



(51) International Patent Classification:
C12N 15/113 (2010.01) *A61K 31/7088* (2006.01)

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:
PCT/EP2017/084458

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(22) International Filing Date:
22 December 2017 (22.12.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/438,644 23 December 2016 (23.12.2016) US
17306240.7 22 September 2017 (22.09.2017) EP

(71) Applicants: UNIVERSITE DE STRASBOURG [FR/FR]; 4 rue Blaise Pascal, 67000 STRASBOURG (FR). CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE [FR/FR]; 3 rue Michel Ange, 75016 PARIS (FR). INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE [FR/FR]; 101 rue de Tolbiac, 75013 PARIS (FR). DYNACURE [FR/FR]; Parc d'Innovation 650, boulevard Gonthier d'Andernach, 67400 ILLKIRCH GRAFFENSTADEN (FR).

(72) Inventors: LAPORTE, Jocelyn; 18, rue de la Hohwart, 67100 STRASBOURG (FR). COWLING, Belinda; 28, rue de Schirrhein, 67240 KALTENHOUSE (FR). CHARLET-BERGUERAND, Nicolas; 2, Rue Sedillot, 67000 STRASBOURG (FR). NEY, Michel; 4, rue de Morteaux, 92160 Antony (FR).

(74) Agent: CABINET BECKER ET ASSOCIES; 25, rue Louis le Grand, 75002 PARIS (FR).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: DYNAMIN 2 INHIBITOR FOR THE TREATMENT OF MYOTONIC DYSTROPHY

(57) Abstract: The present disclosure relates to an inhibitor of Dynamin 2 or composition comprising the same for use in the treatment of myotonic dystrophy.



DYNAMIN 2 INHIBITOR FOR THE TREATMENT OF MYOTONIC DYSTROPHY

FIELD OF THE INVENTION

5 The present disclosure relates to an inhibitor of Dynamin 2 or composition comprising the same for use in the treatment of myotonic dystrophy.

BACKGROUND OF THE INVENTION

10 Myotonic dystrophy (DM), the most common inherited muscular dystrophy in adults, is an autosomal disorder characterized by skeletal muscle progressive atrophy, weakness and myotonia, heart conduction defects and others features, such as cataract, insulin resistance and cognitive dysfunctions. Muscle weakness and atrophy are the first cause of death in DM.

 Currently, no treatment exists for curing DM, nor for alleviating muscular symptoms thereof. There is thus an urgent need for therapeutic compounds for treating this disease.

15 DM encompasses two genetically distinct forms. Myotonic dystrophy type 1 (DM1) and its severe congenital form (CDM1) are caused by an expansion of CTG repeats in the 3'-untranslated region of the DMPK gene, while myotonic dystrophy of type 2 (DM2) is caused by an expansion of CCTG repeats within the first intron of the CNBP gene.

 The molecular origins of muscle weakness and atrophy, which are the major
20 contributors of death in DM, remain poorly defined. Various splicing alterations have been observed in skeletal muscle of individuals with DM. However, splicing changes in the dystrophin (*DMD*), sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase SERCA1 (*ATP2A1*), ryanodine receptor 1 (*RYR1*), calcium channel Cav1.1 (*CACNA1S*) and the exon 11 of the bridging integrator 1 (*BINI*) are associated with only limited changes of skeletal muscles
25 structure and function (Rau et al., Abnormal splicing switch of DMD's penultimate exon compromises muscle fiber maintenance in myotonic dystrophy. Nat Commun. 2015 May 28;6:7205). Mutations in the *BINI* gene cause centronuclear myopathy (CNM) which shares common histopathological features with DM1. *BINI*, also named amphiphysin 2, is a protein specialized in membrane curvature, which function is regulated by tissue-specific alternative
30 splicing. For example, neuronal-specific inclusion of exon 7 and of the successive exons 13 to 16, which are encoding a clathrin and AP2 binding domain, generates a splicing form of *BINI* involved in endocytosis. In contrast, in skeletal muscles, inclusion of the muscle-specific exon 11 that encodes a phosphoinositide binding domain, generates an isoform of *BINI* that induces plasma membrane tubular invaginations and is consequently crucial for the generation and the

maintenance of the T-tubule network. T-tubules are specialized membrane structures fundamental for excitation-contraction (E-C) coupling, and decreased expression of *BIN1* in animal models leads to T-tubules alterations, muscle atrophy and weakness.

Dynamins are large GTPase proteins that play important roles in membrane trafficking and endocytosis, and in actin cytoskeleton assembly. Dynamin proteins contain an N-terminal GTPase domain, middle domain, PH domain (phosphoinositide binding), GED (GTPase effector domain), and a PRD (Proline-rich domain) for protein-protein interactions. Three human dynamins have been identified; dynamin 1, expressed exclusively in neurons, dynamin 3 predominantly in brain and testis, and dynamin 2 (DNM2) which is ubiquitously expressed. Different heterozygous DNM2 mutations have been identified in tissue-specific diseases: Autosomal Dominant Centronuclear Myopathy which affects skeletal muscle, and autosomal dominant Charcot-Marie-Tooth peripheral neuropathy. However, no link between DM and DNM2 has been described so far.

It is herein provided a novel therapeutic approach for the treatment of DM by reducing DNM2 expression or activity.

SUMMARY OF THE INVENTION

In a first aspect, the present invention concerns an inhibitor of Dynamin 2 for use in the treatment of DM.

The present invention also concerns a pharmaceutical composition comprising an inhibitor of Dynamin 2 and a pharmaceutically acceptable carrier/excipient for use in the treatment of DM.

The present invention further concerns a method for the treatment of DM, wherein the method comprises the step of administering into a subject in need of such treatment a therapeutically efficient amount of a Dynamin 2 inhibitor.

Finally, the present invention concerns the use of a Dynamin 2 inhibitor for the preparation of a pharmaceutical composition for the treatment of DM.

Actually, the inventors have identified by RNA sequencing various splicing changes in skeletal muscle samples of DM1 individuals. Among these alterations, an abnormal inclusion of *BIN1* exon 7 was identified. This exon was previously described only in the nervous system where it contributes to the endocytosis regulatory function of *BIN1*. It was found that alternative splicing of *BIN1* exon 7 is regulated by MBNL1 and MBNL2, the splicing regulators titrated by expanded CUG and CCUG repeats in DM. Also, it was found that exon 7 reinforces the interaction of BIN1 with dynamin 2 (DNM2), a GTPase protein promoting membrane fission

and whose dominant mutations cause CNM. Ectopic expression of BIN1 with exon 7 in mouse skeletal muscle causes characteristic pathological features of CNM or DM, namely T-tubules alterations, abnormally centrally localized nuclei, muscle fiber atrophy and weakness. Finally, expression of BIN1 with its exon 7 in mouse skeletal muscle promotes abnormal recruitment of DNM2 to T-tubules and leads to morphological alterations of this specialized muscle excitation-contraction coupling compartment. Altogether, and without being bound to any theory, it seems that the *BIN1* exon 7 alternative splicing participates to muscle wasting in DM notably by reinforcing the interaction of BIN1 with DNM2 and that inhibition of DNM2 could thereby be a therapeutic approach for the treatment of DM.

Moreover, the inventors have found that treatment of a recognized murine model of DM, the DMSXL mice, with antisense oligonucleotide targeting specifically the *Dnm2* pre-mRNA leads to a strong decrease in the level of DNM2 protein (to about 40% of normal level), and an increase in grip strength of hind paws at 1 month of age. Thus decreasing DNM2 *in vivo* appears to have a positive effect on a DM model.

The Dynamin 2 inhibitor is preferably selected from the group consisting of an antibody directed against Dynamin 2, a nucleic acid molecule interfering specifically with Dynamin 2 expression, a nucleic acid or a nuclease engineered to target the DNM2 gene and to deliver nucleases using genome editing therapy, and a small molecule inhibiting the Dynamin 2 activity, expression or function. In a preferred embodiment, the Dynamin 2 inhibitor is selected from the group consisting of a nucleic acid molecule interfering specifically with Dynamin 2 expression. In a particular embodiment, the Dynamin 2 inhibitor is an RNAi, an antisense nucleic acid or a ribozyme interfering specifically with Dynamin 2 expression.

In a more specific embodiment, the Dynamin 2 inhibitor is a siRNA, shRNA or an antisense snRNA. In another particular embodiment, the dynamin 2 inhibitor is a DNA, mRNA or a nuclease engineered to target the DNM2 gene and to deliver nucleases using genome editing therapy

A further object of the invention relates to a method of screening for identifying a compound useful for the treatment of DM comprising:

- a) Providing or obtaining a candidate compound; and
- b) Determining whether said candidate compound inhibits the activity/expression of Dynamin 2,
- c) Selecting said candidate compound if it inhibits the activity/expression of Dynamin 2.

The method for screening or identifying a molecule suitable for the treatment of DM can optionally further comprise the step of administering *in vivo*, *ex vivo* or *in vitro* the selected

molecule in a DM non-human animal model or a part thereof (tissue or cells) and analyzing the effect thereof on the muscle cells phenotype, on the myopathy onset or progression.

These and other objects and embodiments of the invention will become more apparent after the detailed description of the invention.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Identification of splicing alterations in DM1 skeletal muscle.

(A) Scatter plot illustrating the difference of splicing (Δ -psi) between control (n = 3) and DM1 (n = 3) skeletal muscle samples versus Z-score predicted by MISO. Boxes indicate cassette exons significantly included or excluded in DM1 muscles (Δ -psi > 0.4 and Z-score > 1.5). (B) RNA-sequencing reads coverage of *BIN1* exons 6 to 8 in control and DM1 muscle samples. (C) RT-PCR validation of splicing alterations predicted by RNA-sequencing in skeletal muscle samples of control individuals (CTRL, black) and DM1 patients (DM1, red). The percentage of exon inclusion is indicated at the bottom of each gel. Molecular sizes are indicated in base pairs on the left of each gel. (D) Graphical representation of the ratio of the percentage of splicing alteration in DM1 over control.

Figure 2: *BIN1* exon 7 is included in skeletal muscle of DM1 individuals and models.

(A) Left panel, RT-PCR analysis of *BIN1* exon 7 inclusion in human skeletal muscle samples of control adult (CTRL), non-DM fetuses (18, 27 and 28 weeks), congenital DM1 fetuses (CDM1 of 18, 28 and 33 weeks), adults DM1 and DM2 individuals. The percentage of exon 7 inclusion is indicated at the bottom of the gel. Molecular sizes are indicated in base pairs on the left of the gel. Right panel, graphical dot representation of the percentage of *BIN1* mRNA including exon 7 in skeletal muscle samples of adult control individuals (n=7) (CTL), foetal controls (n=3), CDM1 (n=6) and adult DM1 (n=7) and DM2 (n=10) individuals. (B) Correlation between alterations of the alternative splicing of *ATP2A1* exon 22 and *BIN1* exon 7 in skeletal muscle samples of DM1 individuals (splicing alterations from Nakamori et al., 2013). (C) Immunoblotting against *BIN1* exon 7, *BIN1* exon 17 or GAPDH in skeletal muscle from control and DM1 individuals. Molecular sizes are indicated in kilodalton on the left of the gels. (D) Upper panel, RT-PCR analysis of *BIN1* exon 7 inclusion in 0, 3, 5 and 7 days differentiated muscle cell cultures originating from biopsies of control or DM1 individuals. Lower panel, quantification of the percentage of *BIN1* mRNA including exon 7. Bars indicate s.e.m with n=2 independent cell cultures. (E) Immunoblotting against *BIN1* exon 7, *BIN1* exon 17 or GAPDH in differentiated muscle cells cultures derived from control or DM1 individuals. (F) Left panel, RT-PCR analysis of *BIN1* exon 7 inclusion in tibialis anterior muscles of control

non-transgenic or HSA^{LR} mice. The percentage of exon 7 inclusion is indicated at the bottom of the gel. Molecular sizes are indicated in base pairs on the left of the gel. Right panel, quantification of the percentage of *BIN1* mRNA including exon 7. Bars indicate s.e.m with n=4 different mice in each groups. Student's t-test, *** indicates $P < 0.0001$.

Figure 3: MBNL1 regulates BIN1 exon 7 alternative splicing.

(A) Schematic representation of *BIN1* minigene encompassing exons 6 to 8 with their introns 6 and 7. Primers for RT-PCR analysis of exon 7 inclusion are indicated by red arrows. (B) Upper panel, RT-PCR analysis of *BIN1* minigene exon 7 inclusion in Neuro2A cells co-transfected with *BIN1* minigene and with either a plasmid expressing 960 CTG repeats, MBNL1, CUGBP1, or with siRNA directed against *Mbnl1* and *Mbnl2* mRNAs (si*Mbnl1*+2) or *Celf1* (encoding Cugbp1; si*Celf1*) mRNA. Lower panel, percentage of *BIN1* minigene exon 7 inclusion. Bars indicate s.e.m with n=3 independent transfection experiments. Student's t-test, ** indicates $P < 0.005$, *** $P < 0.0001$. (C) Upper panel, schematic representation of the *BIN1* RNA sequence tested in gel-shift assays. Size of RNA fragments and affinity of MBNL1 are indicated. Lower panel, hill plots of MBNL1 binding to *BIN1* RNA fragments. Bars indicate s.e.m with n=2 independent experiments. (D) Sequence comparison of WT and mutated *BIN1* exon 7 minigenes. MBNL1 binding sites (YGC sequences) are indicated in bold, mutations are indicated by red arrowheads. (E) Splicing analysis as in (B) but with a mutant minigene where MBNL1 YGC RNA binding sites described in (D) are mutated in YaC. (F) Left panel, RT-PCR analysis of *Bin1* exon 7 inclusion in skeletal muscle samples of wild-type, single *Mbnl1* knockout mice (*Mbnl1*^{-/-}), double *Mbnl1* homozygous and *Mbnl2* heterozygous knockout mice (*Mbnl1*^{-/-}; *Mbnl2*^{+/-}) and double *Mbnl1* homozygous and skeletal muscle specific-*Mbnl2* homozygous knockout mice (Myo-Cre DKO). Right panel, percentage of *Bin1* exon 7 inclusion. Bars indicate s.e.m with n=3 different mice in each groups. Student's t-test, *** indicates $P < 0.0001$. (G) Correlation between splicing inclusion of *BIN1* exon 7 and the inferred level of active MBNL in skeletal muscle samples of DM1 individuals ([MBNL] inferred from Wagner et al., 2016).

Figure 4: Expression of BIN1 with its exon 7 in mouse skeletal muscle induces T-tubules alterations, muscle atrophy and weakness.

(A) Hematoxylin and eosin and NADH-PH staining of mouse tibialis anterior (TA) muscles injected for 3 months with AAV2/9-BIN1 isoforms with or without exons 7 and 11. Scale bar = 200 μ m. (B) Left panel, muscle fiber area of mouse TA muscles injected for 3 months with AAV2/9-BIN1 isoforms with or without exons 7 and 11. Right panel, quantification of small

(< 2600 μm^2) versus large (> 3000 μm^2) muscle fibers. (C) Percentage of fibers with centrally located nuclei in mouse TA muscles injected for 3 months with AAV2/9-BIN1 isoforms with or without exons 7 and 11. (D to F) Muscle absolute maximal force (D), specific maximal force (E) and fatigue resistance (F) were assessed *in situ* in mouse TA muscles injected for 3 months with AAV2/9-BIN1 isoforms with or without exons 7 and 11. (G) Left panel, transmission electron microscopy of potassium ferrocyanide stained mouse TA muscles injected for 3 months with AAV2/9-BIN1 isoforms with or without exons 7 and 11. Black arrowheads indicate normal T-tubules, red arrowheads indicate altered T-tubules. Scale bar = 1 μm . Right panel, quantification of normal and abnormal T-tubules. For all experiments, bars indicate s.e.m with n=3 independent injections in each groups. Student's t-test, * indicates $P < 0.05$.

Figure 5: Exon 7 increases BIN1 interaction with DNM2.

(A) Upper panel, schematic representation of BIN1 exons structure and protein domains. BAR, Bin/Amphiphysin/Rvs domain; PI, phosphoinositide binding domain; SH3, Src Homology 3 domain. Middle upper panel, immunoblotting against endogenous DNM2 co-immunoprecipitated by HA-tagged BIN1 with or without exon 7 and 11 and its C-terminal SH3 domain. Middle lower panel, immunoblotting against endogenous DNM2 and exogenous BIN1 in COS-1 cell lysates transfected with HA-tagged BIN1 with or without exon 7 and 11 and its C-terminal SH3 domain. Lower panel, quantification of immunoprecipitated DNM2. Bars indicate s.e.m with n=3 independent transfections in each groups. Student's t-test, * indicates $P < 0.05$, *** $P < 0.0001$. (B) Upper panel, schematic representation of DNM2 exons structure, protein domains and splicing isoforms. GTPase, GTPase domain; MR, middle region. GED, GTPase effector domain; PH, pleckstrin homology domain; PRD, proline rich domain. Middle panel, immunoblotting against HIS tag of BIN1 BAR domain with or without exon 7 pull-downed by the various GST-DNM2 isoforms. *Glutathione* beads used as negative binding controls are noted as X. Lower panel, immunoblotting against HIS and GST tags of HIS-tagged BIN1 BAR domain with or without exon 7 and of the various GST-tagged DNM2 isoforms. (C) Left panel, representative confocal images of immunofluorescence labeling of endogenous Dnm2 and Flag-tagged BIN1 in mouse TA longitudinal sections of muscles injected for 3 months with AAV2/9-BIN1 isoforms with or without exons 7 and 11. Scale bar = 5 μm . Right panel, fluorescence intensity measurements of DNM2 (red) and Flag-BIN1 (green).

Figure 6: Dnm2 antisense oligonucleotide (ASO) injection into DMSXL mice - (A) DNM2 protein level in tibialis anterior (DynO: ASO as control; Dyn101: ASO1) – 1 month. (B) Grip strength test: hind paws - 1 month

DETAILED DESCRIPTION

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

5 The Dynamin 2 is encoded by the *DNM2* gene (Gene ID 1785). More precisely, the *DNM2* gene is located from base pair 10,919,884 to base pair 10,942,586 on chromosome 19 (GRCh37/hg19 release) or from 10,718,053 to 10,831,910 base pairs on the NC_000019.10 location (GRCh38/hg19). The dynamin 2 gene or gene products are also known by other names, including but not limited to CMTDI1, CMTDIB, DI-CMTB, DYN2, DYN2_HUMAN,
10 dynamin II, DYNII.

Dynamin 2 inhibitors

As used herein, the term “Dynamin 2 inhibitor” refers to any molecule able to decrease specifically the expression of Dynamin 2 or inhibit the Dynamin 2 activity or function.

15 Preferably, such a Dynamin 2 inhibitor is a direct inhibitor, meaning that it interacts directly with either the Dynamin 2 protein or a nucleic acid encoding said Dynamin 2 or a part thereof. The Dynamin 2 inhibitors according to the invention are capable of inhibiting or decreasing the functional activity of Dynamin 2 *in vivo* and/or *in vitro*. The inhibitor may inhibit the functional activity of Dynamin 2 by at least about 20% or at least about 30%, preferably by at least about
20 50%, preferably by at least about 70, 75 or 80%, still preferably by at least about 85, 90, or 95%. In particular, the inhibitor may inhibit Dynamin 2 expression by at least about 10%, preferably by at least about 30%, 35%, 40%, 45%, preferably by at least about 50%, preferably by at least about 70, 75 or 80%.

A Dynamin 2 inhibitor of the invention may act by blocking and/or inhibiting the
25 activity or function of Dynamin 2. This may for example be achieved by inhibiting the enzymatic activity of Dynamin 2. Functional or enzymatic activity of Dynamin 2 may be readily assessed by one skilled in the art according to known methods by testing for example the GTPase activity or the function of Dynamin 2 in clathrin-mediated endocytosis (Macia E. et al., Dynasore, a cell-permeable inhibitor of dynamin: Developmental cell 10, 839-850, June 2006).
30 For inhibitors of GTPase activity or lipid binding, subcellular localization, clathrin mediated endocytosis, synaptic vesicle endocytosis, one can use the method described in McCluskey et al, Traffic, 2013 ; McGeachie et al, ACS Chem Biol, 2013. For Dynamin 2 GTPase activity, oligomerisation, lipid binding, one can use the methods described in Wang et al J Biol Chem 2010; or Kenniston and Lemmon, Embo J, 2010.

The Dynamin 2 inhibitor of the invention may also act by blocking and/or inhibiting the Dynamin 2 expression (including transcription, splicing, transcript maturation, or translation). The decrease or inhibition of Dynamin 2 expression can be evaluated by any means known to those skilled in the art including but not limited to assessing the level of Dynamin 2 protein using for instance Western Blot analysis or ELISA, for example using an Anti-Dynamin 2 antibody, and/or assessing the level of mRNA for Dynamin 2 using any available technique such as quantitative PCR for example.

The Dynamin 2 inhibitor is preferably selected from the group consisting of an antibody directed against Dynamin 2, a nucleic acid molecule interfering specifically with Dynamin 2 expression, and a small molecule inhibiting the Dynamin 2 enzymatic activity (i.e., inhibition of the GTPase activity), expression (such as by inhibiting promoter, splicing or translation), or function (such as inhibition of oligomerisation, activation, lipid binding, or partner binding).

According to a particular embodiment, the Dynamin 2 inhibitor is selected from the group consisting of an antibody directed against Dynamin 2 or a nucleic acid molecule (or nucleotide) interfering specifically with Dynamin 2 expression. In a preferred embodiment, the Dynamin 2 inhibitor is selected from the group consisting of a nucleic acid molecule interfering specifically with Dynamin 2 expression. According to the invention, the nucleic acid molecule interfering specifically with Dynamin 2 expression is usually a non-naturally occurring nucleic acid. In a particular embodiment, the Dynamin 2 inhibitor is a RNAi, an antisense nucleic acid or a ribozyme interfering specifically with Dynamin 2 expression.

In a particular embodiment, the Dynamin 2 inhibitor is a siRNA or shRNA.

In the present invention, the nucleic acid is complementary to a gene or transcripts coding for Dynamin 2 so that the nucleic acid is able to hybridize to a gene or transcripts coding for Dynamin 2. It is understood that the nucleic acid according to the invention does not need to have 100% complementarity with the target sequence to hybridize. In particular, a nucleic acid with a degree of complementarity at least equal to approximately 90% is capable of hybridizing. Preferably, the degree of complementarity between the nucleic acid according to the invention and the target sequence is equal to at least 95%, 96%, 97%, 98%, 99% or 100%.

The term "complementary" or "complementarity" refers to the ability of polynucleotides to form base pairs with another polynucleotide molecule. Base pairs are typically formed by hydrogen bonds between nucleotide units in antiparallel polynucleotide strands. Complementary polynucleotide strands can base pair in the Watson-Crick manner (e.g., A to T, A to U, C to G), or in any other manner that allows for the formation of duplexes. As persons skilled in the art are aware, when using RNA as opposed to DNA, uracil rather than thymine is

the base that is considered to be complementary to adenosine. However, when a U is denoted in the context of the present invention, the ability to substitute a T is implied, unless otherwise stated. Perfect complementarity or 100 percent complementarity refers to the situation in which each nucleotide unit of one polynucleotide strand can bind to a nucleotide unit of a second polynucleotide strand. Less than perfect complementarity refers to the situation in which some, but not all, nucleotide units of two strands can bind with each other. For example, for two 20-mers, if only two base pairs on each strand can bind with each other, the polynucleotide strands exhibit 10 percent complementarity. In the same way, if 18 base pairs on each strand can be bonded with each other, the polynucleotide strands exhibit 90 percent complementarity.

As used herein, the term "iRNA", "RNAi" or "interfering RNA" means any RNA which is capable of down-regulating the expression of the targeted transcript or gene and thus the corresponding protein. It encompasses small interfering RNA (siRNA), double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and short hairpin RNA (shRNA) molecules. RNA interference designates a phenomenon by which dsRNA specifically suppresses expression of a target gene at post-transcriptional level. In normal conditions, RNA interference is initiated by double-stranded RNA molecules (dsRNA) of several thousands of base pair length. *In vivo*, dsRNA introduced into a cell is cleaved into a mixture of short dsRNA molecules called siRNA. The enzyme that catalyzes the cleavage, Dicer, is an endo-RNase that contains RNase III domains (Bernstein, Caudy et al. 2001 Nature. 2001 Jan 18;409(6818):363-6). In mammalian cells, the siRNAs produced by Dicer are 21-23 bp in length, with a 19 or 20 nucleotides duplex sequence, two-nucleotide 3' overhangs and 5'-triphosphate extremities (Zamore, Tuschl et al. Cell. 2000 Mar 31;101(1):25-33; Elbashir, Lendeckel et al. Genes Dev. 2001 Jan 15;15(2):188-200; Elbashir, Martinez et al. EMBO J. 2001 Dec 3;20(23):6877-88). According to a particular embodiment, iRNAs do not encompass microRNAs.

A number of patents and patent applications have described, in general terms, the use of siRNA molecules to inhibit gene expression, for example, WO 99/32619. RNA interference therapy by siRNA and shRNA is also detailed in the review by Z. Wang et al., Pharm Res (2011) 28:2983-2995.

siRNA or shRNA are usually designed against a region 19-50 nucleotides downstream the translation initiator codon, whereas 5'UTR (untranslated region) and 3'UTR are usually avoided. The chosen siRNA or shRNA target sequence should be subjected to a BLAST search against EST database to ensure that the only desired gene is targeted. Various products are commercially available to aid in the preparation and use of siRNA or shRNA.

In a preferred embodiment, the RNAi molecule is a siRNA of at least about 10-40 nucleotides in length, preferably about 15-30 base nucleotides.

siRNA or shRNA can comprise naturally occurring RNA, synthetic RNA, or recombinantly produced RNA, as well as altered RNA that differs from naturally-occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end of the molecule or to one or more internal nucleotides of the siRNA, including modifications that make the siRNA resistant to nuclease digestion.

Some Dynamin 2 inhibitory nucleic acids are commercially available. One can cite for example, but not limited to: Abnova-Novus Biologicals, Dynamin 2 RNAi with references: H00001785-R05-H00001785-R08; Santa Cruz Biotechnology, Dynamin II siRNA (h) with reference: sc-35236, Dynamin II (h)-PR with reference: sc-35236-PR, Dynamin II shRNA Plasmid (h) with reference: sc-35236-SH, Dynamin II shRNA (h) Lentiviral Particles with reference: sc-35236-V).

In a particular embodiment, the nucleic acid molecule interfering specifically with Dynamin 2 is a nucleic acid interfering specifically with at least one part of the full length muscle human cDNA sequence of dynamin 2 (as shown in SEQ ID No 1, transcript variant 1 (NM_001005360.2)(exon 10a, 13ter) with 12b added, which is a human and mouse muscle specific exon (12B) in DN2). According to this embodiment, and more specifically, the RNAi molecule is a siRNA or shRNA of at least about 10-40 nucleotides in length, preferably about 15-30 base nucleotides iRNA. In a particular embodiment, siRNA or shRNA targets at least one exon of Dynamin2 mRNA, and more specifically at least one of exon 1, 4, 5, 11, 12, 12b, 13, 14, 15, 17 and 21 of Dynamin2 mRNA.

In a particular embodiment, the nucleic acid molecule specifically interfering with Dynamin 2 comprises or consists of a sequence selected from the group consisting of

- iRNA sequence of SEQ ID No 2 : 5'- AAGGACATGATCCTGCAGTTCAT - 3' (or shRNA seq N°C, below),

- iRNA sequence of SEQ ID No 3: 5'- AAGAGGCTACATTGGCGTGGTGA- 3'

- iRNA sequence of SEQ ID No 4: 5'- AGGTGGACACTCTGGAGCTCTCC - 3',

- iRNA sequence of SEQ ID No 5: 5'- AAGAAGTACATGCTGCCTCTGGA - 3',

- iRNA sequence of SEQ ID No 6: 5'- AACGTCTACAAGGACCTGCGGCA - 3',

- iRNA sequence of SEQ ID No 7: 5'- AGGAGAACACCTTCTCCATGGAC - 3',

- iRNA sequence of SEQ ID No 8: 5'- AACTGTTACTATACTGAGCAG - 3',

- iRNA sequence of SEQ ID No 9: 5'- TGCCAACTGTTACTATACT - 3',

- iRNA sequence of SEQ ID No 10: 5' - GAAGAGCTGATCCCGCTGG -3'
- iRNA sequence of SEQ ID No 11: 5' - GCACGCAGCTGAACAAGAA -3'
- iRNA sequence of SEQ ID No 12: 5'-GGACTTACGACGGGAGATC-3'
- iRNA sequence of SEQ ID No 13: 5' -GGATATTGAGGGCAAGAAG-3'
- 5 - iRNA sequence of SEQ ID No 14: 5'-GGACCAGGCAGAAAACGAG-3'
- iRNA sequence of shRNA 15: 5'- GCGAATCGTCACCACTTAC-3'

shRNA against DNM2	Target sequence	Dnm2 Exon target	SEQ ID No :
A	AACCGCGGGATGGAAG AGCT	1	16
B	AACTTGACCCTCATCG ACCTC	4	17
C	AAGGACATGATCCTGC AGTTCAT	4	2
D	TCGGTGTTCATCACCAA GCT	5	18
E	TGCCAACTGTTTCTATA CT	12b	19
F	AACTGTTTCTATACTGA GGAG	12b	20
G	TTTCTATACTGAGGAG CTGGT	12b	21
H	GCACGCAGCTGAACAA GAA	13	22
I	AAGAAGTACATGCTGC CTCTGGA	15	23
J	AACACCTTCTCCATGG ACCC	17	24
K	CCATTATCCGCCCAGC CGAGC	21	25

Antisense nucleic acid can also be used to down-regulate the expression of Dynamin 2. The antisense nucleic acid can be complementary to all or part of a sense nucleic acid encoding Dynamin 2, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence, and it is thought to interfere with the translation of the target mRNA. The antisense nucleic acids used in the invention interfere specifically with Dynamin 2 expression.

According to an embodiment, the antisense nucleic acid is a RNA molecule complementary to a target mRNA encoding Dynamin 2.

According to another embodiment, the antisense nucleotide denotes a single stranded nucleic acid sequence, either DNA or RNA, which is complementary to a part of a pre-mRNA encoding Dynamin 2. In particular, the antisense nucleotide of the present invention is designed to block a splice acceptor (SA) site and/or an exon splicing enhancer (ESE) and/or a branch point in the Dynamin2 pre-mRNA and/or any sequence which could modulate pre-mRNA splicing, i.e. it is designed to be complementary to a part of the Dynamin 2 pre-mRNA

comprising an SA, an ESE, a branch point sequence or any sequence which could modulate pre-mRNA splicing. More specifically, the antisense nucleotide is used for inducing exon-skipping within a Dynamin 2 pre-mRNA, thereby leading to a frameshift which produces a truncated cDNA containing a premature stop codon in the resulting mRNA. This strategy thus allows the reduction of the level of DNM2 protein. In a particular embodiment, the antisense nucleotide is used for inducing exon-skipping within a Dynamin 2 pre-mRNA. For example, the implemented antisense nucleotide is designed to specifically induce exon 2 or exon 8 skipping. In a particular embodiment, the antisense nucleotide of the present invention is able to induce the inclusion of a premature stop codon in the human DNM2 mRNA. Skipping of exon 2 or exon 8 was shown to lead to an absence of the Dynamin 2 protein (as mentioned in “Reducing dynamin 2 expression rescues X-linked centronuclear myopathy”. Cowling BS, Chevremont T, Prokic I, Kretz C, Ferry A, Coirault C, Koutsopoulos O, Laugel V, Romero NB, Laporte J., J Clin Invest. 2014 Mar 3;124(3):1350-63. doi: 10.1172/JCI71206. Epub 2014 Feb 24; and Tinelli E, Pereira JA, Suter U. Hum Mol Genet. 2013 Nov 1;22(21):4417-29. doi: 10.1093/hmg/ddt292. Epub 2013 Jun 27).

In a particular embodiment, the antisense nucleotide is designed to specifically induce DNM2 exon 2 or exon 8 skipping, and comprises or consists of one of the following sequences: U7-Ex2 (target skipping of DNM2 exon 2 with an antisense U7 snRNA), comprising the following sequence:

SEQ ID No 26: GTCACCCGGAGGCTCTCATTCTGCAGCTC

U7-Ex8 (target skipping of DNM2 exon 8 with an antisense U7 snRNA), comprising the following sequence:

SEQ ID No 27: ACACACTAGAGTTGTCTGGTGGAGCCCGCATCA

An antisense nucleic acid can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. Particularly, antisense RNA molecules are usually 15-50 nucleotides in length. An antisense nucleic acid for use in the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. Particularly, antisense RNA can be chemically synthesized, produced by *in vitro* transcription from linear (e.g. PCR products) or circular templates (e.g., viral or non-viral vectors), or produced by *in vivo* transcription from viral or non-viral vectors. Antisense nucleic acid may be modified to have enhanced stability, nuclease resistance, target specificity and improved pharmacological properties. For example, antisense nucleic acid may include modified nucleotides or/and backbone designed to increase the physical stability of the duplex formed between the antisense and sense nucleic acids.

In the context of the invention “Ribozymes” are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes can be used to catalytically cleave mRNA transcripts to thereby inhibit translation of the protein encoded by the mRNA. Ribozyme molecules specific for functional Dynamin 2 can be designed, produced, and administered by methods commonly known to the art (see e.g., Fanning and Symonds (2006) *RNA Towards Medicine (Handbook of Experimental Pharmacology)*, ed. Springer p. 289-303).

Genome editing can also be used as a tool according to the invention. Genome editing is a type of genetic engineering in which DNA is inserted, replaced, or removed from a genome using artificially engineered nucleases, or “molecular scissors”. The nucleases create specific double-stranded break (DSBs) at desired locations in the genome, and harness the cell’s endogenous mechanisms to repair the induced break by natural processes of homologous recombination (HR) and non-homologous end-joining (NHEJ). There are currently four families of engineered nucleases being used: Zinc finger nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), the CRISPR/Cas system (more specifically Cas9 system, as described by P. Mali et al., in *Nature Methods*, vol. 10 No. 10, October 2013), or engineered meganuclease re-engineered homing endonucleases. Said nucleases can be delivered to the cells either as DNAs or mRNAs, such DNAs or mRNAs are engineered to target the DNM2 gene, according to the invention. According to an embodiment, Dynamin 2 inhibitor is a DNA or mRNA engineered to target the DNM2 gene and to deliver nucleases using genome editing therapy or is a nuclease engineered to target the DNM2 using genome editing therapy.

The nucleotides as defined above used according to the invention can be administered in the form of DNA precursors or molecules coding for them.

For use *in vivo*, the nucleotides of the invention may be stabilized, via chemical modifications, such as phosphate backbone modifications (e.g., phosphorothioate bonds). The nucleotides of the invention may be administered in free (naked) form or by the use of delivery systems that enhance stability and/or targeting, e.g., liposomes, or incorporated into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, bioadhesive microspheres, or proteinaceous vectors, or in combination with a cationic peptide. They can also be coupled to a biomimetic cell penetrating peptide. The nucleotides of the invention can also be modified with a range of hydrophobic modified products (phosphoramidites and CPGs) used in the synthesis of oligonucleotides, including cholesterol (made from an entirely plant-

derived source), tocopherol (vitamin E), and palmitate modifiers (such as a fatty acid derivative of palmitic acid; 5'-Palmitate-C6-CE Phosphoramidite; this can be incorporated at the 5'-end of the oligonucleotide of interest). These have been shown to have potential use in the delivery of oligonucleotides into cells. They may also be administered in the form of their precursors or encoding DNAs. Chemically stabilized versions of the nucleotides also include "Morpholinos" (phosphorodiamidate morpholino oligomers - PMO), 2'-O-Methyl oligomers, AcHN-(RXRRBR)2XB peptide-tagged PMO (R, arginine, X, 6-aminohexanoic acid and B, ®-alanine) (PPMO), tricyclo-DNAs, or small nuclear (sn) RNAs. The latter forms of nucleotides that may be used to this effect are small nuclear RNA molecules including U1, U2, U4, U4atac, U5, U7, U11, and U12 (or other UsnRNPs), preferably U7snRNA (as identified above for SEQ ID No 26 and 27, in particular in combination with a viral transfer method based on, but not limited to, lentivirus, retrovirus, adenovirus or adeno-associated virus. All these techniques are well known in the art.

The nucleic acid molecule interfering specifically with Dynamin 2 expression of the invention may be delivered *in vivo* alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the nucleotide to the cells and preferably cells expressing DNM2. Preferably, the vector transports the nucleotide to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, and other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the nucleotides of the invention. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: lentivirus such as HIV-1, retrovirus, such as moloney murine leukemia virus, adenovirus, adeno-associated virus; SV40-type viruses; Herpes viruses such as HSV-1 and vaccinia virus. One can readily use other vectors not named herein but known in the art. Among the vectors that have been validated for clinical applications and that can be used to deliver the nucleotides, lentivirus, retrovirus and Adeno-Associated Virus (AAV) show a greater potential for exon skipping strategy or mRNA degradation.

As used herein, the term "antibody" is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE, and humanized or chimeric antibody. In certain embodiments, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and they are most easily manufactured. The term "antibody" is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')₂, single domain antibodies (DABs),

Fv, scFv (single chain Fv), and the like. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies are also well known in the art (See, e.g., Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*, ed., Cold Spring Harbor Laboratory).

5 A “humanized” antibody is an antibody in which the constant and variable framework region of one or more human immunoglobulins is fused with the binding region, e.g. the CDR, of an animal immunoglobulin. “Humanized” antibodies contemplated in the present invention are chimeric antibodies from mouse, rat, or other species, bearing human constant and/or variable region domains, bispecific antibodies, recombinant and engineered antibodies and
10 fragments thereof. Such humanized antibodies are designed to maintain the binding specificity of the non-human antibody from which the binding regions are derived, but to avoid an immune reaction against the non-human antibody.

A “chimeric” antibody is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable
15 region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, e.g., an enzyme, toxin, hormone, growth factor, drug, etc.; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

20 Antibodies directed against Dynamin 2 are commercially available, such as antibodies sold or made by Novus Biologicals: catalogue numbers: Dynamin 2 Antibody NB300-617, Dynamin 2 Antibody NBP2-16244, Dynamin 2 Antibody (6C9) H00001785-M01, by Santa Cruz Biotechnology: catalogue number: sc-81150, sc-6400, sc-166525, sc-166669, sc-166526, by BD-Biosciences: anti-DNM2 (mouse ab, 610264), or by IGBMC-Illkirch: anti-DNM2 :
25 R2679, R2680, R2865, R2866, R2640, or R2641.

In another particular embodiment, the Dynamin 2 inhibitor is a small molecule inhibiting the Dynamin 2 enzymatic activity or function.

As used herein, the term “small molecule inhibiting Dynamin 2 activity, expression or function” refers to small molecule that can be an organic or inorganic compound, usually less
30 than 1000 daltons, with the ability to inhibit or reduce the activity, expression or function of Dynamin 2. This small molecule can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi and viruses) or from a library of synthetic molecules. Small molecules inhibiting Dynamin 2 activity, expression or function can be identified with the method described in this document.

Dynamin inhibitors are described in Harper CB et al., Trends Cell Biol. 2013 Feb;23(2):90-101. Review. In a particular embodiment, such molecule is selected from the group consisting of:

- Dynasore (a non-competitive, cell-permeable semicarbazone compound inhibitor of Dynamin 1 and Dynamin 2. - N° CAS 304448-55-3), its chemical name is 3-Hydroxynaphthalene-2-carboxylic acid (3,4-dihydroxybenzylidene)hydrazide,
- Hydroxy-Dynasore (a highly potent inhibitor of dynamin 2 ($IC_{50} = 2.6 \mu M$)) (Hydroxy-Dynasore is a cell-permeable hydroxylated analog of Dynamin Inhibitor, Dynasore - N° CAS 1256493-34-1), its chemical name is 3-Hydroxy-N'-[(2,4,5-trihydroxyphenyl)methylidene]naphthalene-2-carbohydrazide,
- Tetradecyltrimethylammonium bromide (N° CAS 1119-97-7), sold under the name MiTMAB™ (ab120466) by Abcam (a Cell permeable dynamin 1 and dynamin 2 inhibitor ($IC_{50} = 8.4 \mu M$ for inhibition of dynamin II). It targets the pleckstrin homology (PH) (lipid binding) domain. It inhibits receptor-mediated and synaptic vesicle endocytosis (IC_{50} values 2.2 μM),
- Phthaladyn-23 (a cell-permeable phthalimide compound that is reported to inhibit Dynamin 2 GTPase activity ($IC_{50} = 63 \mu M$)), the chemical name of Phthaladyn-23 is 4-Chloro-2-((2-(3-nitrophenyl)-1,3-dioxo-2,3-dihydro-1H-isoindole-5-carbonyl)-amino)-benzoic acid,
- Dynole 34-2, it is a Dynamin inhibitor V (scbt.com) and acts on GTPase activity, non-competitive for GTP, chemical name of Dynole 34-2 is 2-Cyano-N-octyl-3-[1-(3-dimethylaminopropyl)-1H-indol-3-yl]acrylamide,
- M-divi 1 (mitochondrial division inhibitor, $IC_{50} = 10 \mu M$) (scbt.com), the chemical name of M-divi-1 is 3-(2,4-Dichloro-5-methoxyphenyl)-2-sulfanylnquinazolin-4(3H)-one,
- Iminodyn-22/17 (scbt.com) (Iminodyn 22 : $IC_{50} = 390 nM$ acting on a GTPase allosteric site and displays uncompetitive antagonism with respect to GTP), the chemical name of Iminodyn 22 is *N,N'*-(Propane-1,3-diyl)bis(7,8-dihydroxy-2-imino-2H-chromene-3-carboxamide), the chemical name of Iminodyn 17 is *N,N'*-(Ethane-1,2-diyl)bis(7,8-dihydroxy-2-imino-2H-chromene-3-carboxamide).
- OcTMAB, i.e., OctadecylTriMethylAmmonium Bromide, (abcam.com), it targets the PH domain,
- Dynamin inhibitory peptide (Tocris Biosciences 1774): with aminoacid sequence: SEQ ID No 28: QVPSRPNRAP,
- Dyngo-4a ($IC_{50} = 2.5 \mu M$), it acts on a GTPase allosteric site, chemical name of Dyngo-4a is 3-Hydroxy-N'-[(2,4,5-trihydroxyphenyl)methylidene]naphthalene-2-carbohydrazide,

-RTIL-13 (IC₅₀ -2.3μM), it is a norcantharidin scaffold targeting the PH domain, chemical name of RTIL-13 is 4-(N,N-Dimethyl-N-octadecyl-N-ethyl)-4-aza-10-oxatricyclo-[5.2.1]decane-3,5-dione bromide.

5 Uses of Dynamin 2 inhibitors

The invention relates to a method for treating DM by administering a therapeutically effective amount of a Dynamin 2 inhibitor as defined above to patients in need thereof, and to the uses of such Dynamin 2 inhibitor in the treatment of DM. It also relates to the use of a Dynamin 2 inhibitor for the manufacture of a pharmaceutical composition for the treatment of
10 DM. It relates to a Dynamin 2 inhibitor for use in the treatment of DM.

Moreover, the present invention relates to a pharmaceutical composition comprising a Dynamin 2 inhibitor, and optionally a pharmaceutically acceptable carrier, in particular for use in the treatment of DM.

In a particular embodiment of the invention, the disease to be treated is Myotonic Dystrophy
15 (DM), more specifically DM1 (such as mild or severe form thereof), its congenital form CDM1 (also known as Steinert disease), or DM2, more particularly by increasing or improving muscle force of DM patients and/or by improving the resistance of muscles to contraction induced injury.

As used herein, the term “therapeutically effective amount” is intended an amount of
20 therapeutic agent, administered to a patient that is sufficient to constitute a treatment of a DM. In a particular embodiment, the therapeutically effective amount to be administered is an amount sufficient to reduce the Dynamin 2 expression, activity or function in a level allowing prevention or decrease of muscle fiber atrophy or in a level allowing prevention or decrease of the number of abnormally centrally localized nuclei of affected cells or in a level allowing
25 inhibition of abnormal recruitment of DNM2 to T-tubules, and preventing or reducing thereby morphological alterations of this specialized muscle excitation-contraction coupling compartment. The amount of Dynamin 2 inhibitor to be administered can be determined by standard procedure well known by those of ordinary skill in the art. Physiological data of the patient (e.g. age, size, and weight), the routes of administration and the disease to be treated
30 have to be taken into account to determine the appropriate dosage, optionally compared with subjects that do not present DM. One skilled in the art will recognize that the amount of Dynamin 2 inhibitor or of a vector containing or expressing the nucleic acid interfering specifically with Dynamin 2 expression to be administered will be an amount that is sufficient to induce amelioration of unwanted DM symptoms or to induce alleviation of one or more

symptoms or characteristics of DM, including skeletal muscle progressive atrophy, weakness and myotonia, heart conduction defects and/or others features, such as cataract, insulin resistance and/or cognitive dysfunctions. An alleviation of one or more symptoms or characteristics may be assessed by any of the following assays on a myogenic cell or muscle cell from a patient: altered calcium uptake by muscle cells, altered collagen synthesis, altered morphology, altered lipid biosynthesis, altered oxidative stress, and/or improved muscle fiber function, integrity, and/or survival. These parameters are usually assessed using immunofluorescence and/or histochemical analyses of cross sections of muscle biopsies. An alleviation of one or more symptoms or characteristics may also be assessed by any of the following assays on the patient himself: skeletal muscle progressive atrophy, weakness and myotonia, heart conduction defects or others features, such as cataract, insulin resistance and cognitive dysfunctions, or improvement of the quality of life. Each of these assays is known to the skilled person. For each of these assays, as soon as a detectable improvement or prolongation of a parameter measured in an assay has been found, it will preferably mean that one or more symptoms of Myotonic Dystrophy has been alleviated in an individual using the method of the invention. Detectable improvement or prolongation is preferably a statistically significant improvement or prolongation. Alternatively, the alleviation of one or more symptom(s) of Myotonic Dystrophy may be assessed by measuring an improvement of a muscle fiber function, integrity and/or survival as later defined herein. The improvement of muscle fiber function, integrity and/or survival may be assessed using at least one of the following assays: a detectable decrease of creatine kinase in blood, a detectable decrease of abnormally centrally localized nuclei of muscle cells and/or a relocalization of nuclei of muscle cells into their periphery in a biopsy cross-section of a muscle suspected to be dystrophic, and/or a detectable increase of the homogeneity of the diameter of muscle fibers in a biopsy cross-section of a muscle suspected to be dystrophic. Each of these assays is known to the skilled person. The amount of Dynamin 2 inhibitor or of a vector containing or expressing the nucleic acid interfering specifically with Dynamin 2 expression may vary inter alia depending on such factors as the type of selected dynamin 2 inhibitors, the gender, age, weight, overall physical condition of the patient, etc. and may be determined on a case by case basis. The amount may also vary according to other components of a treatment protocol (e.g. administration of other medicaments, etc.). Generally, when the Dynamin 2 inhibitor is a nucleic acid, a suitable dose is in the range of from about 50 mg/week to 1,500 mg/week. If a viral-based delivery of the nucleic acid is chosen, suitable doses will depend on different factors such as the virus that is employed, the route of delivery (intramuscular, intravenous, intra-arterial or other), but may

typically range from 10^{-9} to 10^{-15} viral particles/kg. If the inhibitor is a small molecule inhibiting the Dynamin 2 activity, expression or function, each unit dosage may contain, for example, from 2 to 300 mg/kg of body weight, particularly from 5 to 100 mg/kg of body weight. If the inhibitor is an antibody, each unit dosage may contain, for example, from 0.1 to 20 mg/kg of body weight, particularly from 4 to 10 mg/kg of body weight. Those of skill in the art will recognize that such parameters are normally worked out during clinical trials. Further, those of skill in the art will recognize that, while disease symptoms may be completely alleviated by the treatments described herein, this need not be the case. Even a partial or intermittent relief of symptoms may be of great benefit to the recipient. In addition, treatment of the patient may be a single event, or the patient is administered with the Dynamin 2 inhibitor on multiple occasions, that may be, depending on the results obtained, several days apart, several weeks apart, or several months apart, or even several years apart. A treatment in a method according to the invention may have a duration of at least one week, at least one month, at least several months, at least one year, at least 2, 3, 4, 5, 6 years or more. The frequency of administration may be ranged between at least once a week or once in a two weeks, or three weeks or four weeks or five weeks or a longer time period.

Each Dynamin 2 inhibitor as defined herein for use according to the invention may be suitable for any type of administration, preferably systemic administration. It can be direct administration to a cell, tissue and/or an organ *in vivo* of individuals affected by or at risk of developing DM. It may be administered directly *in vivo*, *ex vivo* or *in vitro*. An oligonucleotide as used herein 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 DM, and may be administered directly or indirectly *in vivo*, *ex vivo* or *in vitro*. As myotonic dystrophy has a pronounced phenotype in muscle cells, it is preferred that said cells are muscle cells, it is further preferred that said tissue is a muscular tissue and/or it is further preferred that said organ comprises or consists of a muscular tissue. A preferred organ is the heart. Preferably said cells are cells of an individual suffering from DM.

A Dynamin 2 inhibitor as defined herein (which can be a molecule or oligonucleotide or equivalent thereof) can be delivered as is to a cell. When administering said inhibitor to an individual, it is preferred that it is dissolved in a solution that is compatible with the delivery method. For intravenous, subcutaneous, intramuscular, intrathecal and/or intraventricular administration, it is preferred that the solution is a physiological salt solution. Particularly preferred for a method of the invention is the use of an excipient that will further enhance delivery of said inhibitor as defined herein, to a cell and into a cell, preferably a muscle cell.

The pharmaceutical composition of the invention is formulated in accordance with standard pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopaedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York) known by a person skilled in the art. Preferably, Dynamin 2 inhibitor as defined herein is dissolved in a solution that is compatible with the delivery method. For intravenous, subcutaneous, intramuscular, intrathecal and/or intraventricular administration, it is preferred that the solution is a physiological salt solution.

More generally, possible pharmaceutical compositions include those suitable for oral, rectal, mucosal, topical (including transdermal, buccal and sublingual), or parenteral (including subcutaneous, intramuscular, intravenous, intra-arterial and intradermal) administration. According to a particular embodiment, pharmaceutical compositions of the invention are suitable for a systemic administration. For these formulations, conventional excipient can be used according to techniques well known by those skilled in the art.

More particularly, in order to provide a localized therapeutic effect, specific muscular administration routes are preferred. In particular, intramuscular administration is preferred.

Pharmaceutical compositions according to the invention may be formulated to release the active drug substantially immediately upon administration or at any predetermined time or time period after administration.

Within the context of the invention, the term treatment denotes curative, symptomatic, and preventive treatment. As used herein, the term "treatment" of a disease refers to any act intended to extend life span of subjects (or patients) such as therapy and retardation of the disease progression. The treatment can be designed to eradicate the disease, to stop the progression of the disease, and/or to promote the regression of the disease. The term "treatment" of a disease also refers to any act intended to decrease the symptoms associated with the disease, such as hypotonia and muscle weakness or atrophy. Prolongation of time to loss of walking, improvement of muscle strength, improvement of the ability to lift weight, improvement of the time taken to rise from the floor, improvement in the 6 minute walk or nine-meter walking time, improvement in the time taken for four-stairs climbing, improvement of the leg function grade, improvement of the pulmonary function, improvement of cardiac function, or improvement of the quality of life of subjects (or patients) are also within the definition of the term "treatment". More specifically, the treatment according to the invention is intended to delay the appearance of the DM phenotypes or symptoms, ameliorate the motor and/or muscular behavior and/or

lifespan, in particular by improving muscle force and/or resistance to contraction-induced muscle injury.

The subject (or patient) to treat is any mammal, preferably a human being. Preferably the subject is a human patient, whatever its age or sex. New-borns, infants, children are included as well. More preferably, the patient or subject according to the invention is a Myotonic Dystrophy patient or is suspected to be a Myotonic Dystrophy patient (a patient susceptible to develop DM because of his or her genetic background).

Screening of Dynamin 2 inhibitors

The present invention also concerns a method for identifying or screening molecules useful in the treatment of DM, based on the ability of such molecules to inhibit the expression, activity and/or function of Dynamin 2.

In particular, the invention is drawn to a method for screening comprising the steps of:

- a) providing or obtaining a candidate compound; and
- b) determining whether said candidate compound inhibits the activity, function and/or expression of Dynamin 2,
- c) wherein the ability of said candidate compound to inhibit the expression, function or activity of said Dynamin 2 indicates that said candidate compound is indicative of its usefulness for the treatment of DM.

The candidate compound to be tested in the frame of this method may be of any molecular nature, for example it may correspond to a chemical molecule (preferably a small molecule), an antibody, a peptide, a polypeptide, an aptamer, a siRNA, a shRNA, a snRNA, a sense or antisense oligonucleotide, a ribozyme, or a targeted endonuclease.

The ability of said candidate compound to inhibit the expression, activity or function of Dynamin 2 may be tested using any of the methods known to those skilled in the art, such as those identified above or described in the examples.

The method for screening or identifying a molecule suitable for the treatment of DM can optionally further comprise the step of administering *in vivo*, *ex vivo* or *in vitro* selected molecule in DM non-human animal model or a part thereof (tissue or cells, such as muscle tissue or cells) and analyzing the effect on the myopathy onset or progression.

As DM non-human animal models, one can cite the DM1 mouse model, the HSALR mouse developed in Dr. Charles Thornton's laboratory (Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. Mankodi A, Logigian E, Callahan L, McClain C, White R, Henderson D, Krym M, Thornton CA. Science. 2000 Sep 8;289(5485):1769-73). HSALR

mice exhibit aberrant splicing of many genes that are mis-spliced in DM1, including *Cln1* and, consequently, show prominent myotonia. One can also cite the DMSXL model developed in Dr Genevieve Gourdon's laboratory (Molecular, physiological, and motor performance defects in DMSXL mice carrying >1,000 CTG repeats from the human DM1 locus. Huguet A, Medja F, Nicole A, Vignaud A, Guiraud-Dogan C, Ferry A, Decostre V, Hogrel JY, Metzger F, Hoeflich A, Baraibar M, Gomes-Pereira M, Puymirat J, Bassez G, Furling D, Munnich A, Gourdon G. PLoS Genet. 2012;8(11):e1003043. doi: 10.1371/journal.pgen.1003043. Epub 2012 Nov 29). Molecular features of DM1-associated RNA toxicity in DMSXL mice (such as foci accumulation and mis-splicing), were associated with high mortality, growth retardation, and muscle defects (abnormal histopathology, reduced muscle strength, and lower motor performances).

The following examples are given for purposes of illustration and not by way of limitation.

EXAMPLES

MATERIALS AND METHODS

Human samples.

Human samples were skeletal muscle needle biopsies that were sampled with the informed consent of individuals and approved by the Institutional Review Board of the Neuromuscular Research Center of the Tampere University Hospital, of the Hospital Donostia, of the Friedrich-Baur-Institute, of the IRCCS policlinico San Donato and of the Toneyama National Hospital. Non-affected muscle samples (CTL #1 to #4) were purchased at Ambion. CTRL #5 to #7 were patients with amyotrophic lateral sclerosis (ALS) described previously (Nakamori et al., Neurology, 70(9):677–685, 2008). Foetal control and CDM1 samples were described previously (Fugier C, et al. Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. Nat Med. 2011 Jun;17(6):720-5). Adult DM1 and DM2 samples have been described previously (Fugier et al., 2011).

RNA sequencing.

RNA sequencing was performed on distal skeletal muscle samples of adult DM1 and age- and sex-matched controls described previously (Nakamori et al., Neurology 70(9):677–685, 2008). After isolation of total cellular RNA from human skeletal muscles samples, libraries of template molecules were created using TruSeq RNA Sample Preparation v2 Kit (ref. RS-122-2001, Illumina) with modifications in the manufacturer's protocol. Briefly, mRNA was

purified from 1 µg total RNA using oligo-dT magnetic beads and fragmented using divalent cations at 94°C for 8 minutes. The cleaved mRNA fragments were reverse transcribed to cDNA using random primers, then the second strand of the cDNA was synthesized using Polymerase I and RNase H. The next steps of RNA-Seq Library preparation were performed in a fully automated system using SPRIworks Fragment Library System I kit (ref. A84801, Beckman Coulter) with the SPRI-TE instrument (Beckman Coulter). In this system double stranded cDNA fragments were blunted, phosphorylated and ligated to Illumina indexing adapters and fragments in the range of ~200-400 bp were size selected. The automated steps were followed by PCR amplification (30 sec at 98°C; [10 sec at 98°C, 30 sec at 60°C, 30 sec at 72°C] x 12 cycles; 5 min at 72°C), and then surplus PCR primers and adapter dimers were removed by purification using AMPure XP beads (Beckman Coulter). DNA libraries were checked for quality and quantified using 2100 Bioanalyzer (Agilent). The libraries were sequenced on the Illumina HiSeq 2500 as paired-end 2x100 base reads following Illumina's instructions. Image analysis and base calling were done using RTA 1.17.21.3 and CASAVA.

RNA extraction and RT-PCR analysis.

Total RNA was extracted from samples using TRI Reagent (Molecular Research Center), then 500 ng of RNA was reverse transcribed using 10 units of Transcriptor reverse transcriptase (Roche) with 100 ng of random hexaprimers (Invitrogen) in a final volume of 20 µl. Alternative splicing tests were carried out by PCR using 2 µl of cDNA with 2.5 units of Taq DNA polymerase (Roche) with amplification consisting of one denaturation step at 94 °C for 2 min, 26 cycles of amplification 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min and a final step at 72 °C for 5 min using the primer described in supplemental tables 1 and 2. PCR products were resolved by electrophoresis using 8% non-denaturing polyacrylamide gel stained by ethidium bromide, washed 3 times in distilled water and revealed with a Typhoon FLA 9500 fluorescence scanner (GE Healthcare). Gels were subsequently quantified with the ImageQuant TL software and the percentage of exon splicing was determined. The RT-PCR analysis of Mbnl compound KO mouse model was reported previously (Lee 2013 EMBO MM).

Cloning and plasmids.

Full-length BIN1 isoforms with or without its exon 7 and 11 that all contain exon 17 but lack the neuronal exons 13 to 16 were cloned from BIN1 isoform 8 NM_004305.2 (-ex7, +ex11, -Ex13to16, +ex17) into pcDNA3.1 fused to a C-terminal GFP or HA tag or into pAAV2 fused to a C-terminal Flag tag and were described previously (Fugier et al., 2011). Presence of the alternative BIN1 exon 17 was determinant for further recognition by the anti-BIN1 99D

(Abcam) antibody. Truncated BAR domain constructs with or without exon 7 was cloned into pET28 with a 6xHIS tag.

Cell culture and transfection.

All cells were maintained at 37°C, 5% CO₂. Neuro2A cells (ATCC) were cultured in CellBIND Flasks (Corning) in minimum essential medium Eagle (MEM) with Earle's salts containing 10% foetal calf serum supplemented with non-essential amino acids, 1mM sodium pyruvate and 40 µg/ml gentamycin. HEK293 and COS-1 cells (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 1 g/L glucose and 5% foetal calf serum supplemented with 40 µg/ml gentamycin. Immortalized myoblasts originating from control and DM1 patients were grown and differentiated as previously described (Furling et al., 2001). Transfections were carried out in medium containing 0.1% serum without gentamycin using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

BIN1 exon 7 splicing reporter.

BIN1 minigene containing exons 6, 7 and 8, as well as full intron 6 and truncated intron 7 was constructed by PCR using human genomic DNA (Clontech) as template and reading proof Phusion DNA polymerase (Thermo Scientific) with the primers MG1 fwd, SEQ ID No 29: 5'-AAAGCTAGCTCACGCATTGCCAAGCGGG-3' and MG1 rev, SEQ ID No 30: 5'-AAAAGCTTCCCCCAGGAACACTGTGGTGC-3' for sequence from exon 6 to intron 7 and primers MG2 fwd, SEQ ID No 31: 5'-AAAAGCTTGGCCTGAGAACGTGAGGCTGC-3' and MG2 rev, SEQ ID No 32: 5'-AAAAGCTCGAGCTGTTCCACAGGGACGGCAGC-3' for BIN1 sequence from intron 7 to exon 8. PCR products were cloned side by side into pcDNA3.1+ vector (Clontech) using NheI with HindIII restriction sites for the first part of the minigene (MG1) and HindIII with XhoI for the second part (MG2). Mutations were performed by primer-directed PCR mutagenesis. Neuro2A cells in 6-well plates were transfected using Lipofectamine 2000 (Invitrogen) with 500 ng of WT or mutated minigene with or without 1.5 µg of DT960, 1.5 µg of v5-MBNL1 40 kDa, 200 pmol of siMbnl1 and siMbnl2 (Dharmacon), 1.5 µg of tgCUGBP1 or 200 pmol of siCelf1 (encoding Cugbp1). RNA were extracted 24 hours after transfection and RT-PCR was performed using MG-splicing fwd SEQ ID No 33: 5'-AGAGAACCCACTGCTTACTGGC-3' and MG-splicing rev SEQ ID No 34: 5'-AGATGGCTGGCAACTAGAAGGC-3' primers, localized within the pcDNA3.1 vector to avoid amplification of endogenous Bin1.

DMSXL mouse line.

Male and female homozygous DMSXL mice (Huguet et al 2012), were injected with antisense oligonucleotides targeting the mouse DNM2 mRNA, at 25mg/kg by intraperitoneal injection on postnatal day 1 (day of birth), day 4, day 8, then once per week until day 28. Mice were weighed weekly, and analyzed for muscle force, grip strength test (on hind limbs) and whole body force. Two days after the last injection mice were sacrificed by cervical dislocation. Tissue biopsies were dissected for mRNA and protein analysis for DNM2, antisense oligonucleotide concentration, and for histological analysis. Blood and serum samples were collected for analysis. Antisense oligonucleotides (ASO) used in these studies were synthesized in IONIS Pharmaceuticals. They were 16 nucleotides in length and chemically modified with phosphorothioate in the backbone and cEt modifications on the wings with a deoxy gap (3-10-3 design). Oligonucleotides were synthesized using an Applied Biosystems 380B automated DNA synthesizer (PerkinElmer Life and Analytical Sciences-Applied Biosystems, Waltham, Massachusetts) and purified. A total of 500 ASOs were prescreened in b. END cells. The three best ASO candidates that reduce Dnm2 level have been selected. In addition, a random control ASO sequence was used as control. The used sequences of ASOs (antisense oligonucleotides) targeting the mouse DNM2 mRNA (“Antisense oligonucleotide-mediated Dnm2 knockdown prevents and reverts myotubular myopathy in mice.” Tasfaout H, Buono S, Guo S, Kretz C, Messaddeq N, Booten S, Greenlee S, Monia BP, Cowling BS, Laporte J. Nat Commun. 2017 Jun 7;8:15661. doi: 10.1038/ncomms15661) were the following:

	Sequence	Targeted region
ASO-1 (Dyn101-m) (targeting DNM2)	GGCATAAGGTCACGGA (SEQ ID No 35)	Exon 17
ASO-C (DYN0-m) (control)	GGCCAATACGCCGTCA (SEQ ID No 36)	No homology to mouse genome

Adeno-Associated Virus (AAV2/9) production and muscle injection.

All animal work was performed with approval from the IGBMC/ICS Animal Care Committee and of the French agency for research on animal (DGRI) authorization number 00608.02 and APAFIS#2596-2015110511036862. Recombinant AAV2/9 BIN1 were generated by tri-transfection of HEK293 cells with pAAV2-BIN1 with or without its exon 7 and 11, p5E18-VD29, and pAD-DELTA-F16. Recombinant vectors were purified by double cesium chloride ultracentrifugation gradients from cell lysates, followed by dialysis and concentration against sterile PBS. Particles were quantified by real time PCR and vector titers

are expressed as viral genomes per ml (vg/ml). Tibialis anterior (TA) muscles of 5 to 7-week-old mice (129PAS, Charles River) were injected with either 40 μ L of sterile PBS or 1x10¹⁰ viral genomes of AAV-BIN1. Animals were housed in a temperature-controlled room (19–22°C) with a 12:12-hours light/dark cycle. One or three months after AAV injection, mice were

5 either perfused with PFA or sacrificed by cervical dislocation in order to dissect TA muscles which were subsequently frozen in nitrogen-cooled isopentane for histology or treated for TEM.

Immunofluorescence and muscle histology.

Tibialis anterior muscles from 4% PFA perfused mice were embedded in paraffin and cut into 5 μ m longitudinal muscle sections. Prior to immunofluorescence, sections were

10 deparaffinized two times for 20 min in HistoSol Plus (Shandon) and dehydrated (each step of 5 min) twice in ethanol 100%, twice in ethanol 95%, once in ethanol 80%, once in ethanol 70% and rinsed in PBS. Sections were incubated for 10 min in PBS + 0.5% Triton X-100 and washed three times with PBS. Non-specific sites were blocked in PBS supplemented with 10% foetal calf serum for 1 h. Sections were incubated overnight at 4°C in a humidified chamber with the

15 primary antibody diluted in PBS, 1/200 mouse anti-BIN1 (99D directed against exon 17 of BIN1, Abcam ab181710), 1/200 rabbit anti-DNM2 (R-2865, homemade). Sections were washed twice with PBS before a one-hour incubation with secondary antibodies 1/500 goat anti-mouse Alexa-Fluor 488 (Life Technologies) and 1/500 donkey anti-rabbit DyLight 594 (Thermo Scientific). Sections were rinsed three times before nuclei staining by 10 min

20 incubation with a 1/10,000 DAPI solution, rinsed twice and mounted in Pro-Long media (Molecular Probes). Fluorescent images acquisitions were realized on a Leica SP8 point-scanning confocal system with an HC PL APO CS2 63x/1.40 OIL objective. Fluorescence intensity quantification was measured using the plot profile function in FIJI image analysis software. For histology, muscle sections were stained with H&E or NADH/PH followed by

25 acquisition using a slide scanner NanoZoomer 2HT (Hamamatsu Photonics). Muscle fibers size and nuclei position were analyzed in transversal H&E sections using FIJI image analysis software.

Transmission electron microscopy.

Muscle samples were fixed in 2.5% glutaraldehyde and 2.5% PFA in 0.1 M sodium

30 cacodylate buffer (pH=7.2) for 24h at 4°C, washed in 0.1M cacodylate buffer for 30 minutes and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 hour at 4°C. For selective staining of T-tubules, the samples were post-fixed with 2% OsO₄ + 0.8% K₃Fe(CN)₆ in 0.1 M cacodylate buffer (pH 7.2) for 2 h at 4 °C and incubated with 5% uranyl acetate for 2 h at 4 °C. Samples were dehydrated through graded alcohol (50, 70, 90, and 100%) and

propylene oxide for 30 minutes each, then embedded in Epon 812, cut at semi-thin 2 μ m or ultra-thin 70nm, contrasted with uranyl acetate and lead citrate, examined at 70kv with a Morgagni 268D (Philips) electron microscope and imaged with Mega View III camera (Soft Imaging System).

5 ***In situ* force measurement.**

Mice were anesthetized by intraperitoneal injection of pentobarbital sodium (50-80 mg/kg), immobilized and knee and foot was pin fixed. Tibialis anterior muscle tendon was attached to a servomotor system (Aurora Scientific). Sciatic nerve was stimulated with a bipolar silver electrode using 0.1-ms-duration supramaximal square-wave pulses. Absolute maximal force generated during isometric contractions in response to electrical stimulation (500-ms-duration stimulation train, 100 Hz frequency) was determined at L0 (length at which maximal tension was obtained during the tetanus). Specific maximal force was calculated by dividing absolute maximal force by muscle weight. Fatigue or susceptibility to contraction-induced injury is monitored according to the force drop after a prolonged contraction-induced injury according the following protocol. The sciatic nerve was stimulated for 700 ms (150 Hz frequency) and maximal isometric contraction of the TA muscle was initiated during the first 500 ms. Then, muscle lengthening (10% L0) at a velocity of 5.5 mm/s (0.85 fiber length/s) was imposed during the last 200 ms. All isometric contractions were performed at an initial length L0. Nine lengthening contractions of the TA muscles were recorded, each separated by a 60 ms rest period. Maximal isometric force was measured 1 min after each lengthening contraction and expressed as a percentage of the initial maximal force. After contractile measurements, the animals were euthanized and muscles were dissected and weighed.

BIN1 exon 7 antibody production.

Mouse monoclonal antibody directed against BIN1 containing exon 7 [4A9] was raised against full-length exon 7 peptide (human or mice, SEQ ID No 37: PVSLLLEKAAPQWCQGKLQAHLVAQTNLLRNQ) conjugated with Imject Maleimide Activated Carrier Protein Spin Kit (Pierce) and injected intraperitoneally into two months old female BALB/c mice with 200 μ g of poly(I/C) as adjuvant. Three injections were performed at 2 weeks intervals and four days prior to hybridoma fusion, mice with positively reacting sera were re-injected. Spleen cells were fused with Sp2/0.Agl4 myeloma cells and hybridoma culture supernatants were tested at day 10 by ELISA. Positive cultures were cloned twice on soft agar. Specific hybridomas were established and ascites fluid was prepared by injection of 2x10⁶ EXP6 hybridoma cells into Freund adjuvant-primed BALB/c mice. All animal experimental procedures were performed with approval of IGBMC and ICS Animal Care

Committee.

Western Blotting.

Cells were rinsed with PBS, scraped in PBS, centrifuged at 500g for 5 min and lysed for 30-min on ice in 50 mM Tris-HCl pH 7.5, 0.15 M NaCl, 0.5% NP40 supplemented with protease inhibitor cocktail (Roche). Cell lysates were clarified by centrifugation at 14000g for 10 min and protein concentration was determined by Bradford assay (Biorad). Proteins were denatured 3 min at 95°C with Laemmli buffer, separated on 4-12% bis-Tris gradient gel (NuPAGE) in MOPS SDS running buffer (NuPAGE), transferred on 0.45 µm nitrocellulose membranes (Whatman Protan) in 25 mM Tris, 190 mM Glycine, 20% ethanol and blocked overnight with 5% non-fat dry milk in Tris Buffer Saline (TBS). Equal loading was monitored by Ponceau red. Primary antibody incubation was performed for 1 hour in TBS-T buffer containing 0.5% non-fat dry milk with 1/2,000 mouse anti-BIN1 [99D] (Abcam ab181710), 1/1,000 mouse anti-BIN1 exon 7 [4A9] (homemade), 1/1,000 mouse anti-GAPDH [6C5] (Santa Cruz sc-32233), 1/2,000 mouse anti-MBNL1 or anti-MBNL2 (kind gift of Prof. Morris, Wolfson Centre for Neuromuscular Disease, UK), 1/2,000 mouse anti-CUGBP1 [3B1] (Sigma 3B1-3D11), 1/8,000 rabbit anti-HA tag (Abcam ab9110), 1/2,000 rabbit anti-DNM2 [R-2865] (homemade), 1/5,000 mouse anti-polyHistidine (Sigma H1029). After washing in TBS-T, blots were incubated for 1 h with 1/4,000 horseradish peroxidase (HRP)-conjugated secondary antibodies (Jackson ImmunoResearch) diluted in TBS-T containing 0.5% non-fat dry milk, washed three times with TBS-T, incubated with Chemiluminescent HRP Substrate (Millipore) and imaged on LAS600 (Amersham).

Co-immunoprecipitation assay.

COS-1 cells in 6-well plates were transfected using Lipofectamine 2000 (Invitrogen) with 1 µg of the different HA-C-terminally tagged BIN1 constructions. After 24 h of transfection, cells were lysed in 50 mM Tris-HCl pH 7.5, 0.15 M NaCl, 0.5% NP40 supplemented with protease inhibitor cocktail (Roche) and clarified by centrifugation at 14000g for 10 min. Immunoprecipitations were performed at 4°C for 1 h using pre-washed Pierce Anti-HA Magnetic Beads (ThermoFisher) in TBS buffer supplemented with 0.05% Tween-20, washed three times, then bound proteins were eluted by 2 min denaturation step at 95°C with Laemmli buffer followed by western blot. Percentage of DNM2 immunoprecipitated was obtained by quantification of the western blots using FIJI image analysis software.

Recombinant protein production and purification.

Escherichia coli BL21(RIL) pRARE competent cells (Invitrogen) were transformed with 20 ng of either pGEX-GST-DNM2 (variants 1 to 4), pGEX-GST-SH3 domain of BIN1,

pET28a-His-BIN1-His (-ex7, -ex11 or +ex7, -ex11), pET28a-His-BAR-His domain of BIN1 with or without its exon 7 or pET28a-GST-MBNL1- Δ 101-His. Bacteria were grown at 37°C in 400 ml of LB medium supplemented with kanamycin (pET-28a) or ampicillin (pGEX). When OD600 reached 0.5 units, cultures were cooled down in ice for 15 min prior addition of 0.5 mM IPTG followed by an overnight incubation at 18°C. His-tagged proteins were purified on Protino Ni-TED Packed Columns (Macherey-Nagel) according to the manufacturer's guidelines and elutions were dialyzed overnight at 4°C in 50 mM Tris-HCl pH 8, 150 mM NaCl, 5 mM MgCl₂. Pellets of bacteria expressing GST-tagged DNM2 proteins were rinsed with PBS and lysed in 50 mM Tris-HCl pH 8; 300 mM NaCl; 5 mM MgCl₂ containing 1% sarkosyl. Sonication was realized for 3 min followed by an overnight incubation of the lysate on a rotating wheel. The lysate was clarified by centrifugation at 20,000 g for 20 min and 4% Triton and 40 mM CHAPS was added. GST-DNM2 protein lysates were added to 1 ml pre-washed Glutathione (GSH) sepharose (GE Healthcare), washed and resuspended into 50 mM Tris-HCl pH 8, 100 mM NaCl, 5 mM MgCl₂ and 0.5% Triton. GST-SH3 domain of BIN1 was purified as above but with final elution in 50 mM Tris-HCl pH 8, 100 mM NaCl, 5 mM MgCl₂, 0.5% Triton and 10mM of reduced glutathione. All purifications steps were realized at 4°C with ice-cold buffers supplemented with protease inhibitor cocktail (Roche).

Analytical ultracentrifugation sedimentation velocity (AUC-SV)

AUC-SV experiments were performed with a ProteomeLab XL-I analytical ultracentrifuge (Beckman Coulter) at 4°C and 50,000 rpm. The samples were diluted in 50 mM Tris, pH 8, 100 mM NaCl, and 1 mM EGTA with protease inhibitors (Roche). Absorbance scans were taken at 280 nm every 3 min for 10 h, and the sedimentation data were analyzed with SEDFIT software (<http://www.analyticalultracentrifugation.com>) with continuous c(s) distribution analysis.

RNA transcription and gel-shift assays.

Templates for transcription were obtained by PCR using as template pCDNA3.1-BIN1 control or mutant minigenes with the forward primer including a T7 promoter sequence. Transcription reactions were performed using T7 transcription kit (Ambion) in presence of 1 μ l of α P³²-CTP (10 μ Ci, 800 Ci per mmole, Perkin Elmer), analyzed on 8% denaturing polyacrylamide and quantified with LS-6500 counter (Beckman). After transcription, 1 unit of DNase I (Invitrogen, Carlsbad, CA) was added, and the sample was incubated for additional 30 min at 37°C. Transcribed RNAs were then purified by micro Bio-Spin 6 chromatography columns (Bio-rad). Sizes and integrity of RNAs were confirmed by gel electrophoresis on a denaturing 6% polyacrylamide gel. For gel-shift, recombinant MBNL1 Δ Cter in a concentration

ranging from 3 to 300 nM was diluted in Buffer D (20 mM Hepes pH 7.9, 100 mM KCl, 0.2 mM EDTA, 0.5 mM DTT, 17.5% glycerol, 0.5 µg/µL BSA, 0.1% NP40), incubated 10 min at 30°C with 2 min 94°C denatured 5,000 cpm of internally αP³²-CTP-labeled RNA in binding buffer (Buffer D supplemented with 1.4 units/µL of RNase Inhibitor and 0.01 µg/µL of tRNA from *E. coli*). The reactions were run on a 6% native acrylamide gel (40:1 Acrylamide/bisacrylamide) and revealed and quantified using a Typhoon FLA 9500 scanner (GE Healthcare). The binding affinities (K_d) of MBNL1 are expressed in nanomolar (nM) and were calculated using the intercept of the fitted curve with the Y-axis, using the following formula $K_d = 10 \text{EXP-}y$.

10 Statistical analysis

All cells experiments are represented as average ± Standard Error of Mean (SEM) with significance determined using nonparametric test (Man-Whitney or Student t-test).

RESULTS

15 Identification of splicing changes in skeletal muscle of DM1 individuals.

To identify novel splicing abnormalities in DM1 muscles, paired-end RNA sequencing (RNA-seq) was performed on polyadenylated RNA extracted from distal skeletal muscle samples of three adult DM1 patients compared to three age-matched control individuals. In total it was obtained 935 and 820 million 100 bp paired-end reads for adult DM1 and control skeletal muscle samples, respectively. MISO bioinformatic analysis, which computes the fraction of mRNA including a given cassette alternative exon (Katz et al., Analysis and design of RNA sequencing experiments for identifying isoform regulation. Nat Methods. 2010 Dec;7(12):1009-15), predicted 422 significant ($\Delta\text{PSI} \geq 0.4$; Z-score ≥ 1.2) alternative splicing changes between control and DM1 muscle samples (**Figure 1A**). This analysis confirmed the abnormal inclusion of *BIN1* exon 7 in DM1 skeletal muscle samples (**Figure 1B**), which was validated by RT-PCR analysis (**Figure 1C**). It was similarly confirmed splicing alterations for 35 other mRNAs (**Figure 1C**), including some identified previously (*INSR*, *ATP2A1*, *RYR1*, *CACNA1S*, *DMD*, *PKM2*, etc.) as well as others that represent additional alterations of alternative splicing in DM1 (*TJAP1*, *STK40*, *MBD1*, *PPP3CC*, *AP2A1*, *SLC2A8*, *DIAPH1*, *SPATS2*, etc.). Bioinformatics analysis revealed that MBNL-binding sites are enriched within and around the top 400 exons found misregulated by MISO in DM1 compared to 2,000 unaltered exons. MBNL motifs are enriched within the exon and in the upstream intron of exons abnormally included in DM1, while they are enriched in the downstream intron of exons repressed in DM1. These results are consistent with the known MBNL splicing regulatory map,

where binding of MBNL upstream of an exon mostly inhibits exon inclusion whereas binding of MBNL downstream of an exon generally stimulates exon inclusion. These results confirm that titration of MBNL proteins is likely the main cause of splicing change in skeletal muscle of individuals with DM1. Interestingly, splicing index calculated as the log of the ratio of exon inclusion in DM1 over control revealed that *BIN1* exon 7 was among the top exons misregulated in DM1 due to its quasi null inclusion in control individuals (**Figure 1D**). As splicing changes in *BIN1* correlates with muscle weakness in DM1, and mutations in the *BIN1* gene cause CNM, a muscle disease with histopathological features similar to DM, it was decided to investigate further the splicing alteration of *BIN1* exon 7 in DM1 muscle.

Altered splicing of *BIN1* exon 7 in skeletal muscle of DM1 individuals and models.

To confirm the abnormal inclusion of *BIN1* exon 7 in skeletal muscle of individuals with DM, its alternative splicing by RT-PCR was investigated in a larger number of samples (**Figure 2A**). Exon 7 was absent from skeletal muscle of control adult individuals. In contrast, exon 7 was abnormally included into *BIN1* mRNA in DM1 with an inclusion of 20% in adults but reaching 50 to 70% in congenital cases. To confirm integrity of these DM2 samples, it was tested by RT-PCR an alternative splicing event (exon 22 of *ATP2A1*, also named SERCA1) known as classically altered in DM. *ATP2A1* splicing was only mildly altered in our DM2 samples compared to DM1, which is consistent with the milder severity and later age of onset of DM2 compared to DM1. Thus, presence of *BIN1* exon 7 grossly correlates with disease severity as it is more included in the severe congenital CDM1 form compared to the adult-onset cases and the milder DM2 form. This is consistent with the correlation between inclusion of *BIN1* exon 7 and muscle weakness identified previously in adult DM1 (Nakamori et al., Splicing biomarkers of disease severity in myotonic dystrophy. *Ann Neurol.* 2013 Dec;74(6):862-72). Taking advantage of this large cohort of DM1 patients (Nakamori et al., 2013), it was noted that the extent of *BIN1* exon 7 abnormal inclusion was correlated with other splicing alteration in DM, such as *ATP2A1* exon 22 or *BIN1* exon 11 (**Figure 2B**). These analyses were performed on mRNA, which may only partly reflect BIN1 protein alteration. To confirm the presence of BIN1 exon 7 at the protein level, a monoclonal antibody specific to the 31 amino acids encoded by *BIN1* exon 7 was developed. Immunoblotting confirmed RT-PCR results with inclusion of exon 7 in BIN1 protein in DM1 compared to control skeletal muscle samples (**Figure 2C**). Expression of *BIN1* exon 7 is normally restricted to the nervous system, where it contributes to the endocytosis regulatory function of BIN1 (Ellis et al., Tissue-specific alternative splicing remodels protein-protein interaction networks. *Mol Cell.* 2012 Jun 29;46(6):884-92). Thus, the abnormal inclusion of exon 7 observed in DM1 muscle samples

may originate from an alteration of the nervous system innervating the skeletal muscles. To discriminate between muscle cell and non-muscle cell autonomous mechanisms, the alternative splicing of *BIN1* exon 7 in cultures of muscle cells derived from control or DM1 individuals was tested. RT-PCR assays revealed that inclusion of exon 7 is negligible in control muscle cultures but reaches 50 to 60% in differentiated DM1 muscle cells (**Figure 2D**). Immunoblotting confirmed specific inclusion of exon 7 in BIN1 protein in cultures of DM1 myotubes compared to control myotubes (**Figure 2E**). As control, alternative splicing of *ATP2A1* exon 22 and *BIN1* exon 11 were also altered in DM1 muscle cells compared to control cells. Finally, it was tested misregulation of *Bin1* exon 7 in a mouse model of DM1 expressing expanded CUG repeats only in skeletal muscle (HSA^{LR} mice, Mankodi, A. et al. Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. Science 289, 1769–1773 (2000), thus excluding any potential side effect from changes in others tissues than muscle. Mild inclusion of *Bin1* exon 7 was observed only in HSA^{LR} mice and not in control non-transgenic animals (**Figure 2F**). As control, alternative splicing of *Atp2a1* exon 22 was also altered in HSA^{LR} mice.

Overall, these results suggest that inclusion of exon 7 of *BIN1* is misregulated in DM1 skeletal muscle.

Alternative splicing of *BIN1* exon 7 is regulated by MBNL proteins.

Expanded CUG repeats alter pre-mRNA alternative splicing through dysfunction of the CUGBP1 and MBNL proteins. To clarify the mechanisms underlying the abnormal inclusion of *BIN1* exon 7 in DM1, it was constructed a minigene containing human *BIN1* exons 6 to 8 (**Figure 3A**), which was co-expressed in Neuro2A cells with different vectors expressing either expanded CUG repeats, MBNL1 or CUGBP1 or siRNA targeting *Mbnl* or *Celf1* mRNA (**Figure 3B**). Over-expression of ~1000 CUG repeats reproduced DM1 situation and induced exon 7 inclusion. Similarly, depletion of endogenous *Mbnl1* and *Mbnl2* expression by siRNA mimicked the effect of CUG repeats and promoted exon 7 inclusion, whereas over-expression of MBNL1 inhibited it (**Figure 3B**). In contrast, over-expression or depletion of CUGBP1 had a minor impact on the alternative splicing of *BIN1* exon 7. To determine whether MBNL regulation of *BIN1* exon 7 is direct or indirect, it was tested whether MBNL1 binds to *BIN1* pre-mRNA. Gel-shift assays revealed that purified recombinant GST-tagged MBNL1 binds to UGC intronic RNA sequences located upstream of *BIN1* exon 7 (**Figure 3C**). Mutation of these UGC motifs abolished MBNL1 binding (**Figure 3D**), as well as the inhibitory effect of MBNL1 on a mutant *BIN1* minigene (**Figure 3E**). Interestingly, mutation of MBNL1 binding sites also abolished the responsiveness of the *BIN1* exon 7 minigene to expanded CUG repeats (**Figure**

3E), demonstrating that the pathogenic effect of expanded CUG repeats on *BIN1* splicing mis-regulation requires functional MBNL1 binding sites. Immunoblotting analysis confirmed MBNL1 and CUGBP1 overexpression and efficient siRNA-mediated knockdown of endogenous Mbnl1, Mbnl2 or Cugbp1 levels. Next, it was assessed *Bin1* exon 7 inclusion in

5 *Mbnl* knockout mice. RT-PCR analysis shows a drastic increase of the inclusion of *Bin1* exon 7 in skeletal muscle samples of *Mbnl1* and *Mbnl2* double knockout mice (Myo-CRE DKO; **Figure 3F**). In contrast, *Bin1* exon 7 is not misregulated in skeletal muscles of the sole Mbnl1 knockout mice (*Mbnl1*^{-/-}) or in compound mice with no Mbnl1 and reduced level of Mbnl2 (*Mbnl1*^{-/-}, *Mbnl2*^{+/-}), suggesting that repression of *BIN1* exon 7 is remarkably sensitive to low

10 quantity of Mbnl proteins (**Figure 3F**). Finally, splicing alteration of *BIN1* exon 7 was assessed versus a metric computing the degree of active MBNL1 in DM1 tissue. Importantly, inclusion of *BIN1* exon 7 was negatively correlated to the quantity of MBNL inferred to be active in DM1 skeletal muscle samples (**Figure 3G**). Consistent with the inclusion of *Bin1* exon 7 only in *Mbnl1* and *Mbnl2* double knockout mice, inclusion of *BIN1* exon 7 required a quasi-complete

15 depletion of inferred active MBNL1 in individuals with DM1.

Overall, these results establish that MBNL1 inhibits the inclusion of *BIN1* exon 7.

Expression of BIN1 with exon 7 leads to muscle weakness and atrophy in mouse.

To address a potential progressive or long-term effect of *BIN1* exon 7 expression, adeno-associated virus (AAV2/9) expressing the different splicing forms of *BIN1* was injected in the

20 tibialis anterior (TA) muscles of adult wild type mice. To investigate the pathological role of *BIN1* exon 7, but also of its exon 11, which is partly excluded in DM1 (Fugier C, et al.), it was tested two splicing forms expressed in DM1 both with exon 7 and with or without exon 11 (+7-11 and +7+11) that were compared to the control *BIN1* muscle isoform containing exon 11 but lacking its exon 7 (-7+11). It was controlled by RT-qPCR and histological analyses that ectopic

25 expression of any *BIN1* splicing forms induced no overt toxicity with absence of inflammation or regeneration 1 or 3 months post-AAV injection (**Figure 4A**). Importantly, expression of both *BIN1* isoforms expressed in DM1 and containing exon 7 (+7-11 or +7+11), but not the control *BIN1* isoform (-7+11), reproduced some key pathological alterations characteristic of DM1. First, muscle fiber areas were reduced upon expression of *BIN1* with its exon 7 (with or without

30 exon 11), while the control splicing form of *BIN1* deprived of exon 7 did not induce any muscle fiber atrophy (**Figure 4B**). Expression of *BIN1* with exon 7 (+7-11 or +7+11), but not the control *BIN1* isoform (-7+11), also slightly increased the number of abnormally centrally localized nuclei (**Figure 4C**). Importantly, both maximum strength, specific force and fatigue resistance were decreased upon expression of *BIN1* with its exon 7 compared to contralateral

PBS injected muscle or to muscle injected with control BIN1 without exon 7 (**Figures 4D, 4E and 4F**). Finally, as BIN1 is involved in generation of the muscle T-tubule network, it was investigated further these structures. Electron microscopy analyses revealed that ~30 to 60% of T-tubules were abnormal with longitudinally orientated, disorganized and irregular membrane structures upon expression of BIN1 isoforms with its exon 7 (+7-11 or +7+11) compared to injection of control BIN1 isoform (**Figure 4G**). Of interest, the inclusion or exclusion of BIN1 exon 11 had only a limited pathogenic effect compared to the presence of exon 7. These experiments were repeated upon a shorter time period of ectopic BIN1 expression. Similarly to the 3 months period, expression of BIN1 isoforms for 1 month did not induce any overt toxicity, muscle regeneration or inflammation. In contrast to the 3 months expression, expression of the splicing form of *BIN1* with exon 7 and without its exon 11 (+7-11) induced only mild muscle fiber atrophy and slight abnormal nuclei localization upon a one-month expression period. Similarly, it was observed that expression of BIN1 with its exon 7 during one month induced no major muscle weakness, compared to the three months period. In contrast, a decrease in the resistance to muscle fatigue and alterations of T-tubules were already present upon one-month expression of BIN1 with its exon 7. Thus, alteration of the T-tubules network is an early onset defect that precedes apparition of muscle fiber atrophy and weakness. It was also noted that the splicing form of *BIN1* including exon 7 but lacking exon 11 (+7-11) induced slightly more alterations than the splicing form with both exons 7 and 11 (+7+11), confirming that loss of exon 11 is deleterious for T-tubule structures (Fugier et al., 2011, *infra*). As control, expression of the normal BIN1 isoform (-7+11) had no deleterious effects. Overall, these results indicate that expression of BIN1 with its exon 7 is pathogenic for muscle structure and function and that these alterations progress with time.

Exon 7 increases BIN1 interaction with DNM2.

Expression of BIN1 with its exon 7 induces T-tubules alteration, muscle fiber atrophy and weakness, questioning what are the molecular alterations induced by presence of this exon. Exon 7 of *BIN1* encodes 31 amino acids that form an alpha-helix extruded from the crescent-shaped BAR domain. A previous report showed that BIN1 exon 7 is interacting with DNM2, a large GTPase protein that constricts membrane vesicular necks and which is mutated in the autosomal dominant form of CNM (Ellis JD, et al., Tissue-specific alternative splicing remodels protein-protein interaction networks. *Mol Cell*. 2012 Jun 29; 46(6):884-92). However, other reports indicated that BIN1 interacts with DNM2 through binding of BIN1 C-terminal atypical SH3 domain with DNM2 PRD domain. By co-immunoprecipitation experiments it was found that both models are correct as deletion of either exon 7 or SH3 domain of BIN1 abolishes

interaction of BIN1 with endogenous DNM2 (**Figure 5A**). Interaction of BIN1 exon 7 to DNM2 may be indirect through a third protein partner or any other mechanism. To test whether BIN1 exon 7 directly interacts with DNM2, it was produced and purified the BAR domain of BIN1 with or without exon 7 but deleted of its C-terminal SH3 domain. *In vitro* binding assays indicated that purified recombinant GST-tagged full length DNM2 was able to pull down purified recombinant HIS-tagged BAR domain of BIN1 with its exon 7, but not control BAR domain without exon 7 (**Figure 5B**). As positive control, GST-DNM2 was also able to pull down full length HIS-BIN1 with its exon 7 (**Figure 5B**). *DNM2* is regulated by alternative splicing of its cassette exon 10 and mutually exclusive exons 13a and 13b, it was found that all four splicing forms of DNM2 equally interact with the BAR domain of BIN1 with its exon 7 (**Figure 5B**). These results demonstrate that the exon 7 reinforces the interaction of BIN1 with DNM2. The BAR domain of BIN1 without exon 7 assembles in dimers. However, it was observed that the BAR domain of BIN1 including its exon 7 forms mainly tetramers (data not shown). Beyond DNM2, it has been described that the SH3 domain of BIN1 can also interact with its own BAR domain to induce an inhibitory conformation preventing deleterious association of BIN1 to non-specific membranes. Thus, it was tested whether presence of exon 7 may modulate this mechanism. GST pull down assays demonstrated that inclusion of the exon 7 impairs interaction of BIN1 SH3 domain with its own BAR domain. These data indicate that presence of exon 7 alters BIN1 at multiple levels, notably by potentially reducing its auto-inhibitory regulation while reinforcing its interaction with DNM2.

Expression of BIN1 with its exon 7 alters DNM2 localization.

To investigate further the pathological consequences of DNM2 interaction with BIN1 exon 7, it was analyzed the localization of endogenous DNM2 in skeletal muscle of mice injected with AAV2/9 expressing either the control or the DM1 splicing forms of BIN1. Immunofluorescence followed by confocal microscopy of muscle longitudinal sections indicated that DNM2 is, as reported previously, localized as a single band centered on the muscle Z-line and bordered by the T-tubule marker BIN1 in control non-injected mice or in muscle injected with the control form of BIN1 (-7+11). In contrast, in muscle injected with *BIN1* with its exon 7 (either +7-11 or +7+11), DNM2 localization was altered and changed from a single band to a double band co-localizing with BIN1 (**Figure 5C**). As control, the localization of α -actinin (*Actn1*), which is a marker of muscle fiber Z lines, was neither altered in control nor in BIN1 with exon 7 injected muscles (data not shown). Overall, these results indicate that the *in vitro* increase binding of BIN1 with its exon 7 to DNM2 translates into an *in vivo* abnormal tethering of DNM2 to skeletal muscle T-tubules, which may alter these

membrane structures.

***Dnm2* ASO injection into DMSXL mice**

As shown on **Figure 6**, there is a significant reduction in the DNM2 protein expression, and a trend for improved grip strength after Dyn101-m ASOs (antisense oligonucleotide: ASO-1) treatment.

DISCUSSIONS

Thus, these results suggest that the abnormal inclusion of *BIN1* exon 7 in skeletal muscle of DM1 individuals results in the pathological tethering of DNM2 to T-tubules, where its membrane remodeling activity may lead to T-tubules alterations and disorganization, ultimately resulting in progressive muscle fiber atrophy and weakness.

It was also found in the present study that expressing BIN1 with its exon 7 consistently leads to muscle fiber atrophy, muscle weakness and abnormally located nuclei, demonstrating that inclusion of exon 7 in *BIN1* is an important pathogenic event in DM.

CLAIMS

- 5 1. An inhibitor of Dynamin 2 for use in the treatment of Myotonic Dystrophy.
2. An inhibitor of Dynamin 2 for use according to claim 1, wherein the dynamin 2 inhibitor is selected from the group consisting of an antibody directed against Dynamin 2, a nucleic acid molecule interfering specifically with Dynamin 2 expression, a nucleic acid or a nuclease engineered to target the DNM2 gene and to deliver nucleases using genome editing therapy, and a small molecule inhibiting the Dynamin 2 activity, expression or function.
- 10 3. The inhibitor of Dynamin 2 for use according to anyone of claims 1 and 2, wherein the dynamin 2 inhibitor is selected from the group consisting of an antibody directed against Dynamin 2, or a nucleic acid molecule interfering specifically with Dynamin 2 expression.
- 15 4. The inhibitor of Dynamin 2 for use according to anyone of claims 1, 2, and 3, wherein the Dynamin 2 inhibitor is a RNAi, an antisense nucleic acid or a ribozyme interfering specifically with Dynamin 2 expression, preferably the Dynamin 2 inhibitor is a siRNA or shRNA.
- 20 5. The inhibitor of Dynamin 2 for use according to anyone of claims 1-4, wherein the Dynamin 2 inhibitor is an antisense nucleotide inducing exon-skipping within or degradation of a Dynamin 2 pre-mRNA.
6. The inhibitor of Dynamin 2 for use according to claim 5, wherein the Dynamin 2 inhibitor is an antisense nucleotide designed to specifically induce DNM2 exon 2 or exon 8 skipping, and preferably comprises or consists of one of the following sequences:
- 25 U7-Ex2 (target skipping of DNM2 exon 2), comprising the following sequence:
SEQ ID No 26: GTCACCCGGAGGCTCTCATTCTGCAGCTC
U7-Ex8 (target skipping of DNM2 exon 8), comprising the following sequence:
SEQ ID No 27: ACACACTAGAGTTGTCTGGTGGAGCCCGCATCA.
- 30 7. The inhibitor of Dynamin 2 for use according to anyone of claims 1-6, wherein the dynamin 2 inhibitor is a nucleic acid molecule specifically interfering with Dynamin 2 comprises or consists of a sequence selected from the group consisting of SEQ ID 2-25.

8. The inhibitor of Dynamin 2 for use according to anyone of claims 1-7, wherein the dynamin 2 inhibitor is a DNA, mRNA or a nuclease engineered to target the DNM2 gene and to deliver nucleases using genome editing therapy.
9. The inhibitor of Dynamin 2 for use according to anyone of claims 1-8, for use in the treatment of Myotonic Dystrophy, more particularly Myotonic Dystrophy 1 (such as mild or severe form thereof), or its severe congenital form, or Myotonic Dystrophy 2.
10. A method for identifying or screening molecules useful in the treatment of myotonic dystrophy, comprising the steps of:
 - a. providing or obtaining a candidate compound; and
 - b. determining whether said candidate compound inhibits the activity, function and/or expression of Dynamin 2,
 - c. selecting said candidate compound if it inhibits the activity/expression/function of Dynamin 2.
11. The method according to claim 10, wherein it further comprises the step of administering *in vitro* the selected molecule in a muscular dystrophy non-human animal model of Myotonic Dystrophy or a part thereof and analyzing the effect on the myopathy onset or progression.
12. A pharmaceutical composition comprising an inhibitor of Dynamin 2 and a pharmaceutically acceptable carrier/excipient for use in the treatment of myotonic dystrophy.
13. The pharmaceutical composition according to claim 12, wherein the inhibitor of Dynamin 2 is as defined or identified by one of claims 2 to 11.

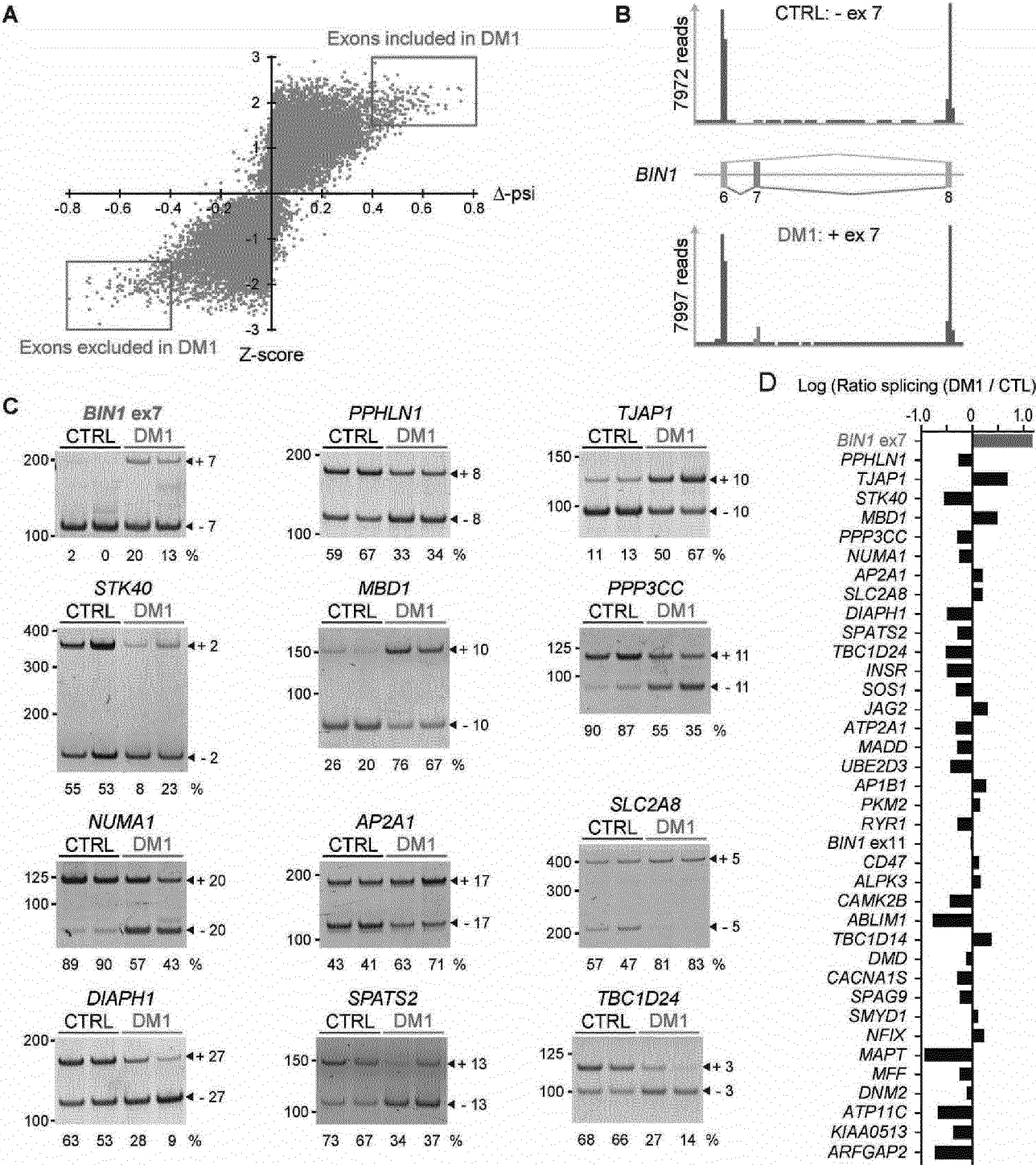


FIGURE 1

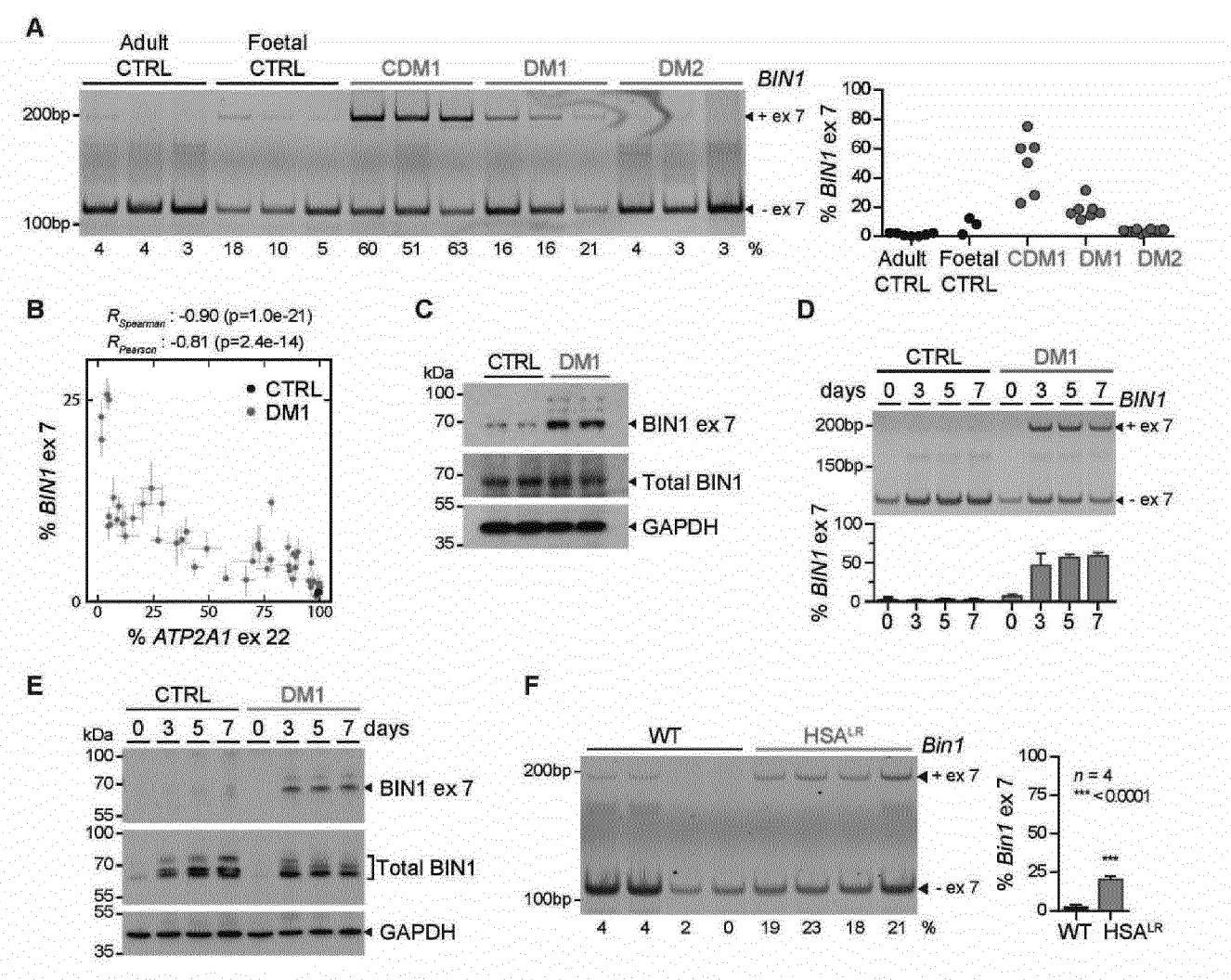


FIGURE 2

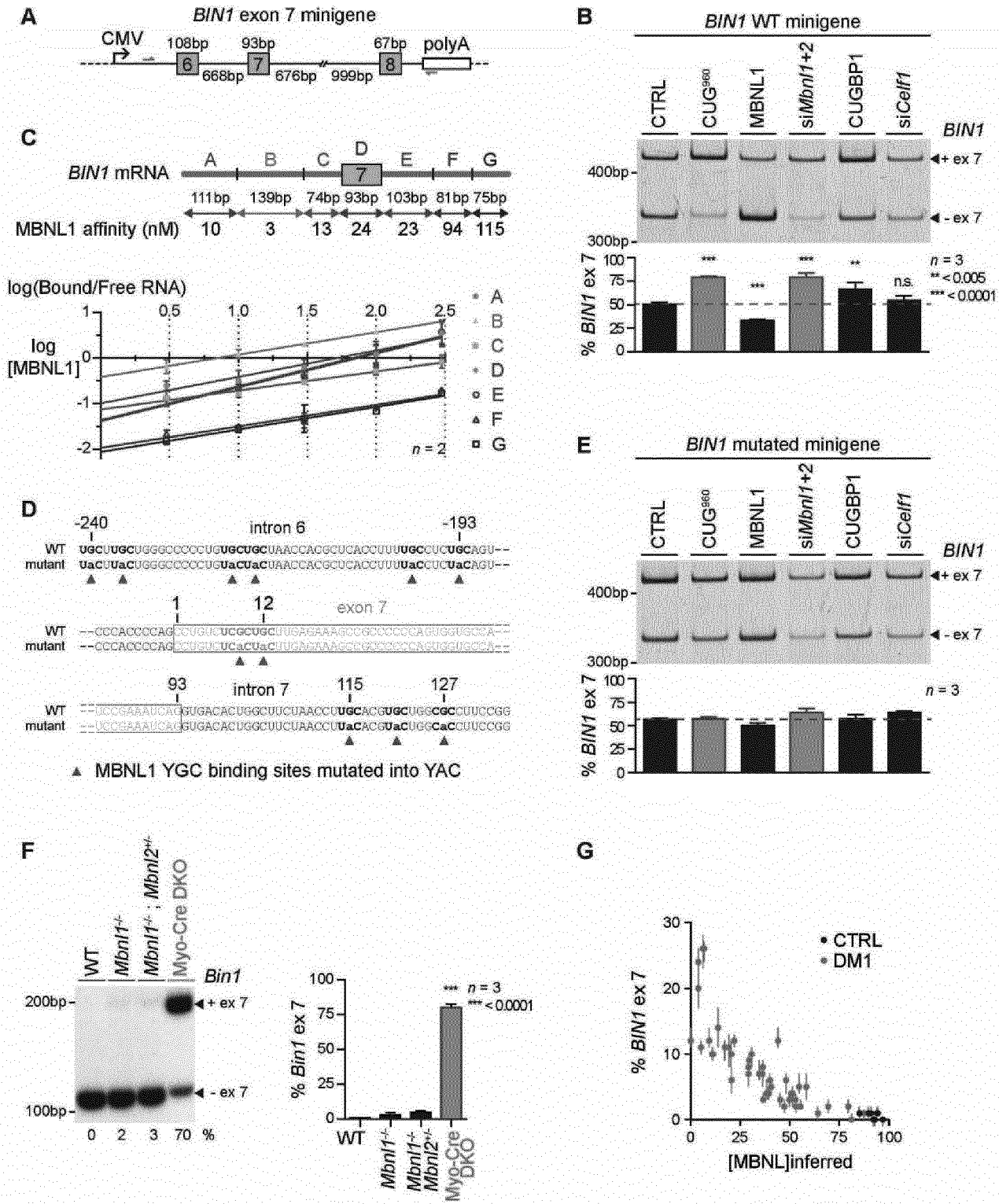


FIGURE 3

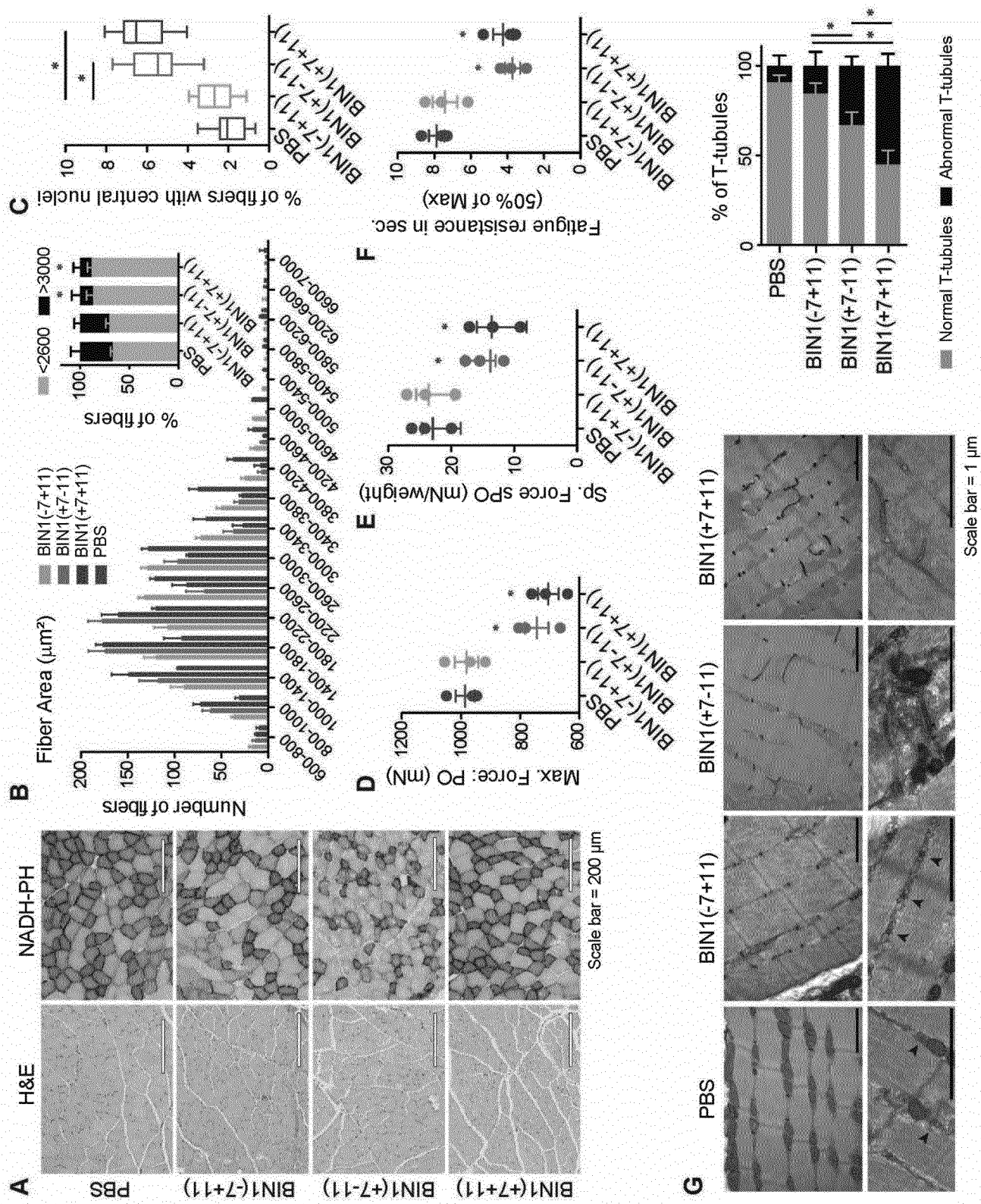


FIGURE 4

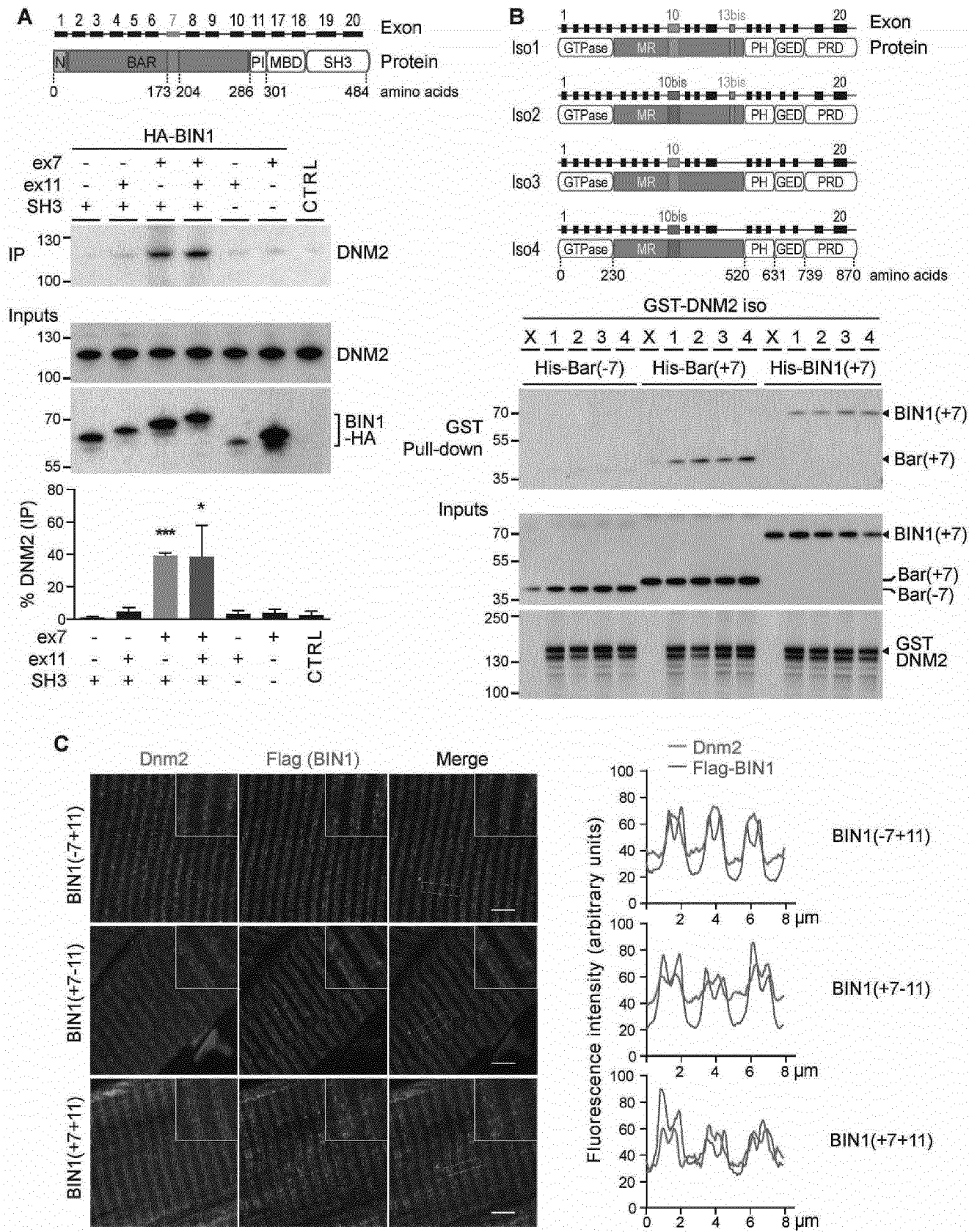


FIGURE 5

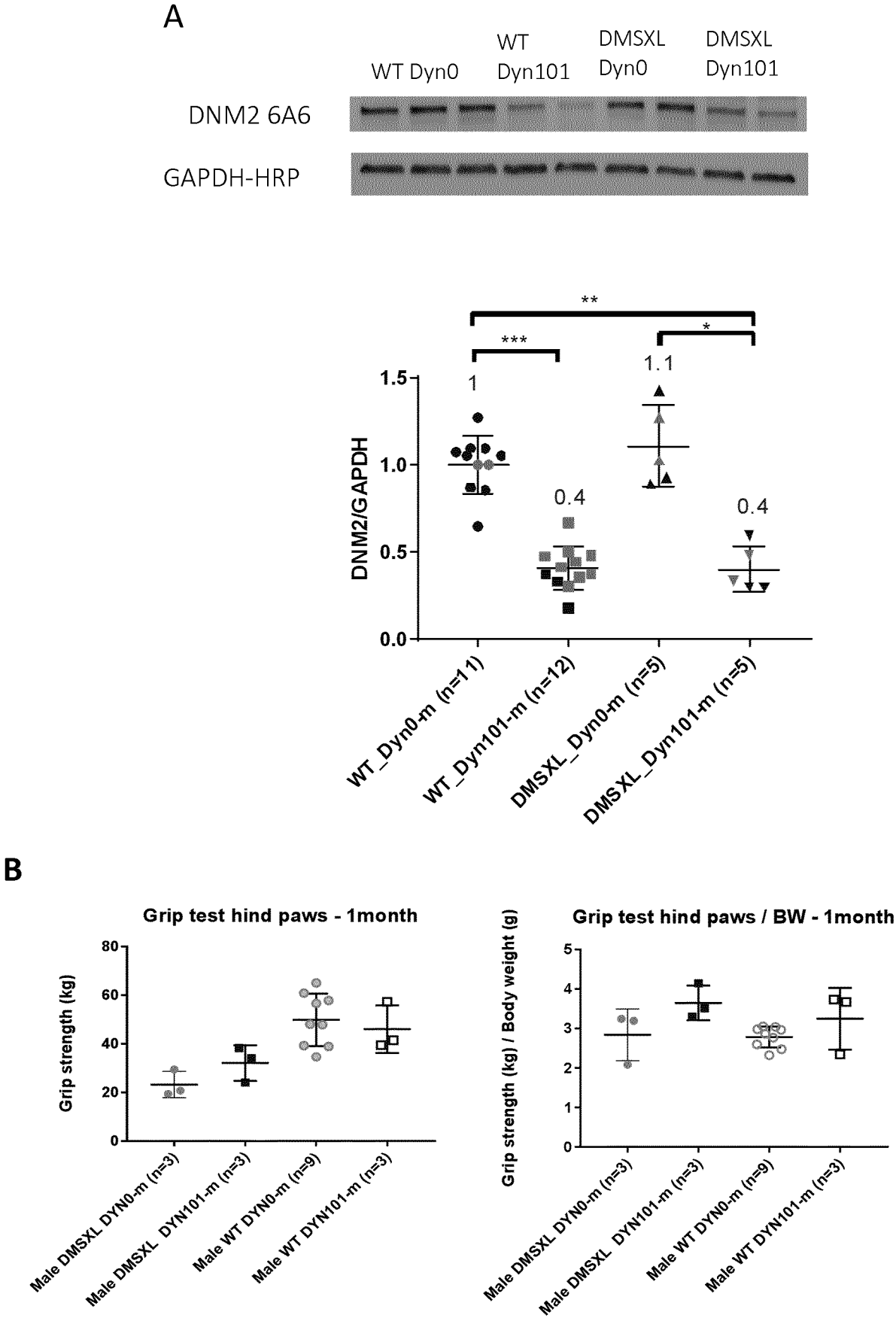


FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/084458

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/7088
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)
C12N A61K

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 3 085 785 A1 (UNIVERSITÉ DE STRASBOURG [FR]; INST NAT SANTÉ ET DE LA RECH MÉDICALE []) 26 October 2016 (2016-10-26) claims 6, 10	1-13
X	----- WO 2015/055859 A1 (UNIV STRASBOURG [FR]; CENTRE NAT RECH SCIENT [FR]; INST NAT SANTE RECH) 23 April 2015 (2015-04-23) claims 1, 9, 13	1-13
A	----- WO 2013/019623 A2 (HUTCHINSON FRED CANCER RES [US]; TAPSCOTT STEPHEN J [US]; GENG LINDA []) 7 February 2013 (2013-02-07) claims 3, 7	1-13
	----- -/-	



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

27 March 2018

Date of mailing of the international search report

05/04/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Franz, Cerstin

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/084458

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2011/113889 A1 (ASS INST DE MYOLOGIE [FR]; GARCIA LUIS [FR]; FURLING DENIS [FR]; BELEY) 22 September 2011 (2011-09-22) claims 1, 6 -----	1-13
A	WO 2005/086925 A2 (CFPH LLC [US]; ASHER JOSEPH M [US]; LUTNICK HOWARD W [US]) 22 September 2005 (2005-09-22) claim 3 -----	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/084458

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 3085785	A1	26-10-2016	AU 2016251973 A1 09-11-2017
		CA 2983211 A1 27-10-2016	
		CN 107532169 A 02-01-2018	
		EP 3085785 A1 26-10-2016	
		EP 3286317 A1 28-02-2018	
		WO 2016170162 A1 27-10-2016	
WO 2015055859	A1	23-04-2015	AU 2014336070 A1 19-05-2016
		CA 2927240 A1 23-04-2015	
		CN 105940108 A 14-09-2016	
		EP 2862928 A1 22-04-2015	
		EP 3058071 A1 24-08-2016	
		JP 2016540732 A 28-12-2016	
		US 2016264976 A1 15-09-2016	
		WO 2015055859 A1 23-04-2015	
WO 2013019623	A2	07-02-2013	CA 2843636 A1 07-02-2013
		EP 2736531 A2 04-06-2014	
		US 2014242093 A1 28-08-2014	
		WO 2013019623 A2 07-02-2013	
WO 2011113889	A1	22-09-2011	CA 2792696 A1 22-09-2011
		EP 2547768 A1 23-01-2013	
		ES 2566553 T3 13-04-2016	
		JP 5894543 B2 30-03-2016	
		JP 2013521791 A 13-06-2013	
		US 2013045538 A1 21-02-2013	
		WO 2011113889 A1 22-09-2011	
WO 2005086925	A2	22-09-2005	AU 2005221161 A1 22-09-2005
		AU 2011253538 A1 08-12-2011	
		CA 2559859 A1 22-09-2005	
		CA 2943959 A1 22-09-2005	
		EP 1735065 A2 27-12-2006	
		JP 5036530 B2 26-09-2012	
		JP 2007528279 A 11-10-2007	
		NZ 549745 A 30-04-2008	
		US 2005202874 A1 15-09-2005	
		US 2009298580 A1 03-12-2009	
		US 2012225704 A1 06-09-2012	
		US 2014221064 A1 07-08-2014	
		WO 2005086925 A2 22-09-2005	