(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
02 November 2017 (02.11.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/187319 A1

(51) International Patent Classification:
A61K 38/17 (2006.01) *A61P 7/06* (2006.01)
C07K 14/705 (2006.01) *A61P 21/00* (2006.01)

(21) International Application Number:
PCT/IB2017/052345

(22) International Filing Date:
24 April 2017 (24.04.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/326,914 25 April 2016 (25.04.2016) US

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

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title:** NOPE FOR TREATMENT OF PATHOLOGICAL MUSCLE LOSS AND WEAKNESS

(57) **Abstract:** Methods of treating muscle wasting and other disorders with NOPE extracellular domain (ECD) polypeptides and NOPE ECD fusion molecules are provided.

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NOPE FOR TREATMENT OF PATHOLOGICAL MUSCLE LOSS AND WEAKNESS

TECHNICAL FIELD

[001] Methods of treating muscle wasting and other disorders with Neighbor of PuncE11 (NOPE), such as NOPE extracellular domain (ECD) polypeptides and NOPE ECD fusion molecules are provided.

BACKGROUND

[002] Skeletal muscle loss and physical weakness are a common feature of many conditions of disuse, such as bed rest and aging, neuromuscular injury, such as ALS and spinal cord injury, and chronic disease including cancer, diabetes, and heart failure. Muscle loss decreases physical function, but also negatively impacts patient prognosis and increases mortality (Cohen et al. Nature Rev Drug Disc 2015. 14, 58-74). Therefore, there exists a need for effective therapies for the treatment or prophylaxis of diseases associated with decreases in any one or a combination of muscle mass, muscle strength, and muscle function.

[003] The transforming growth factor-beta (TGF-beta) superfamily contains a variety of secreted growth factors that share common sequence elements and structural motifs. Myostatin, also known as Growth and Differentiation Factor (GDF-8), is a member of the TGF-β superfamily and is highly conserved across mammals. Myostatin has been characterized as a negative regulator of skeletal muscle mass (McPherron AC, Lawler AM, Lee SJ. 1997. Nature 387(6628):83–90) with genetic inactivation or neutralization of myostatin associated with increased muscle mass during development and adulthood (Schuelke et al., N Engl J Med 2004, 350:2682-8 and Lee SJ Annu Rev Cell Dev Biol. 2004;20:61-86). In addition, circulating levels of myostatin are increased in many conditions of muscle wasting (Han HQ, Mitch WE. Curr Opin Support Palliat Care 2011 Dec; 5(4):334-41 and Elkina et al. J Cachexia Sarcopenia Muscle 2011; 2:143–151). Approaches to inhibit myostatin as a therapeutic strategy in disorders of muscle wasting and weakness have exhibited potential with pharmacological blockade showing prevention or reversal of muscle loss and prolongation of lifespan in various animal models of disease including cancer cachexia and renal failure (as reviewed Curr Opin Drug Discov Devel. 2008 Jul;11(4):487-94. and Int J Biochem Cell Biol. 2013 Oct;45(10):2333-47.). However no current therapies are approved and therefore a need remains.

[004] NOPE is a protein that directly binds and neutralizes myostatin, as well as a closely-related family member GDF11, and provides a novel approach to increase skeletal muscle mass. This may offer an effective therapy for the treatment or prophylaxis of diseases associated with muscle wasting.

SUMMARY

[005] In some embodiments, methods of reducing or delaying muscle wasting in a subject with muscle wasting or at risk of muscle wasting are provided, comprising administering to the subject an effective amount of a NOPE extracellular domain (ECD) polypeptide. In some embodiments, the subject has at least one condition selected from chronic obstructive pulmonary disease (COPD), chronic kidney disease, end stage renal disease, chronic heart failure, cancer, critical illness myopathy, critical illness polyneuropathy, stroke, spinal cord injury, spinal muscular atrophy, multiple sclerosis, progressive multifocal leukoencephalopathy, encephalomyelitis, central pontine myelolysis, adrenoleukodystrophy, Wallerian degeneration, Huntington's disease, Parkinson's disease, traumatic brain injury, Alexander's disease, Pelizaeus Merzbacher disease, globoid cell leucodystrophy, and sarcopenia.

[006] In some embodiments, methods of treating muscle injury are provided, comprising administering to a subject with muscle injury an effective amount of a NOPE extracellular domain (ECD) polypeptide. In some embodiments, the muscle injury is selected from surgery-related muscle injury, traumatic muscle injury, work-related skeletal muscle injury, and overtraining-related muscle injury.

[007] In some embodiments, methods of treating a muscle degenerative disorder are provided, comprising administering to a subject with a muscle degenerative disorder an effective amount of a NOPE extracellular domain (ECD) polypeptide. In some embodiments, the muscle degenerative disorder is selected from muscular dystrophy, myotonic dystrophy, polymyositis, and dermatomyositis. In some embodiments, the muscle degenerative disorder is muscular dystrophy. In some embodiments, the muscular dystrophy is selected from Duchenne muscular dystrophy, Becker muscular dystrophy, congenital muscular dystrophy (Fukuyama), Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy, and fascioscapulohumeral muscular dystrophy. In some embodiments, the muscle degenerative disorder is myotonic dystrophy. In some embodiments, the myotonic dystrophy is selected from myotonic dystrophy type I, myotonic dystrophy type II, and congenical myotonia.

[008] In various embodiments, treating comprises at least one of delaying progression of muscle wasting, improving the subject's 6 minute walking distance (6MWD), reducing physical decline, delaying occurrence of life-changing events, improving independence, reducing hospitalizations, and delaying the need for an assisted living arrangement. In some embodiments, the subject's 6MWD is increased by at least 10 meters, or at least 20 meters, or at least 30 meters after 12 months of treatment. In some embodiments, the method comprises delaying the need for a wheelchair and/or delaying placement of a ventilator.

[009] In some embodiments, methods of treating amyotrophic lateral sclerosis (ALS) are provided, comprising administering to a subject with ALS an effective amount of a NOPE extracellular domain (ECD) polypeptide. In some embodiments, treating ALS comprises at least one of delaying progression of ALS, reducing physical decline, improving forced vital capacity, slowing the decline in forced vital capacity, slowing the decline in the subject's score on the ALS functional rating scale (ALSFRS), improving the subject's score on the ALSFRS, delaying occurrence of life-changing events, and improving the subject's time of survival. In some embodiments, the method comprises delaying a tracheostomy and/or delaying placement of a percutaneous endoscopic gastrostomy (PEG).

[010] In various embodiments, administration of the NOPE extracellular domain (ECD) polypeptide increases type I slow muscle mass and/or decreases fat mass.

[011] In some embodiments, methods of increasing type I slow muscle mass are provided, comprising administering to a subject an effective amount of a NOPE extracellular domain (ECD) polypeptide.

[012] In some embodiments, methods of decreasing fat mass are provided, comprising administering to a subject an effective amount of a NOPE extracellular domain (ECD) polypeptide.

[013] In some embodiments, uses of a NOPE extracellular domain (ECD) polypeptide for reducing or delaying muscle wasting in a subject with muscle wasting or at risk of muscle wasting are provided. In some embodiments, the subject has at least one condition selected from chronic obstructive pulmonary disease (COPD), chronic kidney disease, end stage renal disease, chronic heart failure, cancer, critical illness myopathy, critical illness polyneuropathy, stroke, spinal cord injury, spinal muscular atrophy, multiple sclerosis, progressive multifocal leukoencephalopathy, encephalomyelitis, central pontine myelolysis, adrenoleukodystrophy, Wallerian degernation, Huntington's disease,

Parkinson's disease, traumatic brain injury, Alexander's disease, Pelizaeus Merzbacher disease, globoid cell leucodystrophy, and sarcopenia.

[014] In some embodiments, uses of a NOPE extracellular domain (ECD) polypeptide for treating muscle injury in a subject are provided. In some embodiments, the muscle injury is selected from surgery-related mulcle injury, traumatic muscle injury, work-related skeletal muscle injury, and overtraining-related muscle injury.

[015] In some embodiments, uses of a NOPE extracellular domain (ECD) polypeptide for treating a muscle degenerative disorder in a subject are provided. In some embodiments, the muscle degenerative disorder is selected from muscular dystrophy, myotonic dystrophy, polymyositis, and dermatomyositis. In some embodiments, treating comprises at least one of delaying progression of muscular dystrophy, improving the subject's 6 minute walking distance (6MWD), reducing physical decline, delaying occurrence of life-changing events, improving independence, reducing hospitalizations, and delaying the need for an assisted living arrangement.

[016] In some embodiments, uses of a NOPE extracellular domain (ECD) polypeptide for treating amyotrophic lateral sclerosis (ALS) in a subject are provided. In some embodiments, treating ALS comprises at least one of delaying progression of ALS, reducing physical decline, improving forced vital capacity, slowing the decline in forced vital capacity, slowing the decline in the subject's score on the ALS functional rating scale (ALSFRS), improving the subject's score on the ALSFRS, delaying occurrence of life-changing events, and improving the subject's time of survival. In some embodiments, administration of the NOPE extracellular domain (ECD) polypeptide increases type I slow muscle mass and/or decreases fat mass.

[017] In some embodiments, uses of a NOPE extracellular domain (ECD) polypeptide for increasing type I slow muscle mass in a subject are provided. In some embodiments, uses of a NOPE extracellular domain (ECD) polypeptide for decreasing fat mass in a subject are provided. In some embodiments, uses of a NOPE extracellular domain (ECD) polypeptide for treating anemia and/or thalassemia in a subject are provided.

[018] In various embodiments, the NOPE extracellular domain (ECD) polypeptide is a NOPE ECD fusion molecule. In some embodiments, the NOPE ECD polypeptide or NOPE ECD fusion molecule is capable of binding myostatin with a K_D of less than 100 nM. In some embodiments, the NOPE ECD polypeptide or NOPE ECD fusion molecule inhibits myostatin-mediated activation of SMAD2/3. In some embodiments, the NOPE ECD polypeptide or NOPE ECD fusion molecule comprises amino acids 25 to 620 of SEQ ID

NO: 1. In some embodiments, the NOPE extracellular domain (ECD) polypeptide is a NOPE ECD fusion molecule. In some embodiments, the NOPE ECD fusion molecule comprises a NOPE ECD polypeptide and a fusion partner. In some embodiments, the fusion partner is an Fc. In some embodiments, the NOPE ECD fusion molecule comprises the sequence of SEQ ID NO: 19.

[019] Any embodiment described herein or any combination thereof applies to any and all methods of the invention described herein.

BRIEF DESCRIPTION OF THE FIGURES

[020] **FIG. 1** shows an alignment of the human and mouse NOPE extracellular domains with signal sequence.

[021] **FIG. 2** shows the structures of the NOPE extracellular domain (ECD)-FC constructs used in the experiments described herein.

[022] **FIG. 3A-D** show (A, B) the change in lean mass in mice administered NOPE ECD-FC by hydrodynamic tail vein transfection, (C, D) the change in lean mass in mice administered purified NOPE ECD-FC protein, as described in Example 2. TA = tibialis anterior muscle; EDL = extensor digitorum longus muscle; gastroc = gastrocnemius muscle.

[023] **FIG. 4A-E** show mouse NOPE ECD-FC binding to myostatin (A) but not to pro-myostatin (B); human NOPE ECD-FC binding to myostatin (C); and ActR2b-FC, a myostatin inhibitor, binding to myostatin (D). The association constant, dissociation constant, and affinity of each protein for myostatin is shown in (E), as described in Example 3.

[024] **FIG. 5** shows inhibition of mysotatin by NOPE ECD-FC constructs, as described in Example 4.

[025] **FIG. 6** shows the change in lean mass in mice administered certain NOPE ECD-FC constructs by hydrodynamic tail vein transfection, as described in Example 4.

DETAILED DESCRIPTION

[026] The present inventors have found that NOPE interacts with myostatin and other ligands and that NOPE extracellular domain (ECD)-FC constructs increase lean mass in mice when administered by either hydrodynamic tail vein transfection or as a purified protein. Administration of a NOPE, such as a NOPE ECD or variations of, may therefore be an effective treatment for conditions involving muscle injury and/or muscle wasting.

[027] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[028] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[029] Exemplary techniques used in connection with recombinant DNA, oligonucleotide synthesis, tissue culture and transformation (*e.g.*, electroporation, lipofection), enzymatic reactions, and purification techniques are known in the art. Many such techniques and procedures are described, *e.g.*, in Sambrook *et al. Molecular Cloning: A Laboratory Manual* (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001)), among other places. In addition, exemplary techniques for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients are also known in the art.

[030] In this application, the use of "or" means "and/or" unless stated otherwise. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim in the alternative only. Unless otherwise indicated, the term "include" has the same meaning as "include, but are not limited to," the term "includes" has the same meaning as "includes, but is not limited to," and the term "including" has the same meaning as "including, but not limited to." Similarly, the term "such as" has the same meaning as the term "such as, but not limited to." Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[031] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[032] The terms "**nucleic acid molecule**" and "**polynucleotide**" may be used interchangeably, and refer to a polymer of nucleotides. Such polymers of nucleotides may contain natural and/or non-natural nucleotides, and include, but are not limited to, DNA, RNA, and PNA. "**Nucleic acid sequence**" refers to the linear sequence of nucleotides that comprise the nucleic acid molecule or polynucleotide.

[033] The terms "**polypeptide**" and "**protein**" are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Such polymers of amino acid residues may contain natural or non-natural amino acid residues, and include, but are not limited to, peptides, oligopeptides, dimers, trimers, and multimers of amino acid

residues. Both full-length proteins and variant proteins are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. Furthermore, for purposes of the present invention, a "polypeptide" refers to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the proteins or errors due to PCR amplification.

[034] A "**native sequence**" polypeptide comprises a polypeptide having the same amino acid sequence as a polypeptide found in nature. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring polypeptide from any mammal. Such native sequence polypeptide can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence" polypeptide specifically encompasses naturally occurring truncated or secreted forms of the polypeptide (*e.g.*, an extracellular domain sequence), naturally occurring variant forms (*e.g.*, alternatively spliced forms) and naturally occurring allelic variants of the polypeptide.

[035] A polypeptide "**variant**" means a biologically active polypeptide having at least about 80% amino acid sequence identity with the native sequence polypeptide of SEQ ID NO: 17 after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Such variants include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the polypeptide. In some embodiments, a variant will have at least about 80% amino acid sequence identity. In some embodiment, a variant will have at least about 90% amino acid sequence identity. In some embodiment, a variant will have at least about 95% amino acid sequence identity with the native sequence polypeptide. In some embodiment, a variant will have at least about 97% amino acid sequence identity with the native sequence polypeptide.

[036] As used herein, "**Percent (%) amino acid sequence identity**" and "**homology**" with respect to a peptide, polypeptide sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for

purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGNTM (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[037] The terms "neighbor of punc E11" and "**NOPE**" include any native NOPE from any vertebrate source, including mammals such as primates (*e.g.* humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term includes full-length, unprocessed NOPE as well as any form of NOPE that results from processing in the cell or any variant thereof that retains the ability to specifically bind myostatin with an affinity (Kd) of less than ≤ 1μM, ≤ 100 nM, or ≤ 10 nM. The term also encompasses naturally occurring variants of NOPE, *e.g.*, splice variants or allelic variants. In some embodiments, NOPE is a human NOPE with an amino acid sequence of SEQ ID NO: 1 (precursor, with signal peptide) or SEQ ID NO: 2 (mature, without signal peptide). A nonlimiting exemplary non-human NOPE is mouse NOPE with an amino acid sequence of SEQ ID NO: 3 (precursor, with signal peptide) or SEQ ID NO: 4 (mature, without signal peptide).

[038] The term "**NOPE extracellular domain**" ("**NOPE ECD**") includes full-length NOPE ECDs, and NOPE ECD variants, and refers to an NOPE polypeptide that lacks the intracellular and transmembrane domains, with or without a signal peptide. In some embodiments, an NOPE ECD inhibits myostatin-mediated signaling. The term "**full-length NOPE ECD**", as used herein, refers to an NOPE ECD that extends to the last amino acid of the extracellular domain, and may or may not include an N-terminal signal peptide, and includes natural splice variants in the extracellular domain. In some embodiments, a full-length human NOPE ECD has the amino acid sequence of SEQ ID NO: 5 (with signal peptide) or SEQ ID NO: 6 (without signal peptide). In some embodiments, a full-length mouse NOPE ECD has the amino acid sequence of SEQ ID NO: 11 (with signal peptide) or SEQ ID NO: 12 (without signal peptide). As used herein, the term "**NOPE ECD variants**" refers to NOPE ECDs that contain amino acid additions, deletions, and substitutions and that remain capable of binding to myostatin. Such variants may be at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical to the parent NOPE ECD. The % identity of two polypeptides can be measured by a similarity score determined by comparing the amino acid sequences of the two polypeptides using the Bestfit program with the default settings for determining similarity. Bestfit uses the local homology algorithm of Smith and

Waterman, *Advances in Applied Mathematics* 2:482-489 (1981) to find the best segment of similarity between two sequences.

[039] The term "**NOPE ECD fusion molecule**" refers to a molecule comprising an NOPE ECD, and one or more "**fusion partners**." In some embodiment, the NOPE ECD and the fusion partner are covalently linked ("**fused**"). If the fusion partner is also a polypeptide ("**the fusion partner polypeptide**"), the NOPE ECD and the fusion partner polypeptide may be part of a continuous amino acid sequence, and the fusion partner polypeptide may be linked to either the N-terminus or the C-terminus of the NOPE ECD. In such cases, the NOPE ECD and the fusion partner polypeptide may be translated as a single polypeptide from a coding sequence that encodes both the NOPE ECD and the fusion partner polypeptide (the "**NOPE ECD fusion protein**"). In some embodiments, the NOPE ECD and the fusion partner are covalently linked through other means, such as, for example, a chemical linkage other than a peptide bond. Many known methods of covalently linking polypeptides to other molecules (for example, fusion partners) may be used. In other embodiments, the NOPE ECD and the fusion partner may be fused through a "**linker**," which is comprised of at least one amino acid or chemical moiety.

[040] In some embodiments, the NOPE ECD polypeptide and the fusion partner are noncovalently linked. In some such embodiments, they may be linked, for example, using binding pairs. Exemplary binding pairs include, but are not limited to, biotin and avidin or streptavidin, an antibody and its antigen, *etc.*

[041] Exemplary fusion partners include, but are not limited to, an immunoglobulin Fc domain, albumin, and polyethylene glycol. The amino acid sequences of nonlimiting exemplary Fc domains are shown in SEQ ID NOs: 26 to 28.

[042] In some embodiments, an NOPE ECD amino acid sequence is derived from that of a non-human mammal. In such embodiments, the NOPE ECD amino acid sequence may be derived from mammals including, but not limited to, rodents (including mice, rats, hamsters), rabbits, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets. NOPE ECD fusion molecules incorporating a non-human NOPE ECD are termed "**non-human NOPE ECD fusion molecules**." Similar to the human NOPE ECD fusion molecules, non-human fusion molecules may comprise a fusion partner, optional linker, and an NOPE ECD. Such non-human fusion molecules may also include a signal peptide. A "**non-human NOPE ECD variant**" refers to NOPE ECDs that contain

amino acid additions, deletions, and substitutions and that remain capable of binding to myostatin from the animal from which the sequence was derived.

[043] The terms "myostatin" and "MSTN" refer to mature myostatin, which, in some embodiments of human myostatin, has the sequence of SEQ ID NO: 20. A nonlimiting exemplary mouse myostatin sequence is shown in SEQ ID NO: 23. The term "pro-mysotatin" refers to the myostatin propeptide, with or without the signal sequence, which, in some embodiments of human pro-myostatin, has the sequence of SEQ ID NO: 21 (with signal sequence) or SEQ ID NO: 22 (without signal sequence). Nonlimiting exemplary mouse pro-myostatin has the sequence of SEQ ID NO: 24 (with signal sequence) or SEQ ID NO: 25 (without signal sequence).

[044] The term "**myostatin activity**" or "**biological activity**" of myostatin, as used herein, includes any biological effect of myostatin. In some embodiments, myostatin activity includes the ability of myostatin to activate SMAD2/3. A nonlimiting exemplary assay for determining myostatin activation of SMAD2/3 is described in Example 4.

[045] In an exemplary embodiment, a NOPE **extracellular domain (ECD)** reduces the amount of detectable binding of NOPE to myostatin by at least 50%. In some embodiments, a NOPE extracellular domain (ECD) polypeptide reduces the amount of detectable binding of NOPE to myostatin by at least 60%, at least 70%, at least 80%, or at least 90%.

[046] The terms "**inhibition**" or "**inhibit**" refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. In some embodiments, by "reduce" or "inhibit" is meant the ability to cause a decrease of 20% or greater. In another embodiment, by "reduce" or "inhibit" is meant the ability to cause a decrease of 50% or greater. In yet another embodiment, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater.

[047] In some embodiments, a NOPE extracellular domain (ECD) polypeptide is considered to "**inhibit myostatin-mediated signaling**" when it reduces SMAD2/3 activation with an IC50 of less than 10 nM using, e.g., the assay described in Example 4 herein. In some embodiments, A NOPE extracellular domain (ECD) polypeptide reduces SMAD2/3 activation with an IC50 of less than 5 nM, or less than 3 nM, or less than 2 nM, or less than 1 nM using e.g., the assay described in Example 4 herein.

[048] The term "**signal peptide**" refers to a sequence of amino acid residues located at the N-terminus of a polypeptide that facilitates secretion of a polypeptide from a

mammalian cell. A signal peptide may be cleaved upon export of the polypeptide from the mammalian cell, forming a mature protein. Signal peptides may be natural or synthetic, and they may be heterologous or homologous to the protein to which they are attached. Exemplary signal peptides include, but are not limited to, the signal peptides of NOPE and myostatin. Exemplary signal peptides also include signal peptides from heterologous proteins. A "**signal sequence**" refers to a polynucleotide sequence that encodes a signal peptide. In some embodiments, a NOPE ECD polypeptide or NOPE ECD fusion molecule lacks a signal peptide. In some embodiments, a NOPE ECD polypeptide or NOPE ECD fusion molecule includes at least one signal peptide, which may be a native NOPE signal peptide or a heterologous signal peptide.

[049] The term "**vector**" is used to describe a polynucleotide that may be engineered to contain a cloned polynucleotide or polynucleotides that may be propagated in a host cell. A vector may include one or more of the following elements: an origin of replication, one or more regulatory sequences (such as, for example, promoters and/or enhancers) that regulate the expression of the polypeptide of interest, and/or one or more selectable marker genes (such as, for example, antibiotic resistance genes and genes that may be used in colorimetric assays, *e.g.*, β-galactosidase). The term "**expression vector**" refers to a vector that is used to express a polypeptide of interest in a host cell.

[050] A "**host cell**" refers to a cell that may be or has been a recipient of a vector or isolated polynucleotide. Host cells may be prokaryotic cells or eukaryotic cells. Exemplary eukaryotic cells include mammalian cells, such as primate or non-primate animal cells; fungal cells, such as yeast; plant cells; and insect cells. Nonlimiting exemplary mammalian cells include, but are not limited to, NSO cells, PER.C6® cells (Crucell), and 293 and CHO cells, and their derivatives, such as 293-6E and DG44 cells, respectively.

[051] The term "**isolated**" as used herein refers to a molecule that has been separated from at least some of the components with which it is typically found in nature or has been separated from at least some of the components with which it is typically produced. For example, a polypeptide is referred to as "isolated" when it is separated from at least some of the components of the cell in which it was produced. Where a polypeptide is secreted by a cell after expression, physically separating the supernatant containing the polypeptide from the cell that produced it is considered to be "isolating" the polypeptide. Similarly, a polynucleotide is referred to as "isolated" when it is not part of the larger polynucleotide (such as, for example, genomic DNA or mitochondrial DNA, in the case of a

DNA polynucleotide) in which it is typically found in nature, or is separated from at least some of the components of the cell in which it was produced, *e.g.*, in the case of an RNA polynucleotide. Thus, a DNA polynucleotide that is contained in a vector inside a host cell may be referred to as "isolated" so long as that polynucleotide is not found in that vector in nature.

[052] The terms "**subject**" and "**patient**" are used interchangeably herein to refer to a human. In some embodiments, methods of treating other mammals, including, but not limited to, rodents, simians, felines, canines, equines, bovines, porcines, ovines, caprines, mammalian laboratory animals, mammalian farm animals, mammalian sport animals, and mammalian pets, are also provided. In some instances, a "subject" or "patient" refers to a subject or patient in need of treatment for a disease or disorder.

[053] The term "**sample**" or "**patient sample**" as used herein, refers to material that is obtained or derived from a subject of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase "**disease sample**" and variations thereof refers to any sample obtained from a subject of interest that would be expected or is known to contain the cellular and/or molecular entity that is to be characterized. By "**tissue or cell sample**" is meant a collection of similar cells obtained from a tissue of a subject or patient. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate (including, for example, bronchoalveolar lavage fluid and induced sputum); blood or any blood constituents; bodily fluids such as sputum, cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue or cell sample is obtained from a disease tissue/organ. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

[054] A "**reference sample**", "**reference cell**", or "**reference tissue**", as used herein, refers to a sample, cell or tissue obtained from a source known, or believed, not to be afflicted with the disease or condition for which a method or composition of the invention is being used to identify. In one embodiment, a reference sample, reference cell or reference tissue is obtained from a healthy part of the body of the same subject or patient in whom a disease or condition is being identified using a composition or method of the invention. In one embodiment, a reference sample, reference cell or reference tissue is obtained from a

healthy part of the body of at least one individual who is not the subject or patient in whom a disease or condition is being identified using a composition or method of the invention. In some embodiments, a reference sample, reference cell or reference tissue was previously obtained from a patient prior to developing a disease or condition or at an earlier stage of the disease or condition.

[055] A condition "**has previously been characterized as having [a characteristic]**" when such characteristic of the condition has been shown in at least a subset of patients with the condition, or in one or more animal models of the condition. In some embodiments, such characteristic of the condition does not have to be determined in the patient to be treated with a NOPE extracellular domain (ECD) polypeptide. The presence of the characteristic in a specific patient who is to be treated using the present methods and/or compositions need not have been determined in order for the patient to be considered as having a condition that has previously been characterized as having the characteristic.

[056] A "**disorder**" or "**disease**" is any condition that would benefit from treatment with a NOPE extracellular domain (ECD) polypeptide of the invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Nonlimiting examples of disorders to be treated herein include cancers, autoimmune diseases, and neurodegenerative diseases.

[057] "**Muscle wasting**" as used herein refers to a decrease in the mass of muscle, which, in some embodiments, accompanies a condition that directly causes muscle wasting and/or results in restricted movement, which can lead to muscle wasting. Nonlimiting examplary conditions that may be associated with muscle wasting include chronic obstructive pulmonary disease (COPD), chronic kidney disease, end stage renal disease, chronic heart failure, cancer, critical illness myopathy, critical illness polyneuropathy, stroke, spinal cord injury, spinal muscular atrophy, multiple sclerosis, progressive multifocal leukoencephalopathy, encephalomyelitis, central pontine myelolysis, adrenoleukodystrophy, Wallerian degeneration, Huntington's disease, Parkinson's disease, traumatic brain injury, Alexander's disease, Pelizaeus Merzbacher disease, globoid cell leucodystrophy, and sarcopenia.

[058] "**Muscle degenerative disorder**" as used herein refers to a disorder that impairs the functioning of muscles either directly (e.g., a pathology affecting muscle) or indirectly (e.g., a pathology affecting nerves or neuromuscular junctions). Nonlimiting

exemplary muscle degenerative disorders include muscular dystrophy, amyotrophic lateral sclerosis, myotonic dystrophy, polymyositis, and dermatomyositis.

[059] "**Treatment**," as used herein, covers any administration or application of a therapeutic for a disease (also referred to herein as a "disorder" or a "condition") in a mammal, including a human, and includes inhibiting the disease or progression of the disease, inhibiting or slowing the disease or its progression, arresting its development, partially or fully relieving the disease, partially or fully relieving one or more symptoms of a disease, or restoring or repairing a lost, missing, or defective function; or stimulating an inefficient process.

[060] The term "**effective amount**" or "**therapeutically effective amount**" refers to an amount of a drug effective to treat a disease or disorder in a subject. In some embodiments, an effective amount refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A therapeutically effective amount of an NOPE extracellular domain (ECD) polypeptide of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the extracellular domain (ECD) polypeptide to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of a NOPE extracellular domain (ECD) polypeptide are outweighed by the therapeutically beneficial effects.

[061] A "**prophylactically effective amount**" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount would be less than the therapeutically effective amount.

[062] A "**pharmaceutically acceptable carrier**" refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a "**pharmaceutical composition**" for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed. For example, if the therapeutic agent is to be administered orally, the carrier may be a gel capsule. If the therapeutic agent is to be administered subcutaneously, the carrier ideally is not irritable to the skin and does not cause injection site reaction.

[063] An "**article of manufacture**" is any manufacture (*e.g.*, a package or container) or kit comprising at least one reagent, *e.g.*, a medicament for treatment of a disease or disorder, or a probe for specifically detecting a biomarker described herein. In some embodiments, the manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

Therapeutic Compositions and Methods

Methods of Treating Diseases

[064] NOPE extracellular domain (ECD) polypeptides (including NOPE ECD fusion molecules) are provided for use in methods of treating humans and other mammals. Methods of treating a disease comprising administering NOPE extracellular domain (ECD) polypeptides to humans and other mammals are provided.

Methods of Treating Muscle Wasting

[065] The present inventors have found that NOPE ECD polypeptides and NOPE ECD fusion molecules increase muscle mass and enhance recovery following nerve crush injury *in vivo*. Further, the present inventors have demonstrated that NOPE binds myostatin *in vitro*, suggesting that NOPE ECD polypeptides and NOPE ECD fusion molecules may be acting as a myostatin ligand trap and inhibiting myostatin-mediated signaling *in vivo*.

[066] In some embodiments, methods of treating muscle wasting are provided, comprising administering an effective amount of a NOPE extracellular domain (ECD) polypeptide to a subject with muscle wasting or at risk of muscle wasting. In some embodiments, methods of treating muscle wasting are provided, comprising administering an effective amount of a NOPE ECD polypeptide or NOPE ECD fusion molecule to a subject with muscle wasting or at risk of muscle wasting. In some embodiments, the subject has a condition that causes muscle wasting. In some embodiments, the subject has a condition that leads to inactivity, resulting in muscle wasting. Nonlimiting exemplary conditions associated with muscle wasting include, but are not limited to, chronic obstructive pulmonary disease (COPD), chronic kidney disease, end stage renal disease, chronic heart failure, cancer, critical illness myopathy, critical illness polyneuropathy, stroke, spinal cord injury, spinal muscular atrophy, multiple sclerosis, progressive multifocal leukoencephalopathy, encephalomyelitis, central pontine myelolysis, adrenoleukodystrophy, Wallerian degeneration, Huntington's disease, Parkinson's disease, traumatic brain injury, Alexander's disease, Pelizaeus Merzbacher disease, globoid cell leucodystrophy, and sarcopenia.

[067] In some embodiments, methods of treating muscular injury are provided, comprising administering an effective amount of a NOPE extracellular domain (ECD) polypeptide to a subject with a muscle injury. In some embodiments, methods of treating muscular injury are provided, comprising administering an effective amount of a NOPE ECD polypeptide or NOPE ECD fusion molecule to a subject with a muscle injury. Many circumstances and events may lead to muscle injury, including, but not limited to, surgery-related muscle injury, traumatic muscle injury, work-related skeletal muscle injury, and overtraining-related muscle injury.

[068] Non-limiting examples of surgery-related muscle injuries include muscle damage due to knee replacement, anterior cruciate ligament (ACL) repair, plastic surgery, hip replacement surgery, joint replacement surgery, tendon repair surgery, surgical repair of rotator cuff disease and injury, and amputation.

[069] Non-limiting examples of traumatic muscle injuries include battlefield muscle injuries, auto accident-related muscle injuries, and sports-related muscle injuries. Traumatic injury to the muscle can include lacerations, blunt force contusions, shrapnel wounds, muscle pulls or tears, burns, acute strains, chronic strains, weight or force stress injuries, repetitive stress injuries, avulsion muscle injury, and compartment syndrome.

[070] In some embodiments, the muscle injury is a traumatic muscle injury and the treatment method provides for administration of at least one high dose of a compound of the invention immediately after the traumatic injury (for example, within one day of the injury) followed by periodic administration of a low dose of a compound of the invention during the recovery period.

[071] Non-limiting examples of work-related muscle injuries include injuries caused by highly repetitive motions, forceful motions, awkward postures, prolonged and forceful mechanical coupling between the body and an object, and vibration. Overtraining-related muscle injuries include unrepaired or under-repaired muscle damage coincident with a lack of recovery or lack of an increase of physical work capacity. In an additional embodiment, the muscle injury is exercise or sports-induced muscle damage resulting including exercise-induced delayed onset muscle soreness (DOMS).

[072] In some embodiments, methods of treating a muscle degenerative disorder are provided, comprising administering an effective amount of a NOPE extracellular domain (ECD) polypeptide to a subject with a muscle degenerative disorder. In some embodiments, methods of treating a muscle degenerative disorder are provided, comprising administering an effective amount of a NOPE ECD polypeptide or NOPE ECD fusion

molecule to a subject with a muscle degenerative disorder. In some embodiments, the subject with the muscle degenerative disorder has experienced muscle wasting. In some embodiments, the subject with the muscle degenerative disorder has been diagnosed with the disorder, but has not yet experienced significant or detectable muscle wasting. Nonlimiting exemplary muscle degenerative disorders include, but are not limited to, muscular dystrophy, myotonic dystrophy, polymyositis, and dermatomyositis. Nonlimiting exemplary muscular dystrophies include, but are not limited to, Duchenne muscular dystrophy, Becker muscular dystrophy, congenital muscular dystrophy (Fukuyama), Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy,, and fascioscapulohumeral muscular dystrophy. Nonlimiting exemplary myotonic dystrophies include, but are not limited to, myotonic dystrophy type I, myotonic dystrophy type II, and congenical myotonia.

[073] In some embodiments, treating muscle wasting, muscular injury, or a muscle degenerative disorder comprises at least one of: delaying progression of muscle wasting, improving the subject's 6 minute walking distance (6MWD), reducing physical decline, delaying occurrence of life-changing events, improving independence, reducing hospitalizations, and delaying the need for an assisted living arrangement. In some embodiments, improving the subject's 6 minute walking distance (6MWD) means that the subject's 6MWD has increased by at least 10 meters, or at least 20 meters, or at least 30 meters after 12 months of treatment. In another embodiment, preservation of a subject's 6MWD would indicate good efficacy in some disorders (e.g., Duchenne muscular dystrophy). Nonlimiting exemplary life-changing events include being placed on a ventilator and/or needing a wheelchair.

[074] In some embodiments, methods of treating amyotrophic lateral sclerosis (ALS) are provided, comprising administering an effective amount of a NOPE extracellular domain (ECD) polypeptide to a subject with ALS. In some embodiments, methods of treating a muscle degenerative disorder are provided, comprising administering an effective amount of a NOPE ECD polypeptide or NOPE ECD fusion molecule to a subject with ALS. A method is considered to treat ALS, in some embodiments, when it results in at least one of: delaying progression of ALS, reducing physical decline, improving forced vital capacity, slowing the decline in forced vital capacity, slowing the decline in the subject's score on the ALS functional rating scale (ALSFRS), improving the subject's score on the ALSFRS, delaying occurrence of life-changing events, and improving the subject's time of survival.

In some embodiments, a method is considered to treat ALS when it results in delaying a tracheostomy and/or delaying placement of a percutaneous endoscopic gastrostomy (PEG).

Routes of Administration and Carriers

[075] In various embodiments, NOPE agonists may be administered subcutaneously or intravenously. In some embodiments, NOPE extracellular domain (ECD) polypeptide may be administered *in vivo* by various routes, including, but not limited to, oral, intra-arterial, parenteral, intranasal, intramuscular, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, by inhalation, intradermal, topical, transdermal, and intrathecal, or otherwise, *e.g.*, by implantation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. In some embodiments, a NOPE extracellular domain (ECD) polypeptide is delivered using gene therapy. As a nonlimiting example, a nucleic acid molecule encoding a NOPE extracellular domain (ECD) polypeptide may be coated onto gold microparticles and delivered intradermally by a particle bombardment device, or "gene gun," *e.g.*, as described in the literature (*see, e.g.*, Tang *et al.*, *Nature* 356:152-154 (1992)).

[076] In various embodiments, compositions comprising a NOPE extracellular domain (ECD) polypeptide (such as NOPE ECD fusion molecule) is provided in formulations with a wide variety of pharmaceutically acceptable carriers (*see, e.g.*, Gennaro, *Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus*, 20th ed. (2003); Ansel *et al.*, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 7th ed., Lippencott Williams and Wilkins (2004); Kibbe *et al.*, *Handbook of Pharmaceutical Excipients,* 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are available. Moreover, various pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Nonlimiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

[077] In various embodiments, compositions comprising a NOPE extracellular domain (ECD) polypeptide may be formulated for injection, including subcutaneous administration, by dissolving, suspending, or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids, or propylene glycol; and if desired, with conventional

additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives. In various embodiments, the compositions may be formulated for inhalation, for example, using pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen, and the like. The compositions may also be formulated, in various embodiments, into sustained release microcapsules, such as with biodegradable or non-biodegradable polymers. A nonlimiting exemplary biodegradable formulation includes poly lactic acid-glycolic acid polymer. A nonlimiting exemplary non-biodegradable formulation includes a polyglycerin fatty acid ester. Certain methods of making such formulations are described, for example, in EP 1 125 584 A1.

[078] Pharmaceutical dosage packs comprising one or more containers, each containing one or more doses of a NOPE extracellular domain (ECD) polypeptide, are also provided. In some embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising a NOPE extracellular domain (ECD) polypeptide, with or without one or more additional agents. In some embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In various embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in some embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In some embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. In some embodiments, a composition of the invention comprises heparin and/or a proteoglycan.

[079] Pharmaceutical compositions are administered in an amount effective for treatment or prophylaxis of the specific indication. The therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical or health condition, the extensiveness of the condition to be treated, or the age of the subject being treated. In some embodiments, a NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 50 μg/kg body weight to about 50 mg/kg body weight per dose. In some embodiments, a NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 100 μg/kg body weight to about 50 mg/kg body weight per dose. In some embodiments, a NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 100 μg/kg body weight to about 20 mg/kg body weight per dose. In some embodiments, a

NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose.

[080] In some embodiments, a NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 10 mg to about 1,000 mg per dose. In some embodiments, a NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 20 mg to about 500 mg per dose. In some embodiments, a NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 20 mg to about 300 mg per dose. In some embodiments, a NOPE extracellular domain (ECD) polypeptide may be administered in an amount in the range of about 20 mg to about 200 mg per dose.

[081] The NOPE extracellular domain (ECD) polypeptide compositions may be administered as needed to subjects. In some embodiments, an effective dose of a NOPE extracellular domain (ECD) polypeptide is administered to a subject one or more times. In various embodiments, an effective dose of a NOPE extracellular domain (ECD) polypeptide is administered to the subject once a month, less than once a month, such as, for example, every two months, every three months, or every six months. In other embodiments, an effective dose of a NOPE extracellular domain (ECD) polypeptide is administered more than once a month, such as, for example, every two weeks, every week, twice per week, three times per week, daily, or multiple times per day. An effective dose of a NOPE extracellular domain (ECD) polypeptide is administered to the subject at least once. In some embodiments, the effective dose of a NOPE extracellular domain (ECD) polypeptide may be administered multiple times, including for periods of at least a month, at least six months, or at least a year. In some embodiments, a NOPE extracellular domain (ECD) polypeptide is administered to a subject as-needed to alleviate one or more symptoms of a condition.

Combination Therapy

[082] A NOPE extracellular domain (ECD) polypeptide according to the invention, including any functional variants thereof, may be administered to a subject in need thereof in combination with other biologically active substances or other treatment procedures for the treatment of diseases. For example, NOPE extracellular domain (ECD) polypeptide may be administered alone or with other modes of treatment. They may be provided before, substantially contemporaneous with, or after other modes of treatment.

[083] In some embodiments, a NOPE extracellular domain (ECD) polypeptide is administered to a subject before, during, or after another mode of treatment, such as exercise and/or physical therapy, or surgery.

[084] For treatment of certain muscular dystrophies, such as Duchenne muscular dystrophy, a method of treatment with a NOPE extracellular domain (ECD) polypeptide may further comprise administering a corticosteroid. For treatment of certain myotonic dystrophies, a method of treatment with a NOPE extracellular domain (ECD) polypeptide may further comprise administering a therapeutic agent selected from phenytoin, procainamide, and quinine. For treatment of amyotrophic lateral sclerosis (ALS), a method of treatment with a NOPE extracellular domain (ECD) polypeptide may further comprise administering riluzole.

NOPE Extracellular Domains (ECDs)

[085] Nonlimiting exemplary NOPE ECDs include full-length NOPE ECDs, and NOPE ECD variants. NOPE ECDs bind to myostatin. In some embodiments, an NOPE ECD inhibits myostatin-mediated activation of SMAD2/3. NOPE ECDs may include or lack a signal peptide. Exemplary NOPE ECDs include, but are not limited to, human NOPE ECDs having amino acid sequences selected from SEQ ID NOs: 5 (with signal peptide) and 6 (without signal peptide), and SEQ ID NO: 8. .

[086] NOPE ECD variants include variants comprising one or more amino acid additions, deletions, and/or substitutions, and that remain capable of binding myostatin. In some embodiments, an NOPE ECD variant sequence is at least 80%, 85%, 90%, 92%, 95%, 97%, 98%, or 99% identical to the corresponding sequence of the parent NOPE ECD.

Fusion Partners and Conjugates

[087] In some embodiments, a NOPE ECD of the present invention may be combined with a fusion partner polypeptide, resulting in a fusion protein. These fusion partner polypeptides may facilitate purification, and may show an increased half-life *in vivo*. Fusion partner polypeptides that have a disulfide-linked dimeric structure due to the IgG portion may also be more efficient in binding and neutralizing other molecules than the monomeric NOPE ECD fusion protein or the NOPE ECD alone. Other suitable fusion partners for NOPE ECDs include, for example, polymers, such as water soluble polymers, the constant domain of immunoglobulins; all or part of human serum albumin (HSA); fetuin A; fetuin B; a leucine zipper domain; a tetranectin trimerization domain; mannose binding protein (also known as mannose binding lectin), for example, mannose binding protein 1; and an Fc region, as described herein and further described in U.S. Patent No. 6,686,179.

[088] A fusion molecule may be prepared by attaching polyaminoacids or branch point amino acids to the NOPE ECD. For example, the polyaminoacid may be a carrier protein that serves to increase the circulation half-life of the NOPE ECD (in addition to the advantages achieved via a fusion molecule). For the therapeutic purpose of the present invention, such polyaminoacids should ideally be those that do not create neutralizing antigenic response, or other adverse responses. Such polyaminoacids may be chosen from serum album (such as HSA), fetuin A, fetuin B, leucine zipper nuclear factor erythroid derivative-2 (NFE2), neuroretinal leucine zipper, tetranectin, or other polyaminoacids, for example, lysines. As described herein, the location of attachment of the polyaminoacid may be at the N-terminus or C-terminus, or other places in between, and also may be connected by a chemical linker moiety to the selected molecule.

Polymers

[089] Polymers, for example, water soluble polymers, may be useful in the present invention to reduce precipitation of the NOPE ECD to which the polymer is attached in an aqueous environment, such as typically found in a physiological environment. Polymers employed in the invention will be pharmaceutically acceptable for the preparation of a therapeutic product or composition.

[090] Suitable, clinically acceptable, water soluble polymers include, but are not limited to, polyethylene glycol (PEG), polyethylene glycol propionaldehyde, copolymers of ethylene glycol/propylene glycol, monomethoxy-polyethylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol (PVA), polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, poly (β-amino acids) (either homopolymers or random copolymers), poly(n-vinyl pyrrolidone) polyethylene glycol, polypropylene glycol homopolymers (PPG) and other polyakylene oxides, polypropylene oxide/ethylene oxide copolymers, polyoxyethylated polyols (POG) (*e.g.*, glycerol) and other polyoxyethylated polyols, polyoxyethylated sorbitol, or polyoxyethylated glucose, colonic acids or other carbohydrate polymers, Ficoll, or dextran and mixtures thereof.

[091] Polymers used herein, for example water soluble polymers, may be of any molecular weight and may be branched or unbranched. In some embodiments, the polymers have an average molecular weight of between 2 kDa and 100 kDa, between 5 kDa and 50 kDa, or between 12 kDa and 25 kDa. Generally, the higher the molecular weight or the more branches, the higher the polymer:protein ratio. Other sizes may also be used, depending on the desired therapeutic profile; for example, the duration of sustained release;

the effects, if any, on biological activity; the ease in handling; the degree or lack of antigenicity; and other known effects of a polymer on a NOPE ECD of the invention.

[092] In some embodiments, the present invention contemplates the chemically derivatized NOPE ECD to include mono- or poly- (*e.g.*, 2-4) PEG moieties. Pegylation may be carried out by any of the pegylation reactions available. There are a number of PEG attachment methods available to those skilled in the art. *See*, for example, EP 0 401 384; Malik *et al.*, *Exp. Hematol.*, 20:1028-1035 (1992); Francis, *Focus on Growth Factors*, 3(2):4-10 (1992); EP 0 154 316; EP 0 401 384; WO 92/16221; WO 95/34326; Chamow, *Bioconjugate Chem.*, 5:133-140 (1994); U.S. Pat. No. 5,252,714; and the other publications cited herein that relate to pegylation.

Markers

[093] NOPE ECDs of the present invention may be fused to marker sequences, such as a peptide that facilitates purification of the fused polypeptide. The marker amino acid sequence may be a hexa-histidine peptide such as the tag provided in a pQE vector (Qiagen, Mississauga, Ontario, Canada), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci.* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the hemagglutinin (HA) tag, corresponds to an epitope derived from the influenza HA protein. (Wilson *et al.*, *Cell* 37:767 (1984)). Any of these above fusions may be engineered using the NOPE ECD of the present invention.

Oligomerization Domain Fusion Partners

[094] In various embodiments, oligomerization offers some functional advantages to a fusion protein, including, but not limited to, multivalency, increased binding strength, and the combined function of different domains. Accordingly, in some embodiments, a fusion partner comprises an oligomerization domain, for example, a dimerization domain. Exemplary oligomerization domains include, but are not limited to, coiled-coil domains, including alpha-helical coiled-coil domains; collagen domains; collagen-like domains; and certain immunoglobulin domains. Exemplary coiled-coil polypeptide fusion partners include, but are not limited to, the tetranectin coiled-coil domain; the coiled-coil domain of cartilage oligomeric matrix protein; angiopoietin coiled-coil domains; and leucine zipper domains. Exemplary collagen or collagen-like oligomerization domains include, but are not limited to, those found in collagens, mannose binding lectin, lung surfactant proteins A and D, adiponectin, ficolin, conglutinin, macrophage scavenger receptor, and emilin.

Antibody Fc Immunoglobulin Domain Fusion Partners

[095] Many Fc domains that may be used as fusion partners are known in the art. In some embodiments, a fusion partner is an Fc immunoglobulin domain. An Fc fusion partner may be a wild-type Fc found in a naturally occurring antibody, or a variant thereof. Nonlimiting exemplary Fc fusion partners include Fcs comprising a hinge and the CH2 and CH3 constant domains of a human IgG, for example, human IgG1, IgG2, IgG3, or IgG4. In some embodiments, an Fc fusion partner comprises a C237S mutation, for example, in an IgG1 constant region. *See, e.g.*, SEQ ID NO: 17. In some embodiments, an Fc fusion partner is a human IgG4 constant region. In some such embodiments, the human IgG4 constant region comprises an S241P mutation. *See, e.g.*, Angal *et al. Mol. Immunol.* 30(1): 105-108 (1993). In some embodiments, an Fc fusion partner comprises a hinge, CH2, and CH3 domains of human IgG2 with a P331S mutation, as described in U.S. Patent No. 6,900,292. Additional exemplary Fc fusion partners also include, but are not limited to, human IgA and IgM. Certain exemplary Fc domain fusion partners are shown in SEQ ID NOs: 26 to 28.

[096] In some embodiments, effector function is not desirable. For example, in some embodiments, effector function may not be desirable in treatments of inflammatory conditions and/or autoimmune disorders. In some such embodiments, a human IgG4 or IgG2 heavy chain constant region is selected or engineered. In some embodiments, an IgG4 constant region comprises an S241P mutation.

Albumin Fusion Partners and Albumin-Binding Molecule Fusion Partners

[097] In some embodiments, a fusion partner is an albumin. Exemplary albumins include, but are not limited to, human serum album (HSA) and fragments of HSA that are capable of increasing the serum half-life or bioavailability of the polypeptide to which they are fused. In some embodiments, a fusion partner is an albumin-binding molecule, such as, for example, a peptide that binds albumin or a molecule that conjugates with a lipid or other molecule that binds albumin. In some embodiments, a fusion molecule comprising HSA is prepared as described, *e.g.*, in U.S. Patent No. 6,686,179.

Exemplary Attachment of Fusion Partners

[098] The fusion partner may be attached, either covalently or non-covalently, to the N-terminus or the C-terminus of the NOPE ECD. The attachment may also occur at a location within the NOPE ECD other than the N-terminus or the C-terminus, for example, through an amino acid side chain (such as, for example, the side chain of cysteine, lysine, serine, or threonine).

[099] In either covalent or non-covalent attachment embodiments, a linker may be included between the fusion partner and the NOPE ECD. Such linkers may be comprised of at least one amino acid or chemical moiety. Exemplary methods of covalently attaching a fusion partner to a NOPE ECD include, but are not limited to, translation of the fusion partner and the NOPE ECD as a single amino acid sequence and chemical attachment of the fusion partner to the NOPE ECD. When the fusion partner and NOPE ECD are translated as single amino acid sequence, additional amino acids may be included between the fusion partner and the NOPE ECD as a linker. In some embodiments, the linker is selected based on the polynucleotide sequence that encodes it, to facilitate cloning the fusion partner and/or NOPE ECD into a single expression construct (for example, a polynucleotide containing a particular restriction site may be placed between the polynucleotide encoding the fusion partner and the polynucleotide encoding the NOPE ECD, wherein the polynucleotide containing the restriction site encodes a short amino acid linker sequence). When the fusion partner and the NOPE ECD are covalently coupled by chemical means, linkers of various sizes may typically be included during the coupling reaction.

[0100] Exemplary methods of non-covalently attaching a fusion partner to a NOPE ECD include, but are not limited to, attachment through a binding pair. Exemplary binding pairs include, but are not limited to, biotin and avidin or streptavidin, an antibody and its antigen, *etc.*

Exemplary Properties of NOPE ECDs and NOPE ECD Fusion Molecules

[0101] In some embodiments, a NOPE ECD or a NOPE ECD fusion molecule binds to myostatin, and inhibits myostatin-mediated SMAD2/3 activation. In some embodiments, NOPE ECD fusion molecule binds to myostatin with a binding affinity (K_D) of less than 50 nM, less than 20 nM, less than 10 nM, less than 1 nM, or less than 0.1 nM. In some embodiments, a NOPE ECD fusion molecule blocks binding of myostatin to native NOPE.

Signal Peptides

[0102] In order for some secreted proteins to express and secrete in large quantities, a signal peptide from a heterologous protein may be desirable. Employing heterologous signal peptides may be advantageous in that a resulting mature polypeptide may remain unaltered as the signal peptide is removed in the ER during the secretion process. The addition of a heterologous signal peptide may be required to express and secrete some proteins.

[0103] Nonlimiting exemplary signal peptide sequences are described, *e.g.*, in the online Signal Peptide Database maintained by the Department of Biochemistry, National

University of Singapore. *See* Choo *et al.*, *BMC Bioinformatics*, 6: 249 (2005); and PCT Publication No. WO 2006/081430.

Co-Translational and Post-Translational Modifications

[0104] In some embodiments, a polypeptide such as a NOPE ECD, is differentially modified during or after translation, for example by glycosylation, sialylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or linkage to an antibody molecule or other cellular ligand. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease; $NABH_4$; acetylation; formylation; oxidation; reduction; and/or metabolic synthesis in the presence of tunicamycin.

[0105] Additional post-translational modifications encompassed by the invention include, for example, N-linked or O-linked carbohydrate chains; processing of N-terminal or C-terminal ends; attachment of chemical moieties to the amino acid backbone; chemical modifications of N-linked or O-linked carbohydrate chains; and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression.

Nucleic Acid Molecules Encoding NOPE extracellular domain (ECD) polypeptide

[0106] In some embodiments, nucleic acid molecules comprising polynucleotides that encode NOPE ECDs or NOPE ECD fusion molecules are provided. Nucleic acid molecules comprising polynucleotides that encode NOPE ECD fusion molecules in which the NOPE ECD and the fusion partner are translated as a single polypeptide are also provided.

[0107] In some embodiments, a polynucleotide encoding a NOPE ECD comprises a nucleotide sequence that encodes a signal peptide, which, when translated, will be fused to the N-terminus of the NOPE ECD. As discussed above, the signal peptide may be the native NOPE signal peptide, or may be another heterologous signal peptide. In some embodiments, the nucleic acid molecule comprising the polynucleotide encoding the gene of interest is an expression vector that is suitable for expression in a selected host cell.

[0108] Nucleic acid molecules may be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell.

Polypeptide Expression and Production Vectors

[0109] Vectors comprising polynucleotides that encode NOPE ECDs are provided. Vectors comprising polynucleotides that encode NOPE ECD fusion molecules are also provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, *etc*.

[0110] In some embodiments, a vector is selected that is optimized for expression of polypeptides in CHO or CHO-derived cells, or in NSO cells. Exemplary such vectors are described, *e.g*., in Running Deer *et al*., *Biotechnol. Prog.* 20:880-889 (2004).

[0111] In some embodiments, a vector is chosen for *in vivo* expression of a NOPE extracellular domain (ECD) polypeptide in animals, including humans. In some such embodiments, expression of the polypeptide or polypeptides is under the control of a promoter or promoters that function in a tissue-specific manner. For example, liver-specific promoters are described, *e.g*., in PCT Publication No. WO 2006/076288.

Host Cells

[0112] In various embodiments, NOPE ECDs and/or fusion molecules comprising any of those may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells, plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S and DG44 cells; PER.C6® cells (Crucell); and NSO cells. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to NOPE ECDs, and/or fusion molecules. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[0113] Introduction of one or more nucleic acids into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc.* Nonlimiting exemplary methods are described, *e.g*., in Sambrook *et al*., *Molecular Cloning, A Laboratory Manual,* 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[0114] In some embodiments, one or more polypeptides may be produced *in vivo* in an animal that has been engineered or transfected with one or more nucleic acid molecules encoding the polypeptides, according to any suitable method.

Purification of Polypeptides

[0115] NOPE ECDs, and fusion molecules comprising any of those may be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include any ligands that bind to NOPE (such as myostatin) or that bind to the fusion partner. Further, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind to an Fc fusion partner to purify a fusion molecule.

[0116] In some embodiments, hydrophobic interactive chromatography, for example, a butyl or phenyl column, is also used for purifying some polypeptides. Many methods of purifying polypeptides are known in the art.

Articles of Manufacture

[0117] In some embodiments, an article of manufacture or a kit containing materials useful for the detection of a biomarker (*e.g.*, NOPE or myostatin) or for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used for treating the condition of choice. In some embodiments, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a NOPE extracellular domain (ECD) polypeptide of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises an additional therapeutic agent. The article of manufacture may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include

other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0118] In some embodiments, the molecules of the present invention can be packaged alone or in combination with other therapeutic compounds as a kit. In one embodiment, the therapeutic compound is an anti-cancer agent. In another embodiment, the therapeutic compound is an immunosuppressive agent. The kit can include optional components that aid in the administration of the unit dose to patients, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, *etc.* Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. The kit may be manufactured as a single use unit dose for one patient, multiple uses for a particular patient (at a constant dose or in which the individual compounds may vary in potency as therapy progresses); or the kit may contain multiple doses suitable for administration to multiple patients ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

EXAMPLES

[0119] The examples discussed below are intended to be purely exemplary of the invention and should not be considered to limit the invention in any way. The examples are not intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (for example, amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1: Construction, expression and purification of certain NOPE-ECD fusion proteins

[0120] Constructs encoding human NOPE (Gene ID 57722) and mouse NOPE (Gene ID 56741) extracellular domain (ECD)-Fc fusion molecules were cloned into and expressed from vector pTT5 (Biotechnology Research Institute, Montreal, Canada). Truncated derivatives of the human or mouse NOPE ECD-Fc fusion molecule were constructed using PCR and conventional mutagenesis techniques. The primary protein sequence and domain structure of the human and mouse NOPE ECD-Fc moiety in the parental construct is shown in Figure 1. Truncated NOPE ECD-Fc derivatives were created

to define domains important for the function of NOPE. Figure 2 lists the various NOPE-Fc Fusion proteins used in these examples with names and brief descriptions.

[0121] For in vivo confirmation experiments (e.g., Example 2), mNOPE (23-956) ECD-Fc containing a human CD33 signal sequence in place of the native signal sequence was cloned into a Bacmam vector for secreted expression in CHO or HEK cells via Bacmam technology. The secreted protein was purified using MabSelectSure Protein A affinity followed by Superdex-200 size exclusion chromatograpy. For in vitro binding experiments (Example 3), production of NOPE-ECD-Fc and its' derivatives was achieved by PEI mediated transient transfection of CHO-3E7 cells grown in CD DG44 (Invitrogen, Grand Island, NY). The secreted proteins were purified over a HiTrap Protein A column (GE Healthcare, Pittsburg, PA).

Example 2: NOPE increases muscle mass *in vivo*

[0122] Experiments were carried out to determine whether hydrodynamic tail vein injection of a DNA vector encoding mNOPE (1-955) ECD-FC has an impact on muscle growth in vivo. Female BalbC mice (Charles River Labs, Wilmington, MA) were randomly assigned into 2 treatment groups of 10 mice each. On day 0, mice were subject to lean mass analysis (EchoMRI®, Houston, TX) to establish baseline measurements. Once baseline measurements were recorded, mice were hydrodynamically transfected with mNOPE (1-955) ECD-FC or Vehicle control. On day 7, 21, 27, lean mass was measured. mNOPE (1-955) ECD-FC exposure demonstrated anabolic effects in lean mass within 7 days of dosing (Figure 3A). The increase in lean mass persisted throughout the course of the study. After 27 days, lean mass organs were harvested and weighed to confirm that the increase in lean mass was muscle specific. The lean mass anabolism translated to a significant increase in skeletal muscle in animals exposed to mNOPE (1-955) ECD-FC, however, liver, a non-muscle lean organ, was not affected (Figure 3B).

[0123] We confirmed these findings with purified protein. mNOPE (23-956) ECD-FC was constructed, transiently expressed and purified as described in Example 1. Female BalbC mice (Charles River Labs, Wilmington, MA) were randomly assigned into 2 treatment groups of 10 mice each. On day 0, mice were subject to lean mass analysis as outlined above to establish baseline measurements. Once baseline measurements were recorded, mice were administered vehicle or 45 mg/kg purified recombinant mNOPE (23-956) ECD-FC protein twice per week. On day 6, 11, 14, and 17, lean mass was measured. NOPE exposure demonstrated an anabolic effect in lean mass within 6 days of the first dose

(Figure 3C). The increase in lean mass persisted throughout the course of the study. After 17 days, organs were harvested and weighed. The lean mass anabolism translated to a significant increase in skeletal muscle and heart weight in animals exposed to mNOPE (23-956) ECD-FC, while liver remained unchanged (Figure 3D). These results confirm our observations by hydrodynamic transfection that systemic exposure to NOPE leads to muscle anabolism in mice.

Example 3: NOPE binds myostatin *in vitro*

[0124] Biacore® T100 surface plasmon resonance (SPR) technology (GE, NY, USA) was used to determine the affinity of human and mouse NOPE-FC for Myostatin and Pro-Myostatin (R&D Systems, Minneapolis, MN). Myostatin was selected to determine if one mechanism of promoting muscle growth *in vivo* was through acting as myostatin ligand trap in circulation. hNOPE (1-957) ECD-FCand mNOPE (1-955) ECD-FC were constructed, transiently expressed and purified as described in example 1.

[0125] Briefly, an anti-human antibody (GE Lifesciences, NY, USA) was linked to a CM4 chip via amine coupling according to manufacturer's instructions. Both hNOPE (1-957) ECD-Fc and mNOPE (1-955) ECD-FC were captured on the chip by interaction with the anti-human antibody. A flowcell without NOPE ECD-FC served as the reference control. Myostatin and pro-myostatin were purchased from R&D Systems (MN, USA). A dose response of myostatin or pro-myostatin was injected then dissociation was monitored over time. The association constant, dissociation constant, and affinity of NOPE ECD-FC protein or Activin Receptor 2B-FC (R&D Systems, MN, USA), a positive control for myostatin binding, was calculated using the Biacore T100 Evaluation software package using the Langmuir 1:1 binding model. The affinity for human NOPE (1-957) ECD-FC and mouse NOPE (1-955) ECD-FC for MSTN are 0.2nM and 0.8nM, respectively while the affinity for the positive control, ActR2b-FC is 2.5nM. NOPE ECD-FC and ActR2b-FC do not bind to pro-MSTN. As shown in Figure 4, the NOPE proteins have similar affinities for myostatin and do not bind pro-myostatin.

Example 4: Structure-function studies identify the functional domains of NOPE

[0126] To understand the functional implication of the interaction between NOPE and myostatin we employed a cell-based reporter assay to test the ability of NOPE to inhibit myostatin signaling. In addition, we created truncation mutants to determine the important functional domains of NOPE as described in Example 1. Cignal Lenti Smad 2/3 Luciferase

(Qiagen) was transformed into HEK293 cells and used to test the ability of the NOPE proteins to inhibit myostatin dependent SMAD2/3 activation. A myostatin dose response curve was generated to determine the EC80 of SMAD2/3 activity. 0.1nM of myostatin, EC80, was challenged with a dose response of mNOPE (1-955) ECD-FC, hNOPE (1-957) ECD-FC, hNOPE (1-427) ECD-FC, hNOPE (1-620) ECD-FC, and hNOPE (427-955) ECD-FC. As demonstrated in Figure 5, the interaction with NOPE results in neutralization of myostatin activity. IC50 of hNOPE (1-957) ECD-FC & hNOPE (1-620) ECD-FC inhibition of myostatin are 0.5 and 0.17nM, respectively. mNOPE (1-955) ECD-FC has an IC50 of 0.068nM that is 7-fold greater than hNOPE (1-957) ECD-FC and 2.5-fold greater than hNOPE (1-620) ECD-FC. hNOPE (1-427) ECD-FC and hNOPE (427-955) ECD-FC did not inhibit myostatin induced Smad 2/3 activation (Figure 5).

[0127] Experiments were carried out to determine whether hydrodynamic tail vein injection the NOPE proteins has an impact on muscle growth *in vivo*. Female BalbC mice (Charles River Labs, Wilmington, MA) were randomly assigned into 4 treatment groups of 10 mice each (Figure 6). On day 0, mice were subject to lean mass analysis as described in example 2 to establish baseline measurements. Once baseline measurements were recorded, mice were hydrodynamically transfected with mNOPE (1-955) ECD-FC, mNOPE (1-619) ECD-FC or mNOPE (623-955) ECD-FC or Vehicle control. On day 7 and 14 lean mass was measured. mNOPE (1-955) ECD-FC as well as mNOPE (1-619) ECD-FC exposure demonstrated anabolic effects in lean mass within 7 days exposure. The increase in lean mass persisted throughout the course of the study. After 14 days, lean mass organs were harvested and weighed to confirm that the increase in lean mass was muscle specific. Lean mass anabolism translates to a significant increase in skeletal muscle in animals exposed to mNOPE (1-955) ECD-FC and mNOPE (1-619) ECD-FC while liver was not affected. mNOPE (623-955) ECD-FC had no effect on lean mass or muscle weight.

TABLE OF SEQUENCES

SEQ ID NO	Description	Sequence
1	Human NOPE precursor (1-1250), with signal peptide	MARGDAGRGR GLLALTFCLL AARGELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVREGLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT PSMHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHSVTGII VGVCLGLLCL LACMCAGLRR SPHRESLPGL SSTATPGNPA LYSRARLGPP SPPAAHELES LVHPHPQDWS PPPSDVEDRA EVHSLMGGGV SEGRSHSKRK ISWAQPSGLS WAGSWAGCEL PQAGPRPALT RALLPPAGTG QTLLLQALVY DAIKGNGRKK SPPACRNQVE AEVIVHSDFS ASNGNPDLHL QDLEPEDPLP PEAPDLISGV GDPGQGAAWL DRELGGCELA APGPDRLTCL PEAASASCSY PDLQPGEVLE ETPGDSCQLK SPCPLGASPG LPRSPVSSSA
2	Human mature NOPE (25-1250), without signal peptide	ELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVREGLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT PSMHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHSVTGII VGVCLGLLCL LACMCAGLRR SPHRESLPGL SSTATPGNPA LYSRARLGPP SPPAAHELES LVHPHPQDWS PPPSDVEDRA EVHSLMGGGV SEGRSHSKRK ISWAQPSGLS WAGSWAGCEL PQAGPRPALT RALLPPAGTG QTLLLQALVY DAIKGNGRKK SPPACRNQVE AEVIVHSDFS ASNGNPDLHL QDLEPEDPLP PEAPDLISGV GDPGQGAAWL

		DRELGGCELA APGPDRLTCL PEAASASCSY PDLQPGEVLE ETPGDSCQLK SPCPLGASPG LPRSPVSSSA
3	Mouse NOPE precursor (1-1252), with signal peptide	MARADTGRGL LVLTFCLLSA RGELPLPQET TVKLSCDEGP LQVILGPEQA VVLDCTLGAT AAGPPTRVTW SKDGDTVLEH ENLHLLPNGS LWLSSPLEQE DSDDEEALRI WKVTEGSYSC LAHSPLGVVA SQVAVVKLAT LEDFSLHPES QIVEENGTAR FECHTKGLPA PIITWEKDQV TVPEESRLIT LPNGVLQILD VQDSDAGSYR CVATNSARQR FSQEASLTVA LRGSLEATRG QDVVIVAAPE NTTVVSGQSV VMECVASADP TPFVSWVRQD GKPISTDVIV LGRTNLLIAS AQPRHSGVYV CRANKPRTRD FATAAAELRV LAAPAISQAP EALSRTRAST ARFVCRASGE PRPALHWLHD GIPLRPNGRV KVQGGGGSLV ITQIGLQDAG YYQCVAENSA GTACAAAPLA VVVREGLPSA PTRVTATPLS SSSVLVAWER PELHSEQIIG FSLHYQKARG VDNVEYQFAV NNDTTELQVR DLEPNTDYEF YVVAYSQLGA SRTSSPALVH TLDDVPSAAP QLTLSSPNPS DIRVAWLPLP SSLSNGQVLK YKIEYGLGKE DQVFSTEVPG NETQLTLNSL QPNKVYRVRI SAGTGAGYGV PSQWMQHRTP GVHNQSHVPF APAELKVRAK MESLVVSWQP PPHPTQISGY KLYWREVGTE EEADGDRPPG GRGDQAWDVG PVRLKKKVKQ YELTQLVPGR LYEVKLVAFN KHEDGYAAVW KGKTEKAPTP DLPIQRGPPL PPAHVHAESN SSTSIWLRWK KPDFTTVKIV NYTVRFGPWG LRNASLVTYY TSSGEDILIG GLKPFTKYEF AVQSHGVDMD GPFGSVVERS TLPDRPSTPP SDLRLSPLTP STVRLHWCPP TEPNGEIVEY LILYSNNHTQ PEHQWTLLTT EGNIFSAEVH GLESDTRYFF KMGARTEVGP GPFSRLQDVI TLQKTFSDSL DVHAVTGIIV GVCLGLLCLL ACMCAGLRRS SHREALPGLS SSGTPGNPAL YTRARLGPPS VPAAHELESL VHPRPQDWSP PPSDVEDKAE VHSLMGGSVS DCRGHSKRKI SWAQAGGPNW AGSWAGCELP QGSGPRPALT RALLPPAGTG QTLLLQALVY DAIKSNGRKK PSPACRNQVE AEVIVHSDFG ASKGCPDLHL QDLEPEEPLT AETLPSTSGA VDLSQGADWL GRELGGCQPT TSGPERLTCL PEAASASCSC SDLQPSTAIE EAPGKSCQPK ALCPLTVSPS LPRAPVSSAQ VP
4	Mouse mature NOPE (23-1252), without signal peptide	ELPLPQET TVKLSCDEGP LQVILGPEQA VVLDCTLGAT AAGPPTRVTW SKDGDTVLEH ENLHLLPNGS LWLSSPLEQE DSDDEEALRI WKVTEGSYSC LAHSPLGVVA SQVAVVKLAT LEDFSLHPES QIVEENGTAR FECHTKGLPA PIITWEKDQV TVPEESRLIT LPNGVLQILD VQDSDAGSYR CVATNSARQR FSQEASLTVA LRGSLEATRG QDVVIVAAPE NTTVVSGQSV VMECVASADP TPFVSWVRQD GKPISTDVIV LGRTNLLIAS AQPRHSGVYV CRANKPRTRD FATAAAELRV LAAPAISQAP EALSRTRAST ARFVCRASGE PRPALHWLHD GIPLRPNGRV KVQGGGGSLV ITQIGLQDAG YYQCVAENSA GTACAAAPLA VVVREGLPSA PTRVTATPLS SSSVLVAWER PELHSEQIIG FSLHYQKARG VDNVEYQFAV NNDTTELQVR DLEPNTDYEF YVVAYSQLGA SRTSSPALVH TLDDVPSAAP QLTLSSPNPS DIRVAWLPLP SSLSNGQVLK YKIEYGLGKE DQVFSTEVPG NETQLTLNSL QPNKVYRVRI SAGTGAGYGV PSQWMQHRTP GVHNQSHVPF APAELKVRAK MESLVVSWQP PPHPTQISGY KLYWREVGTE EEADGDRPPG GRGDQAWDVG PVRLKKKVKQ YELTQLVPGR LYEVKLVAFN KHEDGYAAVW KGKTEKAPTP DLPIQRGPPL PPAHVHAESN SSTSIWLRWK KPDFTTVKIV NYTVRFGPWG LRNASLVTYY TSSGEDILIG GLKPFTKYEF AVQSHGVDMD GPFGSVVERS TLPDRPSTPP SDLRLSPLTP STVRLHWCPP TEPNGEIVEY LILYSNNHTQ PEHQWTLLTT EGNIFSAEVH GLESDTRYFF KMGARTEVGP GPFSRLQDVI TLQKTFSDSL DVHAVTGIIV GVCLGLLCLL ACMCAGLRRS SHREALPGLS SSGTPGNPAL YTRARLGPPS VPAAHELESL VHPRPQDWSP PPSDVEDKAE VHSLMGGSVS DCRGHSKRKI SWAQAGGPNW AGSWAGCELP QGSGPRPALT RALLPPAGTG QTLLLQALVY DAIKSNGRKK PSPACRNQVE AEVIVHSDFG ASKGCPDLHL QDLEPEEPLT AETLPSTSGA VDLSQGADWL GRELGGCQPT TSGPERLTCL PEAASASCSC SDLQPSTAIE EAPGKSCQPK ALCPLTVSPS LPRAPVSSAQ VP

5	Human NOPE extracellular domain (ECD) (1-957), with signal peptide	MARGDAGRGR GLLALTFCLL AARGELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVREGLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT PSMHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHSVT
6	Human NOPE ECD (25-955), without signal peptide	ELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVREGLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT PSMHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHS
7	Human NOPE ECD (25-426), without signal peptide	ELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVRE
8	Human NOPE ECD (25-620), without signal peptide	ELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ

		HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVREGLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT
9	Human NOPE ECD (427-955)	GLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT PSMHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHS
10	Human NOPE ECD (623-955)	MHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHS
11	Mouse NOPE extracellular domain (ECD) (1-956), with signal peptide	MARADTGRGL LVLTFCLLSA RGELPLPQET TVKLSCDEGP LQVILGPEQA VVLDCTLGAT AAGPPTRVTW SKDGDTVLEH ENLHLLPNGS LWLSSPLEQE DSDDEEALRI WKVTEGSYSC LAHSPLGVVA SQVAVVKLAT LEDFSLHPES QIVEENGTAR FECHTKGLPA PIITWEKDQV TVPEESRLIT LPNGVLQILD VQDSDAGSYR CVATNSARQR FSQEASLTVA LRGSLEATRG QDVVIVAAPE NTTVVSGQSV VMECVASADP TPFVSWVRQD GKPISTDVIV LGRTNLLIAS AQPRHSGVYV CRANKPRTRD FATAAAELRV LAAPAISQAP EALSRTRAST ARFVCRASGE PRPALHWLHD GIPLRPNGRV KVQGGGGSLV ITQIGLQDAG YYQCVAENSA GTACAAAPLA VVVREGLPSA PTRVTATPLS SSSVLVAWER PELHSEQIIG FSLHYQKARG VDNVEYQFAV NNDTTELQVR DLEPNTDYEF YVVAYSQLGA SRTSSPALVH TLDDVPSAAP QLTLSSPNPS DIRVAWLPLP SSLSNGQVLK YKIEYGLGKE DQVFSTEVPG NETQLTLNSL QPNKVYRVRI SAGTGAGYGV PSQWMQHRTP GVHNQSHVPF APAELKVRAK MESLVVSWQP PPHPTQISGY KLYWREVGTE EEADGDRPPG GRGDQAWDVG PVRLKKKVKQ YELTQLVPGR LYEVKLVAFN KHEDGYAAVW KGKTEKAPTP DLPIQRGPPL PPAHVHAESN SSTSIWLRWK KPDFTTVKIV NYTVRFGPWG LRNASLVTYY TSSGEDILIG GLKPFTKYEF AVQSHGVDMD GPFGSVVERS TLPDRPSTPP SDLRLSPLTP STVRLHWCPP TEPNGEIVEY LILYSNNHTQ PEHQWTLLTT EGNIFSAEVH GLESDTRYFF KMGARTEVGP GPFSRLQDVI TLQKTFSDSL DVHAVT
12	Mouse NOPE ECD (23-956), without signal peptide	ELPLPQET TVKLSCDEGP LQVILGPEQA VVLDCTLGAT AAGPPTRVTW SKDGDTVLEH ENLHLLPNGS LWLSSPLEQE DSDDEEALRI WKVTEGSYSC LAHSPLGVVA SQVAVVKLAT LEDFSLHPES QIVEENGTAR FECHTKGLPA PIITWEKDQV TVPEESRLIT LPNGVLQILD VQDSDAGSYR CVATNSARQR FSQEASLTVA LRGSLEATRG QDVVIVAAPE NTTVVSGQSV VMECVASADP TPFVSWVRQD GKPISTDVIV LGRTNLLIAS

		AQPRHSGVYV CRANKPRTRD FATAAAELRV LAAPAISQAP EALSRTRAST ARFVCRASGE PRPALHWLHD GIPLRPNGRV KVQGGGGSLV ITQIGLQDAG YYQCVAENSA GTACAAAPLA VVVREGLPSA PTRVTATPLS SSSVLVAWER PELHSEQIIG FSLHYQKARG VDNVEYQFAV NNDTTELQVR DLEPNTDYEF YVVAYSQLGA SRTSSPALVH TLDDVPSAAP QLTLSSPNPS DIRVAWLPLP SSLSNGQVLK YKIEYGLGKE DQVFSTEVPG NETQLTLNSL QPNKVYRVRI SAGTGAGYGV PSQWMQHRTP GVHNQSHVPF APAELKVRAK MESLVVSWQP PPHPTQISGY KLYWREVGTE EEADGDRPPG GRGDQAWDVG PVRLKKKVKQ YELTQLVPGR LYEVKLVAFN KHEDGYAAVW KGKTEKAPTP DLPIQRGPPL PPAHVHAESN SSTSIWLRWK KPDFTTVKIV NYTVRFGPWG LRNASLVTYY TSSGEDILIG GLKPFTKYEF AVQSHGVDMD GPFGSVVERS TLPDRPSTPP SDLRLSPLTP STVRLHWCPP TEPNGEIVEY LILYSNNHTQ PEHQWTLLTT EGNIFSAEVH GLESDTRYFF KMGARTEVGP GPFSRLQDVI TLQKTFSDSL DVHAVT
13	Mouse NOPE ECD (23-619), without signal peptide	ELPLPQET TVKLSCDEGP LQVILGPEQA VVLDCTLGAT AAGPPTRVTW SKDGDTVLEH ENLHLLPNGS LWLSSPLEQE DSDDEEALRI WKVTEGSYSC LAHSPLGVVA SQVAVVKLAT LEDFSLHPES QIVEENGTAR FECHTKGLPA PIITWEKDQV TVPEESRLIT LPNGVLQILD VQDSDAGSYR CVATNSARQR FSQEASLTVA LRGSLEATRG QDVVIVAAPE NTTVVSGQSV VMECVASADP TPFVSWVRQD GKPISTDVIV LGRTNLLIAS AQPRHSGVYV CRANKPRTRD FATAAAELRV LAAPAISQAP EALSRTRAST ARFVCRASGE PRPALHWLHD GIPLRPNGRV KVQGGGGSLV ITQIGLQDAG YYQCVAENSA GTACAAAPLA VVVREGLPSA PTRVTATPLS SSSVLVAWER PELHSEQIIG FSLHYQKARG VDNVEYQFAV NNDTTELQVR DLEPNTDYEF YVVAYSQLGA SRTSSPALVH TLDDVPSAAP QLTLSSPNPS DIRVAWLPLP SSLSNGQVLK YKIEYGLGKE DQVFSTEVPG NETQLTLNSL QPNKVYRVRI SAGTGAGYGV PSQWMQHRT
14	Mouse NOPE ECD (623-956), without signal peptide	HNQSHVPF APAELKVRAK MESLVVSWQP PPHPTQISGY KLYWREVGTE EEADGDRPPG GRGDQAWDVG PVRLKKKVKQ YELTQLVPGR LYEVKLVAFN KHEDGYAAVW KGKTEKAPTP DLPIQRGPPL PPAHVHAESN SSTSIWLRWK KPDFTTVKIV NYTVRFGPWG LRNASLVTYY TSSGEDILIG GLKPFTKYEF AVQSHGVDMD GPFGSVVERS TLPDRPSTPP SDLRLSPLTP STVRLHWCPP TEPNGEIVEY LILYSNNHTQ PEHQWTLLTT EGNIFSAEVH GLESDTRYFF KMGARTEVGP GPFSRLQDVI TLQKTFSDSL DVHAVT
15	Human NOPE ECD (25-955)-Fc fusion molecule, without signal peptide	ELLLPQETTV ELSCGVGPLQ VILGPEQAAV LNCSLGAAAA GPPTRVTWSK DGDTLLEHDH LHLLPNGSLW LSQPLAPNGS DESVPEAVGV IEGNYSCLAH GPLGVLASQT AVVKLATLAD FSLHPESQTV EENGTARFEC HIEGLPAPII TWEKDQVTLP EEPRLIVLPN GVLQILDVQE SDAGPYRCVA TNSARQHFSQ EALLSVAHRG SLASTRGQDV VIVAAPENTT VVSGQSVVME CVASADPTPF VSWVRQDGKP ISTDVIVLGR TNLLIANAQP WHSGVYVCRA NKPRTRDFAT AAAELRVLAA PAITQAPEAL SRTRASTARF VCRASGEPRP ALRWLHNGAP LRPNGRVKVQ GGGGSLVITQ IGLQDAGYYQ CVAENSAGMA CAAASLAVVV REGLPSAPTR VTATPLSSSA VLVAWERPEM HSEQIIGFSL HYQKARGMDN VEYQFAVNND TTELQVRDLE PNTDYEFYVV AYSQLGASRT STPALVHTLD DVPSAAPQLS LSSPNPSDIR VAWLPLPPSL SNGQVVKYKI EYGLGKEDQI FSTEVRGNET QLMLNSLQPN KVYRVRISAG TAAGFGAPSQ WMHHRTPSMH NQSHVPFAPA ELKVQAKMES LVVSWQPPPH PTQISGYKLY WREVGAEEEA NGDRLPGGRG DQAWDVGPVR LKKKVKQYEL TQLVPGRLYE VKLVAFNKHE DGYAAVWKGK TEKAPAPDMP IQRGPPLPPA HVHAESNSST SIWLRWKKPD FTTVKIVNYT VRFSPWGLRN ASLVTYYTSS GEDILIGGLK PFTKYEFAVQ SHGVDMDGPF GSVVERSTLP DRPSTPPSDL RLSPLTPSTV RLHWCPPTEP NGEIVEYLIL YSSNHTQPEH QWTLLTTQGN IFSAEVHGLE SDTRYFFKMG ARTEVGPGPF SRLQDVITLQ EKLSDSLDMH S**GS**EPKSSDK THTCPPCPAP ELLGGPSVFL

		FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
16	Human NOPE ECD (25-426)-Fc fusion molecule, without signal peptide	ELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVRE**GS**EPKSSDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
17	Human NOPE ECD (25-620)-Fc fusion molecule, without signal peptide	ELLLPQ ETTVELSCGV GPLQVILGPE QAAVLNCSLG AAAAGPPTRV TWSKDGDTLL EHDHLHLLPN GSLWLSQPLA PNGSDESVPE AVGVIEGNYS CLAHGPLGVL ASQTAVVKLA TLADFSLHPE SQTVEENGTA RFECHIEGLP APIITWEKDQ VTLPEEPRLI VLPNGVLQIL DVQESDAGPY RCVATNSARQ HFSQEALLSV AHRGSLASTR GQDVVIVAAP ENTTVVSGQS VVMECVASAD PTPFVSWVRQ DGKPISTDVI VLGRTNLLIA NAQPWHSGVY VCRANKPRTR DFATAAAELR VLAAPAITQA PEALSRTRAS TARFVCRASG EPRPALRWLH NGAPLRPNGR VKVQGGGGSL VITQIGLQDA GYYQCVAENS AGMACAAASL AVVVREGLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT**GS**EPKSSDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
18	Human NOPE ECD (427-955)-Fc fusion molecule, without signal peptide	GLPS APTRVTATPL SSSAVLVAWE RPEMHSEQII GFSLHYQKAR GMDNVEYQFA VNNDTTELQV RDLEPNTDYE FYVVAYSQLG ASRTSTPALV HTLDDVPSAA PQLSLSSPNP SDIRVAWLPL PPSLSNGQVV KYKIEYGLGK EDQIFSTEVR GNETQLMLNS LQPNKVYRVR ISAGTAAGFG APSQWMHHRT PSMHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHS**GS**EPKSSDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK

19	Human NOPE ECD (623-955)-Fc fusion molecule, without signal peptide	MHNQSHVP FAPAELKVQA KMESLVVSWQ PPPHPTQISG YKLYWREVGA EEEANGDRLP GGRGDQAWDV GPVRLKKKVK QYELTQLVPG RLYEVKLVAF NKHEDGYAAV WKGKTEKAPA PDMPIQRGPP LPPAHVHAES NSSTSIWLRW KKPDFTTVKI VNYTVRFSPW GLRNASLVTY YTSSGEDILI GGLKPFTKYE FAVQSHGVDM DGPFGSVVER STLPDRPSTP PSDLRLSPLT PSTVRLHWCP PTEPNGEIVE YLILYSSNHT QPEHQWTLLT TQGNIFSAEV HGLESDTRYF FKMGARTEVG PGPFSRLQDV ITLQEKLSDS LDMHS**GS**EPKSSDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK
20	Human myostatin	DFGL DCDEHSTESR CCRYPLTVDF EAFGWDWIIA PKRYKANYCS GECEFVFLQK YPHTHLVHQA NPRGSAGPCC TPTKMSPINM LYFNGKEQII YGKIPAMVVD RCGCS
21	Human pro-myostatin (with signal sequence)	MQKLQLCVYI YLFMLIVAGP VDLNENSEQK ENVEKEGLCN ACTWRQNTKS SRIEAIKIQI LSKLRLETAP NISKDVIRQL LPKAPPLREL IDQYDVQRDD SSDGSLEDDD YHATTETIIT MPTESDFLMQ VDGKPKCCFF KFSSKIQYNK VVKAQLWIYL RPVETPTTVF VQILRLIKPM KDGTRYTGIR SLKLDMNPGT GIWQSIDVKT VLQNWLKQPE SNLGIEIKAL DENGHDLAVT FPGPGEDGLN PFLEVKVTDT PKRSRRDFGL DCDEHSTESR CCRYPLTVDF EAFGWDWIIA PKRYKANYCS GECEFVFLQK YPHTHLVHQA NPRGSAGPCC TPTKMSPINM LYFNGKEQII YGKIPAMVVD RCGCS
22	Human pro-myostatin (without signal sequence)	NENSEQK ENVEKEGLCN ACTWRQNTKS SRIEAIKIQI LSKLRLETAP NISKDVIRQL LPKAPPLREL IDQYDVQRDD SSDGSLEDDD YHATTETIIT MPTESDFLMQ VDGKPKCCFF KFSSKIQYNK VVKAQLWIYL RPVETPTTVF VQILRLIKPM KDGTRYTGIR SLKLDMNPGT GIWQSIDVKT VLQNWLKQPE SNLGIEIKAL DENGHDLAVT FPGPGEDGLN PFLEVKVTDT PKRSRRDFGL DCDEHSTESR CCRYPLTVDF EAFGWDWIIA PKRYKANYCS GECEFVFLQK YPHTHLVHQA NPRGSAGPCC TPTKMSPINM LYFNGKEQII YGKIPAMVVD RCGCS
23	Mouse myostatin	DFG LDCDEHSTES RCCRYPLTVD FEAFGWDWII APKRYKANYC SGECEFVFLQ KYPHTHLVHQ ANPRGSAGPC CTPTKMSPIN MLYFNGKEQI IYGKIPAMVV DRCGCS
24	Mouse pro-myostatin (with signal sequence)	MMQKLQMYVY IYLFMLIAAG PVDLNEGSER EENVEKEGLC NACAWRQNTR YSRIEAIKIQ ILSKLRLETA PNISKDAIRQ LLPRAPPLRE LIDQYDVQRD DSSDGSLEDD DYHATTETII TMPTESDFLM QADGKPKCCF FKFSSKIQYN KVVKAQLWIY LRPVKTPTTV FVQILRLIKP MKDGTRYTGI RSLKLDMSPG TGIWQSIDVK TVLQNWLKQP ESNLGIEIKA LDENGHDLAV TFPGPGEDGL NPFLEVKVTD TPKRSRRDFG LDCDEHSTES RCCRYPLTVD FEAFGWDWII APKRYKANYC SGECEFVFLQ KYPHTHLVHQ ANPRGSAGPC CTPTKMSPIN MLYFNGKEQI IYGKIPAMVV DRCGCS
25	Mouse pro-myostatin (without signal sequence)	EGSER EENVEKEGLC NACAWRQNTR YSRIEAIKIQ ILSKLRLETA PNISKDAIRQ LLPRAPPLRE LIDQYDVQRD DSSDGSLEDD DYHATTETII TMPTESDFLM QADGKPKCCF FKFSSKIQYN KVVKAQLWIY LRPVKTPTTV FVQILRLIKP MKDGTRYTGI RSLKLDMSPG TGIWQSIDVK TVLQNWLKQP ESNLGIEIKA LDENGHDLAV TFPGPGEDGL NPFLEVKVTD TPKRSRRDFG LDCDEHSTES RCCRYPLTVD FEAFGWDWII APKRYKANYC SGECEFVFLQ KYPHTHLVHQ ANPRGSAGPC CTPTKMSPIN MLYFNGKEQI IYGKIPAMVV DRCGCS
26	Fc C237S	EPKSSDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK

27	Exemplary Fc #1	ERKCCVECPP CPAPPVAGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVQFNWYV DGVEVHNAKT KPREEQFNST FRVVSVLTVV HQDWLNGKEY KCKVSNKGLP APIEKTISKT KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV EWESNGQPEN NYKTTPPMLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH EALHNHYTQK SLSLSPGK
28	Exemplary Fc #2	ESKYGPPCPS CPAPEFLGGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLGK

CLAIMS

1. A method of reducing or delaying muscle wasting in a subject with muscle wasting or at risk of muscle wasting, comprising administering to the subject an effective amount of a NOPE extracellular domain (ECD) polypeptide.

2. The method of claim 1, wherein the subject has at least one condition selected from chronic obstructive pulmonary disease (COPD), chronic kidney disease, end stage renal disease, chronic heart failure, cancer, critical illness myopathy, critical illness polyneuropathy, stroke, spinal cord injury, spinal muscular atrophy, multiple sclerosis, progressive multifocal leukoencephalopathy, encephalomyelitis, central pontine myelolysis, adrenoleukodystrophy, Wallerian degeneration, Huntington's disease, Parkinson's disease, traumatic brain injury, Alexander's disease, Pelizaeus Merzbacher disease, globoid cell leucodystrophy, and sarcopenia.

3. A method of treating muscle injury comprising administering to a subject with muscle injury an effective amount of a NOPE extracellular domain (ECD) polypeptide.

4. The method of claim 3, wherein the muscle injury is selected from surgery-related muscle injury, traumatic muscle injury, work-related skeletal muscle injury, and overtraining-related muscle injury.

5. A method of treating a muscle degenerative disorder comprising administering to a subject with a muscle degenerative disorder an effective amount of a NOPE extracellular domain (ECD) polypeptide.

6. The method of claim 5, wherein the muscle degenerative disorder is selected from muscular dystrophy, myotonic dystrophy, polymyositis, and dermatomyositis.

7. The method of claim 6, wherein the muscle degenerative disorder is muscular dystrophy.

8. The method of claim 7, wherein the muscular dystrophy is selected from Duchenne muscular dystrophy, Becker muscular dystrophy, congenital muscular dystrophy (Fukuyama), Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy, and fascioscapulohumeral muscular dystrophy.

9. The method of claim 6, wherein the muscle degenerative disorder is myotonic dystrophy.

10. The method of claim 9, wherein the myotonic dystrophy is selected from myotonic dystrophy type I, myotonic dystrophy type II, and congenical myotonia.

11. The method of any one of the preceding claims, wherein treating comprises at least one of delaying progression of muscle wasting, improving the subject's 6 minute

walking distance (6MWD), reducing physical decline, delaying occurrence of life-changing events, improving independence, reducing hospitalizations, and delaying the need for an assisted living arrangement.

12. The method of claim 11, wherein the subject's 6MWD is increased by at least 10 meters, or at least 20 meters, or at least 30 meters after 12 months of treatment.

13. The method of any one of claims 5 to 12, wherein the method comprises delaying the need for a wheelchair and/or delaying placement of a ventilator.

14. A method of treating amyotrophic lateral sclerosis (ALS), comprising administering to a subject with ALS an effective amount of a NOPE extracellular domain (ECD) polypeptide.

15. The method of claim 14, wherein treating ALS comprises at least one of delaying progression of ALS, selected from the group consisting of: reducing physical decline, improving forced vital capacity, slowing the decline in forced vital capacity, slowing the decline in the subject's score on the ALS functional rating scale (ALSFRS), improving the subject's score on the ALSFRS, delaying occurrence of life-changing events, and improving the subject's time of survival.

16. The method of claim 14 or claim 15, wherein the method comprises delaying a tracheostomy and/or delaying placement of a percutaneous endoscopic gastrostomy (PEG).

17. The method of any one of the preceding claims, wherein administration of the NOPE extracellular domain (ECD) polypeptide increases type I slow muscle mass and/or decreases fat mass.

18. A method of increasing type I slow muscle mass, comprising administering to a subject an effective amount of a NOPE extracellular domain (ECD) polypeptide.

19. A method of decreasing fat mass, comprising administering to a subject an effective amount of a NOPE extracellular domain (ECD) polypeptide.

20. The method of any one of the preceding claims, wherein the NOPE extracellular domain (ECD) polypeptide is a NOPE ECD fusion molecule.

21. The method of claim 20, wherein the NOPE ECD polypeptide or NOPE ECD fusion molecule is capable of binding myostatin with a K_D of less than 100 nM.

22. The method of claim 20 or claim 21, wherein the NOPE ECD polypeptide or NOPE ECD fusion molecule inhibits myostatin-mediated activation of SMAD2/3.

23. The method of any one of claims 20 to 22, wherein the NOPE ECD polypeptide or NOPE ECD fusion molecule comprises amino acids 26 to 620 of SEQ ID NO: 1.

24. The method of claim 20, wherein the NOPE ECD fusion molecule comprises a NOPE ECD polypeptide and a fusion partner.

25. The method of claim 24, wherein the fusion partner is an Fc.

26. The method of claim 25, wherein the NOPE ECD fusion molecule comprises the sequence of SEQ ID NO: 19.

27. Use of a NOPE extracellular domain (ECD) polypeptide for reducing or delaying muscle wasting in a subject with muscle wasting or at risk of muscle wasting.

28. The use of claim 27, wherein the subject has at least one condition selected from chronic obstructive pulmonary disease (COPD), chronic kidney disease, end stage renal disease, chronic heart failure, cancer, critical illness myopathy, critical illness polyneuropathy, stroke, spinal cord injury, spinal muscular atrophy, multiple sclerosis, progressive multifocal leukoencephalopathy, encephalomyelitis, central pontine myelolysis, adrenoleukodystrophy, Wallerian degernation, Huntington's disease, Parkinson's disease, traumatic brain injury, Alexander's disease, Pelizaeus Merzbacher disease, globoid cell leucodystrophy, and sarcopenia.

29. Use of a NOPE extracellular domain (ECD) polypeptide for treating muscle injury in a subject.

30. The use of claim 29, wherein the muscle injury is selected from surgery-related mulcle injury, traumatic muscle injury, work-related skeletal muscle injury, and overtraining-related muscle injury.

31. Use of a NOPE extracellular domain (ECD) polypeptide for treating a muscle degenerative disorder in a subject.

32. The use of claim 31, wherein the muscle degenerative disorder is selected from muscular dystrophy, myotonic dystrophy, polymyositis, and dermatomyositis.

33. The use of any one of claims 27 to 32, wherein treating comprises at least one of delaying progression of muscular dystrophy, selected from the group consisting of: improving the subject's 6 minute walking distance (6MWD), reducing physical decline, delaying occurrence of life-changing events, improving independence, reducing hospitalizations, and delaying the need for an assisted living arrangement.

34. Use of a NOPE extracellular domain (ECD) polypeptide for treating amyotrophic lateral sclerosis (ALS) in a subject.

35. The use of claim 34, wherein treating ALS comprises at least one of delaying progression of ALS, selected from the group consisting of: reducing physical decline, improving forced vital capacity, slowing the decline in forced vital capacity, slowing the decline in the subject's score on the ALS functional rating scale (ALSFRS), improving the subject's score on the ALSFRS, delaying occurrence of life-changing events, and improving the subject's time of survival.

36. The use of any one of claims 27 to 35, wherein administration of the NOPE extracellular domain (ECD) polypeptide increases type I slow muscle mass and/or decreases fat mass.

37. Use of a NOPE extracellular domain (ECD) polypeptide for increasing type I slow muscle mass in a subject.

38. Use of a NOPE extracellular domain (ECD) polypeptide for decreasing fat mass in a subject.

39. Use of a NOPE extracellular domain (ECD) polypeptide for treating anemia and/or thalassemia in a subject.

40. The use of any one of claims 27 to 39, wherein the NOPE extracellular domain (ECD) polypeptide is a NOPE ECD fusion molecule.

41. The use of claim 40, wherein the NOPE ECD polypeptide or NOPE ECD fusion molecule is capable of binding myostatin with a K_D of less than 100 nM.

42. The use of claim 40 or claim 41, wherein the NOPE ECD polypeptide or NOPE ECD fusion molecule inhibits myostatin-mediated activation of SMAD2/3.

43. The use of any one of claims 40 to 42, wherein the NOPE ECD polypeptide or NOPE ECD fusion molecule comprises amino acids 26 to 620 of SEQ ID NO: 1.

44. The use of claim 43, wherein the NOPE ECD fusion molecule comprises a NOPE ECD polypeptide and a fusion partner.

45. The use of claim 44, wherein the fusion partner is an Fc.

46. The use of claim 45, wherein the NOPE ECD fusion molecule comprises the sequence of SEQ ID NO: 19.

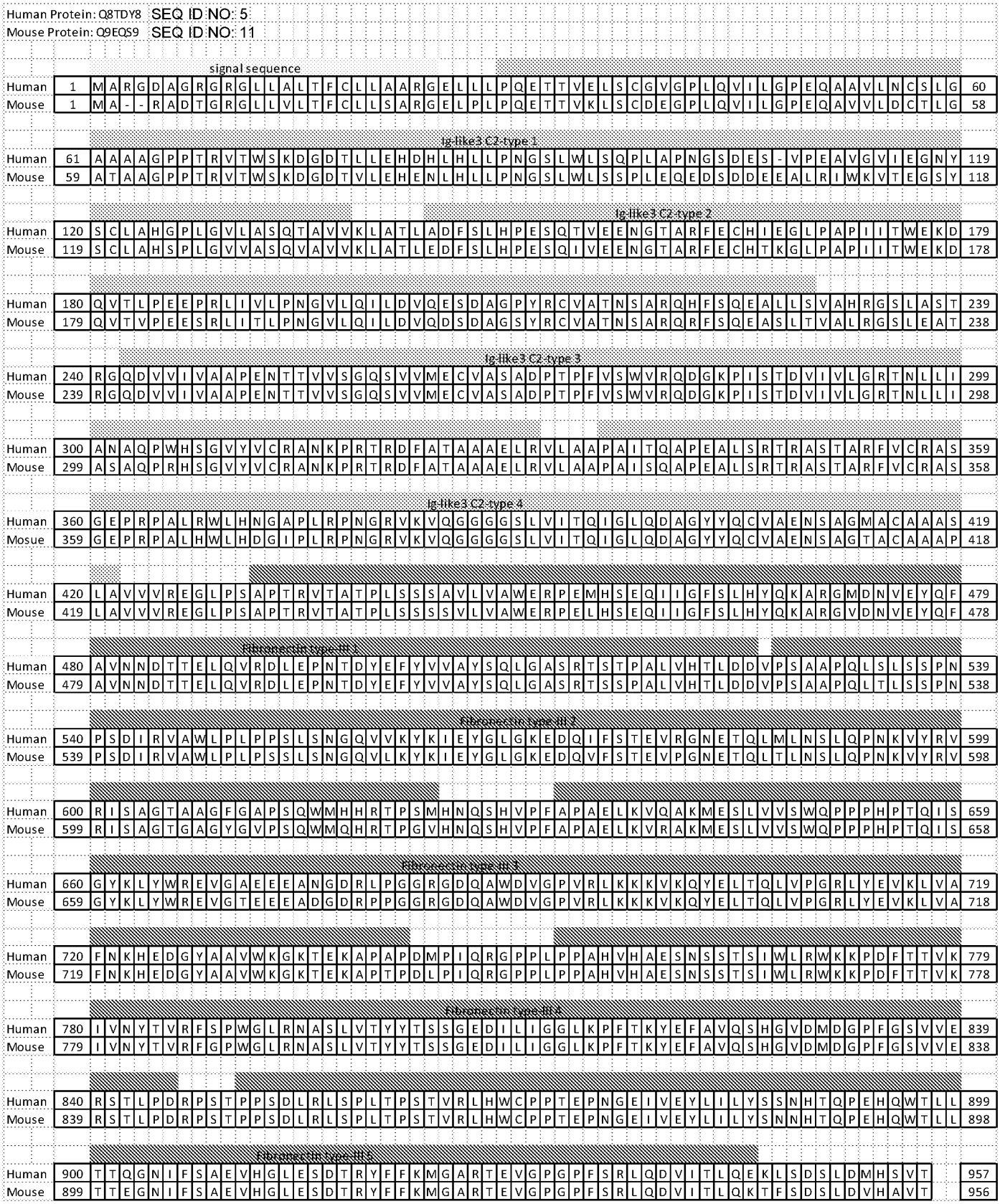
47. A NOPE extracellular domain (ECD) polypeptide or a NOPE ECD fusion molecule, for use in medicine.

48. A NOPE extracellular domain (ECD) polypeptide or a NOPE ECD fusion molecule, for use in treating or preventing muscle wasting.

49. Use of a NOPE extracellular domain (ECD) polypeptide or a NOPE ECD fusion molecule, in the manufacture of a medicament to treat muscle wasting.

50. A NOPE extracellular domain (ECD) polypeptide comprising SEQ ID NO: 19.

Figure 1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Mouse Protein: Q9EQS9 SEQ ID NO: 11
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Ig-like3 C2-type 4
Fibronectin type-III 1
Fibronectin type-III 2
Fibronectin type-III 3
Fibronectin type-III 4
Fibronectin type-III 5
Human 1 MARGDAGRGRGLLALTFCLLAARGELLLPQETTVELSCGVGPLQVILGPEQAAVLNCSLG 60
Mouse 1 MA--RADTGRGLLVLTFCLLSARGELPLPQETTVKLSCDEGPLQVILGPEQAVVLDCTLG 58
Human 61 AAAAGPPTRVTWSKDGDTLLEHDHLHLLPNGSLWLSQPLAPNGSDES-VPEAVGVIEGNY 119
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Mouse 299 ASAQPRHSGVYVCRANKPRTRDFATAAAELRVLAAPAISQAPEALSRTRASTARFVCRAS 358
Human 360 GEPRPALRWLHNGAPLRPNGRVKVQGGGGSLVITQIGLQDAGYYQCVAENSAGMACAAAAS 419
Mosue 359 GEPRPALHWLHDGIPLRPNGRVKVQGGGGSLVITQIGLQDAGYYQCVAENSAGTACAAAAP 418
Human 420 LAVVVREGLPSAPTRVTATPLSSSAVLVAWERPEMHSEQIIGFSLHYQKARGMDNVEYQF 479
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Mouse 839 RSTLPDRPSTPPSDLRLSPLTPSTVRLHWCPPTEPNGEIVEYLILYSNNHTQPEHQWTLL 898
Human 900 TTQGNIFSAEVHGLESDTRYFFKMGARTEVGPGPFSRLQDVITLQEKLSDSLDMHSVT 957
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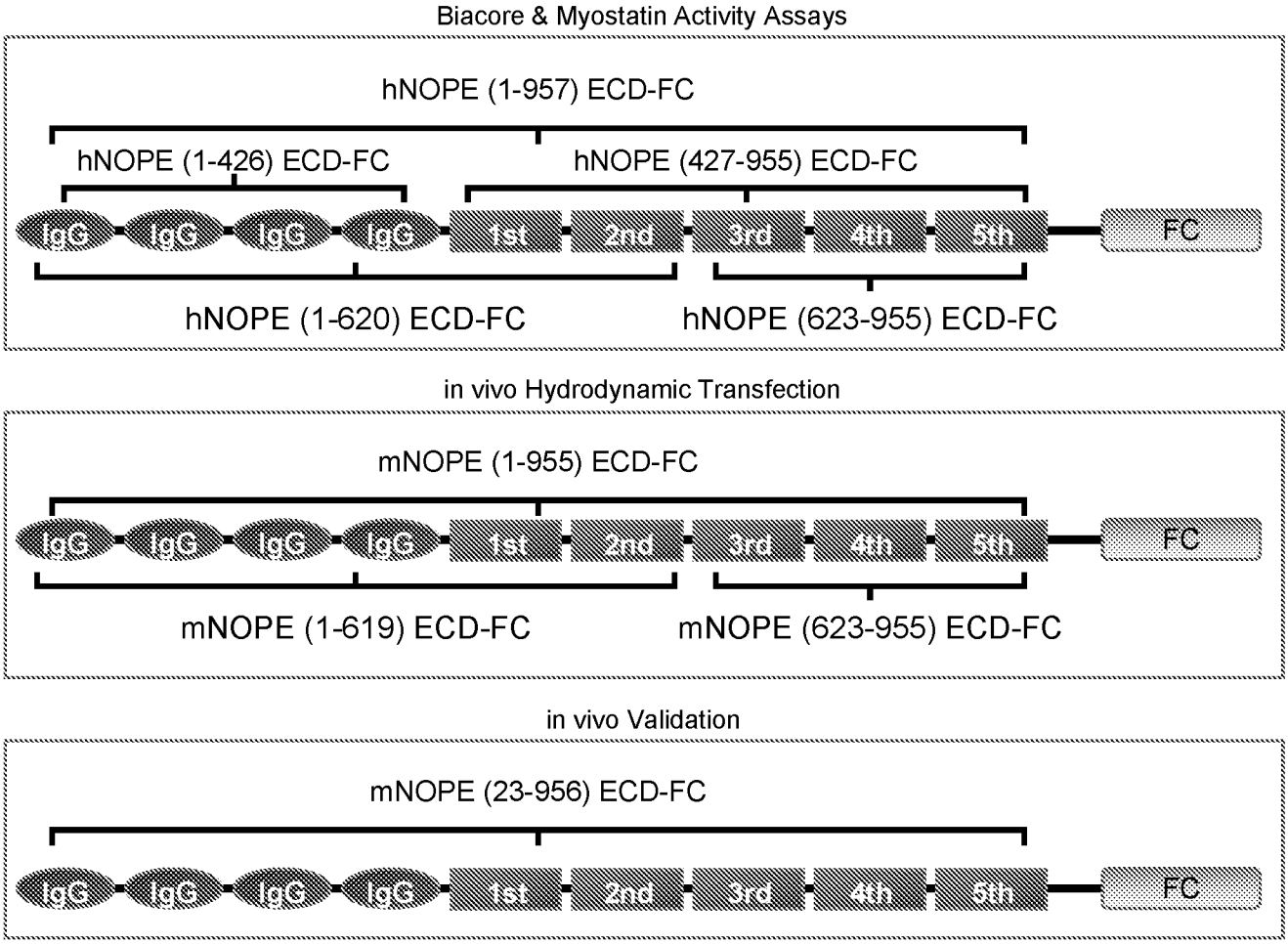
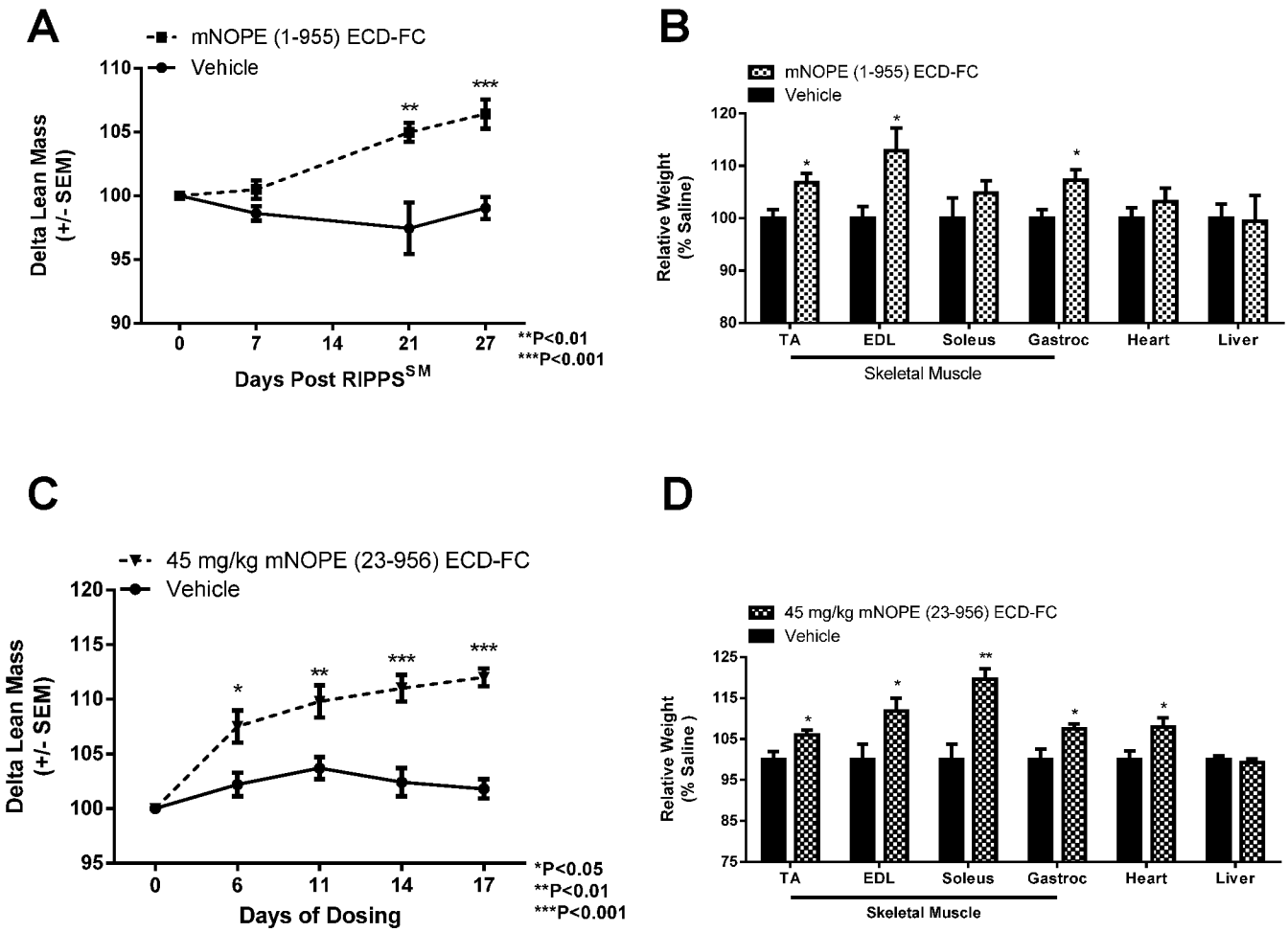
Figure 2



Protein Name	Brief Description	Assay
hNOPE (1-957) ECD-FC	Parental human NOPE extracellular domain (ECD)-FC	biacore & activity assay
hNOPE (1-426) ECD-FC	N terminal IgG domains of human NOPE ECD-FC	biacore & activity assay
hNOPE (1-620) ECD-FC	N terminal IgG domains and first 2 FN3 domains of human NOPE ECD-FC	biacore & activity assay
hNOPE (427-955) ECD-FC	All FN3 domains of human NOPE ECD-FC	biacore & activity assay
mNOPE (1-955) ECD-FC	Parental mouse NOPE extracellular domain (ECD)-FC	hydrodynamic transfection
mNOPE (1-619) ECD-FC	N terminal IgG domains and first 2 FN3 domains of mouse NOPE ECD-FC	hydrodynamic transfection
mNOPE (623-955) ECD-FC	Last 3 FN3 domains of mouse NOPE ECD-FC	hydrodynamic transfection
mNOPE (23-956) ECD-FC	Parental mouse NOPE extracellular domain (ECD)-FC	in vivo validation

Figure 3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mNOPE (1-955) ECD-FC
Vehicle
Delta Lean Mass (+/- SEM)
110
105
100
95
90
0
7
14
21
27
**
***
Days Post RIPPS SM
**P<0.01
***P<0.001
B
mNOPE (1-955) ECD-FC
Vehicle
Relative Weight (% Saline)
120
110
100
90
80
TA
EDL
Soleus
Gastroc
Heart
Liver
Skeletal Muscle
C
45 mg/kg mNOPE (23-956) ECD-FC
Vehicle
Delta Lean Mass (+/- SEM)
120
115
110
105
100
95
0
6
11
14
17
Days of Dosing
*P<0.05
**P<0.01
***P<0.001
D
45 mg/kg mNOPE (23-956) ECD-FC
Vehicle
Relative Weight (% Saline)
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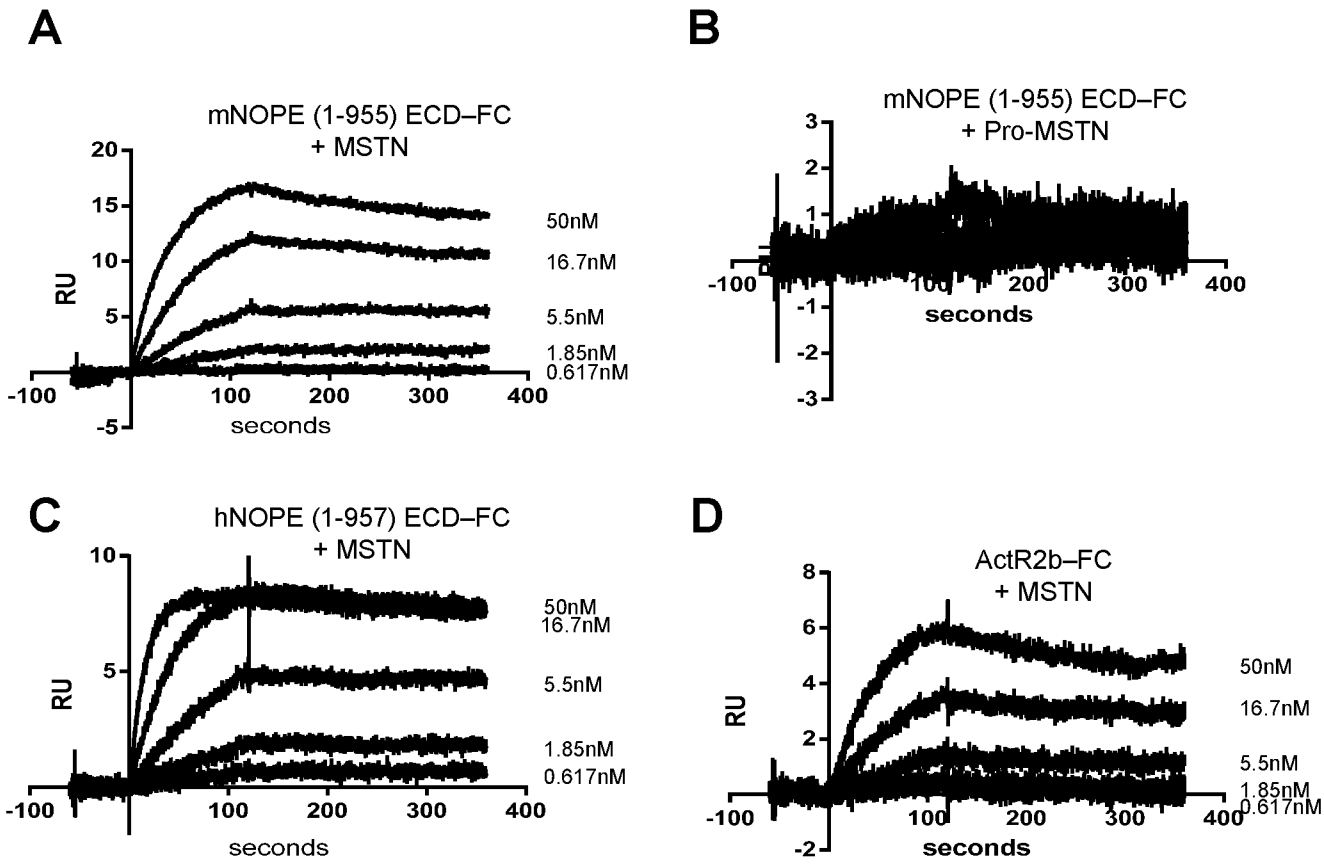
115
105
95
85
75
TA
EDL
Soleus
Gastroc
Heart
Liver
Skeletal Muscle

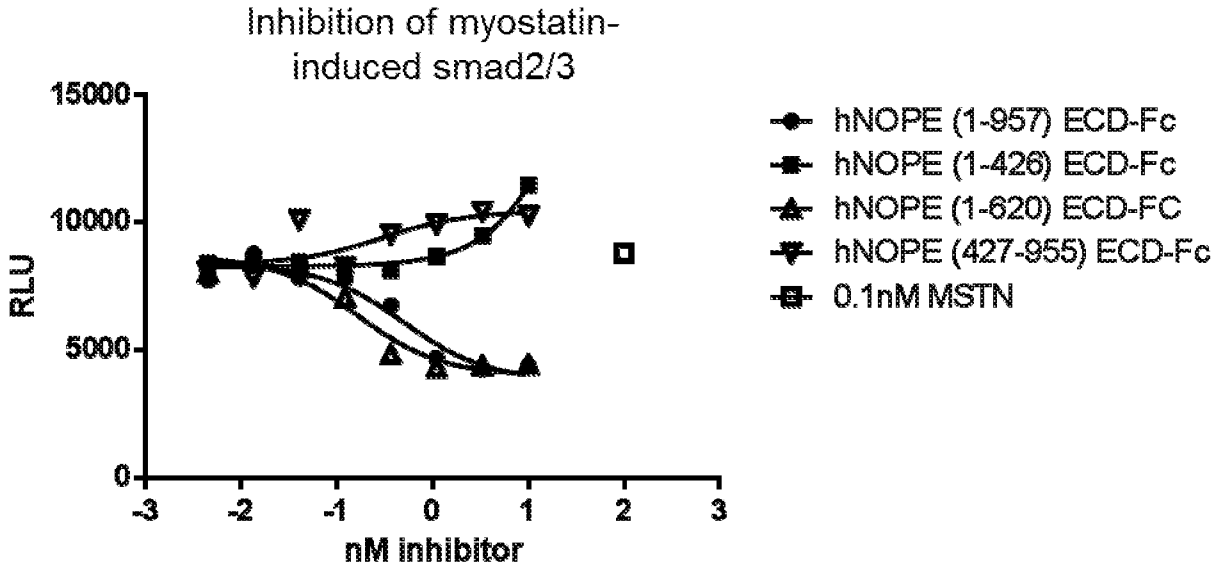
Figure 4



E

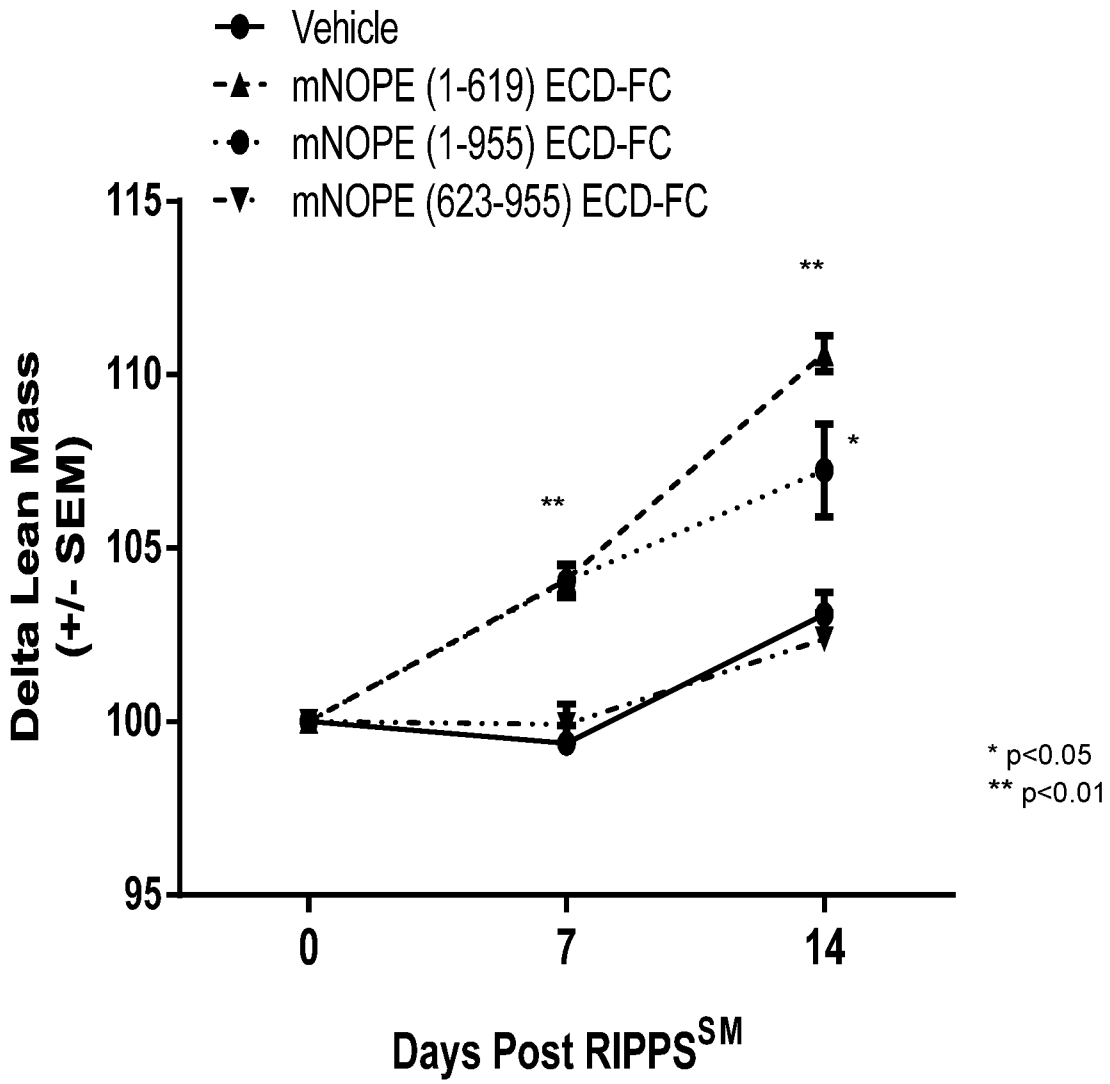
Sample	Ligand	ka (1/Ms)	kd (1/s)	KD (nM)
Myostatin	hNOPE-FC	2.0E+06	3.2E-04	0.2
	mNOPE-FC	6.7E+05	5.8E-04	0.9
	ActR2b-FC	3.9E+05	9.8E-04	2.5
Pro Myostatin	hNOPE-FC	--	--	NB
	mNOPE-FC	--	--	NB
	ActR2b-FC	--	--	NB

Figure 5



Protein	IC50 MSTN inhibition (nM)
mNOPE (1-955) ECD-Fc	0.068
hNOPE (1-957) ECD-Fc	0.50
hNOPE (1-426) ECD-Fc	–
hNOPE (1-620) ECD-Fc	0.17
hNOPE (427-955) ECD-Fc	–

Figure 6


Vehicle
mNOPE (1-619) ECD-FC
mNOPE (1-955) ECD-FC
mNOPE (623-955) ECD-FC
Delta Lean Mass (+/- SEM)
115
110
105
100
95
0
7
14
Days Post RIPPS^SM
**
**
*
* p<0.05
** p<0.01

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/052345

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 C07K14/705 A61P7/06 A61P21/00
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)
A61K C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 2002/102551 A1 (SALBAUM J MICHAEL [US]) 1 August 2002 (2002-08-01) paragraphs [0024], [0049], [0089]; claims 1-5	47,49 1-46,48, 50

X	Anonymous: "Recombinant Mouse Nope Fc Chimera", Fearonetal. Keino-Masuetal. Kolodziejetal. Meyerhardt Vielmetteretal, 1 January 2000 (2000-01-01), XP055385761, Retrieved from the Internet: URL:https://resources.rndsystems.com/pdfs/datasheets/1394-np.pdf [retrieved on 2017-06-27]	50
A	Background	1-49

	-/--	

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

[X] See patent family annex.

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

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

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

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

"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	Date of mailing of the international search report
29 June 2017	21/08/2017

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

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SALBAUM J M ED - COX ROGER D: "GENOMIC STRUCTURE AND CHROMOSOMAL LOCALIZATION OF THE MOUSE GENE PUNC", MAMMALIAN GENOME, SPRINGER NEW YORK LLC, US, vol. 10, no. 2, 1 February 1999 (1999-02-01), pages 107-111, XP000992754, ISSN: 0938-8990, DOI: 10.1007/S003359900953 abstract	1-50
A	SALBAUM J M ET AL: "Cloning and Expression of Nope, a New Mouse Gene of the Immunoglobulin Superfamily Related to Guidance Receptors", GENO, ACADEMIC PRESS, SAN DIEGO, US, vol. 64, no. 1, 15 February 2000 (2000-02-15), pages 15-23, XP004439422, ISSN: 0888-7543, DOI: 10.1006/GENO.2000.6114 abstract; figure 1	1-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2017/052345

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002102551 A1	01-08-2002	AU 1239701 A	16-07-2001
		CA 2396355 A1	12-07-2001
		EP 1246914 A2	09-10-2002
		US 2002102551 A1	01-08-2002
		WO 0149714 A2	12-07-2001