGCCGGGA

CTGCCTCCAGCGTTGCCCGAACCCCCTGGTATCCTGGCCCCGCAACCACCTGACGTCGGTTCGT
CGGACCCGCTGAGCATGGTCGGTCCGAGCCAGGGAAGGTCCCCGTCCTACGCATCCTGA

Codon optimized nucleotide sequence of *homo sapiens* FGF21 – variant 3 (SEQ ID NO: 7)

5 ATGGATTCCGACGAAACTGGATTTGAACATTCAGGGCTGTGGGTCTCTGTGCTGGCTGGACT
GCTGCTGGGGGCTTGTCAGGCTCACCCCATCCCTGACAGCTCCCCTCTGCTGCAGTTCGGAGGA
CAGGTGCGGCAGAGATACCTGTATACCGACGATGCCCAGCAGACAGAGGCACACCTGGAGATCA
GGGAGGACGGAACCGTGGGAGGAGCAGCCGATCAGTCTCCCGAGAGCCTGCTGCAGCTGAAG
GCCCTGAAGCCTGGCGTGATCCAGATCCTGGGCGTGAAGACATCTCGGTTTCTGTGCCAGCGGC
10 CCGACGGCGCCCTGTACGGCTCCCTGCACTTCGATCCCGAGGCCTGTTCTTTTAGGGAGCTGCT
GCTGGAGGACGGCTACAACGTGTATCAGAGCGAGGCACACGGCCTGCCACTGCACCTGCCTGG
CAATAAGTCCCCTCACCGCGATCCAGCACCCAGGGGCCCAGCACGCTTCCTGCCTCTGCCAGGC
CTGCCCCCTGCCCTGCCAGAGCCACCCGGCATCCTGGCCCCCCAGCCTCCAGATGTGGGCTCC
AGCGATCCTCTGTCAATGGTGGGGCCAAGTCAGGGGCGGAGTCCTTCATACGCATCATAA

15 Nucleotide sequence of murine codon-optimized FGF21 (SEQ ID NO: 9)

ATGGAATGGATGAGAAGCAGAGTGGGCACCCTGGGCCTGTGGGTGCGACTGCTGCTGGCTG
TGTTTCTGCTGGGCGTGTACCAGGCCTACCCCATCCCTGACTCTAGCCCCCTGCTGCAGTTTGG
CGGACAAGTGCGGCAGAGATACCTGTACACCGACGACGACCAGGACACCGAGGCCCACCTGGA
AATCCGCGAGGATGGCACAGTCGTGGGCGCTGCTCACAGAAGCCCTGAGAGCCTGCTGGAACT
20 GAAGGCCCTGAAGCCCGGCGTGATCCAGATCCTGGGCGTGAAGGCCAGCAGATTCCTGTGCCA
GCAGCCTGACGGCGCCCTGTACGGCTCTCCTCACTTCGATCCTGAGGCCTGCAGCTTCAGAGAG
CTGCTGCTGGAGGACGGCTACAACGTGTACCAGTCTGAGGCCCACGGCCTGCCCCTGAGACTG
CCTCAGAAGGACAGCCCTAACCAGGACGCCACAAGCTGGGGACCTGTGCGGTTCCTGCCTATGC
CTGGACTGCTGCACGAGCCCCAGGATCAGGCTGGCTTTCTGCCTCCTGAGCCTCCAGACGTGG
25 GCAGCAGCGACCCTCTGAGCATGGTGGAACCTCTGCAGGGCAGAAGCCCCAGCTACGCCTCTT
GA

Nucleotide sequence of CAG promoter (SEQ ID NO: 27)

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
30 GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGT
CAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAG
TACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
TATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGA
GCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTA
35 TTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGGGCGCGCGCCAGGCGGG
GCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAG
AGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAG
CGAAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGC
CTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACG
40 GCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTG
CGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGC

GTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGCCCGGCGGCTGTGAGC
GCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGG
GGGCGGTGCCCCGCGGTGCGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTG
CGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAACCCCCCCTGCACCCCC
5 CTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCG
GGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCG
CCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTC
GAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTC
CTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGC
10 GCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTC
GCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCT
TCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCT
CTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAG

Nucleotide sequence of CMV promoter (SEQ ID NO: 28)

15 GTGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCC
AAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAA
AATGTCGTAACAACTGCGATCGCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTG
GGAGGTCTATATAAGCAGAGCT

Nucleotide sequence of CMV enhancer (SEQ ID NO: 29)

20 GGCATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGT
CAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAG
TCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
25 TACGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG

CMV promoter and CMV enhancer sequence (SEQ ID NO: 34)

GGCATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGT
30 CAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAG
TCCGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCT
TACGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGT
TTTGGCAGTACACCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCC
CATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAA
35 CTGCGATCGCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATA
AGCAGAGCT

AAV2 5’ ITR (SEQ ID NO: 30)

GCGCGCTC GCTCGCTCAC TGAGGCCGCC CGGGCAAAGC
CCGGGCGTCG GGCGACCTTT GGTCGCCCGG CCTCAGTGAG CGAGCGAGCG
40 CGCAGAGAGG GAGTGGCCAA CTCCATCACT AGGGGTTCCT

AAV2 3’ ITR (SEQ ID NO: 31)

AGGAACCCCT AGTGATGGAG TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCACTGAGG CCGGGCGACC AAAGGTCGCC CGACGCCCGG GCTTTGCCCG GGCGGCCTCA GTGAGCGAGC GAGCGCGC

Rabbit β-globin polyadenylation signal (3' UTR and flanking region of rabbit beta-globin, including polyA signal) (SEQ ID NO: 33)

GATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACTTCTG GCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAG GACATATGGGAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATA TGCCCATATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGAGGTCATCAGTATATGAAACAGC CCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTG TTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCT CCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGGAGATC

miRT sequences

miRT-122a (SEQ ID NO: 12): 5’ CAAACACCATTGTCACACTCCA 3’, target for the microRNA-122a (Accession Number to the miRBase database MI0000442), which is expressed in the liver.

miRT-152 (SEQ ID NO: 14): 5’ CCAAGTTCTGTCATGCACTGA 3’, target for the microRNA-152 (MI0000462), which is expressed in the liver.

miRT-199a-5p (SEQ ID NO: 15): 5’ GAACAGGTAGTCTGAACACTGGG 3’, target for the microRNA 199a (MI0000242), which is expressed in the liver.

miRT-199a-3p (SEQ ID NO: 16): 5’ TAACCAATGTGCAGACTACTGT 3’, target for the microRNA-199a (MI0000242), which is expressed in the liver.

miRT-215 (SEQ ID NO: 17): 5’ GTCTGTCAATTCATAGGTCAT 3’, target for the microRNA-215 (MI0000291), which is expressed in the liver.

miRT-192 (SEQ ID NO: 18): 5’ GGCTGTCAATTCATAGGTCAG 3’, target for the microRNA-192 (MI0000234), which is expressed in the liver.

miRT-148a (SEQ ID NO: 19): 5’ ACAAAGTTCTGTAGTGCACTGA 3’, target for the microRNA-148a (MI0000253), which is expressed in the liver.

miRT-194 (SEQ ID NO: 20): 5’ TCCACATGGAGTTGCTGTTACA 3’, target for the microRNA-194 (MI0000488), which is expressed in the liver.

miRT-133a (SEQ ID NO: 21): 5’ CAGCTGGTTGAAGGGGACCAAA 3’, target for the microRNA-133a (MI0000450), which is expressed in the heart.

miRT-206 (SEQ ID NO: 22): 5’ CCACACACTTCCTTACATTCCA 3’, target for the microRNA-206 (MI0000490), which is expressed in the heart.

miRT-1 (SEQ ID NO: 13): 5’ TTACATACTTCTTTACATTCCA 3’, target for the microRNA-1 (MI0000651), which is expressed in the heart.

miRT-208a-5p (SEQ ID NO: 23): 5’ GTATAACCCGGGCCAAAAGCTC 3’, target for the microRNA-208a (MI0000251), which is expressed in the heart.

miRT-208a-3p (SEQ ID NO: 24): 5’ ACAAGCTTTTTGCTCGTCTTAT 3’, target for the microRNA-208a (MI0000251), which is expressed in the heart.

miRT-499-5p (SEQ ID NO: 25): 5' AAACATCACTGCAAGTCTTAA 3', target for the microRNA-499 (MI0003183), which is expressed in the heart.

pAAV-CAG-moFGF21-dmiRT (SEQ ID NO: 35)

1 AGTGAGCGAG CGAGCGCGCA GCTGCATTAA TGAATCGGCC AACGCGCGGG
5 51 GAGAGGCGGT TTGCGTATTG GGCGCTCTTC CGCTTCCTCG CTCACTGACT
101 CGCTGCGCTC GGTCGTTCGG CTGCGGCGAG CGGTATCAGC TCACTCAAAG
151 GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT
201 GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC
251 TGGCGTTTTT CCATAGGCTC CGCCCCCCTG ACGAGCATCA CAAAAATCGA
10 301 CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA GATACCAGGC
351 GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG ACCCTGCCGC
401 TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT
451 CATAGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA
501 GCTGGGCTGT GTGCACGAAC CCCCCGTTCA GCCCGACCGC TGCGCCTTAT
15 551 CCGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATCGCCA
601 CTGGCAGCAG CCACTGGTAA CAGGATTAGC AGAGCGAGGT ATGTAGGCGG
651 TGCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC ACTAGAAGAA
701 CAGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA
751 GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT
20 801 TTTTGTTTGC AAGCAGCAGA TTACGCGCAG AAAAAAAGGA TCTCAAGAAG
851 ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACTCA
901 CGTTAAGGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT TCACCTAGAT
951 CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT ATATATGAGT
1001 AAACTTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC ACCTATCTCA
25 1051 GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA
1101 GATAACTACG ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA
1151 TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAACCAG
1201 CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCCTC
1251 CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG
30 1301 TTAATAGTTT GCGCAACGTT GTTGCCATTG CTACAGGCAT CGTGGTGTCA
1351 CGCTCGTCGT TTGGTATGGC TTCATTCAGC TCCGGTTCCC AACGATCAAG
1401 GCGAGTTACA TGATCCCCCA TGTTGTGCAA AAAAGCGGTT AGCTCCTTCG
1451 GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG
1501 GTTATGGCAG CACTGCATAA TTCTCTTACT GTCATGCCAT CCGTAAGATG
35 1551 CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA
1601 TGCGGCGACC GAGTTGCTCT TGCCCGGCGT CAATACGGGA TAATACCGCG
1651 CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG
1701 GCGAAAACTC TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC
1751 CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT
40 1801 TCTGGGTGAG CAAAAACAGG AAGGCAAAAT GCCGCAAAAA AGGGAATAAG
1851 GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT CAATATTATT

1901 GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT
1951 ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT
2001 GCCACCTGAC GTCTAAGAAA CCATTATTAT CATGACATTA ACCTATAAAA
2051 ATAGGCGTAT CACGAGGCCC TTTCGTCTCG CGCGTTTCGG TGATGACGGT
5 2101 GAAAACCTCT GACACATGCA GCTCCCGGAG ACGGTCACAG CTTGTCTGTA
2151 AGCGGATGCC GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA GCGGGTGTTG
2201 GCGGGTGTCG GGGCTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG
2251 AGAGTGCACC ATATGCGGTG TGAAATACCG CACAGATGCG TAAGGAGAAA
2301 ATACCGCATC AGGCGATTCC AACATCCAAT AAATCATACA GGCAAGGCAA
10 2351 AGAATTAGCA AAATTAAGCA ATAAAGCCTC AGAGCATAAA GCTAAATCGG
2401 TTGTACCAAA AACATTATGA CCCTGTAATA CTTTTGCGGG AGAAGCCTTT
2451 ATTTCAACGC AAGGATAAAA ATTTTTAGAA CCCTCATATA TTTTAAATGC
2501 AATGCCTGAG TAATGTGTAG GTAAAGATTC AAACGGGTGA GAAAGGCCGG
2551 AGACAGTCAA ATCACCATCA ATATGATATT CAACCGTTCT AGCTGATAAA
15 2601 TTCATGCCGG AGAGGGTAGC TATTTTTGAG AGGTCTCTAC AAAGGCTATC
2651 AGGTCATTGC CTGAGAGTCT GGAGCAAACA AGAGAATCGA TGAACGGTAA
2701 TCGTAAAACT AGCATGTCAA TCATATGTAC CCCGGTTGAT AATCAGAAAA
2751 GCCCCAAAAA CAGGAAGATT GTATAAGCAA ATATTTAAAT TGTAAGCGTT
2801 AATATTTTGT TAAAATTCGC GTTAAATTTT TGTTAAATCA GCTCATTTTT
20 2851 TAACCAATAG GCCGAAATCG GCAAAATCCC TTATAAATCA AAAGAATAGA
2901 CCGAGATAGG GTTGAGTGTT GTTCCAGTTT GGAACAAGAG TCCACTATTA
2951 AAGAACGTGG ACTCCAACGT CAAAGGGCGA AAAACCGTCT ATCAGGGCGA
3001 TGGCCCACTA CGTGAACCAT CACCCTAATC AAGTTTTTTG GGGTCGAGGT
3051 GCCGTAAAGC ACTAAATCGG AACCCTAAAG GGAGCCCCCG ATTTAGAGCT
25 3101 TGACGGGGAA AGCCGGCGAA CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA
3151 AGGAGCGGGC GCTAGGGCGC TGGCAAGTGT AGCGGTCACG CTGCGCGTAA
3201 CCACCACACC CGCCGCGCTT AATGCGCCGC TACAGGGCGC GTACTATGGT
3251 TGCTTTGACG AGCACGTATA ACGTGCTTTC CTCGTTAGAA TCAGAGCGGG
3301 AGCTAAACAG GAGGCCGATT AAAGGGATTT TAGACAGGAA CGGTACGCCA
30 3351 GAATCCTGAG AAGTGTTTTT ATAATCAGTG AGGCCACCGA GTAAAAGAGT
3401 CTGTCCATCA CGCAAATTAA CCGTTGTCGC AATACTTCTT TGATTAGTAA
3451 TAACATCACT TGCCTGAGTA GAAGAACTCA AACTATCGGC CTTGCTGGTA
3501 ATATCCAGAA CAATATTACC GCCAGCCATT GCAACGGAAT CGCCATTCGC
3551 CATTCAGGCT GCGCAACTGT TGGGAAGGGC GATCGGTGCG GGCCTCTTCC
35 3601 ACTGAGGCCC AGCTGCGCGC TCGCTCGCTC ACTGAGGCCG CCCGGGCAAA
3651 GCCCGGGCGT CGGGCGACCT TTGGTCGCCC GGCCTCAGTG AGCGAGCGAG
3701 CGCGCAGAGA GGGAGTGGCC AACTCCATCA CTAGGGGTTC CTTGTAGTTA
3751 ATGATTAACC CGCCATGCTA CTTATCTACT CGACATTGAT TATTGACTAG
3801 TTATTAATAG TAATCAATTA CGGGGTCATT AGTTCATAGC CCATATATGG
40 3851 AGTTCCGCGT TACATAACTT ACGGTAAATG GCCCGCCTGG CTGACCGCCC
3901 AACGACCCCC GCCCATTGAC GTCAATAATG ACGTATGTTC CCATAGTAAC

3951 GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGAGTAT TTACGGTAAA
4001 CTGCCCACTT GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT
4051 ATTGACGTCA ATGACGGTAA ATGGCCCGCC TGGCATTATG CCCAGTACAT
4101 GACCTTATGG GACTTTCCTA CTTGGCAGTA CATCTACGTA TTAGTCATCG
5 4151 CTATTACCAT GGTCGAGGTG AGCCCCACGT TCTGCTTCAC TCTCCCCATC
4201 TCCCCCCCCT CCCCACCCCC AATTTTGTAT TTATTTATTT TTTAATTATT
4251 TTGTGCAGCG ATGGGGGCGG GGGGGGGGGG GGGGCGCGCG CCAGGCGGGG
4301 CGGGGCGGGG CGAGGGGCGG GGCGGGGCGA GGCGGAGAGG TGCGGCGGCA
4351 GCCAATCAGA GCGGCGCGCT CCGAAAGTTT CCTTTTATGG CGAGGCGGCG
10 4401 GCGGCGGCGG CCCTATAAAA AGCGAAGCGC GCGGCGGGCG GGAGTCGCTG
4451 CGTTGCCTTC GCCCCGTGCC CCGCTCCGCG CCGCCTCGCG CCGCCCGCCC
4501 CGGCTCTGAC TGACCGCGTT ACTCCCACAG GTGAGCGGGC GGGACGGCCC
4551 TTCTCCTCCG GGCTGTAATT AGCGCTTGGT TTAATGACGG CTTGTTTCTT
4601 TTCTGTGGCT GCGTGAAAGC CTTGAGGGGC TCCGGGAGGG CCCTTTGTGC
15 4651 GGGGGGAGCG GCTCGGGGGG TGCGTGCGTG TGTGTGTGCG TGGGGAGCGC
4701 CGCGTGCGGC TCCGCGCTGC CCGGCGGCTG TGAGCGCTGC GGGCGCGGCG
4751 CGGGGCTTTG TGCGCTCCGC AGTGTGCGCG AGGGGAGCGC GGCCGGGGGC
4801 GGTGCCCCGC GGTGCGGGGG GCTGCGAGGG GAACAAAGGC TGCGTGCGGG
4851 GTGTGTGCGT GGGGGGGTGA GCAGGGGGTG TGGGCGCGTC GGTCGGGCTG
20 4901 CAACCCCCCC TGCACCCCCC TCCCCGAGTT GCTGAGCACG GCCCGGCTTC
4951 GGGTGCGGGG CTCCGTACGG GGCGTGGCGC GGGGCTCGCC GTGCCGGGCG
5001 GGGGGTGGCG GCAGGTGGGG GTGCCGGGCG GGGCGGGGCC GCCTCGGGCC
5051 GGGGAGGGCT CGGGGGAGGG GCGCGGCGGC CCCCGGAGCG CCGGCGGCTG
5101 TCGAGGCGCG GCGAGCCGCA GCCATTGCCT TTTATGGTAA TCGTGCGAGA
25 5151 GGGCGCAGGG ACTTCCTTTG TCCCAAATCT GTGCGGAGCC GAAATCTGGG
5201 AGGCGCCGCC GCACCCCCTC TAGCGGGCGC GGGGCGAAGC GGTGCGGCGC
5251 CGGCAGGAAG GAAATGGGCG GGGAGGGCCT TCGTGCGTCG CCGCGCCGCC
5301 GTCCCCTTCT CCCTCTCCAG CCTCGGGGCT GTCCGCGGGG GGACGGCTGC
5351 CTTCGGGGGG GACGGGGCAG GGCGGGGTTC GGCTTCTGGC GTGTGACCGG
30 5401 CGGCTCTAGA GCCTCTGCTA ACCATGTTCA TGCCTTCTTC TTTTTCCTAC
5451 AGCTCCTGGG CAACGTGCTG GTTATTGTGC TGTCTCATCA TTTTGGCAAA
5501 GAATTGATTA ATTCGAGCGA ACGCGTCGAG TCGCTCGGTA CGATTTAAAT
5551 TGAATTGGCC TCGAGCGCAA GCTTGAGCTA GCGCCACCAT GGAATGGATG
5601 AGAAGCAGAG TGGGCACCCT GGGCCTGTGG GTGCGACTGC TGCTGGCTGT
35 5651 GTTTCTGCTG GGCGTGTACC AGGCCTACCC CATCCCTGAC TCTAGCCCCC
5701 TGCTGCAGTT TGGCGGACAA GTGCGGCAGA GATACCTGTA CACCGACGAC
5751 GACCAGGACA CCGAGGCCCA CCTGGAAATC CGCGAGGATG GCACAGTCGT
5801 GGGCGCTGCT CACAGAAGCC CTGAGAGCCT GCTGGAACTG AAGGCCCTGA
5851 AGCCCGGCGT GATCCAGATC CTGGGCGTGA AGGCCAGCAG ATTCCTGTGC
40 5901 CAGCAGCCTG ACGGCGCCCT GTACGGCTCT CCTCACTTCG ATCCTGAGGC
5951 CTGCAGCTTC AGAGAGCTGC TGCTGGAGGA CGGCTACAAC GTGTACCAGT

6001 CTGAGGCCCA CGGCCTGCCC CTGAGACTGC CTCAGAAGGA CAGCCCTAAC
6051 CAGGACGCCA CAAGCTGGGG ACCTGTGCGG TTCCTGCCTA TGCCTGGACT
6101 GCTGCACGAG CCCCAGGATC AGGCTGGCTT TCTGCCTCCT GAGCCTCCAG
6151 ACGTGGGCAG CAGCGACCCT CTGAGCATGG TGGAACCTCT GCAGGGCAGA
5 6201 AGCCCCAGCT ACGCCTCTTG AGAATGCGGG CCCGGTACCC CCGACGCGGC
6251 CGCTAATTCT AGATCGCGAA CAAACACCAT TGTCACACTC CAGTATACAC
6301 AAACACCATT GTCACACTCC AGATATCACA AACACCATTG TCACACTCCA
6351 AGGCGAACAA ACACCATTGT CACACTCCAA GGCTATTCTA GATCGCGAAT
6401 TACATACTTC TTTACATTCC AGTATACATT ACATACTTCT TTACATTCCA
10 6451 GATATCATTA CATACTTCTT TACATTCCAA GGCGAATTAC ATACTTCTTT
6501 ACATTCCAAG GCTACCTGAG GCCCGGGGGT ACCTCTTAAT TAACTGGCCT
6551 CATGGGCCTT CCGCTCACTG CCCGCTTTCC AGTCGGGAAA CCTGTCGTGC
6601 CAGTCAGGTG CAGGCTGCCT ATCAGAAGGT GGTGGCTGGT GTGGCCAATG
6651 CCCTGGCTCA CAAATACCAC TGAGATCTTT TTCCCTCTGC CAAAAATTAT
15 6701 GGGGACATCA TGAAGCCCCT TGAGCATCTG ACTTCTGGCT AATAAAGGAA
6751 ATTTATTTTC ATTGCAATAG TGTGTTGGAA TTTTTTGTGT CTCTCACTCG
6801 GAAGGACATA TGGGAGGGCA AATCATTTAA AACATCAGAA TGAGTATTTG
6851 GTTTAGAGTT TGGCAACATA TGCCCATATG CTGGCTGCCA TGAACAAAGG
6901 TTGGCTATAA AGAGGTCATC AGTATATGAA ACAGCCCCCT GCTGTCCATT
20 6951 CCTTATTCCA TAGAAAAGCC TTGACTTGAG GTTAGATTTT TTTTATATTT
7001 TGTTTTGTGT TATTTTTTTC TTTAACATCC CTAAAATTTT CCTTACATGT
7051 TTTACTAGCC AGATTTTTCC TCCTCTCCTG ACTACTCCCA GTCATAGCTG
7101 TCCCTCTTCT CTTATGGAGA TCCCTCGACC TGCAGCCCAA GCTGTAGATA
7151 AGTAGCATGG CGGGTTAATC ATTAACTACA AGGAACCCCT AGTGATGGAG
25 7201 TTGGCCACTC CCTCTCTGCG CGCTCGCTCG CTCACTGAGG CCGGGCGACC
7251 AAAGGTCGCC CGACGCCCGG GCTTTGCCCG GGCGGCCTCA GTGAGCGAGC
7301 GAGCGCGCAG CTGGCGTAA

AAV2 5' ITR: 3615-3742 bp
30 CAG promoter: 3782-5452 bp
Mus musculus codon-optimized FGF21 (moFGF21): 5589-6221 bp
dmiRT (4 copies of the miRT-122a and 4 copies of the miRT-1): 6254-6514 bp
Rabbit β-globin polyA signal (3' UTR and 3' flanking region of rabbit beta-globin, including polyA signal): 6674-6764 bp
35 AAV2 3' ITR: 7181-7308 bp

pAAV-CAG-moFGF21 (SEQ ID NO: 46)

1 AGTGAGCGAG CGAGCGCGCA GCTGCATTAA TGAATCGGCC AACGCGCGGG GAGAGGCGGT
61 TTGCGTATTG GGCGCTCTTC CGCTTCCTCG CTCACTGACT CGCTGCGCTC GGTCGTTCGG
40 121 CTGCGGCGAG CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC AGAATCAGGG
181 GATAACGCAG GAAAGAACAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG

241 GCCGCGTTGC TGGCGTTTTT CCATAGGCTC CGCCCCCCTG ACGAGCATCA CAAAAATCGA
301 CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA GATACCAGGC GTTTCCCCCT
361 GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG ACCCTGCCGC TTACCGGATA CCTGTCCGCC
421 TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT CATAGCTCAC GCTGTAGGTA TCTCAGTTCG
5 481 GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA GCCCGACCGC
541 TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA CTTATCGCCA
601 CTGGCAGCAG CCACTGGTAA CAGGATTAGC AGAGCGAGGT ATGTAGGCGG TGCTACAGAG
661 TTCTTGAAGT GGTGGCCTAA CTACGGCTAC ACTAGAAGAA CAGTATTTGG TATCTGCGCT
721 CTGCTGAAGC CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC
10 781 ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG AAAAAAAGGA
841 TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA CGAAAACTCA
901 CGTTAAGGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT TCACCTAGAT CCTTTTAAAT
961 TAAAAATGAA GTTTTAAATC AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC
1021 CAATGCTTAA TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT
15 1081 GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC TGGCCCCAGT
1141 GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC AATAAACCAG
1201 CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCCTC CATCCAGTCT
1261 ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT
1321 GTTGCCATTG CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC
20 1381 TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA AAAAGCGGTT
1441 AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT ATCACTCATG
1501 GTTATGGCAG CACTGCATAA TTCTCTTACT GTCATGCCAT CCGTAAGATG CTTTTCTGTG
1561 ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT
1621 TGCCCGGCGT CAATACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA AGTGCTCATC
25 1681 ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT GAGATCCAGT
1741 TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT CACCAGCGTT
1801 TCTGGGTGAG CAAAAACAGG AAGGCAAAAT GCCGCAAAAA AGGGAATAAG GGCGACACGG
1861 AAATGTTGAA TACTCATACT CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT
1921 TGTCTCATGA GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG
30 1981 CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT CATGACATTA
2041 ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTCG CGCGTTTCGG TGATGACGGT
2101 GAAAACCTCT GACACATGCA GCTCCCGGAG ACGGTCACAG CTTGTCTGTA AGCGGATGCC
2161 GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA GCGGGTGTTG GCGGGTGTCG GGGCTGGCTT
2221 AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC ATATGCGGTG TGAAATACCG
35 2281 CACAGATGCG TAAGGAGAAA ATACCGCATC AGGCGATTCC AACATCCAAT AAATCATACA
2341 GGCAAGGCAA AGAATTAGCA AAATTAAGCA ATAAAGCCTC AGAGCATAAA GCTAAATCGG
2401 TTGTACCAAA AACATTATGA CCCTGTAATA CTTTTGCGGG AGAAGCCTTT ATTTCAACGC
2461 AAGGATAAAA ATTTTTAGAA CCCTCATATA TTTTAAATGC AATGCCTGAG TAATGTGTAG
2521 GTAAAGATTC AAACGGGTGA GAAAGGCCGG AGACAGTCAA ATCACCATCA ATATGATATT
40 2581 CAACCGTTCT AGCTGATAAA TTCATGCCGG AGAGGGTAGC TATTTTTGAG AGGTCTCTAC
2641 AAAGGCTATC AGGTCATTGC CTGAGAGTCT GGAGCAAACA AGAGAATCGA TGAACGGTAA
2701 TCGTAAAACT AGCATGTCAA TCATATGTAC CCCGGTTGAT AATCAGAAAA GCCCCAAAAA
2761 CAGGAAGATT GTATAAGCAA ATATTTAAAT TGTAAGCGTT AATATTTTGT TAAAATTCGC
2821 GTTAAATTTT TGTTAAATCA GCTCATTTTT TAACCAATAG GCCGAAATCG GCAAAATCCC
45 2881 TTATAAATCA AAAGAATAGA CCGAGATAGG GTTGAGTGTT GTTCCAGTTT GGAACAAGAG

2941 TCCACTATTA AAGAACGTGG ACTCCAACGT CAAAGGGCGA AAAACCGTCT ATCAGGGCGA
3001 TGGCCCACTA CGTGAACCAT CACCCTAATC AAGTTTTTTG GGGTCGAGGT GCCGTAAAGC
3061 ACTAAATCGG AACCCTAAAG GGAGCCCCCG ATTTAGAGCT TGACGGGGAA AGCCGGCGAA
3121 CGTGGCGAGA AAGGAAGGGA AGAAAGCGAA AGGAGCGGGC GCTAGGGCGC TGGCAAGTGT
5 3181 AGCGGTCACG CTGCGCGTAA CCACCACACC CGCCGCGCTT AATGCGCCGC TACAGGGCGC
3241 GTACTATGGT TGCTTTGACG AGCACGTATA ACGTGCTTTC CTCGTTAGAA TCAGAGCGGG
3301 AGCTAAACAG GAGGCCGATT AAAGGGATTT TAGACAGGAA CGGTACGCCA GAATCCTGAG
3361 AAGTGTTTTT ATAATCAGTG AGGCCACCGA GTAAAAGAGT CTGTCCATCA CGCAAATTAA
3421 CCGTTGTCGC AATACTTCTT TGATTAGTAA TAACATCACT TGCCTGAGTA GAAGAACTCA
10 3481 AACTATCGGC CTTGCTGGTA ATATCCAGAA CAATATTACC GCCAGCCATT GCAACGGAAT
3541 CGCCATTCGC CATTCAGGCT GCGCAACTGT TGGGAAGGGC GATCGGTGCG GGCCTCTTCC
3601 ACTGAGGCCC AGCTGCGCGC TCGCTCGCTC ACTGAGGCCG CCCGGGCAAA GCCCGGGCGT
3661 CGGGCGACCT TTGGTCGCCC GGCCTCAGTG AGCGAGCGAG CGCGCAGAGA GGGAGTGGCC
3721 AACTCCATCA CTAGGGGTTC CTTGTAGTTA ATGATTAACC CGCCATGCTA CTTATCTACT
15 3781 CGACATTGAT TATTGACTAG TTATTAATAG TAATCAATTA CGGGGTCATT AGTTCATAGC
3841 CCATATATGG AGTTCCGCGT TACATAACTT ACGGTAAATG GCCCGCCTGG CTGACCGCCC
3901 AACGACCCCC GCCCATTGAC GTCAATAATG ACGTATGTTC CCATAGTAAC GCCAATAGGG
3961 ACTTTCCATT GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCACTT GGCAGTACAT
4021 CAAGTGTATC ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA ATGGCCCGCC
20 4081 TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA CATCTACGTA
4141 TTAGTCATCG CTATTACCAT GGTCGAGGTG AGCCCCACGT TCTGCTTCAC TCTCCCCATC
4201 TCCCCCCCCT CCCCACCCCC AATTTTGTAT TTATTTATTT TTTAATTATT TTGTGCAGCG
4261 ATGGGGGCGG GGGGGGGGGG GGGGCGCGCG CCAGGCGGGG CGGGGCGGGG CGAGGGGCGG
4321 GGCGGGGCGA GGCGGAGAGG TGCGGCGGCA GCCAATCAGA GCGGCGCGCT CCGAAAGTTT
25 4381 CCTTTTATGG CGAGGCGGCG GCGGCGGCGG CCCTATAAAA AGCGAAGCGC GCGGCGGGCG
4441 GGAGTCGCTG CGTTGCCTTC GCCCCGTGCC CCGCTCCGCG CCGCCTCGCG CCGCCCGCCC
4501 CGGCTCTGAC TGACCGCGTT ACTCCCACAG GTGAGCGGGC GGGACGGCCC TTCTCCTCCG
4561 GGCTGTAATT AGCGCTTGGT TTAATGACGG CTTGTTTCTT TTCTGTGGCT GCGTGAAAGC
4621 CTTGAGGGGC TCCGGGAGGG CCCTTTGTGC GGGGGGAGCG GCTCGGGGGG TGCGTGCGTG
30 4681 TGTGTGTGCG TGGGGAGCGC CGCGTGCGGC TCCGCGCTGC CCGGCGGCTG TGAGCGCTGC
4741 GGGCGCGGCG CGGGGCTTTG TGCGCTCCGC AGTGTGCGCG AGGGGAGCGC GGCCGGGGGC
4801 GGTGCCCCGC GGTGCGGGGG GCTGCGAGGG GAACAAAGGC TGCGTGCGGG GTGTGTGCGT
4861 GGGGGGGTGA GCAGGGGGTG TGGGCGCGTC GGTCGGGCTG CAACCCCCCC TGCACCCCCC
4921 TCCCCGAGTT GCTGAGCACG GCCCGGCTTC GGGTGCGGGG CTCCGTACGG GGCGTGGCGC
35 4981 GGGGCTCGCC GTGCCGGGCG GGGGGTGGCG GCAGGTGGGG GTGCCGGGCG GGGCGGGGCC
5041 GCCTCGGGCC GGGGAGGGCT CGGGGGAGGG GCGCGGCGGC CCCCGGAGCG CCGGCGGCTG
5101 TCGAGGCGCG GCGAGCCGCA GCCATTGCCT TTTATGGTAA TCGTGCGAGA GGGCGCAGGG
5161 ACTTCCTTTG TCCCAAATCT GTGCGGAGCC GAAATCTGGG AGGCGCCGCC GCACCCCCTC
5221 TAGCGGGCGC GGGGCGAAGC GGTGCGGCGC CGGCAGGAAG GAAATGGGCG GGGAGGGCCT
40 5281 TCGTGCGTCG CCGCGCCGCC GTCCCCTTCT CCCTCTCCAG CCTCGGGGCT GTCCGCGGGG
5341 GGACGGCTGC CTTCGGGGGG GACGGGGCAG GGCGGGGTTC GGCTTCTGGC GTGTGACCGG
5401 CGGCTCTAGA GCCTCTGCTA ACCATGTTCA TGCCTTCTTC TTTTTCCTAC AGCTCCTGGG
5461 CAACGTGCTG GTTATTGTGC TGTCTCATCA TTTTGGCAAA GAATTGATTA ATTCGAGCGA
5521 ACGCGTCGAG TCGCTCGGTA CGATTTAAAT TGAATTGGCC TCGAGCGCAA GCTTGAGCTA
45 5581 GCGCCACCAT GGAATGGATG AGAAGCAGAG TGGGCACCCT GGGCCTGTGG GTGCGACTGC

5641 TGCTGGCTGT GTTTCTGCTG GGCGTGTACC AGGCCTACCC CATCCCTGAC TCTAGCCCCC
5701 TGCTGCAGTT TGGCGGACAA GTGCGGCAGA GATACCTGTA CACCGACGAC GACCAGGACA
5761 CCGAGGCCCA CCTGGAAATC CGCGAGGATG GCACAGTCGT GGGCGCTGCT CACAGAAGCC
5821 CTGAGAGCCT GCTGGAACTG AAGGCCCTGA AGCCCGGCGT GATCCAGATC CTGGGCGTGA
5 5881 AGGCCAGCAG ATTCCTGTGC CAGCAGCCTG ACGGCGCCCT GTACGGCTCT CCTCACTTCG
5941 ATCCTGAGGC CTGCAGCTTC AGAGAGCTGC TGCTGGAGGA CGGCTACAAC GTGTACCAGT
6001 CTGAGGCCCA CGGCCTGCCC CTGAGACTGC CTCAGAAGGA CAGCCCTAAC CAGGACGCCA
6061 CAAGCTGGGG ACCTGTGCGG TTCCTGCCTA TGCCTGGACT GCTGCACGAG CCCCAGGATC
6121 AGGCTGGCTT TCTGCCTCCT GAGCCTCCAG ACGTGGGCAG CAGCGACCCT CTGAGCATGG
10 6181 TGGAACCTCT GCAGGGCAGA AGCCCCAGCT ACGCCTCTTG AGAATGCGGG CCCGGTACCC
6241 CCGACGCGGC CTAACTGGCC TCATGGGCCT TCCGCTCACT GCCCGCTTTC CAGTCGGGAA
6301 ACCTGTCGTG CCAGTCAGGT GCAGGCTGCC TATCAGAAGG TGGTGGCTGG TGTGGCCAAT
6361 GCCCTGGCTC ACAAATACCA CTGAGATCTT TTTCCCTCTG CCAAAAATTA TGGGGACATC
6421 ATGAAGCCCC TTGAGCATCT GACTTCTGGC TAATAAAGGA AATTTATTTT CATTGCAATA
15 6481 GTGTGTTGGA ATTTTTTGTG TCTCTCACTC GGAAGGACAT ATGGGAGGGC AAATCATTTA
6541 AAACATCAGA ATGAGTATTT GGTTTAGAGT TTGGCAACAT ATGCCCATAT GCTGGCTGCC
6601 ATGAACAAAG GTTGGCTATA AAGAGGTCAT CAGTATATGA AACAGCCCCC TGCTGTCCAT
6661 TCCTTATTCC ATAGAAAAGC CTTGACTTGA GGTTAGATTT TTTTTATATT TTGTTTTGTG
6721 TTATTTTTTT CTTTAACATC CCTAAAATTT TCCTTACATG TTTTACTAGC CAGATTTTTC
20 6781 CTCCTCTCCT GACTACTCCC AGTCATAGCT GTCCCTCTTC TCTTATGGAG ATCCCTCGAC
6841 CTGCAGCCCA AGCTGTAGAT AAGTAGCATG GCGGGTTAAT CATTAACTAC AAGGAACCCC
6901 TAGTGATGGA GTTGGCCACT CCCTCTCTGC GCGCTCGCTC GCTCACTGAG GCCGGGCGAC
6961 CAAAGGTCGC CCGACGCCCG GGCTTTGCCC GGGCGGCCTC AGTGAGCGAG CGAGCGCGCA
7021 GCTGGCGTAA

25

AAV2 5' ITR: 3601-3742 bp

CAG promoter: 3779-5423 bp

Mus musculus codon-optimized FGF21 (moFGF21): 5588-6221 bp

Rabbit β-globin polyA signal (3' UTR and 3' flanking region of rabbit beta-globin, including polyA
30 signal): 6315 -6833 bp

AAV2 3' ITR: 6892-7024 bp

Mini-CMV : cmv intermediate early promoter (SEQ ID NO: 36)

TATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
35 GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT
GGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCA
AGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAA
ATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTAT
ATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTAACTGGCTTATCGAAATTAATACGAC
40 TCACTATAGGGAGACCCAAGCTT

Nucleotide sequence of EF1α promoter (SEQ ID NO: 37)

GGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGG
GAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATG

TCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGC
CGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCC
CGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACTGGCTGCA
GTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCT
TAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTG
CGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTT
TGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCA
CACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATG
TTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTG
GCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGG
CTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGG
AGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAA
AGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGG
CACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGC
GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAA
TTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTT
CAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA

Nucleotide sequence of RSV promoter (SEQ ID NO: 38)

CATGTTTGACAGCTTATCATCGCAGATCCGTATGGTGCACTCTCAGTACAATCTGCTCTGATG
CCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAG
CAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTA
GGCGTTTTGCGCTGCTTCGCGATGTACGGGCCAGATATTCGCGTATCTGAGGGGACTAGGGTGT
GTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTT
CGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTA
ACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAG
TAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTGGACGAAC
CACTAAATTCCGCATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCATTTG
ACCATTCACCACATTGGTGTGCACCTCCAAGCTGGGTACCAGCT

Synapsin 1 promoter (SEQ ID NO: 39)

ctgcgctctcaggcacgacacgactcctccgctgcccaccgcagactgaggcagcgctgagtcgccggcgccgcagcgcagatggtcg
cgcccgtgccccctatctcgcgcctcgcgtggtgcggtccggctgggccggcggcggcgcggacgcgaccaaggtggccgggaagggg
agtttgcgggggaccggcgagtgacgtcagcgcgccttcagtgctgaggcggcggtggcgcgcgccgccaggcgggggcgaaggcactgt
ccgcggtgctgaagctggcagtgcgcacgcgcctcgccgcatcctgtttcccctccccctctctgataggggatgcgcaatttggggaatgggg
gttgggtgcttgtccagtgggtcggggtcggtcgtcaggtaggcacccccaccccgcctcatcctggtcctaaacccacttgcact

Calcium/calmodulin-dependent protein kinase II (CaMKII) promoter (SEQ ID NO: 40)

taacattatggccttaggtcacttcatctccatggggttcttcttctgattttctagaaaatgagatgggggtgcagagagcttcctcagtgacctg
cccagggtcacatcagaaatgtcagagctagaacttgaactcagattactaatcttaaattccatgccttgggggcatgcaagtacgatatacag
aaggagtgaactcattagggcagatgaccaatgagtttaggaaagaagagtccagggcagggtacatctacaccacccgcccagccctgg
gtgagtccagccacgttcacctcattatagttgcctctctccagtcctaccttgacgggaagcacaagcagaaactgggacaggagccccagg
agaccaaatcttcatggtccctctgggaggatgggtggggagagctgtggcagaggcctcaggaggggccctgctgctcagtggtgacagat
aggggtgagaaagcagacagagtcattccgtcagcattctgggtctgtttggtacttcttctcacgctaaggtggcggtgtgatatgcacaatggc

taaaaagcagggagagctggaaagaaacaaggacagagacagaggccaagtcaaccagaccaattcccagaggaagcaaagaaac
cattacagagactacaaggggggaagggaaggagagatgaattagcttcccctgtaaaccttagaacccagctgttgccagggcaacggggc
aatacctgtctcttcagaggagatgaagttgccagggtaactacatcctgtctttctcaaggaccatcccagaatgtggcacccactagccgttac
catagcaactgcctctttgccccacttaatcccatcccgtctgttaaaagggccctatagttggaggtgggggaggtaggaagagcgatgatca
5 cttgtggactaagtttgttcgcatccccttctccaaccccctcagtacatcaccctgggggaacagggtccacttgctcctgggcccacacagtcct
gcagtattgtgtatataaggccagggcaaagaggagcaggttttaaagtgaaaggcaggcaggtgttggggaggcagttaccggggcaacg
ggaacagggcgtttcggaggtggttgccatggggacctggatgctgacgaaggctcgcgaggctgtgagcagccacagtgccctgctcaga
agccccaagctcgtcagtcaagccggttctccgtttgcactcaggagcacgggcaggcgagtggcccctagttctgggggcagcgggg

Glial fibrillary acidic protein (GFAP) promoter (SEQ ID NO: 41)

10 cgcgtgatctaacatatcctggtgtggagtaggggacgctgctctgacagaggctcgggggcctgagctggctctgtgagctggggagga
ggcagacagccaggccttgtctgcaagcagacctggcagcattgggctggccgcccccagggcctcctcttcatgcccagtgaatgactca
ccttggcacagacacaatgttcggggtgggcacagtgcctgcttcccgccgcaccccagcccccctcaaatgccttccgagaagcccattgag
caggggggcttgcattgcaccccagcctgacagcctggcatcttgggataaaagcagcacagccccctaggggctgcccttgctgtgtggcgcc
accggcggtggagaacaaggctctattcagcctgtgcccaggaaaggggatcaggggatgcccaggcatggacagtgggtggcaggggg
15 ggagaggagggctgtctgcttcccagaagtccaaggacacaaatgggtgaggggagagctctccccatagctgggctgcggcccaacccc
accccctcaggctatgccagggggtgttgccaggggcacccgggcatcgccagtctagcccactccttcataaagccctcgcatcccaggag
cgagcagagccagagcaggttggagaggagacgcatcacctccgctgctcgcggggtctagagtcga

Nestin promoter (SEQ ID NO: 42)

gaaggcagcccccggaggtcaaaggctgggcacgcgggaggagaggccagagtcagaggctgcgggtatctcagatatgaaggaa
20 agatgagagaggctcaggaagaggtaagaaaagacacaagagaccagagaagggagaagaattagagagggaggcagaggaccgc
tgtctctacagacatagctggtagagactgggaggaagggatgaaccctgagcgcatgaagggaaggaggtggctggtggtatatggagga
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25 tccattacatcccgaggctcaggttctgtggtggtcatctctgtgtggcttgttctgtgggcctacctaaagtcctaagcacagctctcaagcagatc
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tccccaccaccacccgcgctcgtacgtgcgtctccgcctgcagctcttgactcatcggggcccccgggtcacatgcgctcgctcggctctatagg
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gccaccggctgagtctgcagtcctccgaaacgggccctct

Homeobox Protein 9 promoter (HB9) promoter (SEQ ID NO: 43)

tgaataaatttaagcaggctaattaatatataaactagctcaatttgtcaagttgatttgtattttagttaattgtgaaagtaattaccacatggtca
aattaacagctttctggaaatgaccaagcctgaggttttatttccttcctgggtgaagaaaattcatttttccaagctcttgatgtgatgaataaaagtc
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gggggatggggctagagtggttagctgatgaattgacaaaaactaatcagctttattgggaaacaggtttaagggcacggacgtgtcaataac
gctcagcctgacccccctcttccattagctaggcaggctgattaga

Tyrosine hydroxylase (TH) promoter (SEQ ID NO: 44)

CTGCTAGGGGCTGCTTCCCAGCTACTCCTCTTGGCTCCGTGGCTTGCCTTCCAGCCTGTGTG
40 CTGTCTGGAGAGCCTTTAAAGCCTCACTTCCACCAACTAGAAGTCTCTCCCCAACCCTGCCCTGA
CCTCAAGTGCACCTCTTCAAAGTCAGGTTTAGCAGCTGCAGCTGGGGGCCCTGAATCCCACCCC

TGCTGTCTTCCTTGAAGACAGAAGTGTTGGGAGCTGAGGATCTGGGCTAGAGACTGGCTGTATG
ATCCAGAGAAGTAGTGTGCTTCTGGGCCTCAGATTTCCCTTCTGTAGAACAGGTTTGTCTGAAAT
GGAGAGGTTGGTGCTCCTCTGCAGGGCCTAGTGGGAGTCACCATGAGTGGTTAAAAGATCCAGC
TTGTCTTTTGGTGAGCTTTGAGAGGAGGTAACAGGGCTGAGTTCTGGAAGCCTGACCAAGGGCA
5 GACTTAAGGGGCCTCTTGGAGTTGTTCTCATCAAATGGGGATGGGACACAGCTAAAGTGCCCAG
GGCTTCTCTGTGCCCACAGATGCTTTAGATCTTGGCACAGTGTGGTCTACCAGCTGTCTCTCTCT
GTGTATATATATGTATTTCATAGACAGTGTACAGTGGCCTGGTTTGTGCTATCAGGCTGGATATGG
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10 CACCCCCACCTAGCTTCTGTTGCAAGCACCTCCAGCCGAGACAAGAGAACGAATTAAAAAGCAAT
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CCCTATGCAGGATTTGTTAGATACAGCTCCGTCCTACCCTGTGCCAGCTGAGCAAACGCCAGGCT
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GGACCCTGTGGTGGGCATGCAGACCTAGGGACTGGGCATAGATAACCTTTGGGTTTGGGCAACA
15 GCCCCCACTCCTCAGGATTGAAGGCTAAGGTGCAGCCAGCTCTGCCTTCATGGTGGGAATGTCT
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CCAGGGATGTGCTGGGCATGTTGAGACAGGAGTGGGCTAAGCCAGCAGAGTTGCTGACCCAGG
AAGAGTTCAGAAAGGGGCATGGAACATGGGGAGGGGTCCATAGTGAGAGAGAGCAGGCAGTGC
AGAGTAAATAGTCCCTGAGCTGGGGGTTATGGGATTTGCAGGAGCTTGCTCAGAGAAGGCAGAG
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GTCAGGTCAGCAGGGGAGGTTAGGGAGTGTTCCCTTTGTACTGACTTAGCATTTATCCTGCTTCT
AGGGGGGAAGGGGGGCCAGTGGGGGATGCACAGCAAGGCAGTGATGTGGCAGGCAGCCTGCG
GGAGCTCCTGGTTCCTGGTGTGAAAAAGCTGGGAAGGAAGAGGGCTGGGTCTGGTAAGTACAG
CAGGCAGTTGGCTCCTGAGAGTCCAAGCCCTGTCTAGAGGGTGGAGTGAGATTTCAGAGGGAGA
25 GCTAAACGGGGTGGGGGCTGGGGAGTCCAGGCTTCTGGCTCCTGCTAATACTCAGTGTGCTGG
GTCCTCAGAACCTCAGGGTGGCCATTTTCAGGGTGAGAGCTCTGTCCTTTGGCACTTCTGCAGAC
TCCAGTATCCAGAGGAATAAAGATGGTACTCTTCCTCAGTTCCCTTAGTGAGAGGACACCTTTCTC
TGAAGGGCTTGGGCAGTTGTCCTGAACCATTGCCTGAAGGAAGGACTTGACTCCAGGGACATAG
AATGGGCTCAGCATAAGTCCCCTGTAGTAGAGAAAGGTCCCCTCTCTGGTCTCCTTAGAGATCCT
30 GTTTCCTTGGCTGAGGAAGCTAGGGTGGATCTTTGTGTAAGTGGGTGTGGATGCTCACTGGAAAT
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GGGGCAGTGAGTAGATAGTATACTTTGTTACATGGGCTGGGGGGACATGGCCTGTGCCCTGGAG
GGGACTTGAAGACATCCAAAAAGCTAGTGAGAGGGCTCCTAGATTTATTTGTCTCCAAGGGCTAT
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40 ATTAGAGAGCTCTAGATGTCTCCTGTCCCAGAACACCAGCCAGCCCCTGTCTTCATGTCGTGTCT
AGGGCGGAGGGTGATTCAGAGGCAGGTGCCTGCGACAGTGGATGCAATTAGATCTAATGGGAC

GGAGGCCTCTCTCGTCCGTCGCCCTCGCTCTGTGCCCACCCCCGCCTCCCTCAGGCACAGCAG
GCGTGGAGAGGATGCGCAGGAGGTAGGAGGTGGGGGACCCAGAGGGGCTTTGACGTCAGCCT
GGCCTTTAAGAGGCCGCCTGCCTGGCAAGGGCCGTGGAGACAGAACTCGGGACCACCAGCTTG
CACT

5 Myelin basic protein (MBP) promoter (SEQ ID NO: 45)

caccgtggctttaacacttagagaaaatgcatcccctctaatcaataagtcatcgacagtgggtagatggaggaacggcagtgcgtagta
ggatgcgtgcaagcatagtctcgtgcatgggtgcatagatcgctgggcaggtggacaaggtgggggtggataaagaagtgggtagatgattg
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atgataaatgggtgatagactggaagggttgtcaaaagaggataagggaagtgtgagctagccgtatttctaaggtcagtaatagagttggga
10 gaagaggttaagttacatccatttaaacctcacacgaagctgagagggaatggacttgctgccgttggtgaggaaagcgttgcatttcccgtgtg
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ccgagccagaggcctccagcggaatccgggagagggattgctcagtgccctgcttccctggactgtaagctgcagaaagatgtgggaagtcc
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20 acagcagcttccgaagaattctgcagtcgacggtaccgcgggcccgggatc

Claims

1. A gene construct comprising a nucleotide sequence encoding a fibroblast growth factor 21 (FGF21), for use in the treatment and/or prevention of a metabolic disorder, wherein the therapy involves expression of the gene construct in the central nervous system (CNS), preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

2. A gene construct for use according to claim 1, wherein the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous promoter.

3. A gene construct for use according to claim 1 or claim 2, wherein the ubiquitous promoter is selected from the group consisting of a CAG promoter and a CMV promoter, preferably wherein the ubiquitous promoter is a CAG promoter.

4. A gene construct for use according to any one of claims 1-3, wherein the gene construct comprises at least one target sequence of a microRNA expressed in a tissue where the expression of FGF21 is wanted to be prevented.

5. A gene construct for use according to any one of claims 1-4, wherein the at least one target sequence of a microRNA is selected from those target sequences that bind to microRNAs expressed in the heart and/or the liver of a mammal.

6. A gene construct for use according to any one of claims 1 to 5, wherein the nucleotide sequence encoding FGF21 is operably linked to a ubiquitous promoter and at least one target sequence of a microRNA expressed in the liver and at least one target sequence of a microRNA expressed in the heart.

7. A gene construct for use according to claim 5 or 6, wherein a target sequence of a microRNA expressed in the heart is selected from SEQ ID NO's: 13 and 21-25 and a target sequence of a microRNA expressed in the liver is selected from SEQ ID NO's: 12 and 14-20.

8. A gene construct for use according to any one of claims 5 to 7, wherein the gene construct comprises a target sequence of microRNA-122a and a target sequence of microRNA-1.

9. A gene construct for use according to any one of claims 1 to 8, wherein the nucleotide sequence encoding FGF21 is selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide comprising an amino acid sequence that has at least 60% sequence identity with the amino acid sequence of SEQ ID NO: 1, 2 or 3;

(b) a nucleotide sequence that has at least 60% sequence identity with the nucleotide sequence of SEQ ID NO: 4, 5, 6, 7, 8, 9, 10 or 11; and

(c) a nucleotide sequence the sequence of which differs from the sequence of a nucleotide sequence of (b) due to the degeneracy of the genetic code.

10. An expression vector comprising a gene construct as described in any one of claims 1 to 9, for use in the treatment and/or prevention of a metabolic disorder, wherein the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

11. An expression vector for use according to claim 10, wherein the expression vector is a viral vector.

12. An expression vector for use according to claim 10 or 11, wherein the expression vector is selected from the group consisting of adenoviral vectors, adeno-associated viral vectors, retroviral vectors, and lentiviral vectors, preferably wherein the expression vector is an adeno-associated viral vector.

13. An expression vector for use according to any one of claims 10 to 12, wherein the expression vector is an adeno-associated viral vector of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, rh10, rh8, Cb4, rh74, DJ, 2/5, 2/1, 1/2 or Anc80, preferably wherein the expression vector is an adeno-associated viral vector of serotype 1, 2 or 9.

14. A pharmaceutical composition comprising a gene construct according to any one of claims 1-9 and/or an expression vector according to any one of claims 10-13, together with one or more pharmaceutically acceptable ingredients, for use in the treatment and/or prevention of a metabolic disorder, wherein the therapy involves expression of the gene construct in the CNS, preferably in the brain, more preferably in the hypothalamus and/or the cortex and/or the hippocampus and/or the cerebellum and/or the olfactory bulb, most preferably in the hypothalamus.

15. A gene construct for use according to any one of claims 1-9 and/or an expression vector for use according to any one of claims 10-13 and/or a pharmaceutical composition for use according to claim 14, wherein the gene construct and/or expression vector and/or pharmaceutical composition is administered by intra-CSF administration.

16. A gene construct for use according to any one of claims 1-9 and/or an expression vector for use according to any one of claims 10-13 and/or a pharmaceutical composition for use according to claim 14, wherein the metabolic disorder is a diabetes and/or obesity.

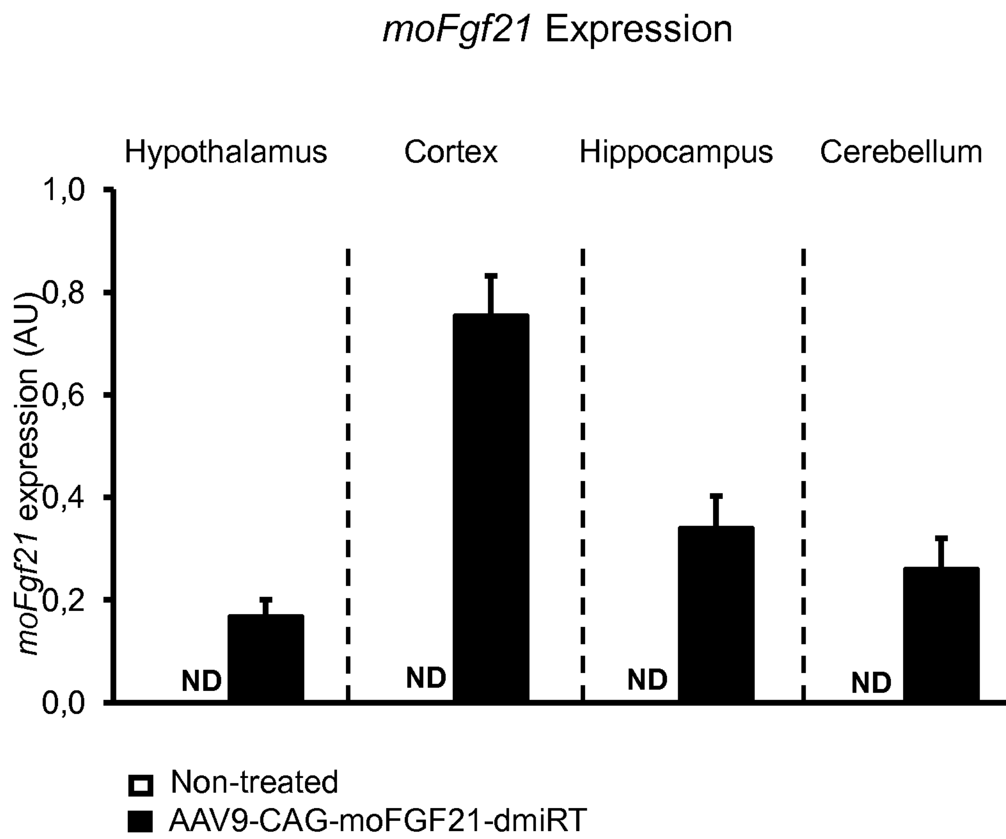

Figure 1

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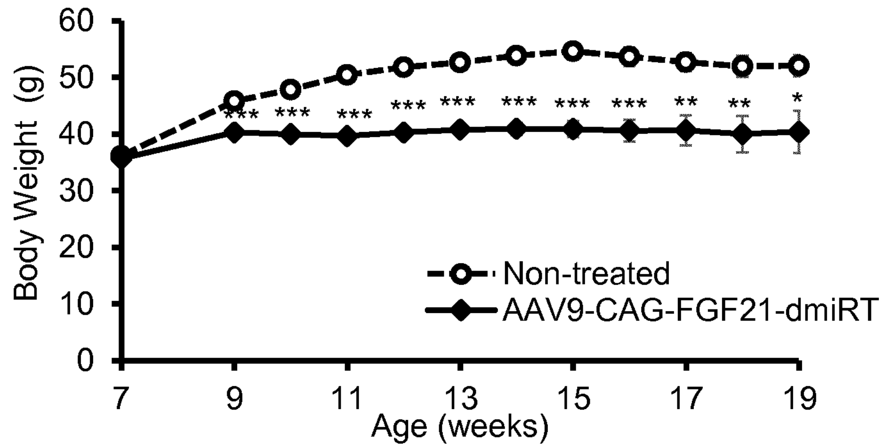

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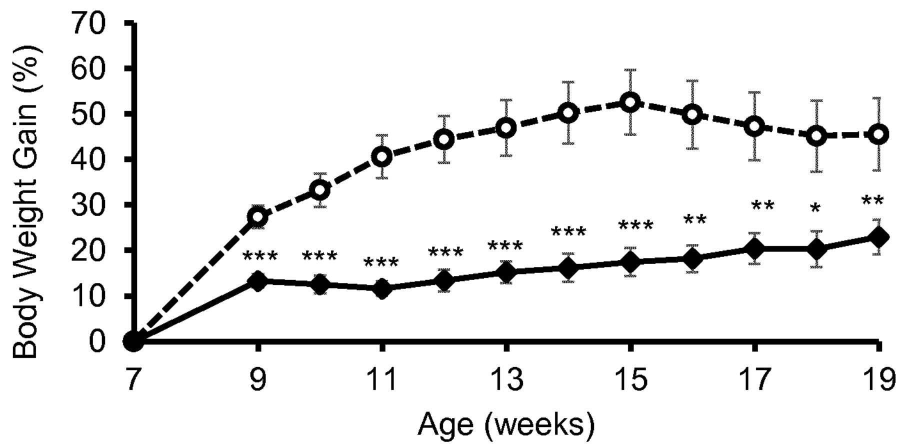

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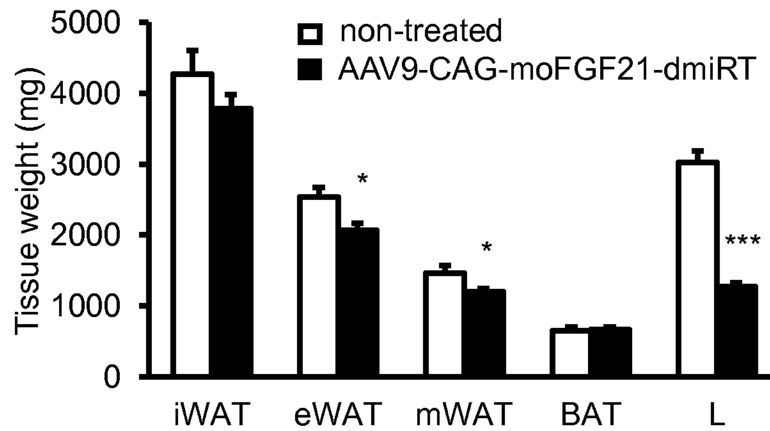

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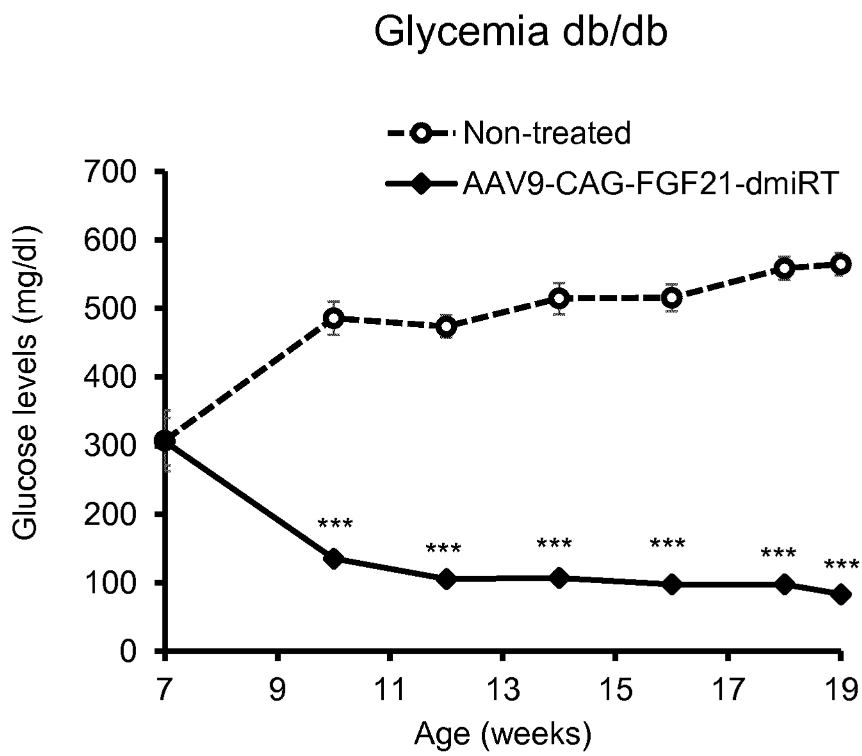

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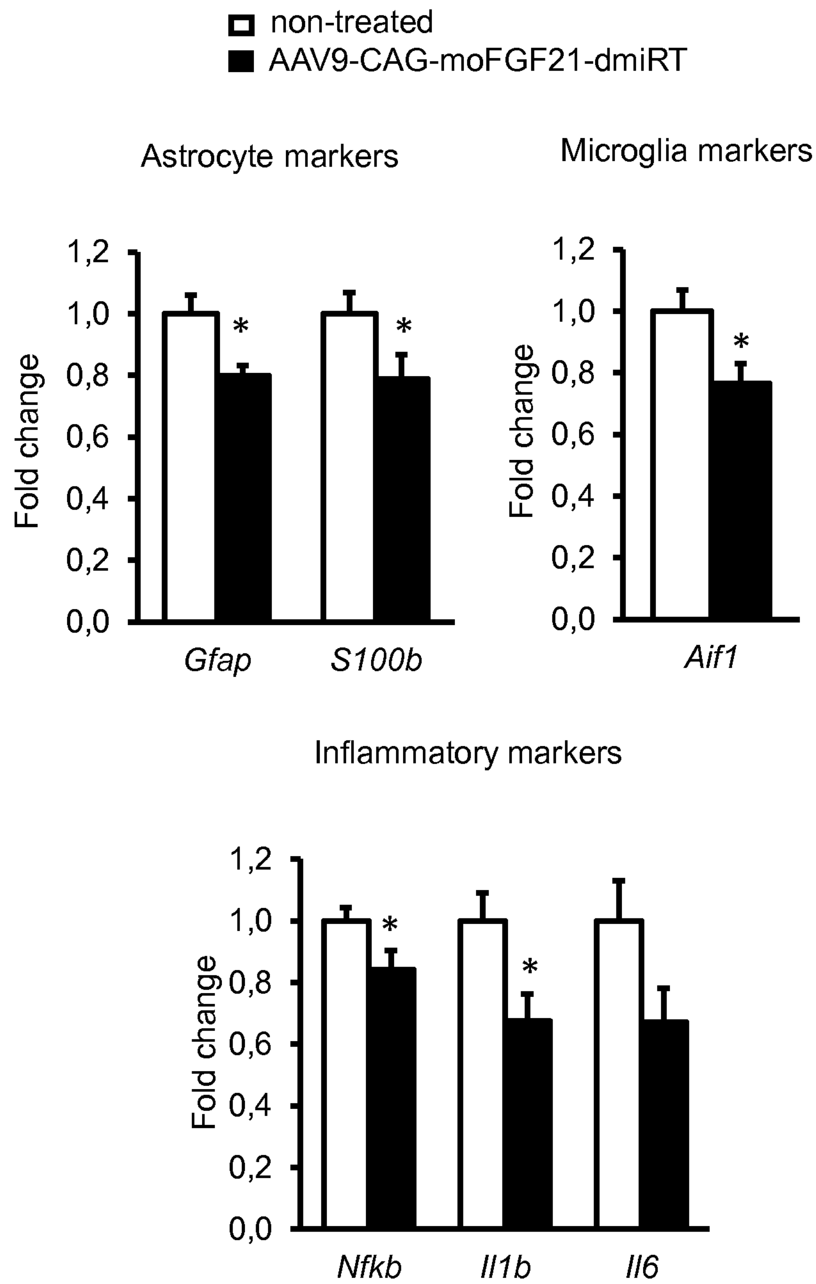

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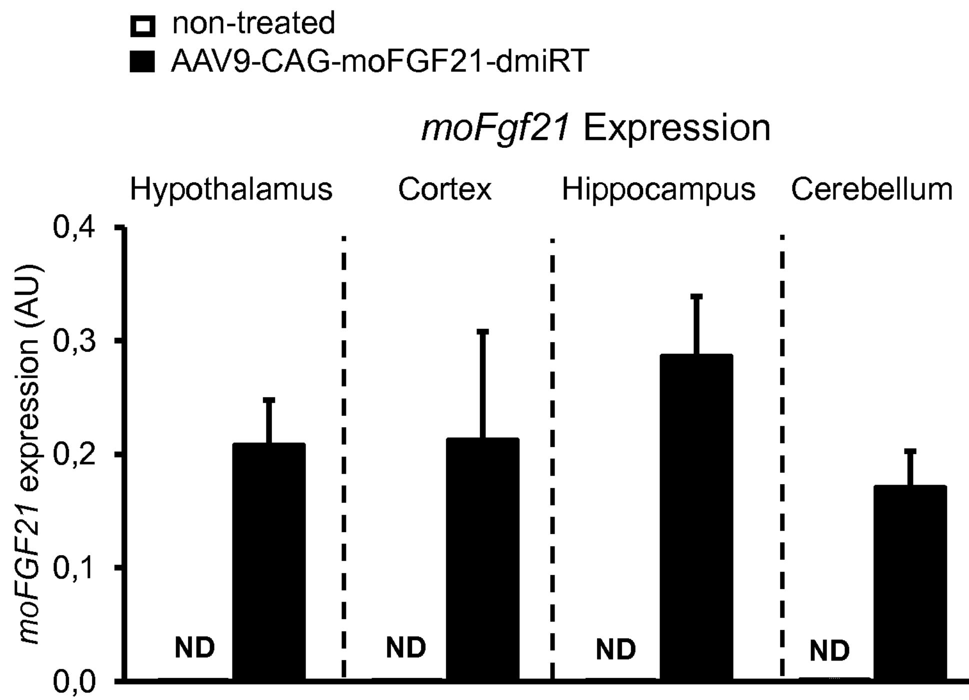

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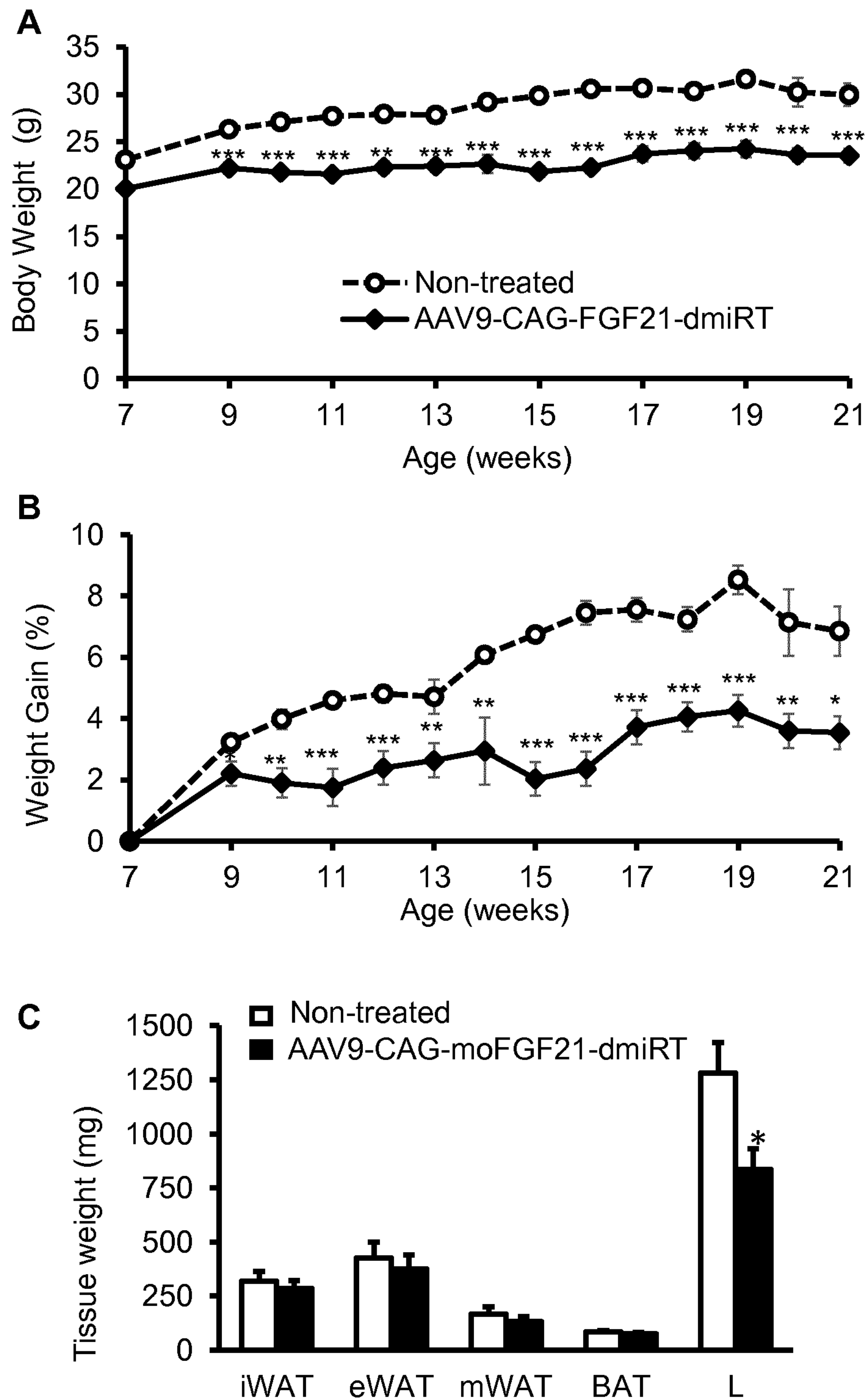
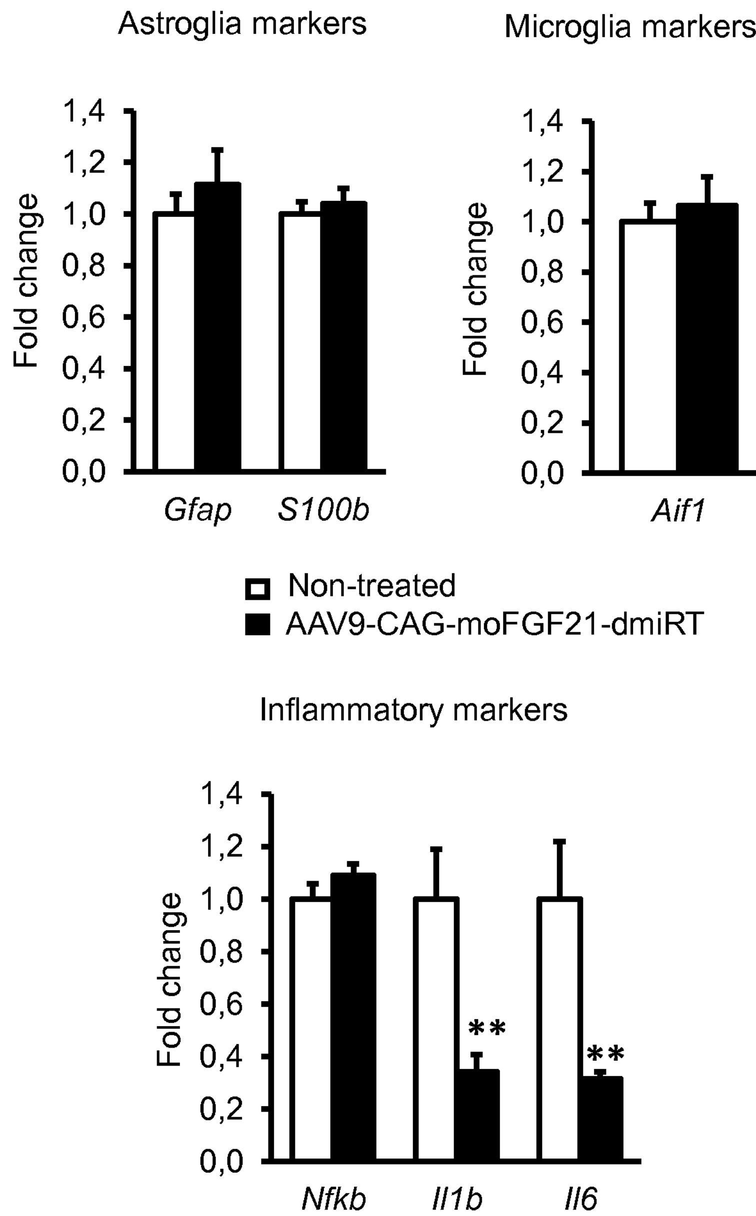


Figure 6



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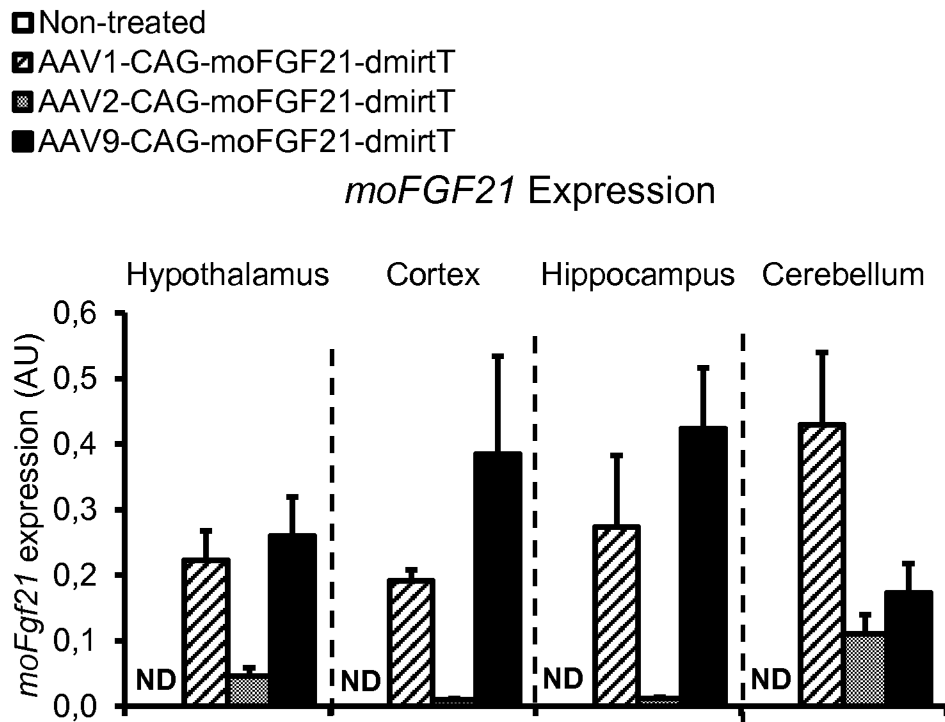

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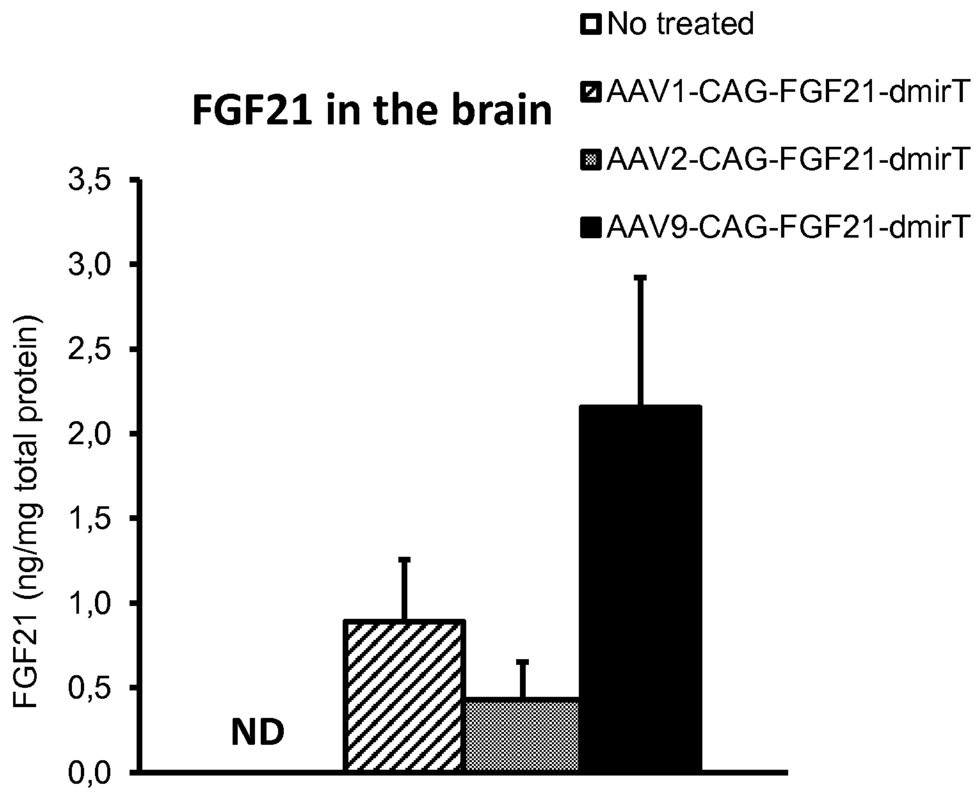

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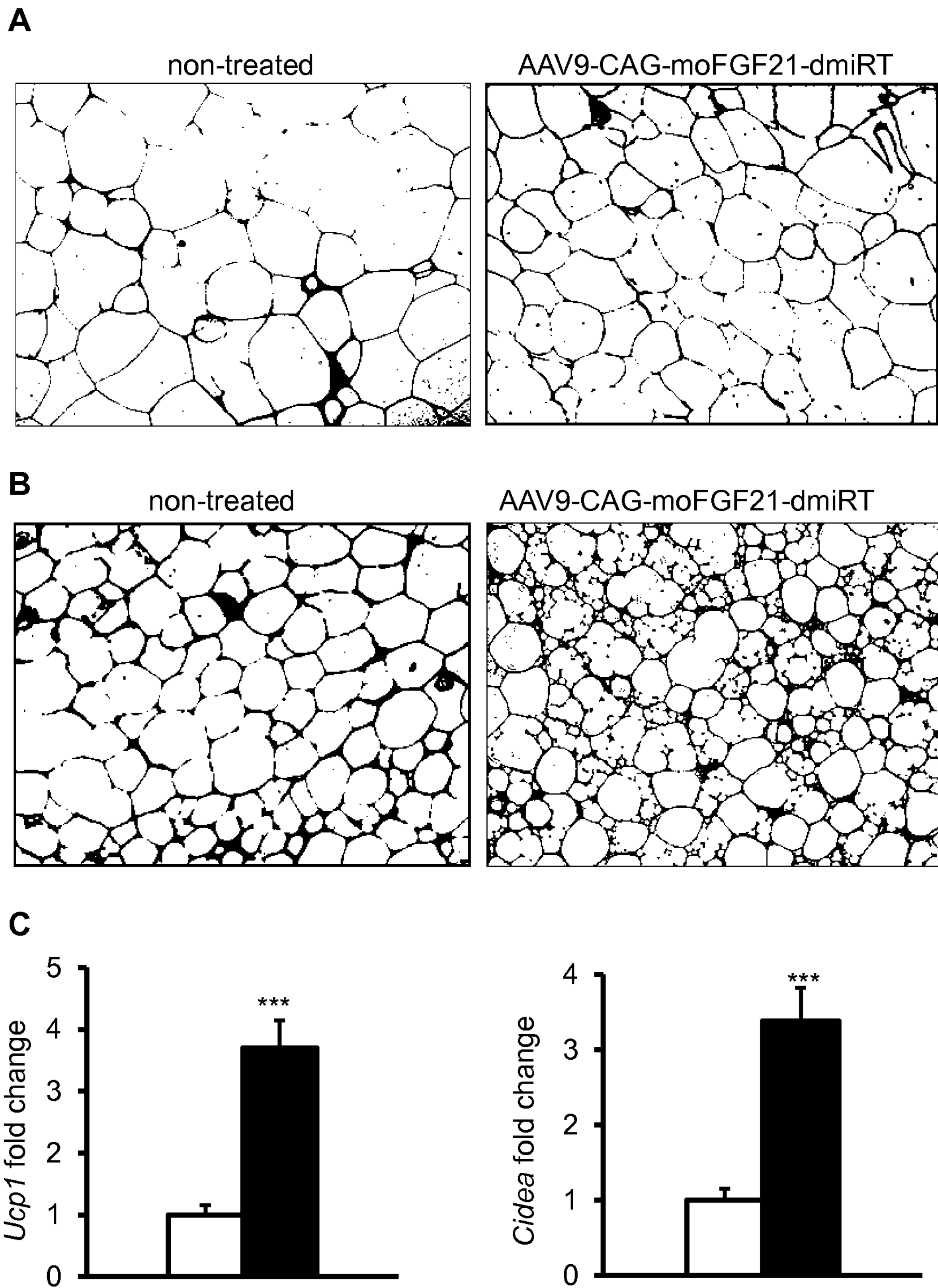

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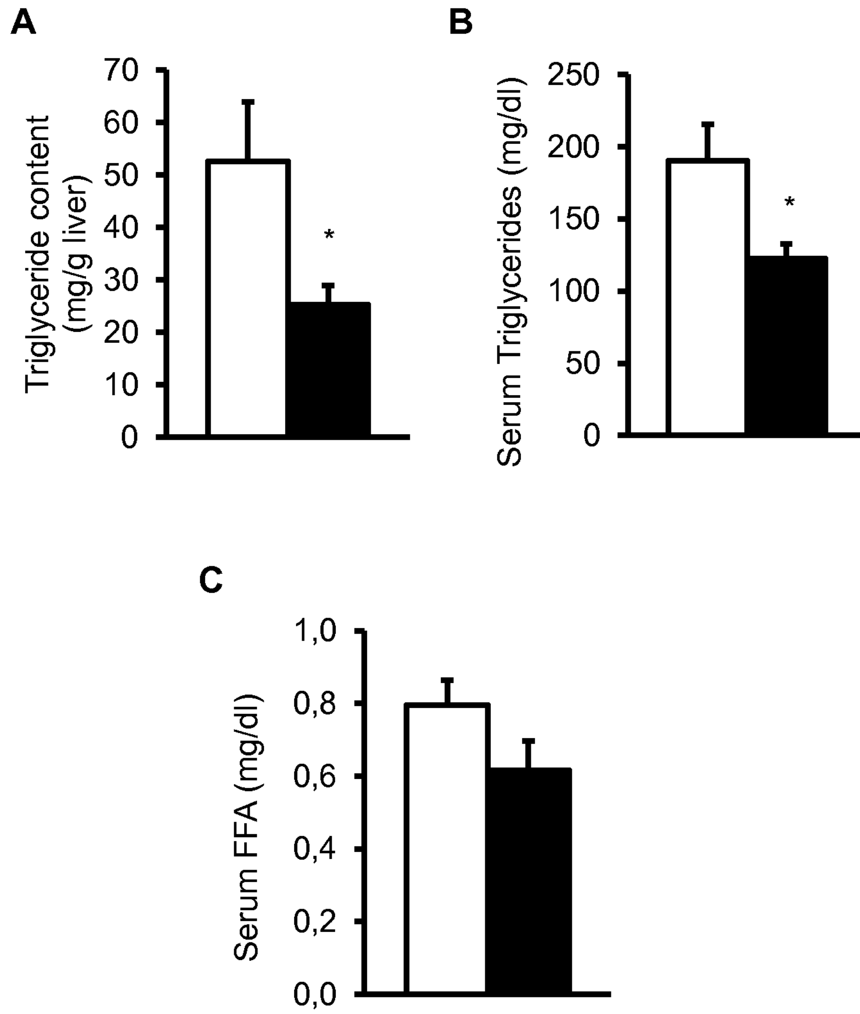

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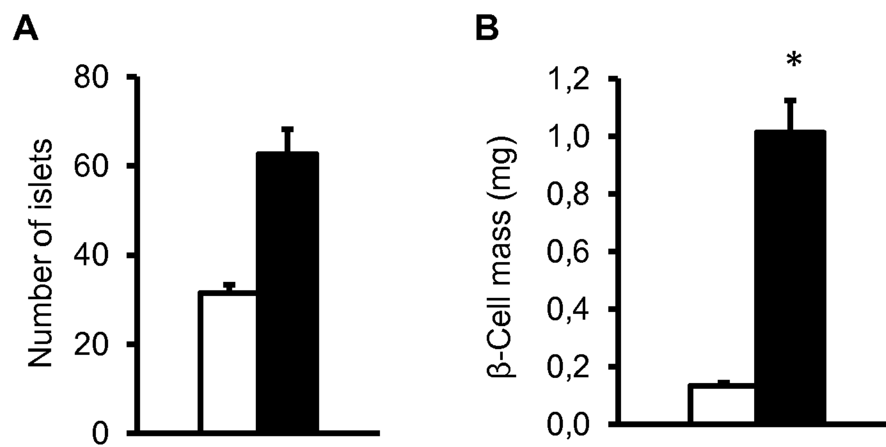

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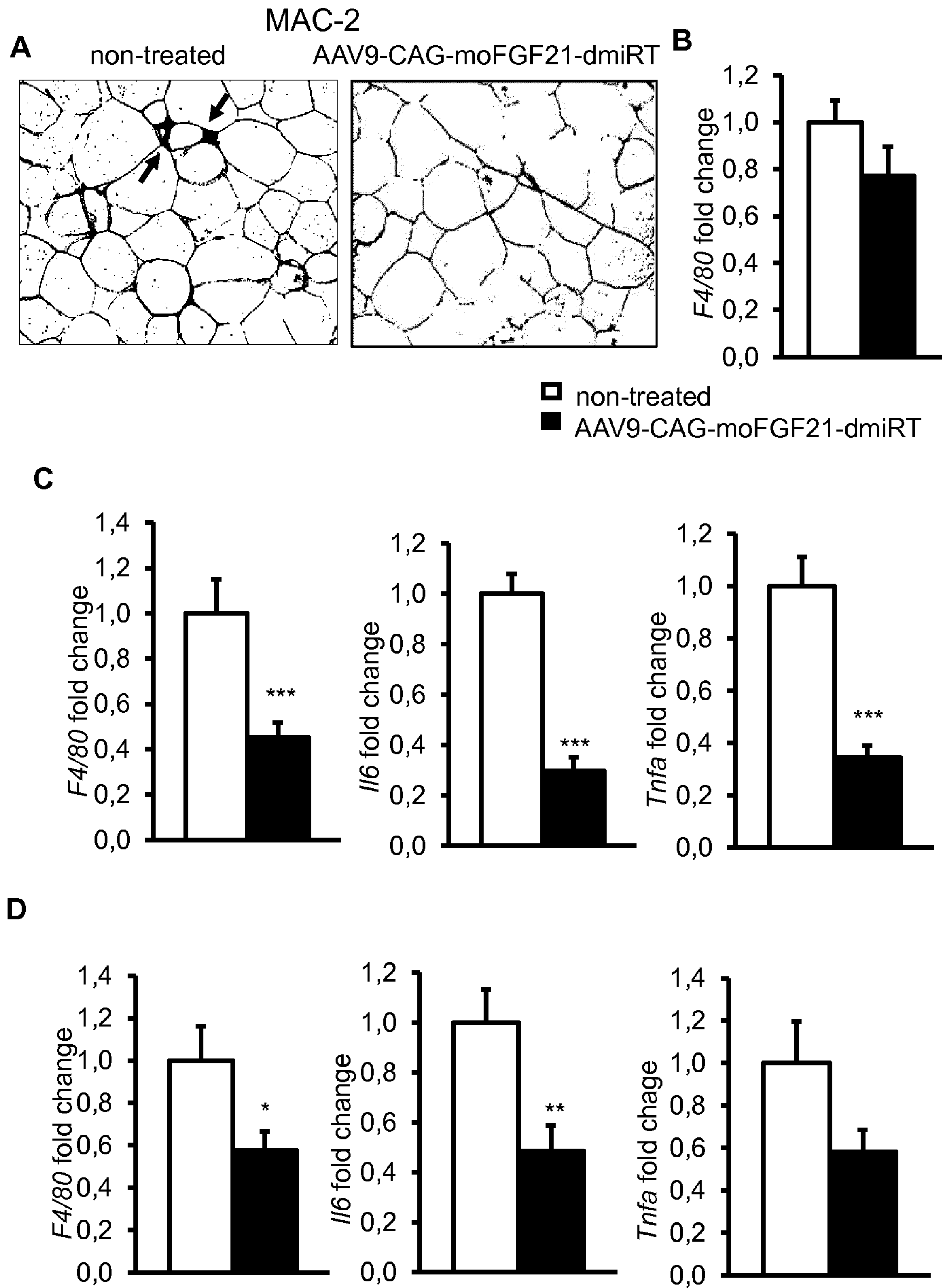


Figure 13

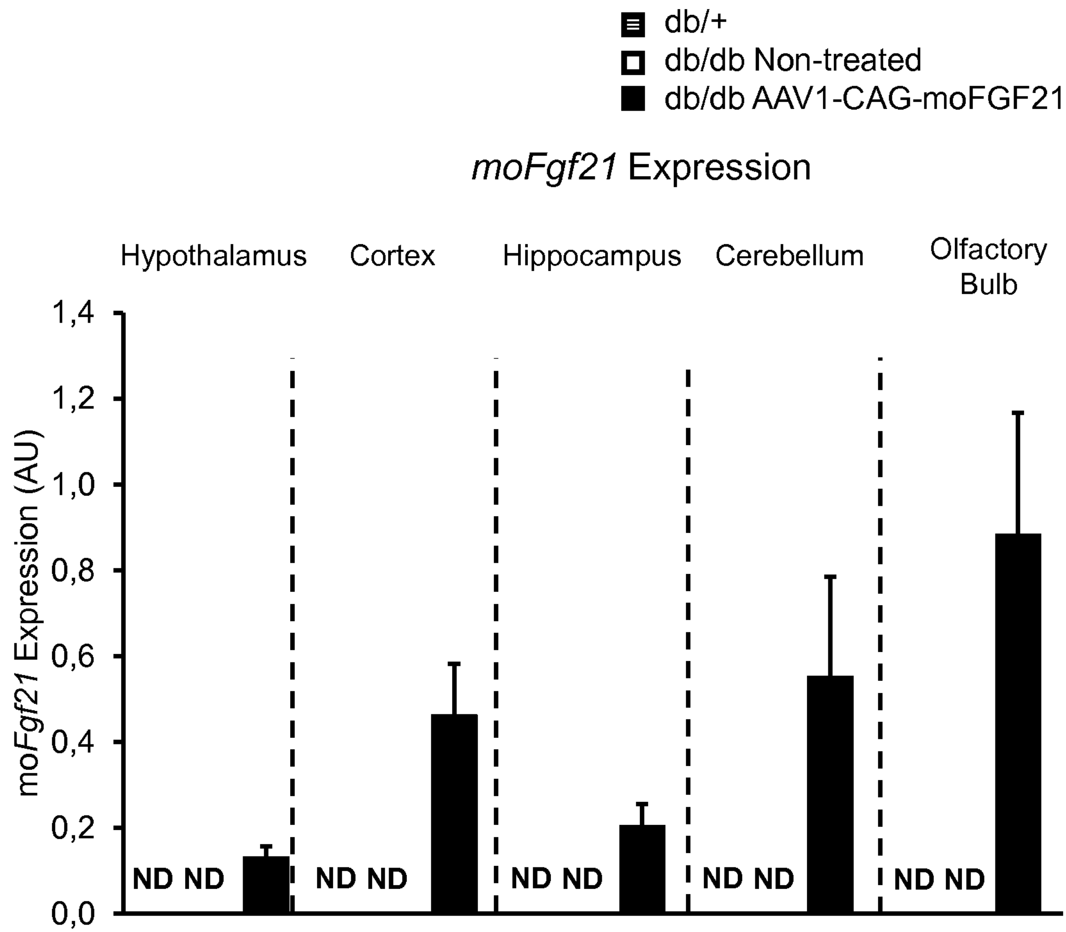
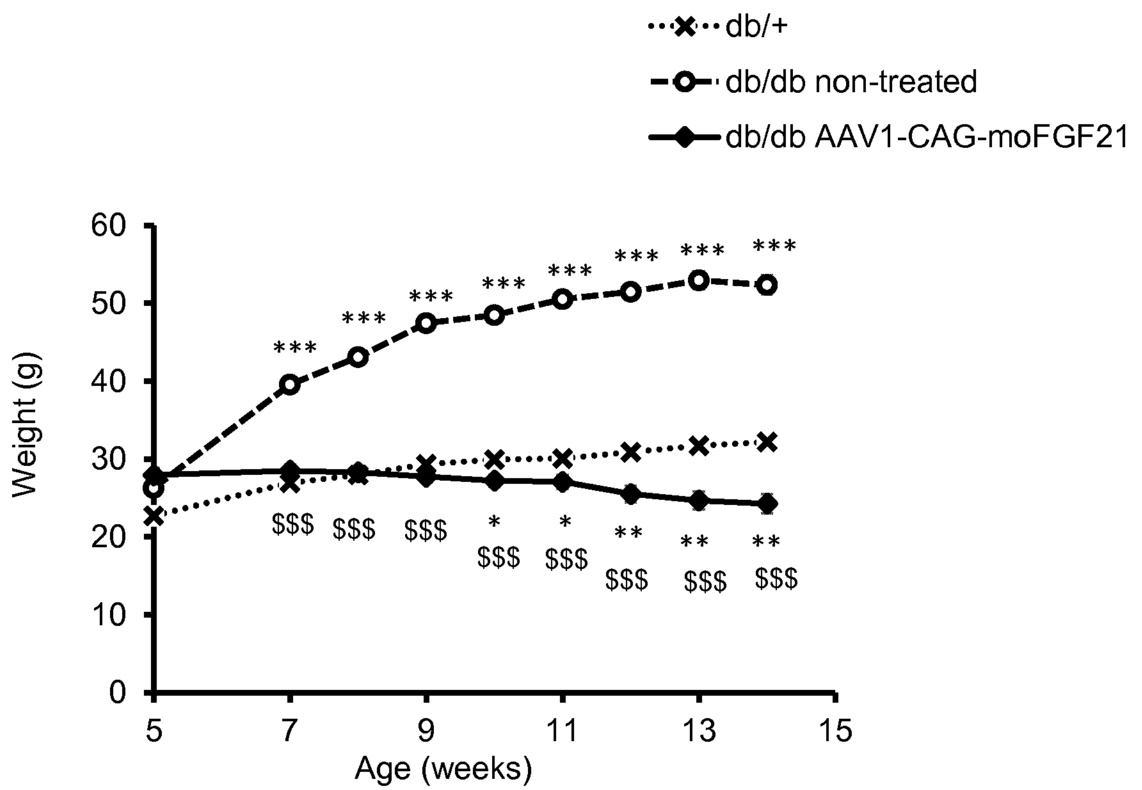


Figure 14



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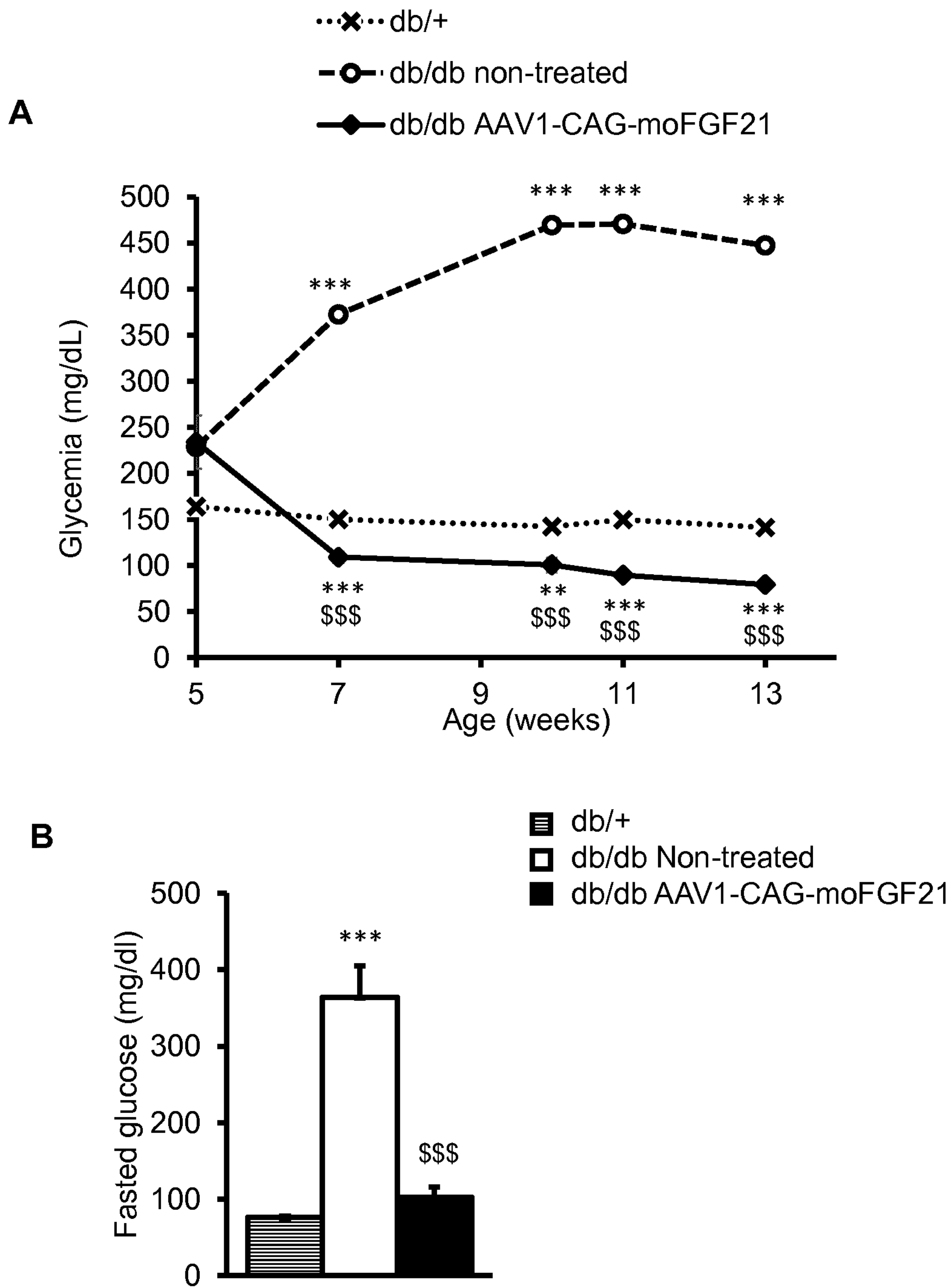

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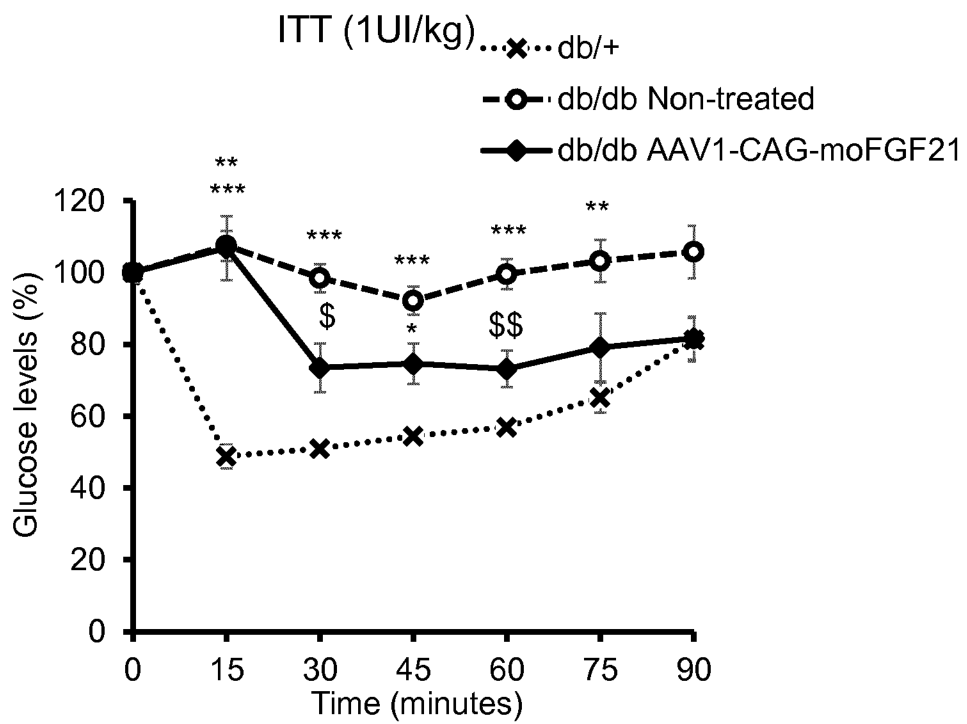

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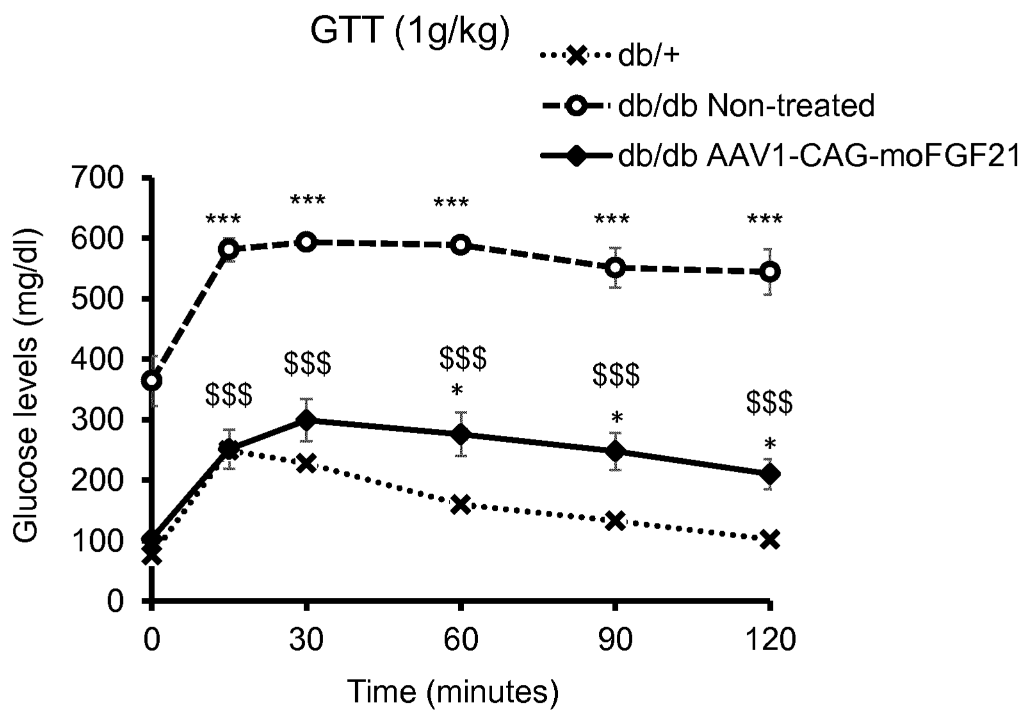

Figure 18

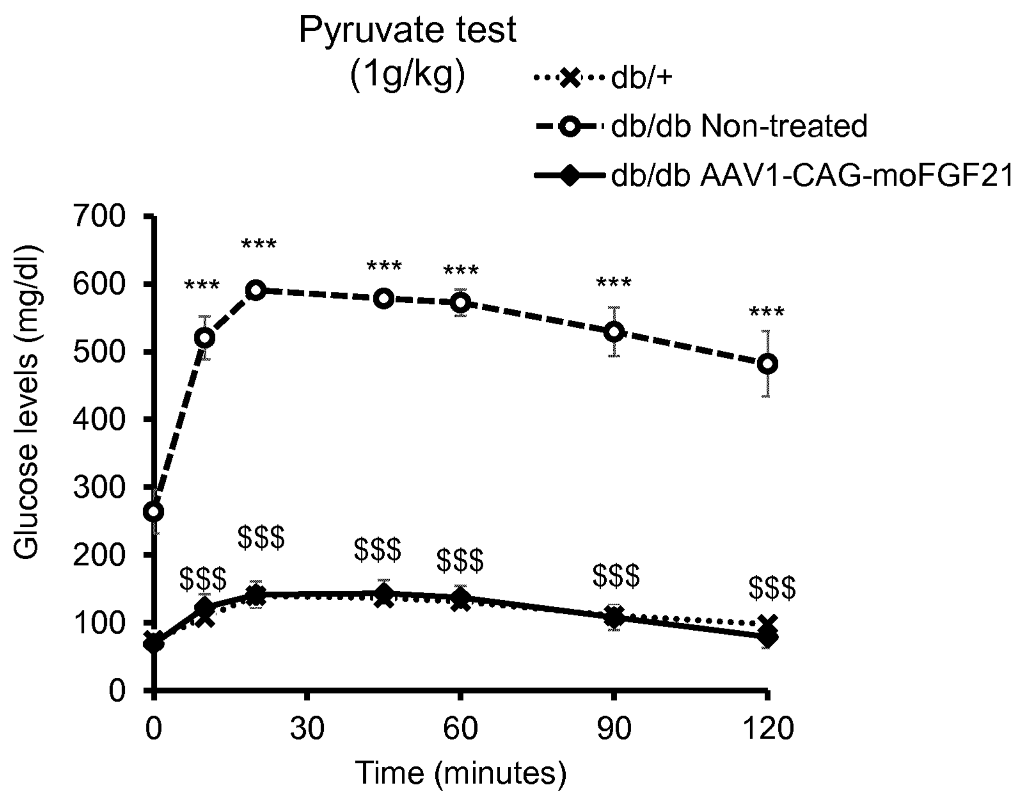

Figure 19

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/082601

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/50 A61K48/00 C12N15/86 C12N15/864
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K C12N

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VERONICA JIMENEZ ET AL: "FGF21 gene therapy as treatment for obesity and insulin resistance", EMBO MOLECULAR MEDICINE (ONLINE), vol. 10, no. 8, 9 July 2018 (2018-07-09), page e8791, XP055568532, DE ISSN: 1757-4684, DOI: 10.15252/emmm.201708791 abstract p. 2 col. 1, 3rd , p. 13 col. 2, 2nd , p. 15 col. 2, last , p. 19 col. 1, last , p. 1 col. 2, 2nd , p. 13 col. 2, 1st , p. 13 col. 2, last , p. 19 col. 2, 3rd , p. 17 col. 1, 3rd -----	1-16

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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

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

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

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

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Date of the actual completion of the international search	Date of mailing of the international search report
10 December 2019	08/01/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Landré, Julien

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	D. A. SARRUF ET AL: "Fibroblast Growth Factor 21 Action in the Brain Increases Energy Expenditure and Insulin Sensitivity in Obese Rats", DIABETES, vol. 59, no. 7, 31 March 2010 (2010-03-31), pages 1817-1824, XP055568048, US ISSN: 0012-1797, DOI: 10.2337/db09-1878 abstract p. 1817 col. 2, 4th , p. 1817 col. 2, 1st , p. 1822 col. 1, 2nd -----	1-16
Y	WO 2009/120978 A2 (UNIV OHIO STATE [US]; DURING MATTHEW J [US]; CAO LEI [US]) 1 October 2009 (2009-10-01) [00008], [00025], [00030], [000136], [000132], [00019], [00031], [00024], [00055], [00069] -----	9
Y	WO 2017/201527 A2 (HARVARD COLLEGE [US]) 23 November 2017 (2017-11-23) the whole document sequences 51, 52, 136 -----	9
Y	US 2018/186849 A1 (MOHAMMADI MOOSA [US] ET AL) 5 July 2018 (2018-07-05) sequences 236, 285, 294, 305, KH509732 the whole document -----	9
Y	WO 2011/127337 A2 (OPKO CURNA LLC [US]; COLLARD JOSEPH [US]; KHORKOVA SHERMAN OLGA [US]) 13 October 2011 (2011-10-13) sequence 1 the whole document -----	9
Y	WO 2017/021893 A1 (NOVARTIS AG [CH]; YOWE DAVID LANGDON [US]) 9 February 2017 (2017-02-09) sequences 1, 2 the whole document -----	9

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

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