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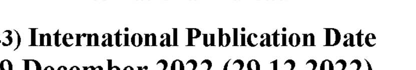

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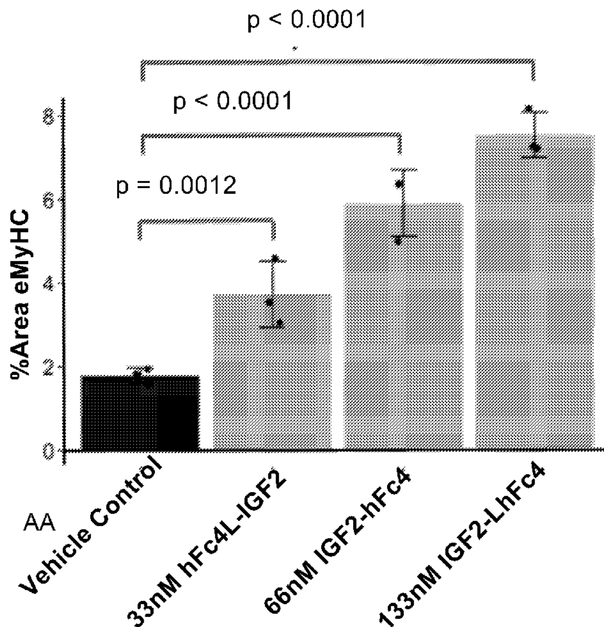
(54) **Title:** REGENERATIVE POLYPEPTIDES AND USES THEREOF

FIG 1A



(57) **Abstract:** Described herein are polypeptides comprising an IGF 2 amino acid sequence and an amino acid sequence from a heterologous polypeptide useful for the treatment of soft-tissue and muscle diseases, disorders, and injuries. Mutations within the IGF2 amino acid sequence improved the stability of the molecule by reducing backbone cleavage. Also described herein are synergistic combinations of an Insulin-like Growth Factor 1 Receptor (IGF1R) agonist and a short chain fatty acid. Also described are methods of treating muscle and soft-tissue diseases comprising administering the polypeptides and/or synergistic compositions.

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[Continued on next page]

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).

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— *with sequence listing part of description (Rule 5.2(a))*

REGENERATIVE POLYPEPTIDES AND USES THEREOF

REFERENCE TO SEQUENCE LISTING, TABLE OR COMPUTER PROGRAM

[0001] The official copy of the Sequence Listing is submitted concurrently with the specification as an ASCII formatted text file via EFS-Web, with a file name of "JTI021_ST25.txt", a creation date of June 7, 2022, and a size of 102 kilobytes. The Sequence Listing filed via EFS-Web is part of the specification and is incorporated in its entirety by reference herein.

BACKGROUND

[0002] As the average life span increases, increasing emphasis is placed upon "healthy aging." Individuals would like to live more active lifestyles as they age, and as a result, many aging disorders can have a significant impact on the quality of life of aging individuals. Treatments directed to regenerative ends have utility for treating aging diseases. Additionally, many treatments for aging disorders can be applicable to younger individuals who have suffered illness, injury, or who possess genetic or developmental defects leading to premature tissue loss, wasting, or weakening.

SUMMARY

[0003] As individuals age, tissue progenitor cells lose their regenerative potential.

[0004] Described herein are polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide useful for the treatment of soft-tissue and muscle diseases, disorders, and injuries. Also described herein mutations within the IGF2 amino acid sequence improved the stability of the molecule by reducing backbone cleavage. Also described herein are mutations within IGF2 sequences that block cleavage of its peptide backbone. Also described are methods of treating muscle and soft-tissue diseases comprising administering the polypeptides and/or synergistic compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0005] **FIG. 1A** depicts purified IGF2-hFcm promoted differentiation of human myoblast cells

[0006] **FIG. 1B** depicts purified IGF2-LhFc4 promoted differentiation of human myoblast cells

[0007] **FIG. 2A** purified HSA-L-IGF2R61A promoted differentiation of human myoblast cells.

[0008] **FIG. 2B** depicts IGF2 and IGF2 receptors were expressed in human myoblast

[0009] **FIG. 2C** depicts IGF2 and IGF2 receptors were expressed in human myoblast

[0010] **FIG. 3A** depicts sodium butyrate enhanced muscle fusion

[0011] **FIG. 3B** depicts sodium butyrate enhanced IGF2 activity

[0012] **FIG. 3C** depicts sodium butyrate enhanced IGF2 activity

[0013] **FIG. 4A** depicts the change in percent area of eMyHC positive cells treated with additional doses of vehicle, IGF2, sodium butyrate, or IGF2 and sodium butyrate

5 [0014] **FIG. 4B** depict the change in percent area of eMyHC positive cells treated with additional doses of vehicle, IGF2, sodium butyrate, or IGF2 and sodium butyrate

[0015] **FIG. 5A** depicts IGF2 enhances MYOG expression in DM1 human myoblast cells.

[0016] **FIG. 6A** depicts IGF2 Receptor was expressed on chondrocyte and osteocytes

[0017] **FIG. 7A** depicts IGF2 treatment promoted proliferation in DM1 human myoblast (32

10 year old caucasian female) cells

[0018] **FIG. 7B** depicts IGF2 treatment promoted fusion in DM1 human myoblast (32 year old caucasian female) cells

[0019] **FIG. 8A** depicts depicts IGF2 enhanced MYH3 and CKM expression in DM1 human myoblast (32 year old caucasian female) cells

15 [0020] **FIG. 8B** depicts depicts IGF2 enhanced ATP1B1 expression in DM1 human myoblast (32 year old caucasian female) cells

[0021] **FIG. 9A** depicts systemic administration of IGF2/sodium butyrate protected against aging induced muscle dysfunction

[0022] **FIG. 9B** depicts an experimental overview of systemic administration of IGF2/NaB

20 protected against aging induced muscle dysfunction as measured by grip strength force

[0023] **FIG. 9C** depicts systemic administration of IGF2/sodium butyrate protected against aging induced muscle dysfunction as measured by both limb grip strength force

[0024] **FIG. 9D** depicts systemic administration of IGF2/sodium butyrate protected against aging induced muscle dysfunction as measured by forelimb force

25 [0025] **FIG. 9E** depicts systemic administration of IGF2/sodium butyrate protected against aging induced muscle dysfunction as measured by treadmill performance

[0026] **FIG. 9F** depicts systemic administration of IGF2/sodium butyrate protected against aging induced muscle dysfunction as measured by running time to exhaustion

[0027] **FIG. 9G** depicts systemic administration of IGF2/sodium butyrate protected against

30 aging induced muscle dysfunction as measured by maximum running speed

[0028] **FIG. 10A** depicts experimental overview for demonstrating chronic, systemic administration of IGF2/sodium butyrate was safe for liver, kidney and pancreas function

[0029] **FIG. 10B** depicts systemic administration of IGF2/sodium butyrate had no adverse effects on white blood cell count, Albumin concentration (**FIG. 10C)**, Creatinine concentration

35 (**FIG. 10D)** and Calcium concentration (**FIG. 10E)**

[0030] **FIG. 11A** depicts an experimental overview of systemic administration of IGF2 and sodium butyrate protecting against Dexamethasone induced muscle atrophy.

[0031] **FIG. 11B** depicts systemic administration of IGF2 and sodium butyrate protected against Dexamethasone induced muscle atrophy as measured by bothlimb force

5 [0032] **FIG. 11C** depicts systemic administration of IGF2 and sodium butyrate protected against Dexamethasone induced muscle atrophy as measured by specific bothlimb force

[0033] **FIG. 11D** depicts systemic administration of IGF2 and sodium Butyrate protected against Dexamethasone induced muscle atrophy as measured by forelimb force

[0034] **FIG. 11E** depicts systemic administration of IGF2 and sodium Butyrate protected
10 against Dexamethasone induced muscle atrophy as measured by specific bothlimb force calculated as the ratio of bothlimb force in mN over the weight

[0035] **FIG. 11F** depicts systemic administration of IGF2 and sodium butyrate protected against Dexamethasone induced muscle atrophy as measured by muscle fiber cross sectional area

[0036] **FIG. 12A** depicts systemic administration of IGF2 and sodium butyrate regenerates
15 and enhances muscle health in the D2-mdx model Duchenne's muscular dystrophy model improving tibialis anterior muscle weight relative to vehicle treatment

[0037] **FIG. 12B** depicts systemic administration of IGF2 and sodium butyrate regenerates and enhances muscle function in the D2-mdx model Duchenne's muscular dystrophy model improving forelimb grip strength relative to vehicle treatment

20 [0038] **FIG. 12C** depicts systemic administration of IGF2 and sodium butyrate protected against regenerates and enhances muscle function in the D2-mdx model Duchenne's muscular dystrophy model improving both limb grip strength relative to vehicle treatment

[0039] **FIG. 12D** depicts systemic administration of IGF2 and sodium butyrate regenerates and enhances muscle function in the D2-mdx model Duchenne's muscular dystrophy model
25 improving distance run on a treadmill relative to vehicle treatment

[0040] **FIG. 12E** depicts systemic administration of IGF2 and sodium butyrate regenerates and enhances muscle function in the D2-mdx model Duchenne's muscular dystrophy model improving distance run on a treadmill relative to vehicle treatment

[0041] **FIG. 12F** depicts systemic administration of IGF2 and sodium butyrate regenerates
30 and enhances muscle function in the D2-mdx model Duchenne's muscular dystrophy model improving distance run on a treadmill relative to vehicle treatment

[0042] **FIG. 13A** depicts HSA-L-IGF2 is cleaved when expressed from CHO cells as visualized on a reducing SDS-PAGE

[0043] **FIG. 13B** depicts that HSA-L-IGF2 is cleaved when expressed from CHO cells as
35 visualized on a reducing SDS-PAGE followed by Western blotting to detect 6xHIS tag, and

depicts cleavage of IGF2 as confirmed by double tagged IGF2 as visualized on a reducing SDS-PAGE followed by Western blotting to detect 6xHIS tag and the antibody constant region, hFc4, tag

[0044] **FIG. 14** depicts IGF2 cleavage with the amino acid sequence tagged on each end (HSA and hFc4) as visualized on a reducing SDS-PAGE followed by Western blotting to detect 6xHIS tag and the hFc4 tag

[0045] **FIG. 15A** depicts double tagged (HSA and hFc4) IGF2 cleavage was blocked by mutations as visualized on a reducing SDS-PAGE

[0046] **FIG. 15B** epicts double tagged (HSA and hFc4) IGF2 cleavage was blocked by mutations as visualized on a reducing SDS-PAGE followed by Western blotting to detect 6xHIS tag and hFc4 tags

[0047] **FIG. 16A** depicts in vitro myogenesis assay results demonstrates HSA-IGF2R61A mutant sequences retain equal activity relative to IGF2 in human DM1 muscle precursors from 32- year-old female

[0048] **FIG. 16B** depicts in vitro myogenesis assay results demonstrates IGF2 displays equal activity relative to HSA-IGF2R61A in human DM1 muscle precursors from 32- year-old female

[0049] **FIG. 17A** depicts in vitro myogenesis assay results demonstrates HSA-IGF2R61A mutant sequences retain equal activity relative to IGF2 in healthy human muscle precursors from 32- year-old female

[0050] **FIG. 17B** depicts in vitro myogenesis assay results demonstrates IGF2 displays equal activity relative to an equimolar amount of HSA-IGF2R61A in healthy human muscle precursors from 32- year-old female

[0051] **FIG. 18A** depicts Non-Compartment Analysis fit of pharmacodynamics data for intravenous administration of the HSA-IGF2R61 in mice which demonstrates significantly improved serum half-life compared to the natural sequence of IGF2

[0052] **FIG. 18B** depicts Non-Compartment Analysis fit of pharmacodynamics data for intravenous administration of the IGF2 and HSA-IGF2R61 in mice.

[0053] **FIG. 19** depicts HSA-IGF2R61A interacts with rhIGFBP3 as demonstrated by mass shifts in size exclusion chromatography by HPLC

[0054] **FIG. 20** depicts a mutant sequence HSA-IGF2R61A retains equal or increased activity to HSA-IGF2 at equimolar concentrations

[0055] **FIG. 21A-F** shows results from an acute injury mouse model. **FIG. 21A** shows the fatigue index for mice receiving 6HIS-HSA-IGF2R61A or mice receiving control (vehicle). **FIG. 21B** shows the force production as measured by specific force frequency for mice receiving 6HIS-HSA-IGF2R61A or mice receiving control (vehicle). **FIG. 21C** shows the force

production as measured by max contraction rate for mice receiving 6HIS-HSA-IGF2R61A or mice receiving control (vehicle). **FIG. 21D** shows the relation rates of mice receiving 6HIS-HSA-IGF2R61A compared mice receiving control (vehicle). **FIG. 21E** shows the regenerative index measured as the number of new muscle fibers per square millimeter for mice receiving 6HIS-HSA-IGF2R61A or mice receiving control (vehicle). **FIG. 21F** shows the muscle mass of mice receiving 6HIS-HSA-IGF2R61A compared mice receiving control (vehicle).

[0056] **FIG. 22A-E** shows results from a sarcopenia mouse model. **FIG. 22A** shows blood glucose normalized to a baseline level for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 22B** shows force generation measured by both limb grip strength (normalized for body weight) for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 22C** shows force generation measured by forelimb grip strength (normalized for body weight) for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 22D** shows recovery of muscle force production for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 22E** shows force frequency, normalized for body weight, for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle).

[0057] **FIG. 23A-B** shows results from a sarcopenia mouse model. **FIG. 23A** shows fiber type distribution for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 23B** shows cross sectional areas (CSA) for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle).

[0058] **FIG. 24A-D** shows results from a muscle dystrophy (DM1) mouse model. **FIG. 24A** shows muscle fiber distribution for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 24B** shows cross sectional areas (CSA) for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 24C** shows change in twitch from baseline for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle). **FIG. 24D** shows change in novel object recognition for mice receiving 6 mg/kg 6HIS-HSA-IGF2R61A and 0.3g/kg of sodium butyrate (NaB) or mice receiving control (vehicle).

DETAILED DESCRIPTION

[0059] In certain aspects disclosed herein is a therapeutically active protein or polypeptide

sequence or derivative or fragment thereof that enhances progenitor cell growth or regeneration or function through activation of a cell surface receptor, and one or more of: a secretion signal a multimerizing component, or a stabilizing component. We modify and combined the sequences of certain polypeptides to create secreted, therapeutically active proteins with applications to muscle and soft tissue regeneration useful to treat acute and chronic muscle wasting diseases or conditions, such as sarcopenia, cachexia, muscular dystrophies, and muscle injury. In certain aspects, disclosed herein is a method of treating individuals with acute and chronic muscle wasting diseases or conditions, such as sarcopenia, cachexia, muscular dystrophies, and muscle injury.

[0001] In certain aspects, disclosed herein is a polypeptide comprising an IGF2 amino acid sequence and a heterologous polypeptide amino acid sequence, wherein the heterologous polypeptide amino acid sequence increases the stability or biological function of the IGF2 amino acid sequence. In certain aspects, disclosed herein is a composition comprising an IGF1R agonist and a short fatty acid chain.

[0060] The secretion signal sequence can either be one naturally occuring with a therapeutically active protein or polypeptide sequence or a different one selected, modified, or created to optimize expression yield through secretion efficiency, processing kinetics, or cell line specific processing Further examples and SEQ IDs are found in the Table of Sequences at the end of this disclosure**.** In certain aspects, the polypeptide may comprise a secretory signal peptide. In certain embodiments, the secretory signal peptide is SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO: 15, or SEQ ID NO: 16. Production of the fusion polypeptides herein in heterologous production systems (e.g., bacteria or yeast) may result in imprecise cleavage of the signal sequence of the fusion polypeptide or non-specific early truncation at the C-terminal end of the fusion polypeptide. Processing of the secretory sequence or N- or C- terminal processing may result in loss of amino acids from either the N- or the C-terminus of the polypeptide.

[0061] There are several polypeptide sequences that can induce a regenerative effect through membrane receptors. Examples from the stem cell secretome selected for their ability to improve muscle and soft tissue regeneration listed in Table of Sequences, including IGF2, and variants thereof. Multimerizing components join two or more other protein components. A multimerizing component can take the form of a linker sequence of amino acids that joins other components tandemly into a single consecutive amino acid sequence. Or multimerizing components can take the form of proteins or protein domains that dimerize, resulting in covalent disulfide linking or non-covalent associations driving dimerization. Examples are disclosed in the Table of Sequences at the end of this disclosure.

[0062] Stabilizing components can reduce degradation rate, increase translational or post-translation folding, reduce unfolding rates, or increase circulating half-life. Examples can include abundant, circulating proteins or fragments thereof such as albumin or the fragment crystallizable (Fc) region from a human antibody. Further examples are disclosed in the Table of Sequences at 5 the end of this disclosure.

Certain definitions

[0063] In the following description, certain specific details are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand 10 that the embodiments provided may be practiced without these details. Unless the context requires otherwise, throughout the specification and claims which follow, the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense, that is, as “including, but not limited to.” As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the 15 content clearly dictates otherwise. It should also be noted that the term “or” is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed embodiments.

[0064] As used herein the term “about” refers to an amount that is near the stated amount by 20 10%.

[0065] As used herein the terms “individual,” “patient,” or “subject” are used interchangeably and refer to individuals diagnosed with, suspected of being afflicted with, or at-risk of developing at least one disease for which the described compositions and method are useful for treating. In certain embodiments the individual is a mammal. In certain embodiments, 25 the mammal is a mouse, rat, rabbit, dog, cat, horse, cow, sheep, pig, goat, llama, alpaca, or yak. In certain embodiments, the individual is a human.

[0066] As used herein the term “treat” or “treating” refers to interventions to a physiological or disease state of an individual designed or intended to ameliorate at least one sign or symptom associated with said physiological or disease state. The skilled artisan will recognize that given a 30 heterogeneous population of individuals afflicted with a disease, not all individuals will respond equally, or at all, to a given treatment.

[0067] As used herein, the term “heterologous” refers to a nucleotide or amino acid sequence that is from a different source (e.g., gene, polypeptide, or organism) compared to the amino acid or nucleotide sequence to which it refers to as being heterologous. Heterologous includes 35 biological sequences derived from different organisms or to sequences derived from different

sources (e.g., genes or proteins) of the same organism. Heterologous sequences include recombinant DNA molecules comprising nucleotide sequences from different sources, fusion proteins comprising amino acid sequences from different sources, and epitope or purification tags of natural or synthetic origin.

5 **[0068]** As used herein, the term “muscle” refers to skeletal muscle, and does not refer to smooth muscle or cardiac muscle.

[0069] As used herein, the term “soft tissue” refers to connective tissues, including without limitations, tendons, ligaments, and cartilage.

[0070] As used herein, the term “mitogenic activity” refers to an activity that induces cell
10 division or proliferation.

[0071] As used herein, the term “fusion promoting activity” refers to activity that promotes the fusion of cells into multinucleated cells, such as the fusion of myocytes into multinucleated myofibers, or advances the differentiation of a terminal differentiating stem or progenitor cells toward a committed cell lineage type, such as the progression of myoblasts into myocytes or the
15 increase in cell size of expanding myofibers.

[0072] 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. Polypeptides, including the provided antibodies and antibody chains and other peptides, e.g., linkers and binding peptides, may include amino acid residues including natural and/or non-natural amino acid residues. The
20 terms also include post-expression modifications of the polypeptide, for example, glycosylation, sialylation, acetylation, phosphorylation, and the like. In some aspects, the polypeptides may contain modifications with respect to a native or natural 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
25 proteins or errors due to PCR amplification.

[0073] Percent (%) sequence identity with respect to a reference polypeptide sequence is the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any
30 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 known for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Appropriate parameters for aligning sequences are able to be determined, including algorithms needed to achieve maximal alignment over the full
35 length of the sequences being compared. For purposes herein, however, % amino acid sequence

identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The
5 ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0074] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the
10 % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows: 100 times the fraction X/Y, where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in
15 that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding
20 paragraph using the ALIGN-2 computer program.

[0075] The polypeptides described herein can be encoded by a nucleic acid. A nucleic acid is a type of polynucleotide comprising two or more nucleotide bases. In certain embodiments, the nucleic acid is a component of a vector that can be used to transfer the polypeptide encoding polynucleotide into a cell. As used herein, the term “vector” refers to a nucleic acid molecule
25 capable of transporting another nucleic acid to which it has been linked. One type of vector is a genomic integrated vector, or “integrated vector,” which can become integrated into the chromosomal DNA of the host cell. Another type of vector is an “episomal” vector, e.g., a nucleic acid capable of extra-chromosomal replication. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as “expression
30 vectors.” Suitable vectors comprise plasmids, bacterial artificial chromosomes, yeast artificial chromosomes, viral vectors and the like. In the expression vectors regulatory elements such as promoters, enhancers, polyadenylation signals for use in controlling transcription can be derived from mammalian, microbial, viral or insect genes. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of
35 transformants may additionally be incorporated. Vectors derived from viruses, such as

lentiviruses, retroviruses, adenoviruses, adeno-associated viruses, and the like, may be employed. Plasmid vectors can be linearized for integration into a chromosomal location. Vectors can comprise sequences that direct site-specific integration into a defined location or restricted set of sites in the genome (e.g., AttP-AttB recombination). Additionally, vectors can comprise sequences derived from transposable elements.

IGF2 Fusion proteins

[0076] Insulin-like growth factor (IGF) ligands IGF1 and IGF2 are involved in many cell signaling and developmental processes. IGF2 is one of the major embryonic growth factors in humans, with minimal expression in adults, transient bursts are localized to skeletal muscle cells transitioning through cell states. Further complexity in its regulation stems from its genomic imprinting, with IGF2 being one of the few proteins that are expressed only from the paternal copy. Its effects are differentially mediated by binding cell surface receptors: insulin receptor, insulin-like growth factor receptor 1 (IGF1R) and insulin-like growth factor receptor 2 (IGF2R). IGF1R activates many signaling including pathways involved in cell proliferation, cell differentiation, and cell survival. IGF2R is involved in attenuating the signaling response. Described herein are certain therapeutically useful IGF2 polypeptides, including IGF2 fusion polypeptides that promote in vivo stability and function of the IGF2 comprising polypeptides, or combinations of IGF2 or IGF2 fusion proteins with IGF binding proteins (IGFBP) such as IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, or IGFBP6.

[0077] In certain aspects described herein are IGF receptor ligand polypeptides. In certain aspects, described herein, are IGF2 polypeptides, that comprise an IGF2 amino acid sequence. In certain embodiments, the IGF2 amino acid sequence is that of a human IGF2 polypeptide. In certain embodiments, the human IGF2 polypeptide comprises amino acids 25 to 91 of SEQ ID NO. 32 (i.e. SEQ ID NO. 29). In certain embodiments, the IGF2 amino acid sequence is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 29. In certain embodiments, the IGF2 amino acid sequence is 100% identical to SEQ ID NO. 29. In certain embodiments, the IGF2 amino acid sequence comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 29, but with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids deleted from the N- or C-terminus.

[0078] In certain embodiments, the IGF2 amino acid sequence is at least about 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 29 and 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids are deleted from the N- or C- terminus of the polypeptide. In certain embodiments, the IGF2 fusion polypeptide amino acid sequence is at least about 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 32 and 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids are deleted from

the N- or C- terminus of the polypeptide. In certain embodiments, the IGF2 fusion polypeptide amino acid sequence is at least about 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 34 and 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids are deleted from the N- or C- terminus of the polypeptide. In certain embodiments, the IGF2 amino acid sequence is at least about 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 39 and 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids are deleted from the N- or C- terminus of the polypeptide.

[0079] In certain IGF2 polypeptides described herein are fusion proteins or polypeptides that may comprise additional heterologous (non-IGF2) amino acid sequences that enhance the expression, stability or function of the IGF2 polypeptide compared to a polypeptide not comprising the heterologous amino acid sequence. These heterologous amino acid sequences may increase the expression of the IGF2 fusion polypeptide from a cell system (e.g., CHO cells or other suitable cell system for bulk production) by 10%, 20%, 25%, 30%, 40%, 50%, 75%, 100%, 150%, 200%, 200%, 400%, 500%, 1,000% or more compared to a polypeptide not comprising the heterologous amino acid sequence. These heterologous amino acid sequences may increase the bioavailability (e.g., increasing the $T_{1/2}$) of the IGF2 polypeptide *in vivo* by 10%, 20%, 25%, 30%, 40%, 50%, 75%, 100%, 150%, 200%, 200%, 400%, 500%, 1,000% or more compared to a polypeptide not comprising the heterologous amino acid sequence. These heterologous amino acid sequences may increase the function (e.g., signaling through an IGF receptor) of the IGF2 polypeptide *in vivo* by 10%, 20%, 25%, 30%, 40%, 50%, 75%, 100%, 150%, 200%, 200%, 400%, 500%, 1,000% or more compared to a polypeptide not comprising the heterologous amino acid sequence.

[0080] Also described herein are IGF receptor ligand fusion polypeptides or polypeptides that comprise an amino acid sequence heterologous to IGF2. In certain embodiments, the IGF receptor ligand fusion is to a heterologous amino acid sequence that promotes the stability or function of the IGF receptor ligand. In certain embodiments, the IGF2 amino acid sequence of the IGF2-heterologous polypeptide fusion protein is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 29. In certain embodiments, the IGF2 amino acid sequence of the fusion protein is 100% identical to SEQ ID NO. 29. In certain embodiments, the IGF2 amino acid sequence of the fusion protein is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 33. In certain embodiments, the IGF2 amino acid sequence of the fusion protein is 100% identical to SEQ ID NO. 33. In certain embodiments, the IGF2 amino acid sequence of the fusion protein is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 41. In certain embodiments, the IGF2 amino acid sequence of the fusion protein is 100% identical to SEQ ID NO. 41. Additional representative sequences can be found in the Table of Sequences at the end of this disclosure.

Secretory signal peptides

[0081] In certain aspects, the fusion polypeptide may comprise a secretory signal peptide. In certain embodiments, the secretory signal peptide is any well known mammalian secretory signal peptide. Production of the fusion polypeptides herein in heterologous production systems (e.g., bacteria or yeast) may result in imprecise cleavage of the signal sequence of the fusion polypeptide or non-specific early truncation at the C-terminal end of the fusion polypeptide. Processing of the secretory sequence or N- or C- terminal processing may result in loss of amino acids from either the N or the C terminus of the polypeptide.

[0082] Multimerizing components join two or more other protein components. A multimerizing component may comprise a linker sequence of amino acids that joins other components that are identical or different into a single consecutive amino acid sequence. Suitable linkers include polypeptide linkers such as a Gly-Ser linker or spacer described herein. A multimerizing component can take the form of proteins or protein domains that multimerize or dimerize, resulting in covalent disulfide linking (e.g., through the addition of one or more *de novo* cysteine residues) or non-covalent associations driving dimerization (e.g., a leucine zipper). In certain embodiments, the multimerizing components may link or multimerize a plurality of IGF2 amino acid sequences. In certain embodiments, the multimerizing components may link or multimerize two IGF2 amino acid sequences. The two IGF2 amino acid sequences may be the same, or different, and selected from any of the IGF2 sequences described herein. In certain embodiments, the multimerizing components may link or multimerize two, three, four, five or more IGF2 amino acid sequences. In certain embodiments, the multimerizing components may link or multimerize an IGF2 amino acid sequence with another polypeptide that provides fusion promoting or proliferation promoting function or increased plasma half-life.

[0083] In some embodiments, the IGF2 amino acid sequence may comprise functional fragments, mutated sequences, or modified polypeptides thereof. The Table of Sequences lists some exemplary fragments, polypeptides and modified polypeptides. In some embodiments, the IGF2 sequence is N-, C-, or O-linked glycosylated. In some embodiments, the IGF2 sequence is glycosylated at one amino acid. In some embodiments, the IGF2 sequence is glycosylated at a site corresponding to Thr96, Thr99, or Thr163.

[0084] IGF family proteins are substrates for a number of proteases for processing during maturation and degrading intracellularly and extracellularly. The M16A family zinc metalloprotease, known as Insulin Degrading Enzyme (IDE) have a high affinity (~100 nM) for IGF2 as a substrate, rapidly degrading it (Malito et al. Cell Mol Life Sci. 2008;65:2574–85). Described herein are mutations within IGF2 that reduce protease mediated cleavage of the IGF2

peptide backbone. In some embodiments, these mutations are within the C-domain, SEQ ID NO. 42. In some embodiments, mutations are specific to altering positively charged amino acids, such as arginine or lysine, to other amino acids. In some embodiments the mutations are the arginine 61 or arginine 64, as SEQ ID NO. 35 or SEQ ID NO. 37. In some embodiments, the mutations alter the positively charged amino acids to other amino acids with lower molecular weights. In some embodiments the mutations mutate the amino acid sequence into one or more alanines, as SEQ ID NO. 34 or SEQ ID NO. 36.

[0085] The IGF2 receptor ligand polypeptides and receptor ligand fusion polypeptides described herein may be encoded by nucleic acids to facilitate production of the receptor ligand polypeptide or fusion polypeptide. These nucleic acids can be compatible with bacterial, yeast, insect, or mammalian expression systems. They may comprise promoters/enhancers (either constructive or inducible), polyadenylation signals, selectable markers (such as antibiotic resistance), origins of replication or other accessory nucleic acid sequences. IGF2 sequences can be used from many organisms. In certain embodiments, the IGF2 sequence comprises a human IGF2 amino acid sequence. In certain embodiments, the IGF2 sequence comprises a cat, dog or a horse IGF2 sequence. In certain embodiments, the IGF2 sequence comprises a mouse, rat, rabbit, dog, cat, horse, cow, sheep, pig, goat, llama, alpaca, yak, or monkey sequence.

<u>IGF2 nucleic acid sequences</u>

[0086] In certain embodiments, the IGF2 nucleic acid sequence is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 17. In certain embodiments, the IGF2 nucleic acid sequence is 100% identical to SEQ ID NO. 17. In certain embodiments, the IGF2 nucleic acid sequence is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 21. In certain embodiments, the IGF2 nucleic acid sequence is 100% identical to SEQ ID NO. 21. In certain embodiments, the IGF2 nucleic acid sequence is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 23. In certain embodiments, the IGF2 nucleic acid sequence is 100% identical to SEQ ID NO. 23. In certain embodiments, the IGF2 amino acid sequence is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 32. In certain embodiments, the IGF2 amino acid sequence is 100% identical to SEQ ID NO. 32.

Heterologous peptides

[0087] The heterologous polypeptide that comprises part of the fusion proteins described herein may comprise, consist, or consist essentially of a fragment of an immunoglobulin molecule, an albumin molecule, a transferrin molecule, an XTEN sequence, a proline-alanine-serine polymer, a homo-amino acid polymer, a glycine-rich sequence, a gelatin-like polymer, an elastin-like peptide, a carboxy-terminal peptide, or combinations thereof.

[0088] In one aspect described herein the therapeutic polypeptide is IGF receptor ligand polypeptide or an IGF2 polypeptide.

[0089] In one aspect described herein the therapeutic polypeptide fused to a heterologous polypeptide amino acid sequence, either directly or through a linker, wherein the heterologous amino acid sequence imparts increased function or stability to the therapeutic polypeptide.

[0090] In one aspect of this invention, the heterologous peptide increases the stability or biological function of the therapeutic amino acid sequence. In certain embodiments, the heterologous sequence may be fused to the therapeutic amino acid sequence at the C-terminus or at the N-terminus of the therapeutic amino acid sequence. In certain aspects, the therapeutic amino acid sequence is fused to a heterologous sequence at the N-terminus. In certain embodiments, the therapeutic amino acid sequence is fused to a heterologous sequence at the C-terminus. In certain embodiments, a flexible linker is used between the therapeutic amino acid sequence and the heterologous sequence at the N terminus. In certain embodiments, a flexible linker is used between the therapeutic amino acid sequence and the heterologous sequence at the C terminus. In certain embodiments, a spacer is used between the therapeutic amino acid sequence and the heterologous sequence at the N terminus. In certain embodiments, a spacer is used between the therapeutic amino acid sequence and the heterologous sequence at the C terminus.

[0091] Heterologous peptides may be used to increase the stability or the biological function of the IGF2 amino acid sequence. Fusion proteins can be used to improve the pharmacokinetics of the biologically active molecules, such as by prolonging the half-life, as discussed in Strohl, "Fusion Proteins for Half-Life Extension of Biologics as a Strategy to Make Biobetters," *BioDrugs* (2015) 29:215-239. Fusing a polypeptide to a molecule or a fragment of a molecule with a long half-life, such as an immunoglobulin, an albumin, or a transferrin increases the half-life of the polypeptide. An XTEN sequence is a repeating amino acid polymer containing the amino acid residues A, E, G, P, S, and T which when fused to a peptide is capable of extending the half-lives of the peptides, while being otherwise inert. Fusing small repeating sequences such as proline-alanine-serine polymers (repeats of proline, alanine and serine), a homo-amino acid polymer sequence such as glycine-rich sequences (G-G-G-S), gelatin-like proteins, and elastin-like sequences (V-P-G-x-G, where x is any amino acid except proline) can also extend the half-life of a polypeptide. Fusing a polypeptide to a carboxy-terminal peptide (CTP) can increase the half-life of the polypeptide in the serum due to the strong negative change of CTP. In certain embodiments, the heterologous polypeptide comprises a fragment of an immunoglobulin molecule, an albumin molecule, a transferrin molecule, an XTEN sequence, a proline-alanine-serine polymer, a homo-amino acid polymer, a glycine-rich sequence, a gelatin-like polymer, an

elastin-like peptide, a carboxy-terminal peptide, or combinations thereof. In certain embodiments the heterologous peptides improving the pharmacokinetics of the biologically active molecule are genetically encoded to produce a fusion protein. In certain embodiments the heterologous peptides improving the pharmacokinetics of the biologically active molecule are covalently or non-covalently associated with the biologically active molecule post translationally. In certain embodiments, that non-covalent association may be driven by a genetically or covalent modified portion of the biologically active molecule.

[0092] Immunoglobulins are large effector molecules produced by the immune system. IgG immunoglobulins possess a plasma half-life of approximately 21 days. When an immunoglobulin fragment is fused to a second polypeptide, this can increase the half-life of the second polypeptide. In some embodiments, the fragment of the immunoglobulin molecule comprises the hinge domain of an IgG, the CH2 domain of an IgG, the CH3 domain of an IgG, or any combination thereof. In some embodiments, the fragment of the immunoglobulin molecule comprises the hinge domain of IgG1, the CH2 domain of IgG1, the CH3 domain of IgG1, or any combination thereof. In some embodiments, the fragment of the immunoglobulin molecule comprises the hinge domain of IgG4, the CH2 domain of IgG4, the CH3 domain of IgG4, or any combination thereof.

[0093] In some circumstances, mutations of the immunoglobulin molecule or fragment may increase the half-life or stability of the immunoglobulin molecule or fragment. In some embodiments, the fragment of the immunoglobulin molecule comprises the hinge domain of IgG1, the CH2 domain of IgG1, the CH3 domain of IgG1, or any combination thereof with one or more of the following amino acid mutations in the immunoglobulin molecule: P329G, L234A and L235A. In some embodiments, the fragment of the immunoglobulin molecule comprises an IgG4 molecule. In some embodiments, the fragment of the immunoglobulin molecule comprises an IgG4 molecule with at least one of the following amino acid mutations in the immunoglobulin molecule: N434A, N434H, T307A/E380A/N434A, M252Y/S254T/T256E, 433K/434F/436H, T250Q, T250F, M428L, M428F, T250Q/M428L, N434S, V308W, V308Y, V308F, M252Y/M428L, D259I/V308F, M428L/V308F, Q311V/N434S, T307Q/N434A, E258F/V427T, S228P, L235E, S228P/L235E/R409K, S228P/L235E, K370Q, K370E, deletion of G446, deletion of K447, and combinations thereof of IgG4 according to the EU numbering system.

[0094] Secretory signal sequences are sequence motifs that target proteins to the secretory pathway in the cell. Secretory sequences may be cleaved from the protein to produce the mature, secreted protein. In some embodiments, the polypeptide comprises a secretory signal sequence. In some embodiments, the polypeptide comprises human IGF2 secretory sequence (SEQ ID NO 9, SEQ ID NO 11, SEQ ID NO 12). In some embodiments, the polypeptide comprises a

secretory signal that is SEQ ID NO. 10, SEQ ID. NO. 13, SEQ ID NO. 14, SEQ ID NO. 15, or SEQ ID NO. 16.

Linkers and spacers

[0095] Linkers or spacers are short amino acid sequences that separate different domains in a single protein, or domains between fusion proteins. As used herein, the term "linker" and spacer" are interchangeable. Linkers can either be rigid or flexible. Rigid linkers may prevent unwanted interactions between different domains. Proline-rich linkers tend to be more rigid, while glycine rich linkers tend to be more flexible. Flexible linkers may allow domains within a single protein to interact. Another use for flexible linkers is to covalently bond protein complexes and binding partners to generate stable protein complexes. Flexible linkers may also be used to promote dimerization. Linkers and spacers are reviewed in Chichili et al, Linkers in the Structural biology of protein-protein interactions, Protein Sci. Feb 2013. 22(2): 153-167.

[0096] The fusion polypeptides described herein may further comprise a linker or a spacer amino acid sequence that separate the therapeutics polypeptide and the heterologous polypeptide. In certain embodiments, the linker or spacer is a peptide linker or spacer. In certain embodiments, the linker or spacer is a flexible linker or spacer. In certain embodiments, the linker is three alanines (AAA). In certain embodiments, the peptide linker is a glycine-serine linker. In certain embodiments, the linker is (in one-letter amino acid code): GGGGS (4GS) or multimers of the 4GS linker, such as repeats of 2, 3, 4, or 5 4GS linkers. In certain embodiments, the glycine-serine linker comprises the amino acid sequence set forth in SEQ ID NO: 43 or 44, or 2, 3, 4, 5, or repeats of SEQ ID NO: 43 or 44. In certain embodiments, the linker comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32 amino acids derived from neither the polypeptide sequences in the Table of Sequences nor the heterologous polypeptide amino acid sequences of Table of Sequences.

[0097] The linker or spacers can be a single amino acid residue or greater in length. In certain embodiments, the peptide linker is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, or 32 amino acids in length. In certain embodiments, the peptide linker has at least one amino acid residue but is no more than 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 amino acid residues in length.

Combinations of an IGF1R agonist and a short fatty acid chain

[0098] In certain aspects, disclosed herein is a composition comprising an IGF1R agonist and a short fatty acid chain. IGF1R signaling activates downstream pathways including pathways involved in cell proliferation, cell differentiation, and cell survival. The two IGF ligands, IGF1 and IGF2, activate IGF1R signaling. Additional peptides that activate IGF1R signaling are INS.

Other agonists of IGF1R include, without limitations, demethylasterriquinone B1, Ginsenoside Rg5, and the human antimicrobial peptide LL-37. In some embodiments, the IGF1R agonist comprises an IGF1R agonistic antibody, an IGF polypeptide or a functional fragment thereof, IGF2 or a functional fragment thereof, insulin, demethylasterriquinone B1, Ginsenoside Rg5, LL-37, or combinations thereof. These compositions comprise an unexpected synergistic effect and are useful for treating the muscle and/or soft-tissue conditions or disorders. This synergistic effect may also be promoted by methods comprising separate administration of an IGF1R agonist and a short fatty acid chain.

[0099] In certain embodiments, the IGF2R agonist is an IGF ligand. In certain embodiments, the IGF1R agonist is IGF2. In certain embodiments, the IGF2 polypeptide is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 29. In certain embodiments, the IGF2 polypeptide is 100% identical to SEQ ID NO. 29. In certain embodiments, the IGF2 polypeptide is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 33. In certain embodiments, the IGF2 polypeptide is 100% identical to SEQ ID NO. 33. In certain embodiments, the IGF2 polypeptide is at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to SEQ ID NO. 34. In certain embodiments, the IGF2 polypeptide is 100% identical to SEQ ID NO. 34.

[00100] In some embodiments, the composition comprises an IGF1R agonist and a short fatty acid chain. Short fatty acid chains include, without limitations, butyrates, a phenylbutyrate, valproic acid, propionic acid, methanoic acid, ethanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid, pentanoic acid, and a multimerized version thereof such as tributyrin. Butyrates include, without limitations, butyric acids, sodium butyrate, methyl butyrate, ethyl butyrate, butyl butyrate, pentyl butyrate, or sodium butyrate. In some embodiments, the short chain fatty acid is a butyrate. In some embodiments, the butyrate is butyric acid. In some embodiments, the butyrate is sodium butyrate. In some embodiment, that short chain fatty acid is a phenylbutyrate, valproic acid, propionic acid, methanoic acid, ethanoic acid, 2-methylpropanoic acid, 3-methylbutanoic acid, pentanoic acid, or a multimerized version thereof such as tributyrin.

[00101] Also described herein are methods comprising administering an IGF1R agonist and a short fatty acid chain. The administration can be in the same composition, separate formulations. When separate formulations are administered, they can be administered effectively simultaneously (e.g., during the same treatment) or separately with an interval of at least 1 hour, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, or more.

Therapeutic Indications

[00102] In certain aspects, the fusion polypeptides comprising an IGF ligand amino acid

sequence and a heterologous polypeptide, compositions comprising an IGF1R agonist and a short fatty acid chain, and the methods described herein, are useful for treating diseases and disorders that involve soft-tissue injury, degradation, or destruction, or for use in treating an individual with an aging disorder, a muscle wasting disorder, a muscle injury, an injury to a connective tissue, or an injury to a non-muscle soft-tissue, or any combination thereof.

[00103] Aging disorders that result in the deterioration and loss of muscle tissue are such disorders. Sarcopenia, for example, is the degenerative loss of skeletal muscle mass quality, and strength and can be associated with aging. Injuries that result in acute muscle damage are other muscle disorders, which are treatable by the polypeptides, compositions and methods described herein. The disorders include muscle ruptures, strains, and contusions. A rupture is a separating of the muscle tissues. Muscle strains are contraction-induced injuries in which muscle fibers tear due to extensive mechanical stress, and can be classified as a grade I, II, or III. Muscle contusions are muscle hematomas. Muscle injury can also be caused by non-mechanical stresses such as cachexia. Cachexia may be caused by malnutrition, cancer, AIDS, coeliac disease, chronic obstructive pulmonary disease, multiple sclerosis, rheumatoid arthritis, congestive heart failure, tuberculosis, familial amyloid polyneuropathy, mercury poisoning (acrodynia), Crohn's disease, untreated/severe type 1 diabetes mellitus, anorexia nervosa, chemotherapy, muscular dystrophy or other genetic diseases which cause immobility, and hormonal deficiencies. Certain disorders that are weaknesses of specific muscles such as dysphagia or facioscapulohumeral muscular dystrophy may also be treated by the polypeptides described herein. Additional soft-tissues disorders that may be treated using the polypeptides comprising an IGF ligand amino acid sequence and compositions comprising an IGF1R agonist and a short fatty acid chain described herein are those that inflict injury to the tendons, ligaments or cartilage.

[00104] In certain embodiments, the muscle wasting disease is a muscular dystrophy. In certain embodiments, the muscular dystrophy comprises a myotonic muscular dystrophy, Duchenne muscular dystrophy, Becker muscular dystrophy, Limb-girdle muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital, muscular dystrophy, oculopharyngeal muscular dystrophy, or distal muscular dystrophy. In certain embodiments, the muscular dystrophy is myotonic dystrophy.

[00105] In certain embodiments, the aging disorder is sarcopenia. In certain embodiments, the muscle wasting disorder is cachexia. In certain embodiments, the cachexia is a result of a cancer, AIDS, end stage kidney disease, or cardiovascular disease. In certain embodiments, the injury is a muscle injury. In certain embodiments, the muscle wasting is atrophy due to limb immobilization or disuse. In certain embodiments, the muscle injury is a strain or a tear. In certain embodiments, the muscle injury is a Grade III strain. In certain embodiments, sarcopenia

contributes to the incidence of the muscle injury. In certain embodiments, the injury is ligament damage. In certain embodiments, the ligament damage is a rupture or a tear. In certain embodiments, the injury is tendon damage. In certain embodiments, the tendon damage is a rupture or a tear. In certain embodiments, the injury is cartilage damage.

5 **[00106]** In certain embodiments, the compositions described herein, are for use in a method of treating myositis. In certain embodiments, the myositis comprises dermatomyositis, polymyositis, necrotizing myopathy (also called necrotizing autoimmune myopathy or immune-mediated necrotizing myopathy), juvenile myositis, or sporadic inclusion-body myositis.

[00107] In certain embodiments, the compositions described herein are for use in a method of 10 treating cartilage related-disorders. In certain embodiments, the cartilage related disorder may be due to tears, injuries, or wear. In certain embodiments, the cartilage-associated disease may be osteoarthritis, osteochondritis dissecans, achondroplasia, or degenerative cartilage lesions.

[00108] In certain embodiments, the compositions described herein are for use in a method of increasing proliferation or promoting survival of a cell associated with soft-tissue damage. In 15 certain embodiments, the polypeptides comprising an IGF ligand amino acid sequence and compositions comprising an IGF1R agonist and a short fatty acid chain described herein are useful in a method of increasing proliferation or promoting survival of any one or more of a muscle cell, a muscle precursor cell, a tenocyte, a tenocyte precursor cell, a chondrocyte, a chondrocyte precursor cell, a mesenchymal stem cell, or a fibroblast.

20 **[00109]** Muscle fibrosis is an excessive accumulation of extracellular matrix components, including collagen. Muscle fibrosis impairs muscle function, negatively affects muscle regeneration after injury, and increases muscle susceptibility to re-injury. In certain embodiments, the compositions described herein are for use in a method of reducing muscle fibrosis. In certain embodiments, the fibrosis is associated with aging, muscular dystrophy, or an 25 injury. In certain embodiments, the IGF ligand is IGF2.

[00110] In order to differentiate into mature muscle cells, myoblasts must fuse and form multinucleated cells. In certain embodiments, the fusion polypeptides comprising an IGF ligand amino acid sequence and a heterologous polypeptide, compositions comprising an IGF1R agonist and a short fatty acid chain, and the methods described herein are for use in a method of 30 increasing myoblast fusion. In certain embodiments, the IGF ligand is IGF2.

[00111] In certain embodiments, the fusion polypeptides comprising an IGF ligand amino acid sequence and a heterologous polypeptide, compositions comprising an IGF1R agonist and a short fatty acid chain, and the methods described herein are for use in a method of increasing muscle mass. In certain embodiments, muscle mass is increased by at least about 1%, 2.5%, 5%, 10%, 35 20%, 30%, 40%, 50% or more than 50%. In certain embodiments, the IGF ligand is IGF2.

[00112] In certain embodiments, the fusion polypeptides comprising an IGF ligand amino acid sequence and a heterologous polypeptide, compositions comprising an IGF1R agonist and a short fatty acid chain, and the methods described herein are for use in a method of increasing grip strength. In certain embodiments, grip strength is increased by at least about 1%, 2.5%, 5%, 10%, 20%, 30%, 40%, 50% or more than 50%. In certain embodiments, the IGF ligand is IGF2.

[00113] In certain embodiments the fusion polypeptides comprising an IGF ligand amino acid sequence and a heterologous polypeptide, compositions comprising an IGF1R agonist and a short fatty acid chain, and the methods described herein are for use in a method of increasing muscle endurance. In certain embodiments, muscle endurance is increased by at least about 1%, 2.5%, 5%, 10%, 20%, 30%, 40%, 50% or more than 50%. In certain embodiments the IGF ligand is IGF2.

Methods of treatment

[00114] In certain aspects, disclosed herein is a method of treating an individual with a disorder comprising administering an IGF1R agonist and a short fatty acid chain to the individual. In some embodiments, the IGF1R agonist and the short fatty acid chain are administered in separate formulations. In some embodiments, the IGF1R agonist and the short fatty acid chain are administered simultaneously. In some embodiments, the IGF1R agonist and the short fatty acid chain are administered at different times.

[00115] In certain aspects, disclosed herein is a method of treating an individual with a disorder comprising administering a polypeptide comprising an IGF ligand amino acid sequence and a butyrate to the individual the disorder. In some embodiments, the polypeptide comprising the IGF ligand amino acid sequence and the butyrate are administered in separate formulations. In some embodiments, the polypeptide comprising the IGF ligand amino acid sequence and the butyrate are administered simultaneously. In some embodiments, the polypeptide comprising the IGF ligand amino acid sequence and the butyrate are administered at different times.

[00116] In certain aspects, disclosed herein is a method of treating an individual with a disorder comprising administering a polypeptide comprising an IGF2 amino acid sequence and a short fatty acid chain to the individual the disorder. In some embodiments, the polypeptide comprising the IGF ligand amino acid sequence and the short fatty acid chain are administered in separate formulations. In some embodiments, the polypeptide comprising the IGF2 amino acid sequence and the short fatty acid chain are administered simultaneously. In some embodiments, the polypeptide comprising the IGF2 amino acid sequence and the short fatty acid chain are administered at different times.

[00117] In certain aspects, disclosed herein is a method of treating an individual with a disorder comprising administering a polypeptide comprising an IGF2 amino acid sequence and a

butyrate to the individual the disorder. In some embodiments, the polypeptide comprising the IGF2 amino acid sequence and the butyrate are administered in separate formulations. In some embodiments, the polypeptide comprising the IGF2 amino acid sequence and the butyrate are administered simultaneously. In some embodiments, the polypeptide comprising the IGF2 amino acid sequence and the butyrate are administered at different times.

[00118] In certain embodiments, the treatment can be administered by any suitable route such as, for example, subcutaneous, intravenous, or intramuscular. In certain embodiments, the treatment is administered on a suitable dosage schedule, for example, weekly, twice weekly, monthly, twice monthly, once every three weeks, or once every four weeks. The treatment can be administered in any therapeutically effective amount. In certain embodiments, the therapeutically effective amount is about 0.001 mg/kg to about 1 mg/kg. In certain embodiments, the therapeutically effective amount is about 0.001 mg/kg to about 0.002 mg/kg, about 0.001 mg/kg to about 0.005 mg/kg, about 0.001 mg/kg to about 0.01 mg/kg, about 0.001 mg/kg to about 0.02 mg/kg, about 0.001 mg/kg to about 0.05 mg/kg, about 0.001 mg/kg to about 0.1 mg/kg, about 0.001 mg/kg to about 0.2 mg/kg, about 0.001 mg/kg to about 0.5 mg/kg, about 0.001 mg/kg to about 1 mg/kg, about 0.002 mg/kg to about 0.005 mg/kg, about 0.002 mg/kg to about 0.01 mg/kg, about 0.002 mg/kg to about 0.02 mg/kg, about 0.002 mg/kg to about 0.05 mg/kg, about 0.002 mg/kg to about 0.1 mg/kg, about 0.002 mg/kg to about 0.2 mg/kg, about 0.002 mg/kg to about 0.5 mg/kg, about 0.002 mg/kg to about 1 mg/kg, about 0.005 mg/kg to about 0.01 mg/kg, about 0.005 mg/kg to about 0.02 mg/kg, about 0.005 mg/kg to about 0.05 mg/kg, about 0.005 mg/kg to about 0.1 mg/kg, about 0.005 mg/kg to about 0.2 mg/kg, about 0.005 mg/kg to about 0.5 mg/kg, about 0.005 mg/kg to about 1 mg/kg, about 0.01 mg/kg to about 0.02 mg/kg, about 0.01 mg/kg to about 0.05 mg/kg, about 0.01 mg/kg to about 0.1 mg/kg, about 0.01 mg/kg to about 0.2 mg/kg, about 0.01 mg/kg to about 0.5 mg/kg, about 0.01 mg/kg to about 1 mg/kg, about 0.02 mg/kg to about 0.05 mg/kg, about 0.02 mg/kg to about 0.1 mg/kg, about 0.02 mg/kg to about 0.2 mg/kg, about 0.02 mg/kg to about 0.5 mg/kg, about 0.02 mg/kg to about 1 mg/kg, about 0.05 mg/kg to about 0.1 mg/kg, about 0.05 mg/kg to about 0.2 mg/kg, about 0.05 mg/kg to about 0.5 mg/kg, about 0.05 mg/kg to about 1 mg/kg, about 0.1 mg/kg to about 0.2 mg/kg, about 0.1 mg/kg to about 0.5 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.2 mg/kg to about 0.5 mg/kg, about 0.2 mg/kg to about 1 mg/kg, or about 0.5 mg/kg to about 1 mg/kg. In certain embodiments, the therapeutically effective amount is about 0.001 mg/kg, about 0.002 mg/kg, about 0.005 mg/kg, about 0.01 mg/kg, about 0.02 mg/kg, about 0.05 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.5 mg/kg, or about 1 mg/kg. In certain embodiments, the therapeutically effective amount is at least about 0.001 mg/kg, about 0.002 mg/kg, about 0.005 mg/kg, about 0.01 mg/kg, about 0.02 mg/kg, about 0.05 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, or about 0.5

mg/kg. In certain embodiments, the therapeutically effective amount is at most about 0.002 mg/kg, about 0.005 mg/kg, about 0.01 mg/kg, about 0.02 mg/kg, about 0.05 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.5 mg/kg, or about 1 mg/kg. In certain embodiments, the therapeutically effective amount is about 0.1 mg/kg to about 50 mg/kg. In certain embodiments, the therapeutically effective amount is about 0.1 mg/kg to about 0.2 mg/kg, about 0.1 mg/kg to about 0.5 mg/kg, about 0.1 mg/kg to about 1 mg/kg, about 0.1 mg/kg to about 2 mg/kg, about 0.1 mg/kg to about 5 mg/kg, about 0.1 mg/kg to about 10 mg/kg, about 0.1 mg/kg to about 20 mg/kg, about 0.1 mg/kg to about 50 mg/kg, about 0.2 mg/kg to about 0.5 mg/kg, about 0.2 mg/kg to about 1 mg/kg, about 0.2 mg/kg to about 2 mg/kg, about 0.2 mg/kg to about 5 mg/kg, about 0.2 mg/kg to about 10 mg/kg, about 0.2 mg/kg to about 20 mg/kg, about 0.2 mg/kg to about 50 mg/kg, about 0.5 mg/kg to about 1 mg/kg, about 0.5 mg/kg to about 2 mg/kg, about 0.5 mg/kg to about 5 mg/kg, about 0.5 mg/kg to about 10 mg/kg, about 0.5 mg/kg to about 20 mg/kg, about 0.5 mg/kg to about 50 mg/kg, about 1 mg/kg to about 2 mg/kg, about 1 mg/kg to about 5 mg/kg, about 1 mg/kg to about 10 mg/kg, about 1 mg/kg to about 20 mg/kg, about 1 mg/kg to about 50 mg/kg, about 2 mg/kg to about 5 mg/kg, about 2 mg/kg to about 10 mg/kg, about 2 mg/kg to about 20 mg/kg, about 2 mg/kg to about 50 mg/kg, about 5 mg/kg to about 10 mg/kg, about 5 mg/kg to about 20 mg/kg, about 5 mg/kg to about 50 mg/kg, about 10 mg/kg to about 20 mg/kg, about 10 mg/kg to about 50 mg/kg, or about 20 mg/kg to about 50 mg/kg. In certain embodiments, the therapeutically effective amount is about 0.1 mg/kg, about 0.2 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 5 mg/kg, about 10 mg/kg, about 20 mg/kg, or about 50 mg/kg. In certain embodiments, the therapeutically effective amount is at least about 0.1 mg/kg, about 0.2 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 5 mg/kg, about 10 mg/kg, or about 20 mg/kg. In certain embodiments, the therapeutically effective amount is at most about 0.2 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 5 mg/kg, about 10 mg/kg, about 20 mg/kg, or about 50 mg/kg.

[00119] In certain embodiments, the individual treated is a mammal. In certain embodiments, the mammal is a mouse, rat, rabbit, dog, cat, horse, cow, sheep, pig, goat, llama, alpaca, or yak. In certain embodiments, the individual is a dog, cat, or a horse. In certain embodiments, the individual to be treated is a human.

Methods of production

[00120] Ther polypeptide comprising an IGF2 ligand amino acid sequence can be purified or synthesized in any suitable manner. A nucleic acid encoding the polypeptide can be cloned into a suitable vector and expressed in a suitable cellular system. In certain embodiments, the cellular system is a prokaryotic cell system. In certain embodiments, the cellular system is a eukaryotic cell system. In certain embodiments, the cellular system is a mammalian cell system. In certain

embodiments, the polypeptide may be expressed from *Eschericia coli*. In certain embodiments, the polypeptide may be expressed from a yeast cell, including without limitations, *Saccharomyces cerevisiae*, *Pichia pastoris*, *Kluyveromyces lactis*, *Hansenula polymorpha*, or *Yarrowia lipolytica*. In certain embodiments, the polypeptide may be expressed from a mouse
5 myeloma cell, including without limitations, NS0, Sp2/0, and FO. In certain embodiments, the polypeptide may be expressed from a chinese hamster ovary (CHO) cell. In certain embodiments, the polypeptide may be expressed by a mammalian cell, including without limitations, a COS cell, a Vero cell, or a BHK cell. In certain embodiments, the polypeptide may be expressed from a human cell, including without limitations a HeLa cell, a HEK-293 cell, a CAP cell, a CAP-T
10 cell, a PER.C6® cell

[00121] The supernatants from such an expression system can be subjected to one or more purification steps involving centrifugation, ultracentrifugation, filtration, diafiltration, tangential-flow filtration, dialysis, chromatography (e.g., cation exchange, ion exchange, hydrophobic interaction, reverse phase, affinity, or size exclusion). The polypeptides can be purified to an
15 extent suitable for human administration. Additionally, polypeptides can be synthesized for inclusion in a formulation to be administered to a human individual. In certain embodiments, the polypeptides can be produced by a suitable peptide synthesis method, such as solid-phase synthesis.

[00122] In certain embodiments, the mammalian expression vector pmax Cloning is used to
20 make C- In certain embodiments, the mammalian expression vector pmax Cloning is used to make C-terminally 6xHis-tagged, StrepII-tagged, and human IgG1 Fc-tagged vectors. The DNA fragments encoding the secreted myogenic factors are amplified by PCR from human open reading frame (ORF) clones, and subsequently inserted into the tagged vectors by In-Fusion cloning technology (Takara Bio Inc.). The expression vectors carrying the secreted myogenic
25 factors are transiently transfected into ExpiCHO-S cells at a density of $6x10^6$ per ml by using ExpiFectamine CHO transfection kit (Thermo Scientific).

[00123] The expressed myogenic factors with different tags in the culture supernatants are affinity-purified by using different purification media. In some embodiments, the polypeptide comprises an Fc region. For these polypeptides a matrix or resin comprising Protein A, Protein
30 G, protein L or any combination thereof can be used. The matrix or resin may suitably be loaded onto a column for ease in batch purification.

Purification of immunoglobulin fusion proteins

[00124] In certain embodiments, the heterologous sequence may comprise an immunoglobulin or a fragment thereof. When the polypeptide comprises an immunoglobulin or a fragment
35 thereof, the polypeptide may be purified by means of protein A, G, or L affinity. Protein A and G

are cell surface proteins found in *Staphylococcus aureus*. They have the property of binding the Fc region of a mammalian antibody, in particular of IgG class antibodies. For use in protein A or G affinity chromatography, protein A or G is coupled to a solid matrix such as crosslinked, uncharged agarose (Sepharose, freed from charged fraction of natural agarose), trisacryl,
5 crosslinked dextran or silica-based materials. Methods for such are commonly known in the art, e.g. coupling via primary amino functions of the protein to a CNBr-activated matrix. Protein A binds with high affinity and high specificity to the Fc portion of IgG, that is the Cγ2-Cγ3 interface region of IgG as described in Langone et al., 1982, supra. In particular, it binds strongly to the human allotypes or subclasses IgG1, IgG2, IgG3 and the mouse allotypes or subclasses
10 IgG2a, IgG2b, IgG3.

[00125] After purification by Protein A, G, or L the bound fraction can be eluted and passed over or through an additional resin or matrix comprising one or more ion exchange columns. The first ion exchanger is generally an anion exchanger resin. The pH of buffer used for loading and running the first ion exchanger is set as to put opposing total change on the Fc comprising fusion
15 polypeptide and the protein A to be separated by means of the ion exchanger in a flow-through mode according to the present invention, taking the pI's of the Fc comprising fusion polypeptide and protein A into account. The mode of operation of a first anion exchanger according to the present invention requires buffer exchange of the acidic or neutralized eluate from the protein A affinity chromatography step with the equilibrium buffer of the first anion exchanger. After the
20 first anion exchanger, the Fc comprising fusion polypeptide is ready for use in applications or may be deemed to require further polishing by customary purification methods. In a further preferred embodiment, the first ion exchange step is followed by a second ion exchange step in which second step the antibody is loaded and bound by the second ion exchange medium and is eluted with a buffer other than the loading buffer, by means of increased salt and/or pH, as an
25 essentially monomeric, non-aggregated antibody.

[00126] In certain embodiments, in the method according to the present invention at least 70%, 80%, or 90% of the Fc comprising fusion polypeptide loaded onto the first ion exchanger can be recovered in the flow-through of the ion-exchanger. In certain embodiments, disregarding glycoforms and eventual processing variants of the same Fc comprising fusion polypeptide, there
30 is only one type of species of Fc comprising fusion polypeptide present in the mixture.

Master cell bank and transgenic cells

[00127] In a certain embodiment, described herein is a master cell bank comprising a cell that comprises a nucleic acid encoding one or more IGF ligand or IGF2 fusion polypeptides integrated into its genome creating a transgenic cell-line. In some embodiments, the master cell
35 bank comprises a plurality of cells that each comprise a nucleic acid encoding an IGF ligand or

IGF2 fusion polypeptide. In certain embodiments, the nucleic acid is maintained extrachromosomally on a plasmid or yeast artificial chromosome. In certain embodiments, the nucleic acid is integrated into a chromosomal location. In certain embodiments, the cell is a yeast cell. In certain embodiments, the yeast is *Pichia pastoris* or *Saccharomyces cerevisiae*. In certain embodiments, the cell is a mammalian cell. In certain embodiments, the mammalian cell is a 293T cell or derivative thereof (e.g., 293T-Rex). In certain embodiments, the cell is a bacterial cell.

[00128] In certain embodiments, the transgenic mammalian, yeast, or bacterial cell is a master cell bank that comprises a cryopreservative suitable for freezing to at least about -80° or below. In certain embodiments, the master cell bank comprises glycerol or DMSO at between about 10 and about 30%, and is suitable for long-term storage at about -80° or below. In certain embodiments, the master cell bank can preserve a transgenic mammalian, yeast, or bacterial strain for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more years.

Pharmaceutically acceptable excipients, carriers, and diluents

[00129] The polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein can be administered in a pharmaceutical composition that comprises one or more pharmaceutically acceptable excipients, carriers, or diluents. The exact components can differ based upon the preferred route of administration. The excipients used in a pharmaceutical composition can provide additional function to the polypeptide by making the polypeptide suitable for a particular route of administration (e.g., intravenous, topical, subcutaneous, or intramuscular), increasing polypeptide stability, increasing penetration of a desired tissue (e.g., muscle or skin), increasing residence time at particular site, increasing solubility, enhancing the efficacy of the polypeptide, and/or reducing inflammatory reactions coincident with administration.

[00130] In certain embodiments, the compositions are included in a pharmaceutical composition with a solubilizing emulsifying, or dispersing agent. In certain embodiments, the solubilizing agent can allow high-concentration solutions of fusion polypeptides that exceed at least about 2 mg/mL, 5 mg/mL, 10 mg/mL, 15 mg/mL, or 20 mg/mL. Carbomers in an aqueous pharmaceutical composition serve as emulsifying agents and viscosity modifying agents. In certain embodiments, the pharmaceutically acceptable excipient comprises or consists of a carbomer. In certain embodiments, the carbomer comprises or consists of carbomer 910, carbomer 934, carbomer 934P, carbomer 940, carbomer 941, carbomer 1342, or combinations thereof. Cyclodextrins in an aqueous pharmaceutical composition serve as solubilizing and stabilizing agents. In certain embodiments, the pharmaceutically acceptable excipient comprises or consists of a cyclodextrin. In certain embodiments, the cyclodextrin comprises or consists of

alpha cyclodextrin, beta cyclodextrin, gamma cyclodextrin, or combinations thereof. Lecithin in a pharmaceutical composition may serve as a solubilizing agent. In certain embodiments, the solubilizing agent comprises or consists of lecithin. Poloxamers in a pharmaceutical composition serve as emulsifying agents, solubilizing agents, and dispersing agents. In certain embodiments, the pharmaceutically acceptable excipient comprises or consists of a poloxamer. In certain embodiments, the poloxamer comprises or consists of poloxamer 124, poloxamer 188, poloxamer 237, poloxamer 338, poloxamer 407, or combinations thereof. Polyoxyethylene sorbitan fatty acid esters in a pharmaceutical composition serve as emulsifying agents, solubilizing agents, surfactants, and dispersing agents. In certain embodiments, the pharmaceutically acceptable excipient comprises or consists of a polyoxyethylene sorbitan fatty acid ester. In certain embodiments, the polyoxyethylene sorbitan fatty acid ester comprises or consists of polysorbate 20, polysorbate 21, polysorbate 40, polysorbate 60, polysorbate 61, polysorbate 65, polysorbate 80, polysorbate 81, polysorbate 85, polysorbate 120, or combinations thereof. Polyoxyethylene stearates in a pharmaceutical composition serve as emulsifying agents, solubilizing agents, surfactants, and dispersing agents. In certain embodiments, the pharmaceutically acceptable excipient comprises or consists of a polyoxyethylene stearate. In certain embodiments, the polyoxyethylene stearate comprises or consists of polyoxyl 2 stearate, polyoxyl 4 stearate, polyoxyl 6 stearate, polyoxyl 8 stearate, polyoxyl 12 stearate, polyoxyl 20 stearate, polyoxyl 30 stearate, polyoxyl 40 stearate, polyoxyl 50 stearate, polyoxyl 100 stearate, polyoxyl 150 stearate, polyoxyl 4 distearate, polyoxyl 8 distearate, polyoxyl 12 distearate, polyoxyl 32 distearate, polyoxyl 150 distearate, or combinations thereof. Sorbitan esters in a pharmaceutical composition serve as emulsifying agents, solubilizing agents, and non-ionic surfactants, and dispersing agents. In certain embodiments, the pharmaceutically acceptable excipient comprises or consists of a sorbitan ester. In certain embodiments, the sorbitan ester comprises or consists of sorbitan laurate, sorbitan oleate, sorbitan palmitate, sorbitan stearate, sorbitan trioleate, sorbitan sesquioleate, or combinations thereof. In certain embodiments, solubility can be achieved with a protein carrier. In certain embodiments the protein carrier comprises recombinant human albumin.

[00131] In certain embodiments, the polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein are formulated to increase stability. Polypeptides in aqueous formulations may require stabilization to prevent degradation. In certain embodiments, the stabilizer comprises pH buffers, salts, amino acids, polyols /disaccharides /polysaccharides, liposomes, surfactants, antioxidants, reducing agents, or chelating agents. In certain embodiments, the stabilizer comprises or consists of a polyol/non-reducing sugar. In certain embodiments, the non-reducing sugar comprises or consists of sucrose,

mannitol, trehalose, raffinose, stachyose, xylitol, starch, verbascose, or combinations thereof. Polypeptides can be encapsulated in liposomes to increase stability. In certain embodiments, the stabilizer comprises or consists of liposomes. In certain embodiments, the liposomes comprise or consists of ipalmitoylphosphatidylcholine (DPPC) liposomes, phosphatidylcholine:cholesterol (PC:Chol) (70:30) liposomes, or dipalmitoylphosphatidylcholine: dipalmitoylphosphatidylserine (DPPC:DPPS) liposomes (70:30). Non-ionic surfactants can increase the stability of a polypeptide. In certain embodiments, the stabilizer comprises or consists of a non-ionic surfactant. In certain embodiments, the non-ionic surfactant comprises or consists of polysorbates (e.g., poly sorbate 80, poly sorbate 20), alkylsaccharides alkyl ethers and alkyl glyceryl ethers, polyoxyethelene (4) lauryl ether; polyoxyethylene cetyl ethers, polyoxyethylene stearyl ethers, sorbitan fatty acid esters, polyoxyethylene fatty acid esters, or combinations thereof. In certain embodiments, the polypeptide is formulated with a protein surfactant, such as recombinant human serum albumin as a stabilizer. Antioxidants or reducing agents can increase the stability of a polypeptide. In certain embodiments, the stabilizer comprises or consists of an antioxidant or reducing agent. In certain embodiments, the reducing agent comprises or consists of dithiothreitol, ethylenediaminetetraacetic acid, 2-Mercaptoethanol, Tris(2-carboxyethyl)phosphine hydrochloride, Tris(hydroxypropyl)phosphine, or combinations thereof. In certain embodiments, the antioxidant comprises or consists of methionine, ascorbic acid, citric acid, alpha tocopherol, sodium bisulfite, ascorbyl palmitate, erythorbic acid, or combinations thereof. Chelating agents can stabilize polypeptides by reducing the activity of proteases. In certain embodiments, the stabilizer comprises or consists of a chelating agent. In certain embodiments, the chelating agent comprises or consists of ethylenediaminetetraacetic acid (EDTA), ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), metal complexes (e.g. Zn-protein complexes), or combinations thereof. Buffer agents can stabilize polypeptides by reducing the acid hydrolysis of polypeptides. In certain embodiments, the stabilizer comprises or consists of a buffer agent. In certain embodiments, the buffer agent comprises or consists sucrose octa-sulfate, ammonium carbonate, ammonium phosphate, boric acid, sodium citrate, potassium citrate, lactic acid, 3-(N-morpholino)propanesulfonic acid (MOPS), 2-(N-morpholino)ethanesulfonic acid (MES), hydroxymethylaminomethane (Tris), calcium carbonate, calcium phosphate or combinations thereof.

[00132] The polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein also may be entrapped in or associated with microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal delivery systems (for example,

liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

[00133] The polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein may be formulated or delivered with an anti-inflammatory agent. In certain embodiments, the anti-inflammatory agent comprises or consists of a corticosteroid. In certain embodiments, the corticosteroid comprises or consists of hydrocortisone, cortisone, ethamethasoneb (Celestone), prednisone (Prednisone Intensol), prednisolone (Orapred, Prelone), triamcinolone (Aristospan Intra-Articular, Aristospan Intralesional, Kenalog), methylprednisolone (Medrol, Depo-Medrol, Solu-Medrol), or dexamethasone (Dexamethasone Intensol). In certain embodiments, the anti-inflammatory comprises or consists of a non-steroidal anti-inflammatory (NSAID). In certain embodiments, the NSAID comprises or consists of aspirin, celecoxib, diclofenac, diflunisal, etodolac, ibuprofen, indomethacin, ketoprofen, ketorolac, nabumetone, naproxen, oxaprozin, piroxicam, salsalate, sulindac, or tolmetin.

[00134] In certain embodiments, the polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein are included in a pharmaceutical composition suitable for intravenous administration comprising one or more pharmaceutically acceptable excipients, carriers, and diluents. In certain embodiments, the polypeptides of the current disclosure are administered suspended in a sterile solution. In certain embodiments, the solution is one commonly used for administration of biological formulations, and comprises, for example, about 0.9% NaCl or about 5% dextrose. In certain embodiments, the solution further comprises one or more of: buffers, for example, acetate, citrate, histidine, succinate, phosphate, potassium phosphate, bicarbonate and hydroxymethylaminomethane (Tris); surfactants, for example, polysorbate 80 (Tween 80), polysorbate 20 (Tween 20), and poloxamer 188; polyol/disaccharide/polysaccharides, for example, glucose, dextrose, mannose, mannitol, sorbitol, sucrose, trehalose, and dextran 40; amino acids, for example, glycine, histidine, leucine, or arginine; antioxidants, for example, ascorbic acid, methionine; or chelating agents, for example, EDTA, or EGTA.

[00135] In certain embodiments, the polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein are included in a pharmaceutical composition suitable for intramuscular or subcutaneous administration comprising one or more pharmaceutically acceptable excipients, carriers, and diluents. Formulations suitable for intramuscular or subcutaneous injection can include physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, and

sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and non-aqueous carriers, diluents, solvents, or vehicles include ethanol, polyols (inositol, propyleneglycol, polyethylene-glycol, glycerol, cremophor and the like) and suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity is maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. Formulations suitable for subcutaneous injection also contain optional additives such as preserving, wetting, emulsifying, and dispensing agents.

[00136] In certain embodiments, the polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein are formulated for topical administration as a cream, gel, paste, ointment, or emulsion. Excipients in a cream, gel, paste, ointment, or emulsion can comprise gelatin, casein, lecithin, gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glyceryl monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene stearates, colloidol silicon dioxide, phosphates, sodium dodecyl sulfate, carboxymethylcellulose calcium, carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethycellulose phthalate, noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, polyvinylpyrrolidone, sugars, and starches.

[00137] The excipient used with the polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein will allow for storage, formulation, or administration of highly concentrated formulations. In certain embodiments, a highly concentrated fusion polypeptide(s) comprises at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 20, 25, 40, 45, 50 or more milligrams per milliliter.

[00138] In certain embodiments, the polypeptides and/or compositions of the current disclosure are shipped/stored lyophilized and reconstituted before administration. In certain embodiments, lyophilized ligand fusion polypeptide formulations comprise a bulking agent such as, mannitol, sorbitol, sucrose, trehalose, and dextran 40. The lyophilized formulation can be contained in a vial comprised of glass. The fusion polypeptides when formulated, whether reconstituted or not, can be buffered at a certain pH, generally less than 7.0. In certain embodiments, the pH can be between 4.5 and 6.5, 4.5 and 6.0, 4.5 and 5.5, 4.5 and 5.0, or 5.0 and 6.0.

Kits

[00139] Also described herein are kits comprising one or more of the polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide

described herein in a suitable container and one or more additional components selected from: instructions for use; a diluent, an excipient, a carrier, and a device for administration.

[00140] In certain embodiments, described herein is a method of preparing a soft tissue or muscle disease or disorder treatment comprising admixing one or more pharmaceutically acceptable excipients, carriers, or diluents and polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide described herein. In certain embodiments, described herein is a method of preparing a soft tissue or muscle disease or disorder treatment for storage or shipping comprising lyophilizing one or more antibodies of the current disclosure.

EXAMPLES

Example 1 – Expression and purification of recombinant proteins

[0001] Mammalian expression plasmids carrying genes with different tags were transiently transfected into CHO cells. The genes were expressed to produce proteins that were subsequently secreted into the culture medium. The proteins in the culture medium were visualized on polyacrylamide gels and their activities were measured by *in vitro* functional assays. Then the recombinant proteins in the culture medium were affinity purified. The purified proteins were visualized on polyacrylamide gels to evaluate the purity and assayed by *in vitro* functional assays to determine their biological activities.

[0002] **Expression vector engineering:** Mammalian expression vector pmax Cloning was used to make C-terminally 6xHis-tagged, StrepII-tagged, and human IgG1 and IgG4 Fc-tagged vectors. The DNA fragments encoding the secreted myogenic factors were amplified by PCR from human open reading frame (ORF) clones, and subsequently inserted into the tagged vectors by In-Fusion cloning technology (Takara Bio Inc.).

[0003] **Expressing secreted myogenic polypeptides:** The expression vectors carrying the secreted myogenic factors were transiently transfected into ExpiCHO-S cells at a density of $6x10^6$ per ml by using ExpiFectamine CHO transfection kit (Thermo Scientific). After 18-22 hours, CHO feed and enhancer were added into the transfected culture. Then the expressed proteins were monitored by SDS-PAGE every 24 hours to achieve maximal expression level. In most of the cases, cell culture was collected at day 4, and cells were spun down. The supernatant was spun down again to get rid of cellular debris. The clarified culture supernatant containing the secreted myogenic factors was stored at -80 °C or immediately processed for use.

[0004] **Measuring expression level of secreted myogenic polypeptides:** To measure the improved expression level of the secreted myogenic factors, three protein analytical techniques were applied: sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting, and enzyme-linked immunosorbent assay (ELISA). Western Blots were performed to

identify the myogenic factors. ELISAs were used to measure the absolute amount of myogenic factors in the culture supernatant.

[0005] **Isolation of engineered myogenic polypeptides:** The expressed myogenic factors with different tags in the culture supernatants were affinity-purified by using different purification media. For Fc-fusion factors, either Protein A magnetic beads (GenScript) or Protein A membrane column (Takara Bio Inc.) were used to specifically bind to the Fc-fusion factors. For 6xHis-tagged factors, NTA-magnetics beads (NEB) were used to isolate the factor.

Example 2 – Purified IGF2-hFcm promoted differentiation of human myoblast cells.

[0006] **FIG 1A:** The suspension CHO cells were transiently transfected with the IGF2-hFcm encoding plasmid. IGF2-hFcm was affinity-purified by Protein A membrane column. The purified IGF2-hFcm was added into the culture of human myoblast cells for 96 hours. Myosin heavy chain (MyHC) was immunostained and imaged by a fluorescence microscope. The percentage area of MyHC of human myoblasts treated with the purified IGF2-hFcm is significantly higher than the percentage area of MyHC of human myoblasts treated with the vehicle control (One-Way ANOVA Tukey Honest Significant Difference, n=2-6).

Condition	%MyHC	SD	p-value
Vehicle control	1.787	0.186	
33nM IGF2-hFcm	3.734	0.790	0.012
66nM IGF2-hFcm	5.922	0.795	3.20E-05
133nM IGF2-hFcm	7.568	0.538	1.46E-06

Example 3 **IGF2-LhFc4 promoted differentiation of human myoblast cells**.

[0007] **FIG 1B**: The suspension CHO cells were transiently transfected with the IGF2-LhFc4 encoding plasmid. IGF2-LhFc4 was affinity-purified by Protein A membrane column. The purified IGF2-LhFc4 was added into the culture of human myoblast cells for 96 hours with daily media change. Myosin heavy chain (MyHC) was immunostained and imaged by a fluorescence microscope. The percentage area of MyHC of human myoblasts treated with the purified IGF2-LhFc4 is significantly higher than the percentage area of MyHC of human myoblasts treated with the vehicle control (One-Way ANOVA Tukey Honest Significant Difference, n=2-6).

Condition	%MyHC	SD	p-value
Vehicle control	1.384	0.285	
hFc4L-IGF2	5.820	0.319	0.011
IGF2-hFc4	6.901	0.537	0.004

IGF2-LhFc4	6.237	1.848	0.007

Example 4 - **Purified HSA-L-IGF2R61A differentiation of human myoblast cells**.

[0008] **FIG 2A:** The suspension CHO cells were transiently transfected with the HSA-L-IGF2R61A encoding plasmid. HSA-L-IGF2R61A was affinity-purified by Protein A membrane column. The purified HSA-L-IGF2R61A was added into the culture of human myoblast cells for 96 hours with daily media change. Myosin heavy chain (MyHC) was immunostained and imaged by a fluorescence microscope. The percentage area of MyHC of human myoblasts treated with the purified HSA-L-IGF2R61A is significantly higher than the percentage area of MyHC of human myoblasts treated with the vehicle control (One-Way ANOVA Tukey Honest Significant Difference, n=2-6).

Condition	%MyHC	SD	p-value
Vehicle control	0.34	0.221	
HSA-IGF2	7.079	2.009	0.013
HSA-IGF2R61A	6.914	2.691	0.014

Example 5 **IGF2 and IGF2 receptors are expressed in human myoblast.**

[0009] Bar graph and quantitation table of IGF2 **(FIG 2B)** and IGF2 receptor **(FIG 2C)** RNASeq expression in young (17-21 year old caucasian males) and aged human myoblast (68-69 year old caucasian males) cell lines. Myoblast were cultured growth media (GM) or 96h in fusion media (FM). Fresh media was added every 24h. Mean±SEM. n=6. Expression are expressed as FPKM. Significant p-values (Young GM ~ Aged GM: 3.54E-04).

FIG 2B

n=6	IGF2 (FPKM)	SEM	p-val (n=6)
Young GM	13.11	3.275	--
Aged GM	3.413	1.12	3.54E-04
Young FM	17.68	6.42	--
Aged FM	13.08	3.67	n.s.

FIG 2C

n=6	IGF2R (FPKM)	SEM	P-val (n=6)
Young GM	74.93	9.45	--
Aged GM	75.01	6.89	n.s.
Young FM	82.35	3.43	--

Aged FM	88.44	9.86	n.s.

Example 6 **Sodium butyrate enhances muscle fusion.**

[0010] Mouse myoblasts were treated with PBS or sodium butyrate at concentrations 0.1 nM, 1 nM, and 10 nM. Myoblasts were cultured for 48 hours, with fresh media added every 24 hours. Cells were pulsed for 2-5 hours with EdU (30 uM), ethanol fixed, stained with Hoescht 3342, immunostained for proliferation -as measured by the percent of cells staining positive for EdU (%EdU)-, and immunostained for differentiation -as measured by the increase in cellular area staining positive for embryonic myosin heavy chain (%eMyHC) relative to the negative controls, which received media and vehicle only. When compared to untreated myoblasts, the cells treated with 1 nM of sodium butyrate had increased rates of fusion, as depicted in **FIG. 3A**. Significance was determined by a p-value less than 0.05 by the one-way ANOVA Tukey Honest Significant Difference test.

[0011] **FIG 3A**: Bar graph of fusion index in response to sodium butyrate (NaBut) compared to vehicle. Myoblast were cultured 48h in the presence of NaBut at indicated dose. Fresh media and NaBut were added every 24h. Mean∓S.D. Table with quantitation of fusion index and p-values also shown. (*p<0.05 by Student's Two-tailed T-test, n=3-5)

Condition	Fusion Index (nuclei/myotube)	p-value
vehicle	7.56	---
NaBut 0.1nM	8.44	n.s.
NaBut 1nM	31.60	2.02E-3
NaBut 10nM	14.00	n.s.

Example 7 Sodium butyrate enhances IGF2 activity.

[0012] Human myoblast cells were treated with either PBS (vehicle), IGF2 (15 ng/mL), sodium butyrate, or IGF2 and sodium butyrate. Fresh media was added every 24 hours. After 96 hours, cells were pulsed for 2-5 hours with EdU (30 uM), ethanol fixed, stained with Hoescht 3342, immunostained for proliferation -as measured by the percent of cells staining positive for EdU (%EdU)-, and immunostained for differentiation -as measured by the increase in cellular area staining positive for embryonic myosin heavy chain (%eMyHC) relative to the negative controls, which received media and vehicle only. The total area of eMyHc positive cells was analyzed, and treated cells were compared to cells treated with the vehicle alone. Cells that had been treated with IGF alone and two conditions in which cells had been treated with IGF2 and sodium butyrate produced a significant increase in the amount of differentiation. There was a significant increase in the total area of eMyHC cells in the cells treated with 1nM and 100nM of

sodium butyrate and IGF2, compared to the cells treated with IGF2 alone. Significance was determined by a p-value less than 0.05 by the one-way ANOVA Tukey Honest Significant Difference test.

[0013] **FIG 3B**: Bar graph of fusion index of mouse myoblast in response to sodium butyrate (NaBut) compared to vehicle. Mouse myoblast were cultured 48h in the presence of NaBut at indicated dose. Fresh media and NaBut were added every 24h. Mean∓S.D. Table with quantitation of fusion index and p-values shown. (*p<0.05 by Student's Two-tailed T-test, n=3-5). Significant p-values (Vehicle ~ IGF2: 0.015 ug/mL: 6.33E-06, Vehicle ~ NaBut: 1 nM IGF2: 0.015 ug/mL: 1.79E-11, Vehicle ~ NaBut: 100 nM IGF2: 0.015 ug/mL: 1.79E-11)

Table of data for FIG 3B

Condition	%eMyHC	SD	p-value
Vehicle	6.813	1.695	-----
IGF2: 0.015 ug/mL	10.843	1.308	
NaBut: 1 nM	2.321	0.374	
NaBut: 10 nM	6.199	1.174	
NaBut: 100 nM	8.341	0.477	
NaBut: 1 nM IGF2: 0.015 ug/mL	28.387	1.036	1.79E-11
NaBut: 10 nM IGF2: 0.015 ug/mL	9.274	0.654	
NaBut: 100 nM IGF2: 0.015 ug/mL	29.239	3.185	1.79E-11

[0014] **FIG 3C** Bar graph quantitation of %Area eMyHC+ human myoblast in response to indicated treatment compared to IGF2 (15 ng/mL). Myoblast were cultured 96h in the presence of IGF2 at indicated dose. Fresh media and IGF2 was added every 24h. Mean∓S.D. (*p<0.05 by One-Way Anova Tukey Honest Significant Difference, n=2-12)

Table of data for FIG 3C

Condition	%eMyHC	SD	p-value
IGF2: 0.015 ug/mL	10.843	1.308	
NaBut: 1 nM IGF2: 0.015 ug/mL	28.387	1.036	6.18E-8
NaBut: 10 nM IGF2: 0.015 ug/mL	9.274	0.654	
NaBut: 100 nM IGF2: 0.015 ug/mL	29.239	3.185	3.50E-3

Example 8 Sodium butyrate enhances IGF2 activity.

[0015] Human myoblast cells were treated with either PBS (vehicle), IGF2 (15 ng/mL),

sodium butyrate, or IGF2 and sodium butyrate. Fresh media was added every 24 hours. After 48 hours, cells were pulsed for 2-5 hours with EdU (30uM), ethanol fixed, stained with Hoescht 3342, immunostained for proliferation -as measured by the percent of cells staining positive for EdU (%EdU)-, and immunostained for differentiation -as measured by the increase in cellular
5 area staining positive for embryonic myosin heavy chain (%eMyHC) relative to the negative controls, which received media and vehicle only. The total area of eMyHc positive cells was analyzed, and treated cells were compared to cells treated with the vehicle alone, as seen in **FIG. 4A.** Myoblasts that had been treated with either 0.03 ug/mL of IGF2 or with IGF2 in combination with sodium butyrate showed a significant increase in the eMyHC+ area when
10 compared to cells cultured with the vehicle alone.

Table of data for Fig 4A

Condition	%eMyHC	SD	p-value
Vehicle	6.813	1.695	---
IGF2: 0.03 ug/mL	16.620	1.301	1.42E-08
NaBut: 1 nM	2.321	0.374	n.s.
NaBut: 10 nM	6.199	1.174	n.s.
NaBut: 100 nM	8.341	0.477	n.s.
NaBut: 1 nM IGF2: 0.03 ug/mL	24.615	0.258	1.79E-11
NaBut: 10 nM IGF2: 0.03 ug/mL	22.821	0.234	1.80E-11
NaBut: 100 nM IGF2: 0.03 ug/mL	28.427	3.136	1.79E-11

[0016] The myoblasts that had been treated with a combination of IGF2 and sodium butyrate were compared to the cells treated with IGF2 alone. There was a significant increase in all cells treated with the combination compared to cells treated with IGF2 alone, as depicted in **FIG 4B**
15 and **Table**. Significance was determined by a p-value less than 0.05 by the one-way ANOVA Tukey Honest Significant Difference test.

Table of data for Fig 4B

Condition	%eMyHC	SD	p-value
IGF2: 0.03 ug/mL	16.620	1.301	---
NaBut: 1 nM IGF2: 0.03 ug/mL	24.615	0.258	1.88E-3
NaBut: 10 nM IGF2: 0.03 ug/mL	22.821	0.234	4.80E-3

NaBut: 100 nM IGF2: 0.03 ug/mL	28.427	3.136	1.87E-3

Example 9 IGF2 enhances MYOG expression in DM1 human myoblast cells.

[0017] **FIG 5A** Bar graph of myogenic gene expression fold change in DM1 human myoblast in response to indicated treatment compared to FM (vehicle). Myoblasts were cultured 48h in the presence of factors (BMP7 50ng/mL, Butyrate 100nM, IGF2 200 ng/mL). Mean∓S.D.
5 Significant p-values (FM ~ IGF2: 4.94E-04, FM ~ IGF2_NaBut: 6.53E-03) **(*p<0.01)** Table of mean and p-value of MYF5, MYOD1, and MYOG (n=3).

Table of data for FIG 5A

Condition	MYF5	MYF5 p-value	MYOD1	MYOD1 p-value	MYOG	MYOG p-value
FM	1.000		1.000		1.000	
BMP7	0.709	n.s.	0.709	n.s.	0.361	n.s.
NaBut	1.128	n.s.	1.095	n.s.	1.020	n.s.
IGF2	0.730	n.s.	1.252	n.s.	2.972	4.94E-04
IGF2 NaBut	0.820	n.s.	1.500	n.s.	3.483	6.53E-03

Example 10 IGF2 Receptor is expressed on chondrocyte and osteocytes

[0018] **FIG 6A**: Bar graph showing IGF2 receptors are expressed on cartilage-associated
10 cells. Data is derived from Ramilowsky et al., Nature 2015.

Table of data for FIG 6A RNA Expression (TPM)

Cell Type	IGF2R
Preadipocyte (Subcutaneous)	27.083
Chondrocyte	47.63
Osteocyte	83.96
Tenocyte	23.12

Example 11 IGF2 treatment promotes proliferation and fusion in DM1 human myoblast (32 year old caucasian female) cells.

[0019] **FIG 7A** Bar graph of %EdU+ human myoblast (32 year old caucasian female) and
15 **FIG 7B** %area MyHC in response to IGF2. Myoblast were cultured 72h for proliferation and 96h for fusion in the presence of indicated factor. Mean∓S.D. Mean∓SD. Significant p-values (EdU: Vehicle ~ IGF2: 6.8E-3, %eMyHC Area: Vehicle ~ IGF2: 1.9E-4) (*p<0.05 by Students Two-

Tailed T-test, n=3-6).

Table of data for FIG 7A

n=3-6	EdU FC	s.d.	p-val
Vehicle	1.0	0.02	
IGF2	2.18	0.32	6.8E-3

Table of data for FIG 7B

n=3	%eMyHC area	s.d.	p-val
Vehicle	0.45	0.02	
IGF2	5.49	0.54	1.9E-4

5 ***Example 12 IGF2 enhances MYH3, CKM, and ATP1B1 expression in DM1 human myoblast (32 year old caucasian female) cells.***

[0020] **FIG 8A** Bar graph of MYH3 and CKM expression fold change in DM1 human myoblast (32 year old caucasian female) in response to indicated treatment compared to vehicle. Myoblasts were cultured 96h in the presence of factors (IGF2 200ng/mL). Mean∓S.D.
10 Significant p-values (MYH3: Vehicle ~ IGF2: 1.13E-03, CKM: Vehicle ~ IGF2: 7.67E-03) **FIG 8B** Bar graph of ATP1B1 expression fold change in DM1 human myoblast (32 year old caucasian female) in response to indicated treatment compared to FM (vehicle). Myoblasts were cultured 48h in the presence of factors (IGF2 200ng/mL). Mean∓S.D. Significant p-values (Vehicle ~ IGF2: 3.11E-05) (*p<0.05 by Students Two-Tailed T-test, n=3).

15 **Table of data for Fig 8A**

	MYH3	p-val	CKM	p-val
Vehicle	1		1	
IGF2	14.833	1.13E-03	5.165	7.67E-03

Table of data for Fig 8B

n=3	ATP1B1	p-val
Vehicle	1	
IGF2	3.01789	3.11E-05

Example 13 Systemic administration of IGF2/NaB protects against aging induced muscle dysfunction.

20 **[0021]** **FIG 9A:** Subcutaneous injection of IGF2(50ug/kg) or NaB (1.2g/kg),

IGF2/NaB(150ug/kg; 1.2g/kg) or vehicle (PBS) were administered to 21-24M old mice for 14 days. Muscle function was assessed at days 13 and 14. (**FIG 9B)** Grip strength force assessed at day 13. The first graph **FIG 9B** shows Bothlimb grip strength force, **** p<0.0001, **p=0.0043, **p=0.001(One-way ANOVA, multiple comparisons). **FIG 9C)** Forelimb force, ****p<0.0001, *p=0.0368, *p=0.0187 (One-way ANOVA, multiple comparisons). **FIG 9D)** Treadmill performance measured at day 14 using an induced treadmill running model set to progressively increase speed 2 m/min every subsequent 2 min. Distance ran shown. ***p=0.0005, *p=0.0459, ****p<0.0001 (One-way ANOVA, multiple comparisons) **FIG 9E)** Time to exhaustion ***p=0.0002, **p=0.0024 (One-way ANOVA, multiple comparisons) **FIG 9F)** Maximum speed ***p=0.0004, **p=0.0013 **FIG 9G)** Work in kj **p=0.0026, **p=0.0035 (One-way ANOVA, multiple comparisons).

Example 14 Systemic administration of IGF2/NaB is safe.

[0022] **FIG 10A)** Subcutaneous injection of vehicle or IGF2/NaB were administered to 21M old mice for 14 days, blood and serum were collected to assess complete blood count and a metabolic panel for liver, kidney and pancreas function. **FIG 10B-E)** 4 representative graphs out of 37 readouts measured showing the white blood cell count (Unpaired t-test, p=0.8020), Albumin concentration (Unpaired t-test, p>0.9999), Creatinine concentration (Unpaired t-test, p=0.5490) and Calcium concentration (Unpaired t-test, p=0.811)**.**

Example 15 Systemic administration of IGF2/But protects against Dexamethasone induced muscle atrophy.

[0023] **FIG 11A)** Dexamethasone (25 mg/kg i.p.) was administered to 12 weeks old mice for 14 days simultaneously with a subcutaneous injection of IGF2/NaB (150ug/kg; 1.2g/kg) or vehicle (PBS). Muscle function was assessed at day 13-14. Grip strength force assessed at day 13, graphs showing **FIG 11B)** bothlimb force and **FIG 11C)** specific bothlimb force measured on Day 13. Specific bothlimb force calculated as the ratio of bothlimb force in mN over the weight in g, *** p=0.0003, ***p=0.0004 (Unpaired t-test). **FIG 11D)** Grip strength force assessed at day 13, graphs showing **FIG 11D)** forelimb force and **FIG 11E)** forelimb specific force measured on Day 13. Specific forelimb force calculated as the ratio of forelimb force in mN over the weight in g, ** p=0.0012, ***p=0.0005 (Unpaired t-test). **FIG 11F)** At day 15, mice were euthanized and TAs were collected for histological analysis, graphs showing muscle fiber size distribution assessed using SMASH software. **p= 0.054, *p= 0.037, and **** p<0.0001 (2-way ANOVA, multiple comparisons).

Example 16 Myogenic activity measurement assay in vitro

Myoblast proliferation assay

[0001] Reduced regeneration from an individual's tissue progenitor cells is a hallmark of age

or disease related dysfunction, therefore assays that measure mitogenic capacity in tissue progenitor cells serve as a read-out for potential success of a treatment. Measuring the increased proliferation rate, degree of differentiation, and cellular survival of treated mouse or human muscle progenitor cells will provide good basis for potentially therapeutic regenerative factors for treating individuals who have suffered illness, injury, or who possess genetic or developmental defects leading to premature tissue loss, wasting, or weakening.

[0002] Mouse muscle progenitor cells (early passage myoblasts) were cultured and expanded in mouse growth medium: Ham's F-10 (Gibco), 20% Bovine Growth Serum (Hyclone), 5 ng/mL FGF2 and 1% penicillin-streptomycin on Matrigel coated plates (1:300 matrigel: PBS), at 37°C and 5% CO2. For experimental conditions, cells were plated at 40,000 cells/well on Matrigel coated 8-well chamber slides in 250-500 µL medium per well (1:100 matrigel: PBS) in mouse fusion medium: DMEM (Gibco) + 2% horse serum (Hyclone). One hour after plating, mouse myoblasts were treated with 50% respective media: Mouse myoblasts were cultured for 24 hours in the above conditions, at 37°C in 10% CO2 incubator. BrdU (300µM) in DMSO was added for 2 hours prior to fixation with cold 70% ethanol and stored at 4°C until staining.

[0003] Human muscle progenitor cells (early passage myoblasts) were cultured and expanded in growth medium: Ham's F-10 (Gibco), 20% Bovine Growth Serum (Hyclone), 5 ng/mL FGF2 and 1% penicillin-streptomycin on Matrigel coated plates (1:300 matrigel: PBS), at 37°C and 5% CO2. For experimental conditions, cells were plated at 40,000 cells/well on Matrigel coated 8-well chamber slides in 250-500 µL medium per well (1:100 matrigel: PBS) in human fusion medium: DMEM (Gibco) + 2% horse serum (Hyclone). One hour after plating, human myoblasts were treated with 50% respective media: Mouse myoblasts were cultured for 24 hours in the above conditions, at 37°C in 10% CO2 incubator. BrdU (300µM) in DMSO was added for 2 hours prior to fixation with cold 70% ethanol and stored at 4°C until staining.

[0004] Testing in the in vitro myogenesis assay described above using healthy human muscle precursors from 32- year-old female demonstrated HSA-IGF2R61A (FIG. 17A) mutant sequences retain equal activity relative to IGF2 (FIG. 17B) at equimolar concentrations across a range of test article concentrations.

[0005] Testing in the in vitro myogenesis assay described above using healthy human muscle precursors from 32-year-old female demonstrated mutant sequence HSA-IGF2R61A retains equal or increased activity to HSA-IGF2 at equimolar concentrations before or after size exclusion purification via HPLC FIG 20.

Quantifying Regenerative Index

[0006] Following permeabilization in PBS + 0.25% Triton X-100, antigen retrieval was performed. Primary staining was performed with primary antibodies including: a species-specific

monoclonal antibody for mouse anti-embryonic Myosin Heavy Chain (eMyHC, hybridoma clone 1.652, Developmental Studies Hybridoma Bank) and Rat-anti-BrdU (Abcam Inc. ab6326). Secondary staining with fluorophore-conjugated, species-specific antibodies (Donkey anti-Rat-488, #712-485-150; Donkey anti-Mouse-488, #715-485-150. Nuclei are visualized by Hoechst staining. Using the Hoechst stain to tally cell numbers, the percent of cells positive for BrdU and eMyHC were tabulated and reported.

Testing in Myotonic Dystrophy muscle precursor cells

[0007] Testing in the in vitro myogenesis assay described above using human DM1 muscle precursors from 32- year-old female demonstrated HSA-IGF2R61A (**FIG. 16A**) mutant sequences retain equal activity relative to IGF2 (**FIG. 16B**) at equimolar concentrations across a range of test article concentrations.

Example 17 Myogenic Gene Profiling for pro-regenerative factors

[0008] Expression of myogenic factors Pax7, Myf5, Myod1, and Myog are key indicators of the functional status of muscle progenitor cells. Factors upregulating of Pax7 and Myf5 indicate rejuvenation of proliferative progenitor cells whereas upregulation of Myod1 and Myog are indicative of muscle myofiber regeneration. A read-out of these gene expressions will provide potential success for any given polypeptide comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide or combinations of an Insulin-like Growth Factor 1 Receptor (IGF1R) agonist and a short chain fatty acid described herein. Measuring myogenic genes in mouse or human muscle progenitor cells treated with factors will provide a good characterization of the therapeutic effect for treating individuals who have suffered injury, or who possess genetic or developmental defects leading to premature tissue loss, wasting, or weakening. As a control, the assay will also be performed on proteins purified from differentiated cells, which result in no in myoblast proliferation, cultured in medium conditioned by differentiated cells, or purified heparin-associated fractions.

[0009] RNA was isolated from each well (RNeasy Mini Kit, Qiagen) and cDNA was obtained by reverse-transcription (High Capacity Reverse Transcription Kit, Thermo Fisher Scientific). Real-time quantitative PCR was performed using QuantStudio3 (Thermo Fisher).

[0010] Aged human myoblasts were cultured in well plates. Culturing the cells with the different medias resulted in differential induction of myogenic gene expression. All factors resulted in changes in at least one myogenic receptor gene at 48 hours and 72 hours when compared to cells cultured in fusion media, as depicted in the Table below. Cells that had been cultured with IGF2 had increases in levels of MYOG at 48 hours and levels of MYOD at 72 hours.

Table: Myogenic transcription factor fold change increase in myoblasts cultured with IGF2

Condition	MYF5 -48h	MYOD1 -48h	MYOG -48h	MYF5 -72h	MYOD1 -72h	MYOG -72h
FM	1.04	1.001	1.013	1.023	1.055	1.092
IGF2	0.409	0.519	5.756	0.708	5.723	0.018

Myogenic gene profiling in human or mouse progenitor cells

[0011] Human or mouse muscle progenitor cells will be plated and cultured as described above for myogenic activity testing. One hour after plating, myoblasts will be treated with respective factors. Myoblasts are analyzed for expression of Pax7, Myf5, Myod1, and Myog to characterize the regenerative effect of treatment with polypeptides comprising an IGF2 amino acid sequence and will be tested to characterize the effects an amino acid sequence from a heterologous polypeptide or combinations of an Insulin-like Growth Factor 1 Receptor (IGF1R) agonist and a short chain fatty acid.

Example 18 In vivo testing of stem cell secreted factors

[0012] Multiple *in vivo* models of muscle degeneration will be tested. Given that polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide or combinations of an Insulin-like Growth Factor 1 Receptor (IGF1R) agonist and a short chain fatty acid described herein have regenerative properties in *in vitro* models, these *in vivo* models will show that similar regenerative and proliferative effects in the context of intact organ systems.

Acute Injury Model

[0013] The experimental groups will be: C57BL/6J male mice, N = 18; Young: 12-13 week old (3-month-old) mice, n=6; Aged: 77-78 week old (18-month-old) mice, n=12. This design will be used to test any single factor identified and validated in *in vitro* assays or polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide or combinations of an Insulin-like Growth Factor 1 Receptor (IGF1R) agonist and a short chain fatty acid.

[0014] On Day 0, mice will be weighed and undergo muscle injury with focal injection of barium chloride ($BaCl_2$,10 µL, 1.2% w/v in saline, Sigma-Aldrich) in the Tibialis anterior (TA; Day 0) of both the right and left hindlegs. Injections of vehicle or factor A (0.1 mg/kg) will be co-administered intramuscularly (i.m) following the $BaCl_2$ into the TA injured hindleg sites, and again 48 hours later on day 2 (i.m.) into the TA injured hindleg sites. Also on day 2, $BaCl_2$ (Ctx; 10 µL, 1.2% w/v in saline, Sigma-Aldrich) was injected into the Gastrocnemius (GA, Day 2, i.m.) muscles of both right and left hind legs. Injections of vehicle or a factor will be sequentially

administered (i.m.) following the BaCl2 into the TA hindleg sites post-injury, and again 48 hours later on day 4 (i.m.) into the GA injured hind leg sites. Bromodeoxyuridine (BrdU) will be administered (100 mg/kg, i.p.) once daily for 3 days, day 2-4, before sacrifice to label proliferating cells.

5 **[0015]** On day 5, animals will be sacrificed, and animal weight recorded followed by collecting 0.5 ml of terminal blood via cardiac puncture which was processed to plasma and stored at 80 °C. We then perfuse the animal with 1xPBS, carefully dissect the skin from the GA/TA muscles of each hind leg and took photos (prior to excision). After excision of exclusively the GA or TA muscle, excised tissue is photographed, weighed, then placed into 25%

10 sucrose in PBS at 4°C for 4 hr rinsed in 1x PBS, immersed in Tissue-TEK OCT and rapidly frozen before storing the muscles tissues frozen at 80 °C. Cryosectioning and H&E will be performed to ensure muscle injury site was appropriately visualized. Muscle tissue composition from new skeletal muscle fibers, fibrotic tissue, and adipose (fat), will be measured. Muscle regeneration, as defined as the number of new myofibers with centrally located nuclei per

15 millimeter, fibrosis as defined as the area of fibrotic scarring, size of the fibers, as defined as the width and area, adipose tissue, as defined by the amount of fat surrounding the muscle, will be measured to assess level of regeneration.

<u>Sarcopenia/Chronic Administration Model</u>

[0016] The experimental design is C57BL/6J male mice, N = 18; Young: 12-13 week old (3-

20 month-old) mice, n=6; Aged: 77-78 week old (18-month-old) mice, n=12. This design can be used to test any single factor identified and validated in *in vitro* assays or complex mixtures of 2 or more factors or synergistic small molecules.

[0017] On Day 0, mice will have the following *in vivo* healthspan measurements will be performed over 1 day as a baseline for age-based parameters: Weight, running wheel

25 performance, grip strength, and horizontal bar. Each assay should be run for 4 trials per assay per animal. These healthspan assays will be repeated on day -1. After one day of rest on day -9, mice will begin 1x daily injections (0.1 mg/kg) of vehicle or factor A for the remainder of the experiment until sacrifice (days -8 to +5, 13 days of dosing). On day -4, 6 days after dosing begins, mice will undergo a repeat of the healthspan assays. On day 0, 5 days prior to sacrifice,

30 mice will undergo muscle injury with focal injection of cardiotoxin (Ctx; 10 µg, Sigma-Aldrich) in the Tibialis anterior (TA; day 0) of the right hindleg only. On day 2, the Gastrocnemius (GA; day 2) muscle of the right hind leg will then receive cardiotoxin (Ctx; 10 µg, Sigma-Aldrich). BrdU will be administered (100 mg/kg, i.p.) once daily for 3 days, day 2-4, before sacrifice. On day +5, prior to take-down, the animals will have an in vivo incapacitance assay run. On day +5,

35 animals will be sacrificed, and animal weight recorded. We will Collect 0.5 ml of blood via

cardiac puncture, process to plasma and store plasma samples at 80 °C. The animals will then be perfused with 1xPBS. Carefully dissect the skin from the GA/TA muscles of each hind leg and take photos (prior to excision). After excision of exclusively the GA or TA muscle, we will weigh the muscles, then place muscles into 25% sucrose in PBS at 4°C for 4 hours, then rinse the muscles in 1x PBS, adding Tissue-TEK OCT and storing the muscles tissues frozen at 80 °C. Perform cryosectioning and H&E, ensuring muscle injury site is appropriately visualized. Carefully excising the inguinal white adipose tissue (WAT) will be weighed.

[0018] Muscle tissue composition, from new skeletal muscle fibers, fibrotic tissue, and adipose (fat), will be measured. Muscle regeneration, as defined as the number of number of new myofibers with centrally located nuclei per millimeter, fibrosis, as defined as the area of fibrotic scarring, size of the fibers, as defined as the width and area, adipose tissue, as defined by the amount of fat surrounding the muscle, will be measured to assess level of regeneration. Weights of the animals during the duration of treatment, as well as healthspan assays including performance on a running wheel (speed, distance, duration), grip strength, and performance on a horizontal bar will take into account the phenotypic outcomes of treatment of the aged animals systemically with the polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide or combinations of an Insulin-like Growth Factor 1 Receptor (IGF1R) agonist and a short chain fatty acid.

[0019] The horizontal bar test will be performed as described previously (Malinowska et al. 2010) at 8 months (n = 6 WT, n = 7 MPS IIIB) and 10 months (n = 3 WT, n = 4 MPS IIIB) of age. In brief, a 300-mm metal wire, 2 mm in diameter, was secured between two posts 320 mm above a padded surface. The mouse will be allowed to grip the center of the wire and the time to fall or reach the side was recorded, and after 2 minutes the test was stopped. Crossing the bar in x seconds will be scored as 240−x, remaining on the bar will be scored as 120, and falling off the bar after y seconds will be recorded as the value of y. The test will be repeated three times as a practice run followed by a 10-min rest prior to three tests where the score was recorded.

[0020] Animals will also have better healthspan outcomes: reduced weight, fat composition, scar tissue around muscles, increased running speed, duration, and distance, increased grip strength, and enhanced performance on the horizontal bar test.

<u>Genetically obese muscle dystrophy model</u>

[0021] Genetically obese (*ob/ob*) mice will be injected with BaCl2 on day 0 in the TA muscle. 3 mice will be treated with vehicle only, 3 mice will be injected with the hPSC factors and 3 mice will be treated with FGF19 (positive control) on day 0 and day 2. On day 5, the mice will be euthanized, the TA muscles perfused with PBS, and dissected**.** Muscles will be then analyzed for regenerative index and fibrotic index.

Methods of testing muscle strength, endurance and function

[0022] **Forelimb and Both limb grip strength test**: After 30 min acclimation, the mice are introduced to the grip strength meter. For forelimb grip strength, the mice held by the tail are allowed to grasp the grip bar with only its forelimbs. For both limb measurements the mice are placed on the grid and allowed to grasp the grid with both limbs. The force generated by each mouse is calculated as the average of 5-6 measurements.

[0023] **Limb endurance test:** Mice are allowed to discover and acclimate the rodent treadmill environment through 2 training sessions of 10 minutes each at 10m/min on separate days prior to the endurance test. For the endurance test, mice are placed in the individual lanes of the rodent treadmill. The speed is gradually increased at 2m/min until exhaustion is reached. Exhaustion is defined as a mouse staying on a grill electrified to deliver a shock of 2Hz, intensity 5 for 3-5 seconds.

[0024] ***In vivo* tetanic force measurement:** Mice are under anesthesia using regulated delivery of isoflurane during the whole process. Following anesthetization, the animal is placed onto a heated chamber with the foot secured on the foot pedal of an Aurora force transducer. The 2 electrodes are placed specifically to stimulate the sciatic nerve. The force generated by the ankle torsion of the animal's hind limb, as opposed to direct force is measured in response to a series of stimulation that includes 50, 100, 150 and 200 Hz.

[0025] ***In situ* tetanic force measurement:** This experiment is performed using Aurora force measurement. Mice are under anesthesia during the whole process. A small incision in the skin around the Anterior Tibialis exposes the Achilles tendon which is connected via surgical suture to the Aurora force transducer through a hook. The force generated by the muscle in response to a series of stimulation that includes 50, 100, 150 and 200 Hz by 2 electrodes placed on the anterior tibialis is recorded.

Example 19 – Mitogenic polypeptide stability in vivo assayed by bioavailability and pharmacokinetics

Bioavailability in tissues

[0026] The bioavailability of the therapeutic polypeptides will be assessed in the target tissues in young mice (10-12 weeks old) and old mice (78 weeks old). For this experiment, 1 cohort of young mice (10-12 weeks old; N=24) and 1 cohort of old mice (78 weeks old; N=24) will receive 1 subcutaneous (SC) injection of a therapeutic composition. 4 young mice (10-12 weeks old; N=6) and 4 old mice (78 weeks old; N=6) will receive 1 SC injection of Vehicle and used as control. 4 mice from each cohort will be euthanized after 30 minutes, 1 hour, 1.5 hours, 2 hours, or 4 hours. At each time point blood will be collected by heart puncture followed by harvesting select tissues, such as the tibialis anterior, gastrocnemius, quadriceps, heart and

diaphragm. The detection and quantitation of the administered therapeutic polypeptides will be detected by enzyme-linked immunosorbent assay (ELISA). The level of therapeutic polypeptides will be compared to the samples collected from mice injected with vehicle to determine tissue level bioavailability.

5 Pharmacokinetics of engineered mitogenic polypeptides

[00141] Murine pharmacokinetics (PK) represents the absorption, distribution, metabolism, and elimination of drugs from the body. The pharmacokinetic profile of the therapeutic polypeptides were determined in mice (10-12 weeks old). Mice were fed ad libitum and housed under controlled conditions of lighting (12-hour light/12-hour dark) and temperature (22-24°C).
10 Mice were allowed to acclimate for 3 days prior to the initiation of the experiment. Intravenous (IV) injection in 10-12 weeks old mice. Engineered mitogenic polypeptides concentrations in the samples were measured by ELISA. Various pharmacokinetics will be calculated as well as the absorption/elimination dynamics following different routes of administration. Non-Compartment Analysis fit of pharmacodynamics data for intravenous administration of the HSA-IGF2R6 (Seq.
15 ID 81) **FIG 18A** in mice which demonstrates significantly improved serum half-life compared to the natural sequence of IGF2 (Seq. ID 76) **FIG 18B**.

Table of data for FIG 18. HSA-IGF2R61A demonstrated increased stability compared to IGF2

Molecule	Half-life	Dose
HSA-IGF2R61A	20.81 hr	10 mg/kg
IGF2	2.85 hr	600 ug/kg

20 ***Example 20- The purified IGF2-hFcm promoted differentiation of myoblast cells***

[0001] Suspension CHO cells were transiently transfected with the IGF2-hFcm encoding plasmid. After four days, the culture supernatants were collected and IGF2-hFcm was affinity-purified by Protein A membrane column. The purified IGF2-hFcm was added into the culture of human myoblast cells. Myosin heavy chain (MyHC) was immunostained and imaged by a
25 fluorescent microscope. After quantification of the stained MyHC, the percentage area of MyHC was calculated as the percent of pixels within the field that are illuminated above background in the stained channel. The percentage of EdU of mouse myoblasts treated with the purified IGF2-hFcm is significantly higher than the percentage of EdU of mouse myoblasts treated with the culture supernatant of CHO cells expressing the empty control vector. Significance was
30 determined by a p-value less than 0.05 by the one-way ANOVA Tukey Honest Significant Difference test.

Table: IGF2 promoted differentiation of myoblast cells

Condition	%MyHC	SD	p_value
Vehicle control	1.787	0.186	
33nM IGF2-hFcm	3.734	0.790	0.012
66nM IGF2-hFcm	5.922	0.795	3.20E-05
133nM IGF2-hFcm	7.568	0.538	1.46E-06

[0002] This example found that the IGF2-fusion protein was able to induce cell proliferation. The IGF2-fusion protein shares *in vitro* properties with the HAPs, which is suggestive of shared *in vivo* properties.

5 ***Example 21 - Modelling treatment of a muscular dystrophy with an IGF2 composition in vitro***

[0003] Muscular dystrophies (MD) encompass a variety of muscular degeneration diseases typically due to genetic mutations in genes encoding proteins responsible for forming and stabilizing skeletal muscle. The phenotypic consequence of these genetic mutations is the progressive loss of muscle mass and strength over time, similar to sarcopenia but with different 10 underlying causes. As HAPs provided phenotypic improvements on sarcopenic muscle, we tested for similar improvements in a model for MD.

[0004] IGF2 was tested individually for its ability to promote proliferation and/or fusion of human muscle progenitor cells from an individual with myotonic dystrophy type 1 (hMD) - a muscular dystrophy caused by mutations in the DMPK1 gene. The effect of IGF2 on myogenic 15 activity was assayed in biological triplicate across a range of concentrations centered around expected physiological levels by adding each factor to hMD myoblasts for 72 hours with daily media changes (DMEM +2% horse serum) and a second pulse of factors at the first media change. After 72 or 96 hours, cells were pulsed for 2-5 hours with EdU (30 uM), ethanol fixed, stained with Hoescht 3342, immunostained for proliferation -as measured by the percent of cells 20 staining positive for EdU (%EdU)-, and immunostained for differentiation -as measured by the increase in cellular area staining positive for embryonic myosin heavy chain (%eMyHC) relative to the negative controls, which received media and vehicle only. Wells were imaged on a Keyence BZ-100 at 4x, the images quantified in Cell Profiler, and the statistics were computed in R. Additionally, RNA was extracted from myoblast and select transcript abundances quantified 25 by qPCR. **FIG.s 7A** and **7B** depicts IGF2 treatment promoted proliferation and differentiation respectively in DM1 human myoblast (32 year old caucasian female) cells. **FIGs 8A** and **8B** depict IGF2 enhanced MYH3, CKM, and ATP1B1 expression in DM1 human myoblast (32 year old caucasian female) cells.

Example 22- Systemic administration of therapeutic polypeptides reverses sarcopenia and protects from muscle injury

[0005] A daily subcutaneous injection of therapeutic polypeptides or vehicle only is administered to 78 week old mice for 14 days. IGF2 is injected at a concentration of 100-1000 μg/kg. In some experiments, treatment groups receive a single therapeutic factor while in other experiments, treatment groups receive a combination of factors. At 7 days, muscle function is assessed using forelimb grip strength and both grip strength. On day 12, 13 and 14, groups 1 and 2 are injected with BrdU intraperitoneally. On days 13-15, all mice are assessed for grip strength and an endurance test to determine max distance and max speed and tetanic force.

[0006] At 15 days, mice in groups 1 and 2 are euthanized and the muscles are analyzed for markers of proliferation and fibrosis. At 15 days, an intramuscular injection of 1.2% of $BaCl_2$ (7ul/TA) is used to generate chemical injury in the TAs of group 3 and group 4. Mice from groups 3 and 4 continue to receive a therapeutic polypeptide injected subcutaneously on days 15-21. They also receive BrdU injections intraperitoneally on days 19, 20, and 21. On day 21, the TA muscles are tested for *in situ* tetanic force. The TA muscles are dissected and assessed for signs of proliferation and fibrosis.

Example 23- Systemic administration of fusion polypeptides reversed induced muscle atrophy.

[0007] 12-week-old mice are divided into 3 treatment groups: group 1 which receives injections only of the vehicle, group 2 which receives injections of dexamethasone, and group 3 which receives injections of dexamethasone and IGF2 fusion polypeptide. Dexamethasone (25 mg/kg i.p.) is administered for 14 days simultaneously with a subcutaneous injection of IGF2 fusion polypeptide.

[0008] At 7 days, mice are assessed for forelimb and both limb grip strength. At days 13-15, mice are assessed for grip strength, *in vivo* tetanic force, and undergo a treadmill endurance test to determine max speed and max distance.

Example 24- Systemic administration of IGF2 fusion polypeptide predicted to improve muscle atrophy in genetically obese mice.

[0009] Thirteen-week old genetically obese mice (*ob/ob*) will be injected subcutaneously with an IGF2 fusion polypeptide for 14 days. At day 7, forelimb and both grip strength will be measured. BrdU is injected on days 12, 13 and 14. On days 13, 14 and 15, forelimb and both limb grip strength and *in vivo* tetanic force will be tested, and an endurance test to determine max distance and max speed is performed. At 14 days, the mice will be euthanized, and the TA muscles dissected. Muscle weight and proliferation will be analyzed.

Example 25- Systemic administration of IGF2 fusion polypeptide predicted to reverse of slow down dystrophic features in 70 weeks old mdx mice

[0010] Another class of human myopathies in need of treatment are the genetic abnormality induced muscular dystrophies, among which Duchenne muscular dystrophy is a rare but fatal case. Old genetically dystrophic (mdx) mice (>15 month old) show similar features to the human Duchenne muscular dystrophy (DMD), notably, a decrease in muscle regeneration leading to muscle wasting. Treatment with IGF2 fusion polypeptide can reverse the dystrophic features of old mdx mice. During the acclimation period, the weight, Forelimb and both limb grip strength as well as *in vivo* tetanic force will be assessed to determine the baseline strength of each mouse. 70 week dystrophic mice (*mdx)* are injected with the IGF2 fusion polypeptide subcutaneously for 14 days. At day 7, forelimb and both grip strength are measured. BrdU is injected on days 12, 13 and 14. On days 13, 14, and 15, forelimb and both limb grip strength and *in vivo* tetanic force are tested, and an endurance test to determine max distance and max speed is performed. The right tibialis anterior and gastrocnemius will be collected, immersed in Tissue-TEK OCT and then flash frozen in chilled isopentane bath precooled in liquid nitrogen and stored at -80°C. Tissue will be sectioned and stained for Laminin to determine the cross sectional area (CSA) of muscle fibers, for eMyHC to measure new fiber formation and for BrdU to assess the proliferation rate. The left anterior tibialis and gastrocnemius will be collected and flash frozen in liquid nitrogen for molecular analysis that include qPCR and western blot.

[0011] IGF2 is predicted to be effective at a concentration of 10-200ug/kg.

Example 26- Systemic administration of IGF2 fusion polypeptide improved dystrophic features in 10 week old mice

[00142] Between 3-6 weeks old, the skeletal muscle of mdx mice undergoes severe necrosis followed by an increase in the activation of satellite cells to promote muscle regeneration. Treatment with IGF2 fusion polypeptide described herein can improve the regeneration process and therefore muscle health. Mice were fed *ad libitum* and housed under controlled conditions of lighting (12-hour light/12-hour dark) and temperature (22-24oC). Mice were allowed to acclimate for 3 days prior to the initiation of the experiment. During the acclimation period, the weight, forelimb and both limb grip strength as well as in vivo tetanic force were assessed to determine the baseline strength of each mouse. 10 week old dystrophic mice (*mdx)* were injected with the IGF2 fusion polypeptide with sodium butyrate subcutaneously for 14 days**.** At day 7, forelimb and both grip strength are measured as described in Example 4. BrdU was injected on days 12, 13 and 14. On days 13, 14 and 15, forelimb and both limb grip strength and *in vivo* tetanic force were tested, and an endurance test to determine max distance and max speed was performed using methods described in Example 4.

[00143] Mice were euthanized. The right tibialis anterior and gastrocnemius were collected, immersed in Tissue-TEK OCT and then flash frozen in a chilled isopentane bath precooled in

liquid nitrogen and stored at -80°C. Tissue was sectioned and stained for Laminin to determine the cross sectional area (CSA) of muscle fibers, for eMyHC to measure new fiber formation and for BrdU to assess the proliferation rate. The left anterior tibialis and gastrocnemius were collected and flash frozen in liquid nitrogen for molecular analysis that include qPCR and western blot.

[00144] IGF2 was effective at improving muscle weight (Unpaired t-test, p=0.0055) **FIG 12A**, bothlimb grip strength (Unpaired t-test, p=0.0011) **12B**, forelimb grip strength (Unpaired t-test, p<0.001) **12C**, treadmill running distance (Unpaired t-test, p=0.0035) **12D,** and treadmill running time to exhaustion (Unpaired t-test, p=0..0023) **12E**, and total work (Unpaired t-test, p=0.0022) **12F** at a concentration of 150 ug/kg with 1.2g/kg sodium butyrate administered subcutaneously every 24 hours.

Example 27- Engineering the sequence of IGF2 to reduce backbone cleavage

[00145] The amino acid backbone of IGF2 is cleaved when expressed from Chinese Hamster Ovary (CHO) cells (**FIG. 13A**) as visualized on a reducing SDS-PAGE and Western blot (**FIG. 13B**). The CHO cells were grown in a 37°C incubator with a humidified atmosphere of 8% CO2 on an orbital shaker platform with a shaking speed 125rpm to reach 4E6 cells/mL, followed by transferring the calculated volume of cells to fresh, pre-warmed ExpiCHO™ Expression Medium in a shake flask. Incubated flasks in a 37°C incubator with a humidified atmosphere of 8% CO2 shook on an orbital shaker platform until cultures reached a density of 4 × 106 – 6 × 106 viable cells/mL.

After mixing each plasmid with 100ul SFM in a microtube; ExpiFectamine (8ul x 10.5 = 84ul) with OptiPro medium (92ul x 10.5 = 966ul) in a microtube; adding 100ul into the plasmid tube and mix by pipetting; After 5min, add 200ul into each well and swirl to mix. mix enhancer (15x10.2=153ul) with feed (600x10.2=6.12ml), add 615ul into each well. Culture supernatant collected at intervals was spun down at low speed and high speed, then frozen at -80deg. Aliquots were analyzed for protein concentration by nanodrop, and then an equivalent amount loaded onto 4-12% SDS-PAGE gels for molecular weight distribution analysis. The cleavage of IGF2 was confirmed by expression of a construct a of double tagged IGF2, n-terminus tagged with human serum albumin, HSA, and c-terminus tagged with human immunoglobulin heavy chain 4, hFc4, **FIG 14**. The sequence of IGF2 was engineered to prevent cleavage by mutation one or more arginines as confirmed by visualization on a reducing SDS-PAGE followed by Western blotting to detect 6xHIS tag **FIG 15B** in and in following purification with mini Protein A columns as visualized on a reducing SDS-PAGE **FIG 15A**.

Example 28- Clinical testing of IGF2 fusion polypeptides

[00146] In physiological conditions IGF2 will bind IGF binding proteins (IGFBPs). The

purpose of this study was to determine if mutant sequences of IGF2 interact with IGFBPs. Retention time across a size exclusions chromatography column is proportional to the size of the molecule. Thus molecular interactions can be ascertained by a corresponding change in retention time on a size exclusions chromatography column. Measuring the retention of HSA-IGF2R61A
5 and rhIGFBP3 separately and as a 1:1 stoichiometric mixture demonstrated evidence of preserved affinity as demonstrated by mass shifts in size exclusion chromatography by HPLC (**FIG 19**).

Example 29-Clinical testing of IGF2 fusion polypeptides

[00147] The purpose of this study is to determine the safety, tolerability, and
10 pharmacokinetics of repeat dosing with multiple dose levels of polypeptides comprising an IGF2 amino acid sequence and an amino acid sequence from a heterologous polypeptide in healthy individuals or individuals diagnosed with sarcopenia, a muscular dystrophy, or recovery from surgery. In certain embodiments, the muscular dystrophy is myotonic dystrophy. In addition, this study will generate data on the physical function, skeletal muscle mass and strength resulting
15 from treatment with IGF2 fusion polypeptides in such individuals. Individuals will be administered placebo or IGF2 fusion polypeptide compositions and monitored for 25 weeks of study. The following primary and secondary outcome measures will be assessed:

Primary Outcome Measures:

[00148] Safety and tolerability as assessed by various measures such as percent of adverse
20 events per study arm.

Secondary Outcome Measures:

[00149] Plasma Pharmacokinetics (Cmax, Tmax, AUC) [Plasma at 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hrs after dosing.]

[00150] Short Physical Performance Battery (SPPB). Change from baseline to week 25.

25 **[00151]** 10-meter walk test. Change from baseline to week 25.

[00152] Change in total lean body mass and appendicular skeletal muscle index measured by Dual-energy X-ray Absorptiometry (DEXA) or MRI from baseline to week 25.

Inclusion Criteria:

[00153] Diagnosis of sarcopenia, a muscular dystrophy, or recovery from surgery; Low
30 muscle mass as confirmed by DXA; Low gait speed; SPPB score less than or equal to 9; Weigh at least 35 kg; with adequate dietary intake as determined by patient interview. Independently ambulatory to 10 meters.

Protocol

[00154] Patients will be i.v.-administered placebo (5% dextrose solution) or treatment article
35 (in 5% dextrose). Starting on day 1, week 1 and repeated every week (day one of weeks 1

through 25). At the end of week 13 and 25 patients will be assessed by the above methods for improvement. Doses will be selected from a traditional 3 + 3 design, and selected as the top two-doses that lack dose-limiting toxicity.

Example 30 – Administration of 6HIS-HSA-L-IGF2R61A Enhances Myogenic Activation, Fusion and Maturation in an Acute Injury Model

[00155] The acute injury model of Example 18 was used. Brown female C57/BL6 (NIA) mice (20 months of age) were injected intramuscularly (IM) in each tibialis anterior with cardiotoxin at day one (10 ug/20 uL) and from day two the mice were given IM injections every other day with 0.4 ug/20 uL of 6HIS-HSA-IGF2R61A (SEQ ID NO: 34) or vehicle (0.4 ug/20 uL of HSA).

[00156] Mice receiving 6HIS-HSA-IGF2R61A showed reduced muscle fatigue compared to control treatment (vehicle) as measured by specific fatigue index. These results are shown in FIG. 21A. Mice receiving the 6HIS-HSA-IGF2R61A showed increased force production relative to control treatment (vehicle) as measured by specific force-frequency. These results are shown in FIG. 21B. Mice receiving 6HIS-HSA-IGF2R61A showed increased force production comparted to control treatment (vehicle) as measured by max contraction rate index. These results are shown in FIG. 21C. Mice receiving 6HIS-HSA-IGF2R61A showed improved relation rates relative to control treatment (vehicle) index. These results are shown in FIG. 21D. Mice receiving 6HIS-HSA-IGF2R61A showed increased regenerative index compared to control treatment (vehicle) as measured by number of new fibers per square millimeter. These results are shown in FIG. 21E. Mice receiving 6HIS-HSA-IGF2R61A showed increased muscle mass relative to control treated (vehicle) mice. These results are shown in FIG. 21F.

Example 31 – Administration of 6HIS-HSA-L-IGF2R61A and Sodium Butyrate Preserved Muscle Function and Fiber Size in a Sarcopenia Model

[00157] The sarcopenia model of Example 18 was used. Brown male C57/BL6 (NIA) mice (83-86 weeks of age) were injected every other day with 6 mg/kg of 6HIS-HSA-IGF2R61A (SEQ ID NO: 34) and 0.3g/kg of sodium butyrate (NaB), or vehicle for 4 weeks to measure muscle regeneration and functional changes.

[00158] Blood glucose was measured in both groups and normalized to the baseline level within each mouse. Treatment with 6HIS-HSA-IGF2R61A and NaB decreased blood glucose relative to control (vehicle) treated mice. These results are shown in FIG. 22A.

[00159] Limb Grip strength was measured in both groups and normalized by body weight within each mouse to determine specific force generation. Treatment with 6HIS-HSA-IGF2R61A and NaB increased specific force generation relative to mice treated with control (vehicle). These results are shown in FIG. 22B. Forelimb Grip strength was measured in both groups and

normalized by body weight within each mouse to determine specific force generation. Treatment with 6HIS-HSA-IGF2R61A and NaB increased specific force generation relative to mice treated with control (vehicle). These results are shown in FIG. 22C. Recovery of muscle force production was measured in both groups. Treatment with 6HIS-HSA-IGF2R61A and NaB increased force generation relative to mice treated with control (vehicle) as measured by twitch force. These results are shown in FIG. 22D. Force-frequency was measured in both groups and normalized by body weight within each mouse to determine specific force frequency. Treatment with 6HIS-HSA-IGF2R61A and NaB increased specific force generation relative to mice treated with control (vehicle). These results are shown in FIG. 22E.

Example 32 – Administration of 6HIS-HSA-L-IGF2R61A and Sodium Butyrate Preserved Muscle Function and Fiber Size in a Sarcopenia Model

[00160] The sarcopenia model of Example 18 was used. Brown male C57/BL6 (NIA) mice (23 months of age) were injected daily with 6 mg/kg of HSA-IGF2R61A (SEQ ID NO: 34) and 0.3g/kg of sodium butyrate(NaB), or vehicle for 2 weeks to measure muscle regeneration and functional changes.

[00161] The fiber type distribution was measured in both groups. The fiber type 2A was lower and type 2B higher in the 6HIS-HSA-IGF2R61A and NaB treated mice compared to the mice treated with control (vehicle). These results are shown in FIG. 23A. The cross-sectional areas (CSA) were measured and plotted by group. The mice treated with 6HIS-HSA-IGF2R61A and NaB showed an increase in muscle tissue with larger CSA values compared to mice treated with control (vehicle). These results are shown in FIG. 23B.

Example 33 – Administration of 6HIS-HSA-L-IGF2R61A and Sodium Butyrate Restored Muscle Function, Fiber Size, and Fiber Composition in a Myotonic Dystrophy (DM1) Mouse Model

[00162] Brown male TRED960I(+/+)/M2rtTA(+/-) mice were fed doxycycline chow from birth, and muscle wasting phenotype was observed by weeks 12-14. Mice were injected daily with 6 mg/kg of 6HIS-HSA-IGF2R61A (SEQ ID NO: 34) and 0.3g/kg of sodium butyrate(NaB) or vehicle for 2 weeks to measure muscle regeneration and functional changes, and post-mortem histology analysis.

[00163] The fiber type distribution was measured in both groups. The fiber types 2A and 2B were significantly different (P-value < 0.05) in the two groups with 2A being decreased and 2B increased in mice treated with 6HIS-HSA-IGF2R61A and NaB compared to mice treated with control (vehicle). These results are shown in FIG. 24A. The cross-sectional areas (CSA) were measured and plotted by group. The mice treated with 6HIS-HSA-IGF2R61A and NaB showed an increase in muscle tissue with larger CSA values compared to mice treated with control

(vehicle). These results are shown in FIG. 24B.

[00164] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

[00165] All publications, patent applications, issued patents, and other documents referred to in this specification are herein incorporated by reference as if each individual publication, patent application, issued patent, or other document was specifically and individually indicated to be incorporated by reference in its entirety. Definitions that are contained in text incorporated by reference are excluded to the extent that they contradict definitions in this disclosure.

Table of Sequences			
Seq ID No	Sequence Name	Description	Sequence
1	spFGF-17	Human FGF-17 secretory signal peptide nucleotide sequence	ATGGGAGCCGCCCGCCTGCTGCCCAACCTCACTCTGTGCTTACAGCTGCTGATTCTCTGCTGTCAA
2	spTHBS1	Human THBS1 secretory signal peptide nucleotide sequence	ATGGGGCTGGCCTGGGGACTAGGCGTCCTGTTCCTGATGCATGTGTGTGGCACC
3	spIGF-2	Human IGF-2 secretory signal peptide nucleotide sequence	ATGGGAATCCCAATGGGGAAGTCGATGCTGGTGCTTCTCACCTTCTTGGCCTTCGCCTCGTGCTGCATTGCT
4	spBMP-7	Human BMP-7 secretory signal peptide nucleotide sequence	ATGCACGTGCGCTCACTGCGAGCTGCGGCGCCGCACAGCTTCGTGGCGCTCTGGGCACCCCTGTTCCTGCTGCGCTCCGCCCTGGCC

5	spALB	Human Albumin secretory signal peptide nucleotide sequence	ATGAAGTGGGTAACCTTTATTTCCCTTCTTTTTCTCTTTAGCTCGGCTTATTCC
6	spAZU1	Human Azurocidin secretory signal peptide nucleotide sequence	ATGACCCGGCTGACAGTCCTGGCCCTGCTGGCTGGTCTGCTGGCGTCCTCGAGGGCC
7	spBM40	Human osteonectin secretory signal peptide nucleotide sequence	ATGAGGGCCTGGATCTTCTTTCTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCA
8	spGAU	Gaussia luciferase secretory signal peptide nucleotide sequence	ATGGGAGTCAAAGTTCTGTTTGCCCTGATCTGCATCGCTGTGGCCGAGGCC
9	spFGF-17	Human FGF-17 secretory signal peptide amino acid sequence	MGAARLLPNLTLCLQLLILCCQ
10	spTHBS1	Human THBS1 secretory signal peptide amino acid sequence	MGLAWGLGVLFLMHVCGT
11	spIGF-2	Human IGF-2 secretory signal peptide amino acid sequence	MGIPMGKSMLVLLTFLAFASCCIA
12	spBMP-7	Human BMP-7 secretory signal peptide amino acid sequence	MHVRSLRAAAPHSFVALWAPLFLLRSALA

13	spALB	Human Albumin secretory signal peptide amino acid sequence	MKWVTFISLLFLFSSAYS
14	spAZU1	Human Azurocidin secretory signal peptide amino acid sequence	MTRLTVLALLAGLLASSRA
15	spBM40	Human osteonectin secretory signal peptide amino acid sequence	MRAWIFFLLCLAGRALAA
16	spGAU	Gaussia luciferase secretory signal peptide amino acid sequence	MGVKVLFALICIAVAEA
17	IGF2	Human IGF2 nucleotide sequence	GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGGT GGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTACTT CAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCGTGGCA TCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCC TGGAGACGTACTGTGCTACCCCCGCCAAGTCCGAG
18	IGF2-linker1-hFcm	Human IGF2-linker1-hFcm nucleotide sequence	GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGGT GGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTACTT CAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCGTGGCA TCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCC TGGAGACGTACTGTGCTACCCCCGCCAAGTCCGAG GGATCGGGATCGGACAAAACTCACACATGCCCACCGTGCCCA GCACCTGAAGCTGCCGGGGGACCGTCAGTCTTCCTCTTCCCC CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAG GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAA TGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA CCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCT GAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT CGGCGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCA GCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGG

			ATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCA AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCA ATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGC TGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGT GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAaAG CCTCTCCCTGTCTCCGGGTAAA
19	IGF2- linker2- hFcm	nucleotide sequence	GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGGT GGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTACTT CAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCGTGGCA TCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCC TGGAGACGTACTGTGCTACCCCCGCCAAGTCCGAG GGATCTGGGAGCGCTGACAAAACTCACACATGCCCACCGTGC CCAGCACCTGAAGCTGCCGGGGGACCGTCAGTCTTCCTCTTC CCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCC TGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAC GTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC CCTCGGCGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCG GGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT CAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAG CAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGT GCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCAC CGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA aAGCCTCTCCCTGTCTCCGGGTAAA
20	6xHis- HSA- linker3- IGF2	His tagged HSA fusion IGF2 with a long linker nucleotide sequence	CACCATCACCATCACCATAGCGGCGATGCACACAAGAGTGAG GTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAG CCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCC ATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTG CAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAA ATCACTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAA CTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAA ACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAAAGAT GACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGAT GTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGA AAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTAT

			GCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTT TTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTT GCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGA GAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAG AGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGA CAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCT GCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATAT CTGTGAAAATCAAGATTCGATCTCCAGTAAACTGAAGGAATGC TGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAG TGGAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGC TGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAG GCAAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAA GAAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAGACTTGC CAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGC AGATCCTCATGAATGCTATGCCAAAGTGTTCGATGAATTTAAA CCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTG AGCTTTTTGAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCT ATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCA ACTCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGC AAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAG AAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCA TGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCAC AGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAA GTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACAT TCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAG ACAAATCAAGAAACAAACTGCACTTGTTGAGCTCGTGAAACAC AAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGAT GATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATA AGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTG CAAGTCAAGCTGCCTTAGGCTTAGGCGGAGGCGGTAGCGGA GGCGGTGGCTCCGGTGGCGGAGGGTCTGCTTACCGCCCCAG TGAGACCCTGTGCGGCGGGGAGCTGGTGGACACCCTCCAGT TCGTCTGTGGGGACCGCGGCTTCTACTTCAGCAGGCCCGCAA GCCGTGTGAGCCGTCGCAGCCGTGGCATCGTTGAGGAGTGC TGTTTCCGCAGCTGTGACCTGGCCCTCCTGGAGACGTACTGT GCTACCCCCGCCAAGTCCGAG

21	6xHis-HSA-linker3-IGF2R61A	His tagged HSA fusion IGF2 R61A mutant with a long linker nucleotide sequence	CACCATCACCATCACCATAGCGGCGATGCACACAAGAGTGAG GTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAG CCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCC ATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTG CAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAA ATCACTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAA CTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAA ACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAAAGAT GACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGAT GTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGA AAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTAT GCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTT TTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTT GCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGA GAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAG AGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGA CAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCT GCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATAT CTGTGAAAATCAAGATTCGATCTCCAGTAAACTGAAGGAATGC TGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAG TGGAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGC TGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAG GCAAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAA GAAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAGACTTGC CAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGC AGATCCTCATGAATGCTATGCCAAAGTGTTCGATGAATTTAAA CCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTG AGCTTTTTGAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCT ATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCA ACTCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGC AAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAG AAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCA TGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCAC AGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAA GTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACAT TCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAG ACAAATCAAGAAACAAACTGCACTTGTTGAGCTCGTGAAACAC AAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGAT GATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATA AGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTG

			CAAGTCAAGCTGCCTTAGGCTTAGGCGGAGGCGGTAGCGGAGGCGGTGGCTCCGGTGGCGGAGGGTCTGCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGGTGGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTACTTCAGCAGGCCCGCAAGCCGTGTGAGCGcTCGCAGCCGTGGCATCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCCTGGAGACGTACTGTGCTACCCCCGCCAAGTCCGAG
22	6xHis-HSA-linker3-IGF2R61Q	His tagged HSA fusion IGF2 R61Q mutant with a long linker nucleotide sequence	CACCATCACCATCACCATAGCGGCGATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCCATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGAAAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTATGCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTCTGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCTGCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATATCTGTGAAAATCAAGATTCGATCTCCAGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAGTGGAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGCTGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAGGCAAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAAGTGTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCAACTCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAGAAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCAC

			AGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAA GTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACAT TCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAG ACAAATCAAGAAACAAACTGCACTTGTTGAGCTCGTGAAACAC AAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGAT GATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATA AGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTG CAAGTCAAGCTGCCTTAGGCTTAGGCGGAGGCGGTAGCGGA GGCGGTGGCTCCGGTGGCGGAGGGTCTGCTTACCGCCCCAG TGAGACCCTGTGCGGCGGGGAGCTGGTGGACACCCTCCAGT TCGTCTGTGGGGACCGCGGCTTCTACTTCAGCAGGCCCGCAA GCCGTGTGAGCCaGCGCAGCCGTGGCATCGTTGAGGAGTGC TGTTTCCGCAGCTGTGACCTGGCCCTCCTGGAGACGTACTGT GCTACCCCCGCCAAGTCCGAG
23	6xHis-HSA-linker3-IGF2R64A	His tagged HSA fusion IGF2 R64A mutant with a long linker nucleotide sequence	CACCATCACCATCACCATAGCGGCGATGCACACAAGAGTGAG GTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAG CCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCC ATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTG CAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAA ATCACTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAA CTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAA ACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAAAGAT GACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGAT GTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGA AAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTAT GCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTT TTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTT GCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGA GAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAG AGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGA CAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCT GCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATAT CTGTGAAAATCAAGATTCGATCTCCAGTAAACTGAAGGAATGC TGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAG TGGAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGC TGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAG GCAAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAA GAAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAGACTTGC CAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGC AGATCCTCATGAATGCTATGCCAAAGTGTTCGATGAATTTAAA

			CCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTG AGCTTTTTGAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCT ATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCA ACTCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGC AAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAG AAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCA TGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCAC AGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAA GTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACAT TCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAG ACAAATCAAGAAACAAACTGCACTTGTTGAGCTCGTGAAACAC AAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGAT GATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATA AGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTG CAAGTCAAGCTGCCTTAGGCTTAGGCGGAGGCGGTAGCGGA GGCGGTGGCTCCGGTGGCGGAGGGTCTGCTTACCGCCCCAG TGAGACCCTGTGCGGCGGGGAGCTGGTGGACACCCTCCAGT TCGTCTGTGGGGACCGCGGCTTCTACTTCAGCAGGCCCGCAA GCCGTGTGAGCCGTCGCAGCGCTGGCATCGTTGAGGAGTGC TGTTTCCGCAGCTGTGACCTGGCCCTCCTGGAGACGTACTGT GCTACCCCCGCCAAGTCCGAG
24	6xHis-HSA-linker3-IGF2R64Q	His tagged HSA fusion IGF2 R64Q mutant with a long linker nucleotide sequence	CACCATCACCATCACCATAGCGGCGATGCACACAAGAGTGAG GTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAG CCTTGGTGTTGATTGCCTTTGCTCAGTATCTTCAGCAGTGTCC ATTTGAAGATCATGTAAAATTAGTGAATGAAGTAACTGAATTTG CAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTGTGACAA ATCACTTCATACCCTTTTTGGAGACAAATTATGCACAGTTGCAA CTCTTCGTGAAACCTATGGTGAAATGGCTGACTGCTGTGCAAA ACAAGAACCTGAGAGAAATGAATGCTTCTTGCAACACAAAGAT GACAACCCAAACCTCCCCCGATTGGTGAGACCAGAGGTTGAT GTGATGTGCACTGCTTTTCATGACAATGAAGAGACATTTTTGA AAAAATACTTATATGAAATTGCCAGAAGACATCCTTACTTTTAT GCCCCGGAACTCCTTTTCTTTGCTAAAAGGTATAAAGCTGCTT TTACAGAATGTTGCCAAGCTGCTGATAAAGCTGCCTGCCTGTT GCCAAAGCTCGATGAACTTCGGGATGAAGGGAAGGCTTCGTC TGCCAAACAGAGACTCAAGTGTGCCAGTCTCCAAAAATTTGGA GAAAGAGCTTTCAAAGCATGGGCAGTAGCTCGCCTGAGCCAG AGATTTCCCAAAGCTGAGTTTGCAGAAGTTTCCAAGTTAGTGA CAGATCTTACCAAAGTCCACACGGAATGCTGCCATGGAGATCT GCTTGAATGTGCTGATGACAGGGCGGACCTTGCCAAGTATAT CTGTGAAAATCAAGATTCGATCTCCAGTAAACTGAAGGAATGC

			TGTGAAAAACCTCTGTTGGAAAAATCCCACTGCATTGCCGAAG TGGAAAATGATGAGATGCCTGCTGACTTGCCTTCATTAGCTGC TGATTTTGTTGAAAGTAAGGATGTTTGCAAAAACTATGCTGAG GCAAAGGATGTCTTCCTGGGCATGTTTTTGTATGAATATGCAA GAAGGCATCCTGATTACTCTGTCGTGCTGCTGCTGAGACTTGC CAAGACATATGAAACCACTCTAGAGAAGTGCTGTGCCGCTGC AGATCCTCATGAATGCTATGCCAAAGTGTTCGATGAATTTAAA CCTCTTGTGGAAGAGCCTCAGAATTTAATCAAACAAAATTGTG AGCTTTTTGAGCAGCTTGGAGAGTACAAATTCCAGAATGCGCT ATTAGTTCGTTACACCAAGAAAGTACCCCAAGTGTCAACTCCA ACTCTTGTAGAGGTCTCAAGAAACCTAGGAAAAGTGGGCAGC AAATGTTGTAAACATCCTGAAGCAAAAAGAATGCCCTGTGCAG AAGACTATCTATCCGTGGTCCTGAACCAGTTATGTGTGTTGCA TGAGAAAACGCCAGTAAGTGACAGAGTCACCAAATGCTGCAC AGAATCCTTGGTGAACAGGCGACCATGCTTTTCAGCTCTGGAA GTCGATGAAACATACGTTCCCAAAGAGTTTAATGCTGAAACAT TCACCTTCCATGCAGATATATGCACACTTTCTGAGAAGGAGAG ACAAATCAAGAAACAAACTGCACTTGTTGAGCTCGTGAAACAC AAGCCCAAGGCAACAAAAGAGCAACTGAAAGCTGTTATGGAT GATTTCGCAGCTTTTGTAGAGAAGTGCTGCAAGGCTGACGATA AGGAGACCTGCTTTGCCGAGGAGGGTAAAAAACTTGTTGCTG CAAGTCAAGCTGCCTTAGGCTTAGGCGGAGGCGGTAGCGGA GGCGGTGGCTCCGGTGGCGGAGGGTCTGCTTACCGCCCCAG TGAGACCCTGTGCGGCGGGGAGCTGGTGGACACCCTCCAGT TCGTCTGTGGGGACCGCGGCTTCTACTTCAGCAGGCCCGCAA GCCGTGTGAGCCGTCGCAGCCaGGGCATCGTTGAGGAGTGC TGTTTCCGCAGCTGTGACCTGGCCCTCCTGGAGACGTACTGT GCTACCCCCGCCAAGTCCGAG
25	IGF2- linker3- hFc4	Human IGF2- linker3-hFc4 nucleotide sequence	GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGGT GGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTACTT CAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCGTGGCA TCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCC TGGAGACGTACTGTGCTACCCCCGCCAAGTCCGAGGGCGGA GGCGGTAGCGGAGGCGGTGGCTCCGGTGGCGGAGGGTCTG AGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGCACCTG AGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAAC CCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGT GCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAG TTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTG GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGC

			AAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCC TCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATG ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTC CGACGGCTCCTTCTTCCTCTACAGCAGGCTCACCGTGGACAA GAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGAT GCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTC CCTGTCTCTGGGTAAA
26	IGF2-hFc4	Human IGF2-hFc4 nucleotide sequence	GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGGT GGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTACTT CAGCAGGCCCGCAAGCCGTGTGAGCCGTCGCAGCCGTGGCA TCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCC TGGAGACGTACTGTGCTACCCCCGCCAAGTCCGAGGAGTCCA AATATGGTCCCCCATGCCCACCCTGCCCAGCACCTGAGTTCC TGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAACCCAAGG ACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGG TGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACT GGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGC CGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGC GTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAG TACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCCATC GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCA CAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCC AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA GAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGG CTCCTTCTTCCTCTACAGCAGGCTCACCGTGGACAAGAGCAG GTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGA GGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTC TCTGGGTAAA
27	hFc4-linker3-IGF2	hFc4-linker3-human IGF2 nucleotide sequence	GAGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGCACCT GAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAA CCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCA GTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAA GACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGT GGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGG CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTC

			CTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCG AGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGAT GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT CTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT CCGACGGCTCCTTCTTCCTCTACAGCAGGCTCACCGTGGACA AGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGA TGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAAGGCGGAGGCGGTAGCGGAGGCGGT GGCTCCGGTGGCGGAGGGTCTGCTTACCGCCCCAGTGAGAC CCTGTGCGGCGGGGAGCTGGTGGACACCCTCCAGTTCGTCT GTGGGGACCGCGGCTTCTACTTCAGCAGGCCCGCAAGCCGT GTGAGCCGTCGCAGCCGTGGCATCGTTGAGGAGTGCTGTTTC CGCAGCTGTGACCTGGCCCTCCTGGAGACGTACTGTGCTACC CCCGCCAAGTCCGAG
28	IGF2R61A-linker3-hFc4	Human IGF2 R61A point mutant-linker3-hFc4 nucleotide sequence	GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGGGAGCTGGT GGACACCCTCCAGTTCGTCTGTGGGGACCGCGGCTTCTACTT CAGCAGGCCCGCAAGCCGTGTGAGCGcTCGCAGCCGTGGCA TCGTTGAGGAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTCC TGGAGACGTACTGTGCTACCCCCGCCAAGTCCGAGGGCGGA GGCGGTAGCGGAGGCGGTGGCTCCGGTGGCGGAGGGTCTG AGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGCACCTG AGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAAC CCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGT GCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAG TTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAG ACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTG GTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGC AAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCC TCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGA GAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATG ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC TACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTC CGACGGCTCCTTCTTCCTCTACAGCAGGCTCACCGTGGACAA GAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGAT GCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTC CCTGTCTCTGGGTAAA
29	IGF2	Human IGF2 amino acid	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVE ECCFRSCDLALLETYCATPAKSE

		sequence	
30	IGF2-linker1-hFcm		AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPAKSEGSGSDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
31	IGF2-linker2-hFcm		AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPAKSEGSGSADKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
32	IGF2 Big	Full-length human IGF2	MGIPMGKSMLVLLTFLAFASCCIAAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPAKSERDVSTPPTVLPDNFPRYPVGKFFQYDTWKQSTQRLRRGLPALLRARRGHVLAKELEAFREAKRHRPLIALPTQDPAHGGAPPEMASNRK
33	6xHis-HSA-linker3-IGF2	His tagged HSA fusion IGF2 with a long linker	HHHHHHSGDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGGGGSAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPAKSE

34	6xHis-HSA-linker3-IGF2R61A	His tagged HSA fusion IGF2 R61A mutant with a long linker	HHHHHHSGDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGGGGSAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSARSRGIVEECCFRSCDLALLETYCATPAKSE
35	6xHis-HSA-linker3-IGF2R61Q	His tagged HSA fusion IGF2 R61Q mutant with a long linker	HHHHHHSGDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGGGGSAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSQRSRGIVEECCFRSCDLALLETYCATPAKSE
36	6xHis-HSA-linker3-IGF2R64A	His tagged HSA fusion IGF2 R64A mutant with a long linker	HHHHHHSGDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL

			RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSK CCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQ TALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAE EGKKLVAASQAALGLGGGGSGGGGSGGGGSAYRPSETLCGGE LVDTLQFVCGDRGFYFSRPASRVSRRSAGIVEECCFRSCDLALL ETYCATPAKSE
37	6xHis-HSA-linker3-IGF2R64Q	His tagged HSA fusion IGF2 R64Q mutant with a long linker	HHHHHHSGDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPF EDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLR ETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMC TAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECC QAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKA WAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDR ADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLL RLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSK CCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQ TALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAE EGKKLVAASQAALGLGGGGSGGGGSGGGGSAYRPSETLCGGE LVDTLQFVCGDRGFYFSRPASRVSRRSQGIVEECCFRSCDLALL ETYCATPAKSE
38	IGF2-linker3-hFc4	Human IGF2-linker3-hFc4	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVE ECCFRSCDLALLETYCATPAKSEGGGGSGGGGSGGGGSESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL SLSLGK
39	IGF2-hFc4	Human IGF2-hFc4	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVE ECCFRSCDLALLETYCATPAKSEESKYGPPCPPCPAPEFLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

40	hFc4-linker3-IGF2	hFc4-linker3-human IGF2	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKGGGGSGGGGSGGGGSAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPAKSE
41	IGF2R61A-linker3-hFc4	Human IGF2 R61A point mutant-linker3-hFc4	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSARSRGIVEECCFRSCDLALLETYCATPAKSEGGGGSGGGGSGGGGSESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
42	IGF2-mutant region	IGF2 region for mutations to reduce cleavage	RGFYFSRPASRVSRRSR
43	Linker 1	A short flexible linker nucleotide sequence	GGATCGGGATCG
44	Linker 2	A short flexible linker nucleotide sequence	GGATCTGGGAGCGCT
45	Linker 3	A long flexible linker nucleotide sequence	GGCGGAGGCGGTAGCGGAGGCGGTGGCTCCGGTGGCGGAGGGTCT
46	hFcm IgG1	Human IgG1 Fc mutant (L234A L235A P329G) nucleotide sequence	GACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGCTGCCGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCGGCGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAA

			GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCC CAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG GCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATG AGGCTCTGCACAACCACTACACGCAGAAaAGCCTCTCCCTGTC TCCGGGTAAA
47	hFc4	Human IgG4 Fc with S228P point mutation nucleotide sequence	GAGTCCAAATATGGTCCCCCATGCCCACCCTGCCCAGCACCT GAGTTCCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCAAAA CCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCA GTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAA GACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGT GGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGG CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTC CTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCG AGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGAT GACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT CTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT CCGACGGCTCCTTCTTCCTCTACAGCAGGCTCACCGTGGACA AGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGA TGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCT CCCTGTCTCTGGGTAAA
48	6xHis	Six Histidine short peptide nucleotide sequence	CACCATCACCATCACCAT
49	StrepII	Strep-Tactin binding peptide nucleotide sequence	TGGAGCCACCCGCAGTTCGAAAAA
50	HSA	Full length of Human Serum Albumin nucleotide sequence	GATGCACACAAGAGTGAGGTTGCTCATCGGTTTAAAGATTTGG GAGAAGAAAATTTCAAAGCCTTGGTGTTGATTGCCTTTGCTCA GTATCTTCAGCAGTGTCCATTTGAAGATCATGTAAAATTAGTGA ATGAAGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTC AGCTGAAAATTGTGACAAATCACTTCATACCCTTTTTGGAGACA AATTATGCACAGTTGCAACTCTTCGTGAAACCTATGGTGAAAT GGCTGACTGCTGTGCAAAACAAGAACCTGAGAGAAATGAATG CTTCTTGCAACACAAAGATGACAACCCAAACCTCCCCCGATTG

			GTGAGACCAGAGGTTGATGTGATGTGCACTGCTTTTCATGACA ATGAAGAGACATTTTTGAAAAAATACTTATATGAAATTGCCAGA AGACATCCTTACTTTTATGCCCCGGAACTCCTTTTCTTTGCTAA AAGGTATAAAGCTGCTTTTACAGAATGTTGCCAAGCTGCTGAT AAAGCTGCCTGCCTGTTGCCAAAGCTCGATGAACTTCGGGAT GAAGGGAAGGCTTCGTCTGCCAAACAGAGACTCAAGTGTGCC AGTCTCCAAAAATTTGGAGAAAGAGCTTTCAAAGCATGGGCAG TAGCTCGCCTGAGCCAGAGATTTCCCAAAGCTGAGTTTGCAG AAGTTTCCAAGTTAGTGACAGATCTTACCAAAGTCCACACGGA ATGCTGCCATGGAGATCTGCTTGAATGTGCTGATGACAGGGC GGACCTTGCCAAGTATATCTGTGAAAATCAAGATTCGATCTCC AGTAAACTGAAGGAATGCTGTGAAAAACCTCTGTTGGAAAAAT CCCACTGCATTGCCGAAGTGGAAAATGATGAGATGCCTGCTG ACTTGCCTTCATTAGCTGCTGATTTTGTTGAAAGTAAGGATGTT TGCAAAAACTATGCTGAGGCAAAGGATGTCTTCCTGGGCATGT TTTTGTATGAATATGCAAGAAGGCATCCTGATTACTCTGTCGT GCTGCTGCTGAGACTTGCCAAGACATATGAAACCACTCTAGAG AAGTGCTGTGCCGCTGCAGATCCTCATGAATGCTATGCCAAA GTGTTCGATGAATTTAAACCTCTTGTGGAAGAGCCTCAGAATT TAATCAAACAAAATTGTGAGCTTTTTGAGCAGCTTGGAGAGTA CAAATTCCAGAATGCGCTATTAGTTCGTTACACCAAGAAAGTA CCCCAAGTGTCAACTCCAACTCTTGTAGAGGTCTCAAGAAACC TAGGAAAAGTGGGCAGCAAATGTTGTAAACATCCTGAAGCAAA AAGAATGCCCTGTGCAGAAGACTATCTATCCGTGGTCCTGAAC CAGTTATGTGTGTTGCATGAGAAAACGCCAGTAAGTGACAGAG TCACCAAATGCTGCACAGAATCCTTGGTGAACAGGCGACCAT GCTTTTCAGCTCTGGAAGTCGATGAAACATACGTTCCCAAAGA GTTTAATGCTGAAACATTCACCTTCCATGCAGATATATGCACAC TTTCTGAGAAGGAGAGACAAATCAAGAAACAAACTGCACTTGT TGAGCTCGTGAAACACAAGCCCAAGGCAACAAAAGAGCAACT GAAAGCTGTTATGGATGATTTCGCAGCTTTTGTAGAGAAGTGC TGCAAGGCTGACGATAAGGAGACCTGCTTTGCCGAGGAGGGT AAAAAACTTGTTGCTGCAAGTCAAGCTGCCTTAGGCTTA
51	Linker 1	A short flexible linker amino acid sequence	GSGS
52	Linker 2	A short flexible linker amino acid sequence	GSGSA

53	Linker 3	A long flexible linker amino acid sequence	GGGGSGGGGSGGGGS
54	hFcm IgG1	Human IgG1 Fc mutant (L234A L235A P329G) amino acid sequence	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
55	hFc4	Human IgG4 Fc with S228P point mutation amino acid sequence	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLGK
56	6xHis	Six Histidine short peptide amino acid sequence	HHHHHH
57	StrepII	Strep-Tactin binding peptide amino acid sequence	WSHPQFEK
58	HSA	Full length of Human Serum Albumin amino acid sequence	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNE VTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMAD CCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEE TFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACL LPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQR FPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICEN QDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVES KDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTL EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEY KFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKR MPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFS ALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKH KPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAAS QAALGL

59	HSA-IGF2R61A	HSA fusion IGF2 R61A	SGDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGGGGSAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSARSRGIVEECCFRSCDLALLETYCATPAKSE
60	pro-HSA-IGF2R61A	pro-HSA fusion IGF2 R61A	RGVFRRSGDAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGLGGGGSGGGGSGGGGSAYRPSETLCGGELVDTLQFVCGDRGFYFSRPASRVSARSRGIVEECCFRSCDLALLETYCATPAKSE

WHAT IS CLAIMED IS:

1. 1. A method of treating a muscle or soft-tissue disorder or condition in an individual comprising administering to the individual a therapeutically effective amount of a polypeptide comprising an HSA-IGF2R61A.

5 2. The method of claim 1, further comprising the step of administering a short chain fatty acid.

3. The method of claim 2, wherein the short chain fatty acid is a butyrate.

4. The method of any one of claims 1-3, wherein the HSA-IGF2R61A comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or 100% identical to the amino acid

10 sequence set forth in SEQ ID NO: 59.

5. The method of claim 4, wherein the IGF2 sequence comprises at least one amino acid that is N-, C-, or O-linked glycosylated.

6. The method of any one of claims 1-5, further comprising the step of increasing a regenerative capability of a myoblast in the subject.

15 7. The method of claim 6, wherein the regenerative capability is a proliferation, a degree of differentiation, or a cellular survival.

8. The method of claim 7, wherein increasing a proliferation of the myoblast produces an increase in new myofibers.

9. The method of claim 7 or 8, wherein the subject has an increase in a muscle

20 regeneration.

10. The method of any one of claims 7-9, wherein a grip strength of the subject is increased.

11. The method of any one of claims 7-10, wherein a weight of muscle in the subject is increased.

25 12. The method of any one of claims 7-11, wherein a forelimb force in the subject is increased.

13. The method of any one of claims 7-12, wherein a lean body mass of the subject is increased.

14. The method of any one of claims 7-13, wherein an appendicular skeletal muscle index

30 of the subject is increased.

15. The method of any one of claims 1-14, wherein the disorder or condition is a sarcopenia.

16. The method of any one of claims 1-14, wherein the disorder or condition is a muscular dystrophy.

17. The method of any one of claims 1-14, wherein the disorder or condition is a result of an obesity, a disease progression, a metabolic disorder, a therapeutic treatment, or a combination thereof.

18. The method of any one of claims 1-14, wherein the disorder or condition is a cachexia.

5 19. The method of claim 17, wherein the metabolic disorder is a diabetes.

20. The method of any one of claims 1-14, wherein the disorder or condition results from a muscle injury, or a limb immobilization.

21. A composition comprising: a 6HIS-HSA-IGF2R61A wherein the 6HIS-HAS-IGF2R61A comprises an amino acid sequence at least about 90%, 95%, 97%, 98%, 99%, or
10 100% identical to the amino acid sequence set forth in SEQ ID NO: 43.

22. A composition comprising: a 6HIS-HSA-IGF2R61A.

23. A nucleic acid comprising a sequence of SEQ ID NO: 43.

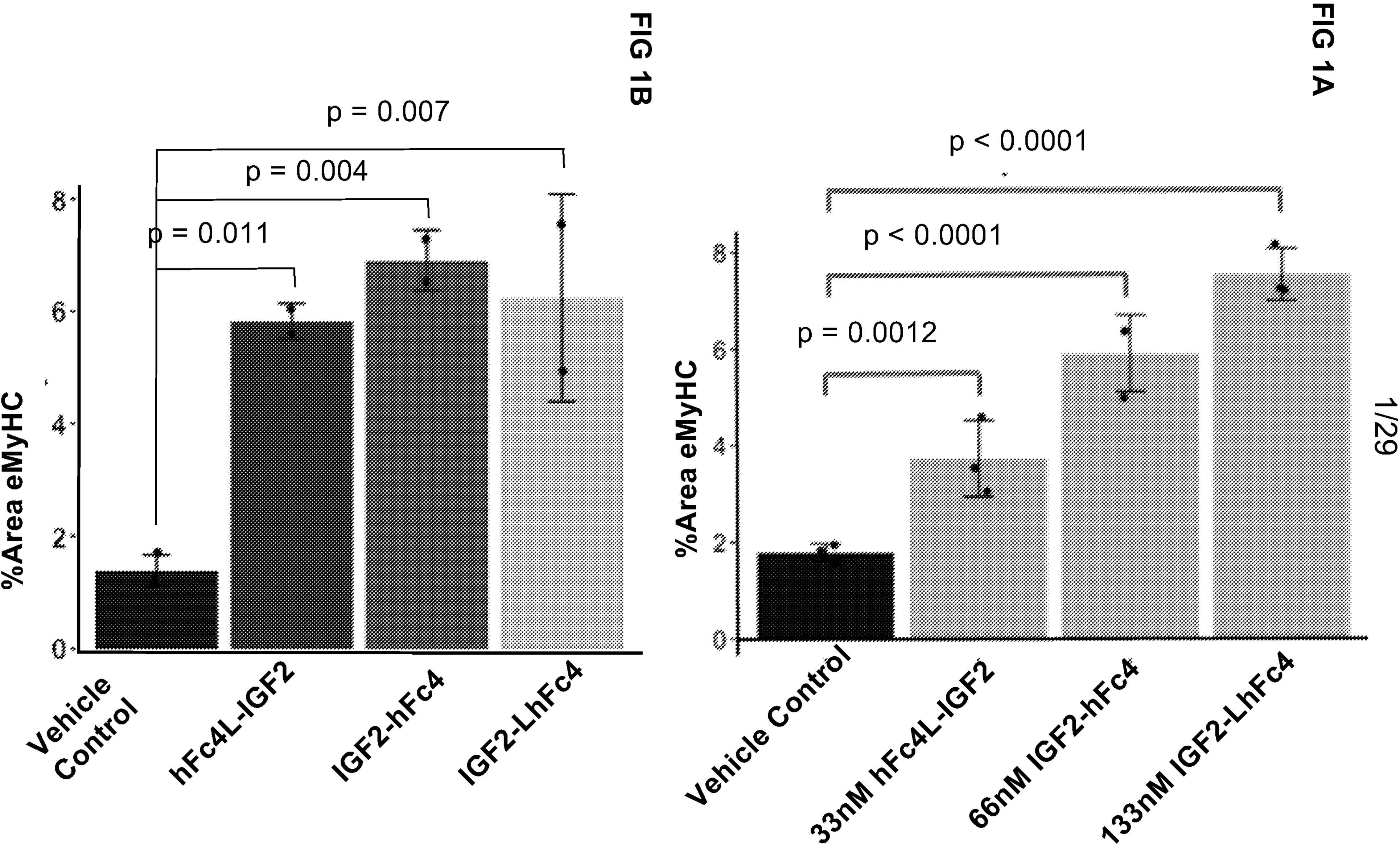
24. The nucleic acid of claim 23, further comprising an expression vector.

25. A nucleic acid comprising a polynucleotide that hybridizes under stringent
15 hybridization conditions to SEQ ID NO: 43.

26. A cell comprising the nucleic acid of any one of claims 23-25.

FIG 1A

FIG 1B



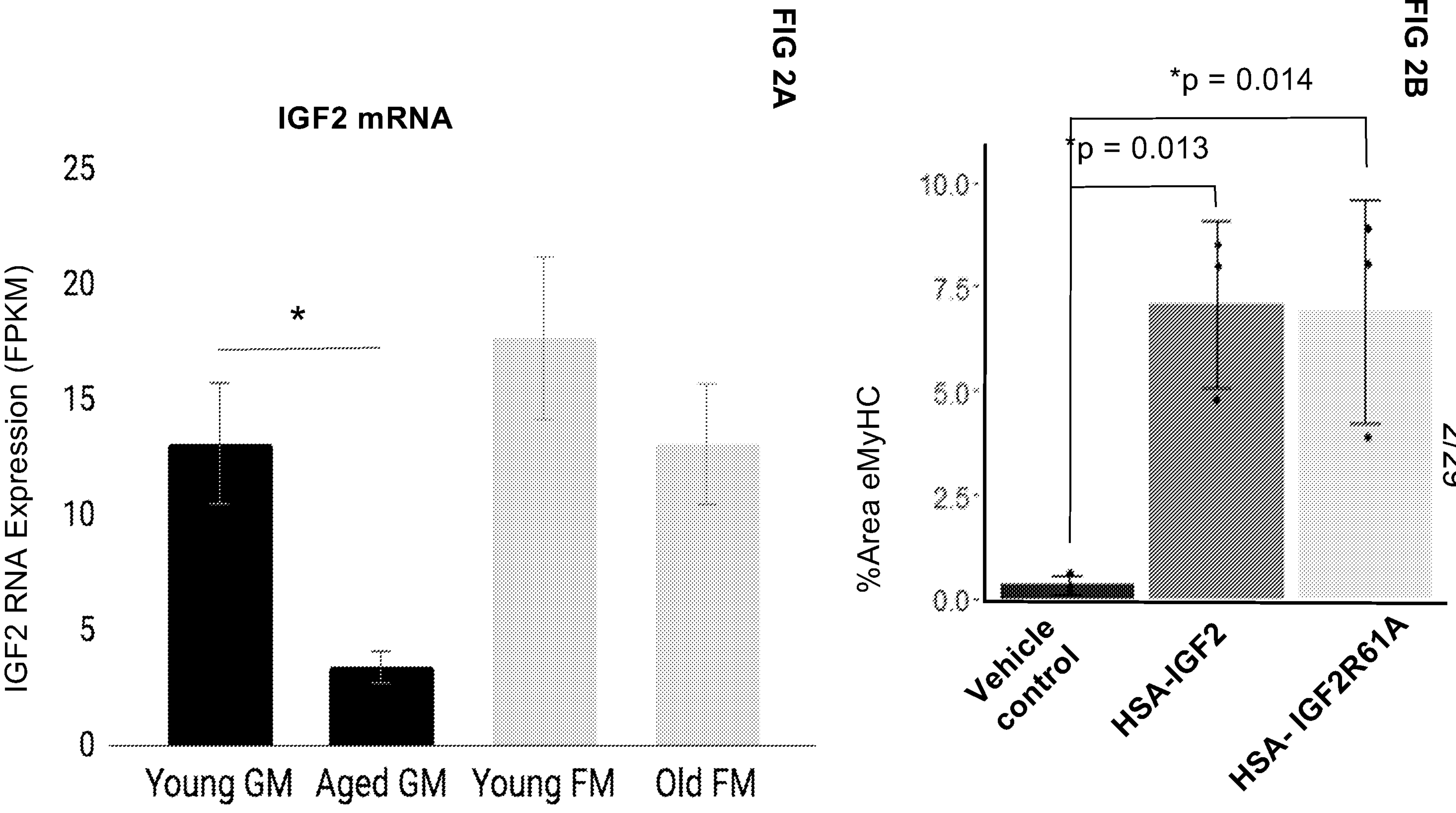


FIG 2A
IGF2 mRNA
IGF2 RNA Expression (FPKM)
25
20
15
10
5
0
*
Young GM
Aged GM
Young FM
Old FM
FIG 2B
*p = 0.014
*p = 0.013
%Area eMyHC
10.0
7.5
5.0
2.5
0.0
Vehicle control
HSA-IGF2
HSA- IGF2R61A

FIG 3A

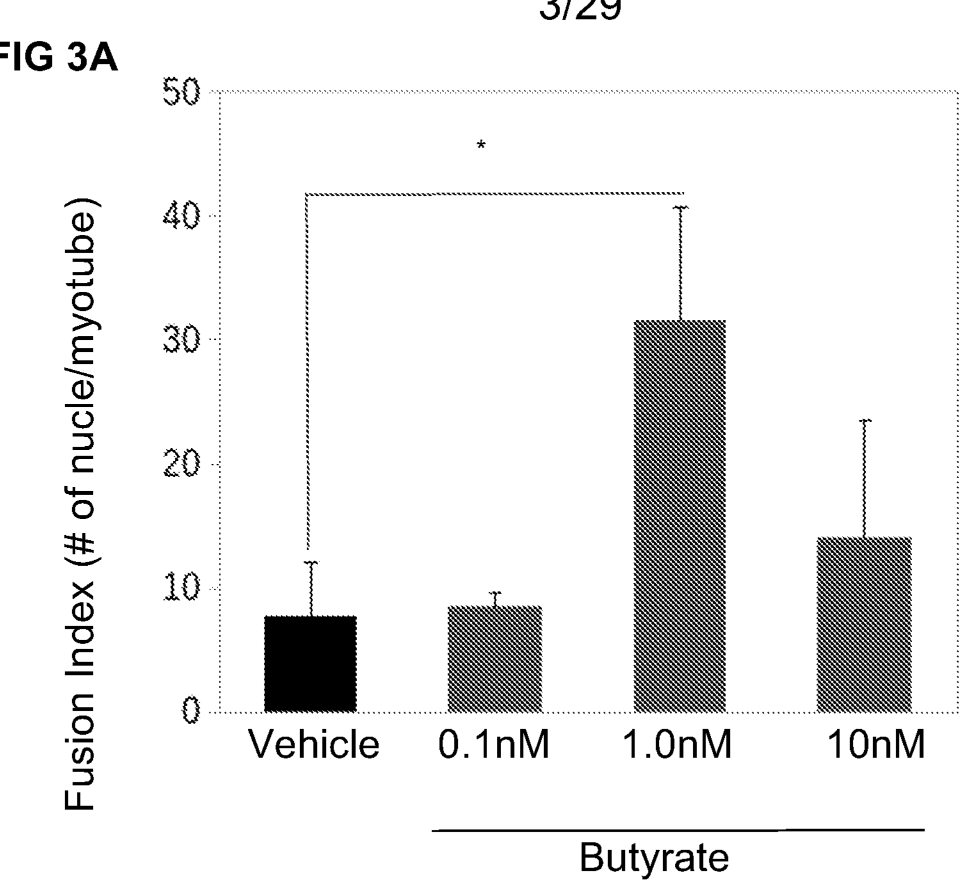
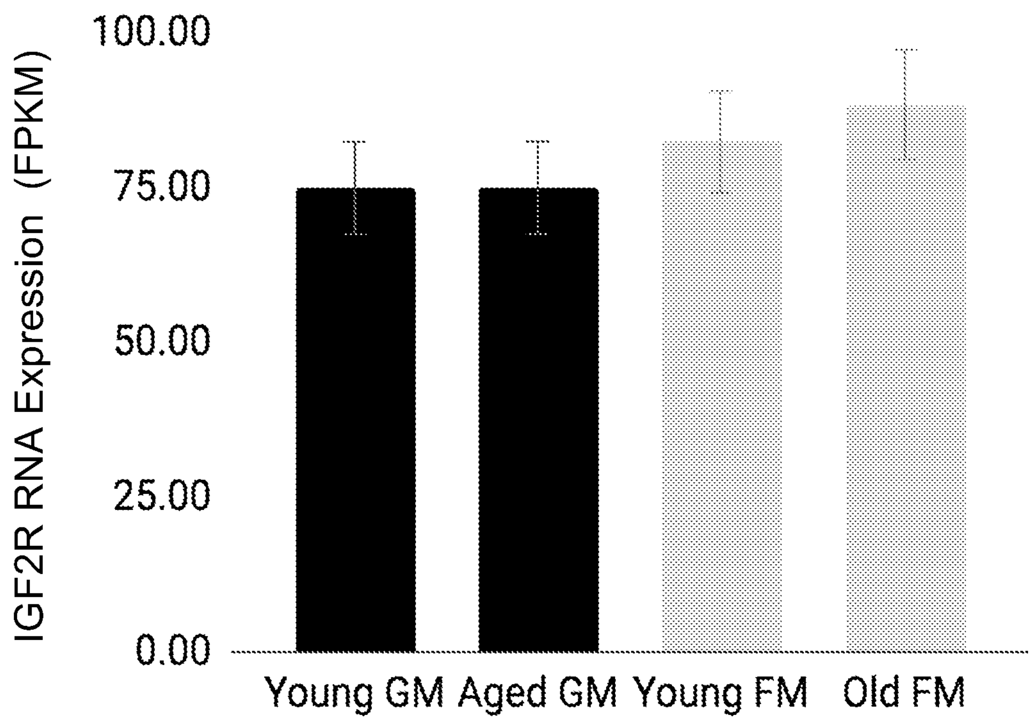


FIG 2C



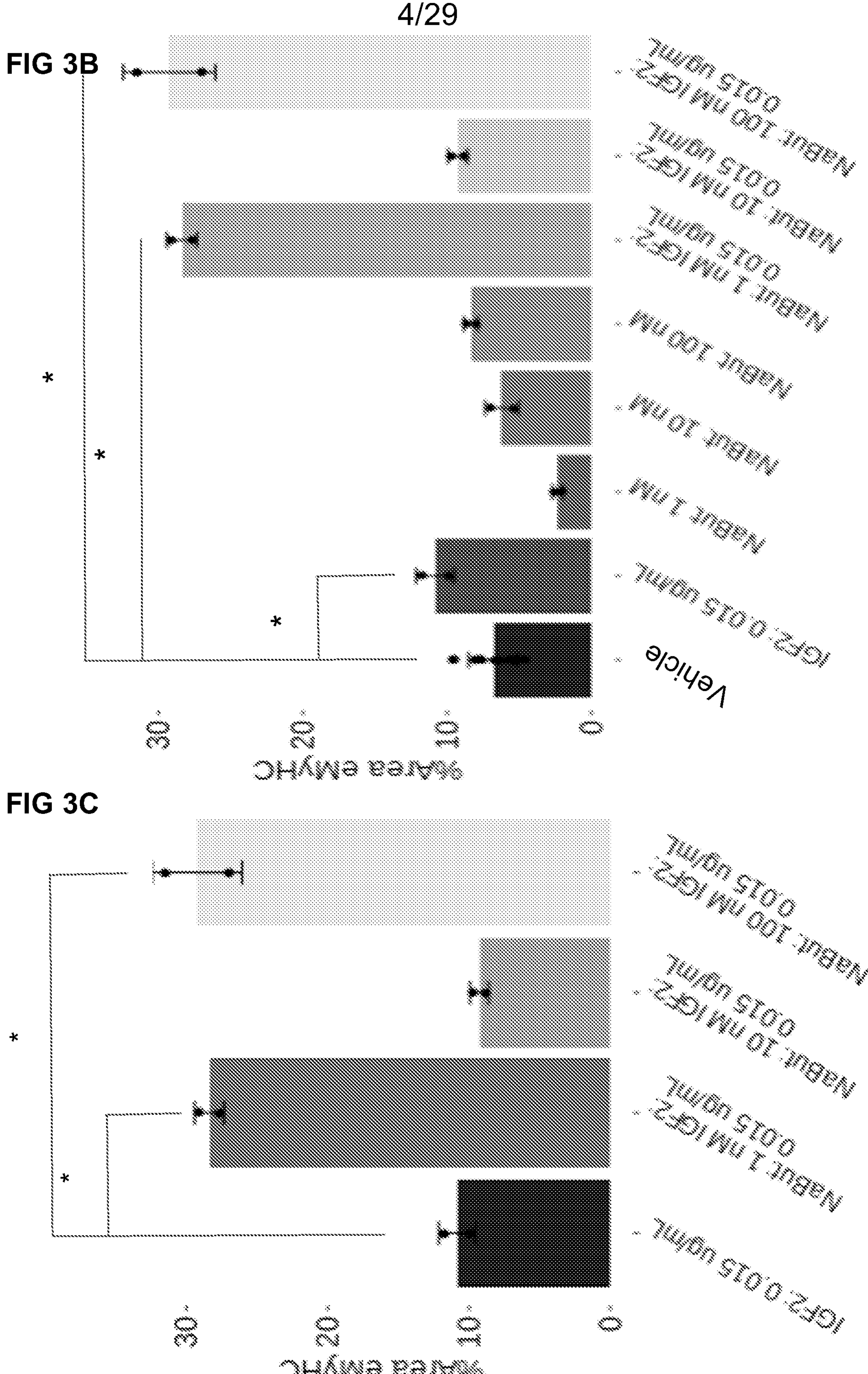


FIG 3B
%Area eMyHC
0
10
20
30
Vehicle
IGF2: 0.015 ug/mL
NaBut: 1 nM
NaBut: 10 nM
NaBut: 100 nM
NaBut: 1 nM IGF2: 0.015 ug/mL
NaBut: 10 nM IGF2: 0.015 ug/mL
NaBut: 100 nM IGF2: 0.015 ug/mL
*
*
*
FIG 3C
%Area eMyHC
0
10
20
30
IGF2: 0.015 ug/mL
NaBut: 1 nM IGF2: 0.015 ug/mL
NaBut: 10 nM IGF2: 0.015 ug/mL
NaBut: 100 nM IGF2: 0.015 ug/mL
*
*

FIG 4A

FIG 4B

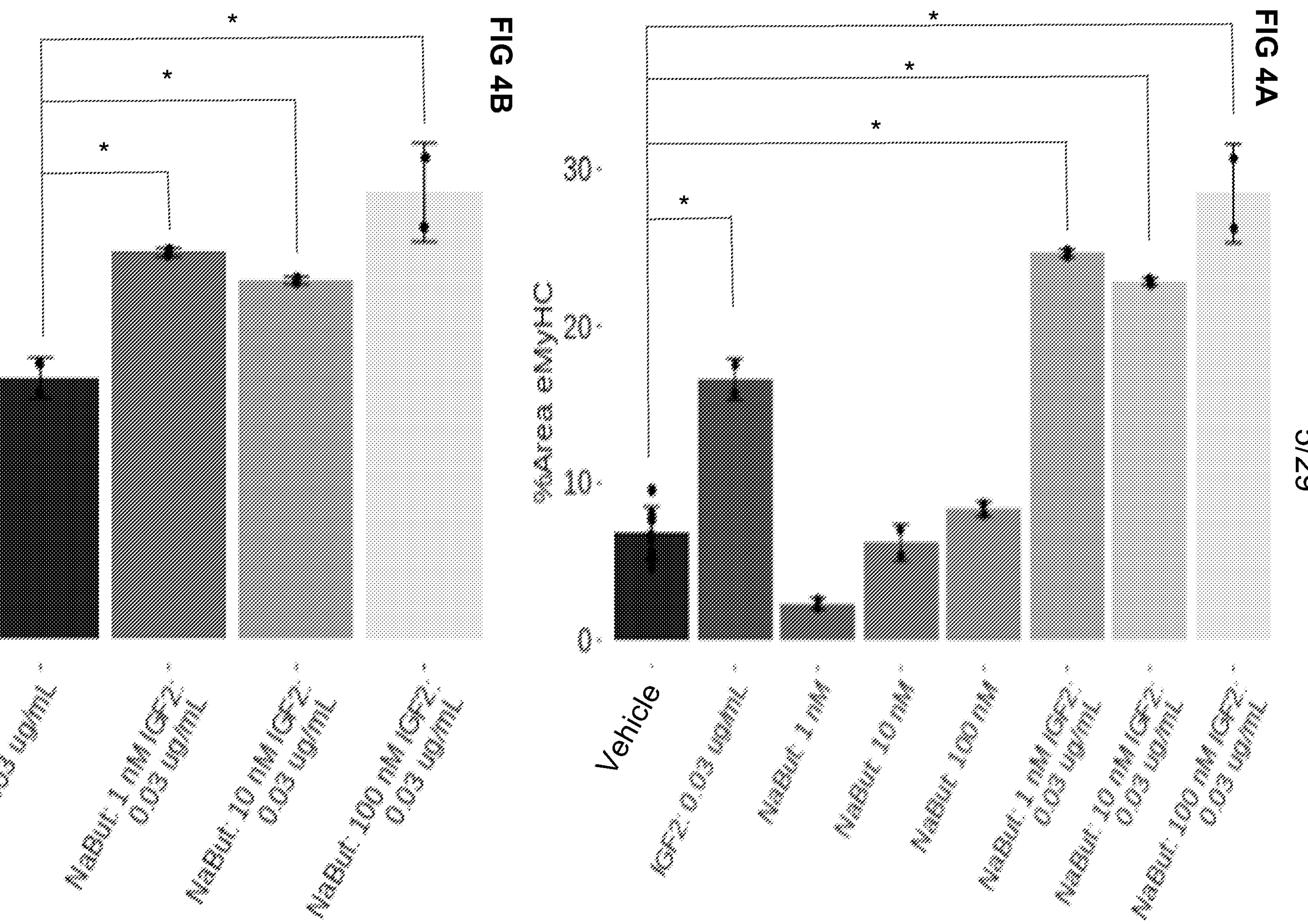


FIG 5A

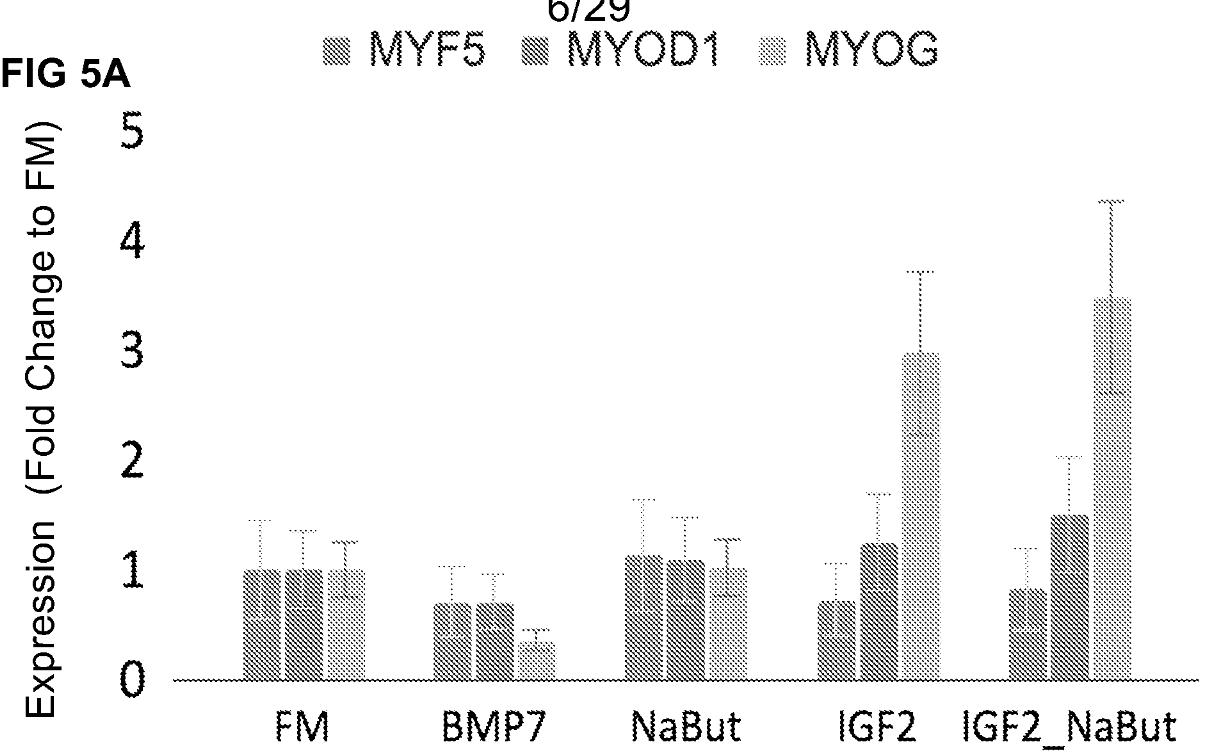
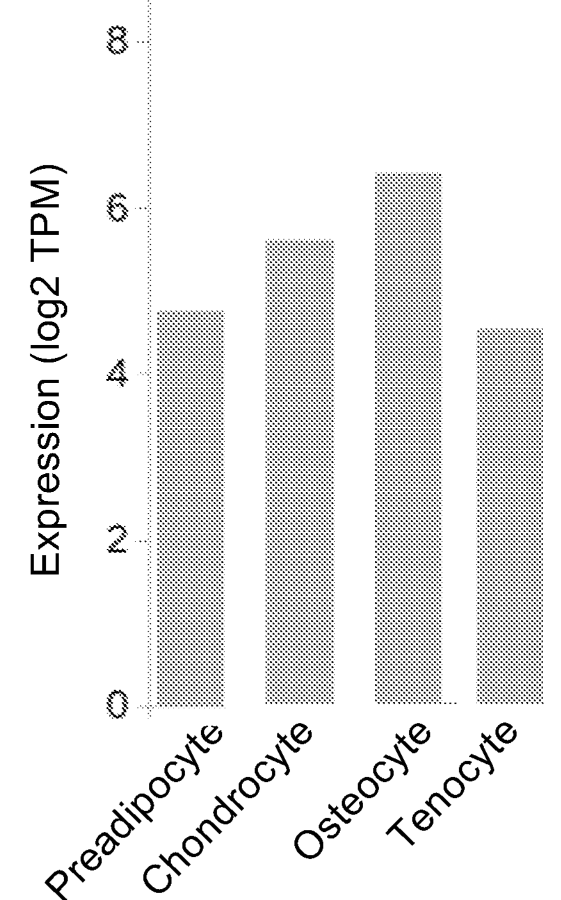


FIG 6A



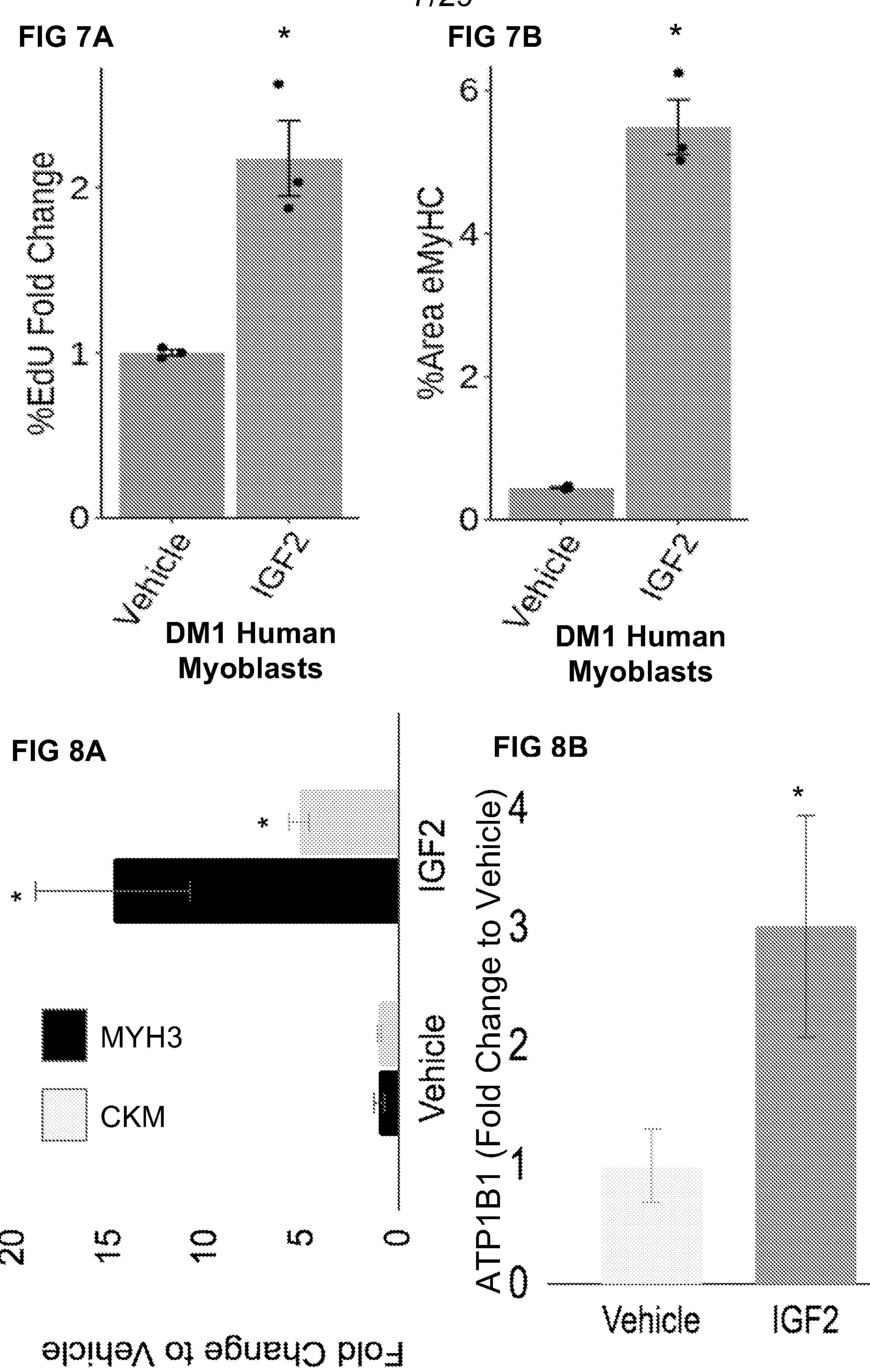


FIG 7A
*
%EdU Fold Change
0
1
2
Vehicle
IGF2
DM1 Human Myoblasts
FIG 7B
*
%Area eMyHC
0
2
4
6
Vehicle
IGF2
DM1 Human Myoblasts
FIG 8A
MYH3
CKM
Vehicle
IGF2
*
*
0
5
10
15
20
Fold Change to Vehicle
FIG 8B
*
ATP1B1 (Fold Change to Vehicle)
0
1
2
3
4
Vehicle
IGF2

FIG 9A

Daily injection of Vehicle or IGF2 or NaB or IGF2/NaB

Days: 1 14

Sarcopenia experiment old 29-24M
Vehicle (N=31): Vehicle
IGF2 (N=8): IGF2 (150ug/kg)
NaB (N=8-10): Sodium Butyrate (1.2ug/kg)
IGF2/But (N=12): IGF2 + Sodium butyrate (NaB)

FIG 9B

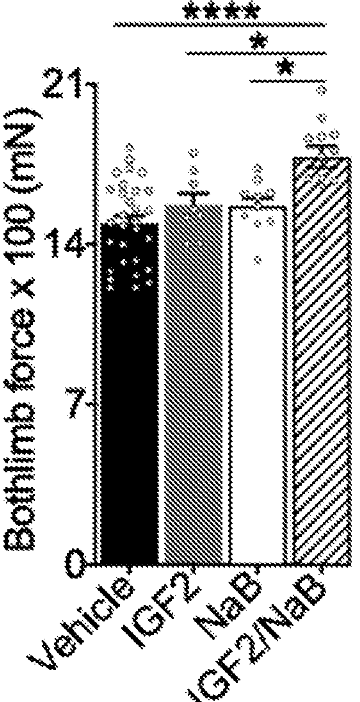


FIG 9C

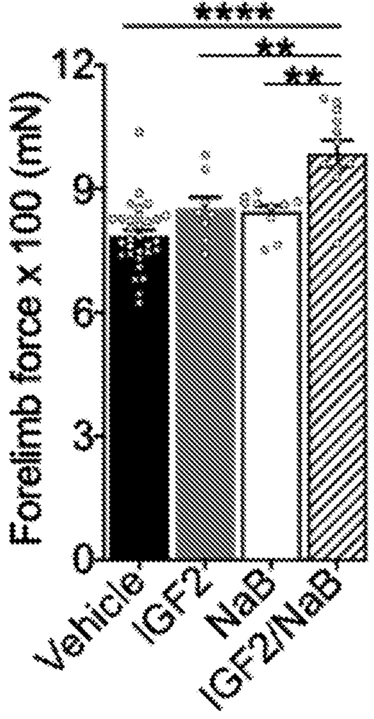


FIG 9D

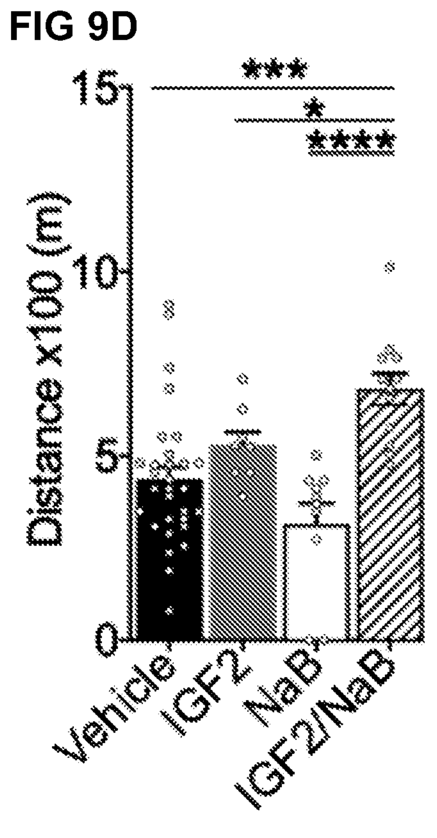


FIG 9E

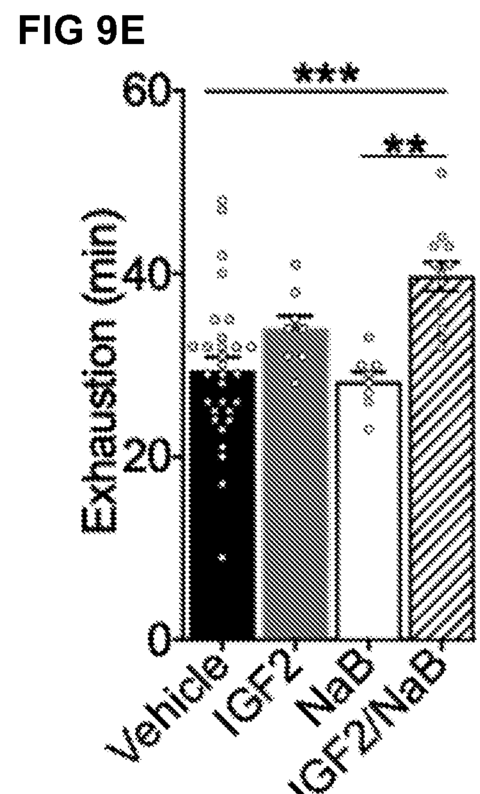


FIG 9F

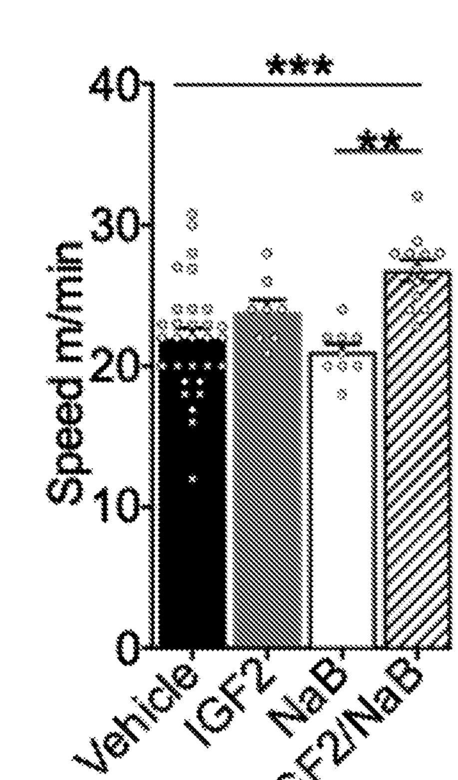


FIG 9G

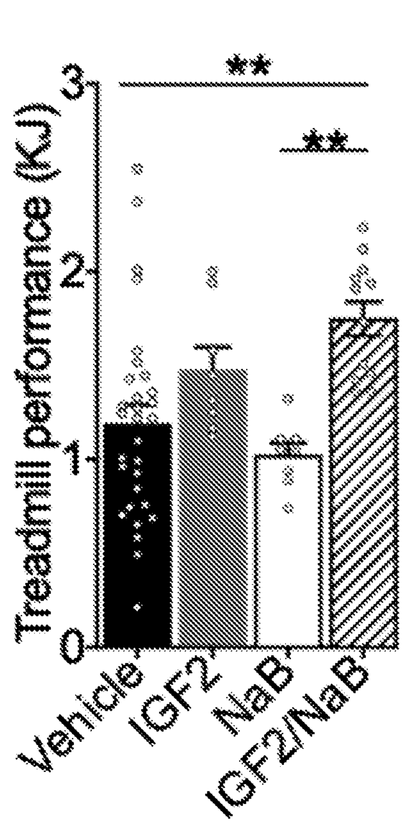


FIG 10A

Sarcopenia experiment old 21M
Vehicle (N=6): Vehicle
IGF2/NaB (N=6): IGF2 (150ug/kg) + Sodium butyrate (1.2 g/kg)

Vehicle, SC or
IGF2/NaB, SC

Safety/ toxicity

Daily injection of Vehicle or IGF2 or IGF2/NaBut

Days: 1 14

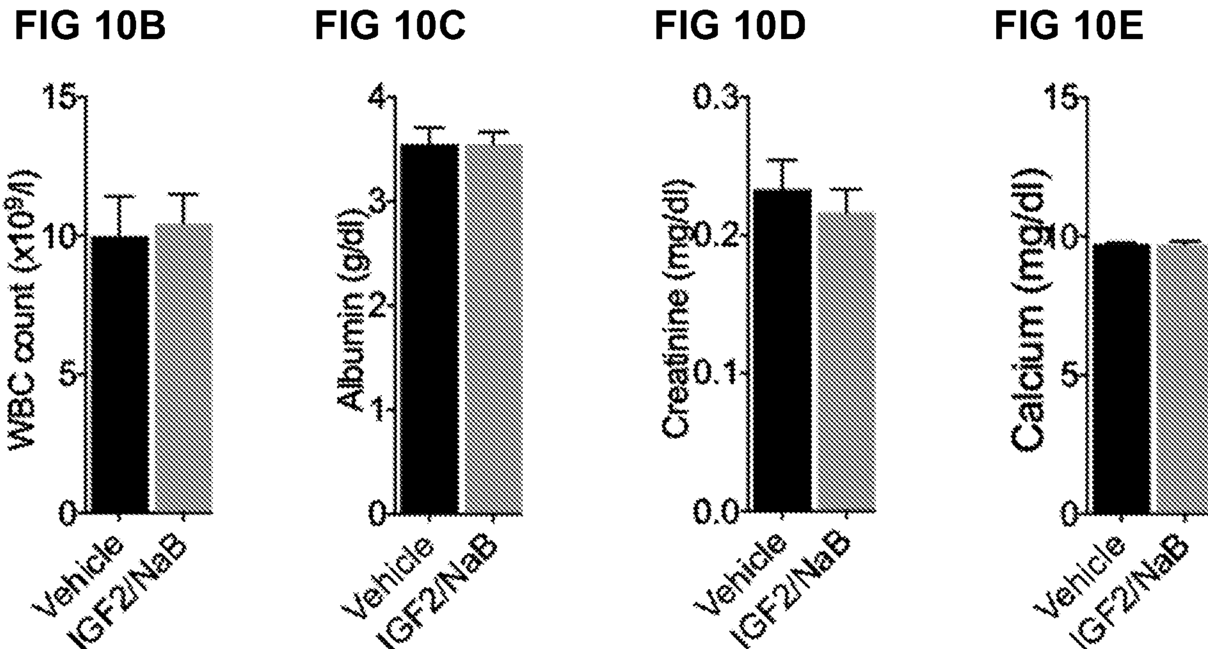


FIG 11A

Dexamethasone 25mg/kg

Vehicle, SC or

IGF2/NaBut, SC

Muscle Function & histology

Daily injection of Dexa with Vehicle or IGF2/NaB

Days:1 14

Atrophy experiment overview: 3M old mice
Vehicle (N=14): Dexamethasone + Vehicle
IGF2/NaB (N=13): Dexamethasone + IGF2 (150ug/kg) + Sodium butyrate (1.2g/kg)

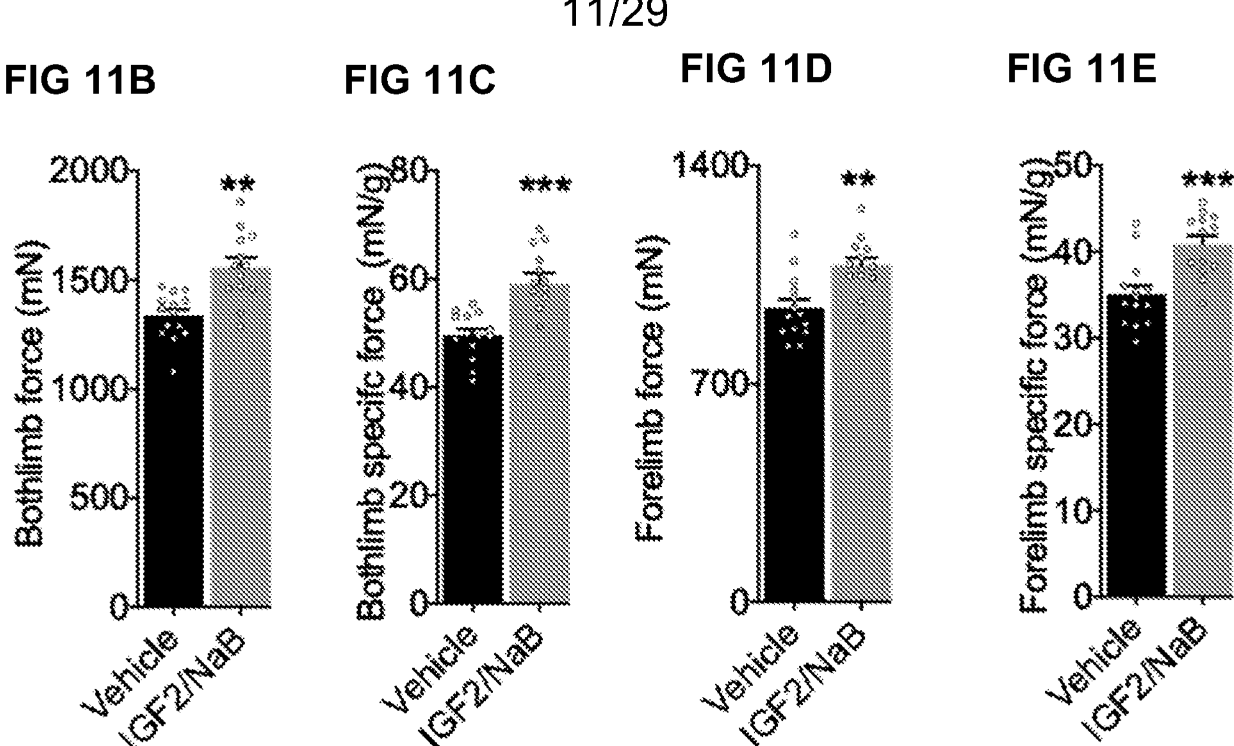
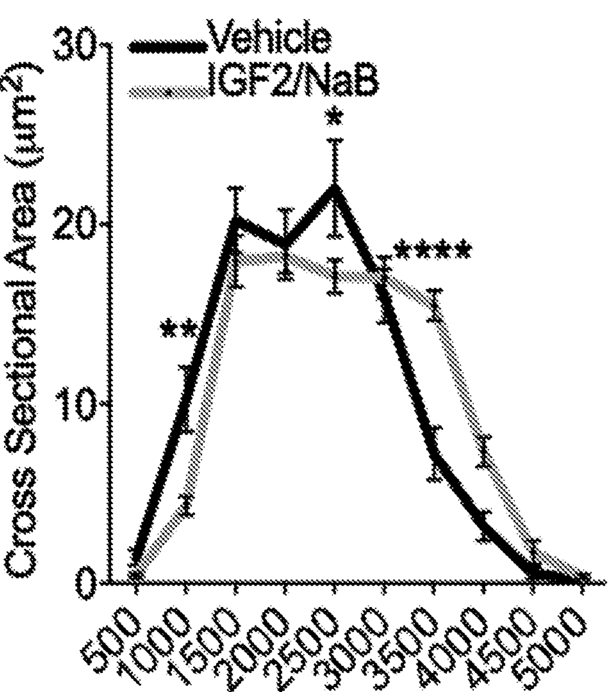


FIG 11F



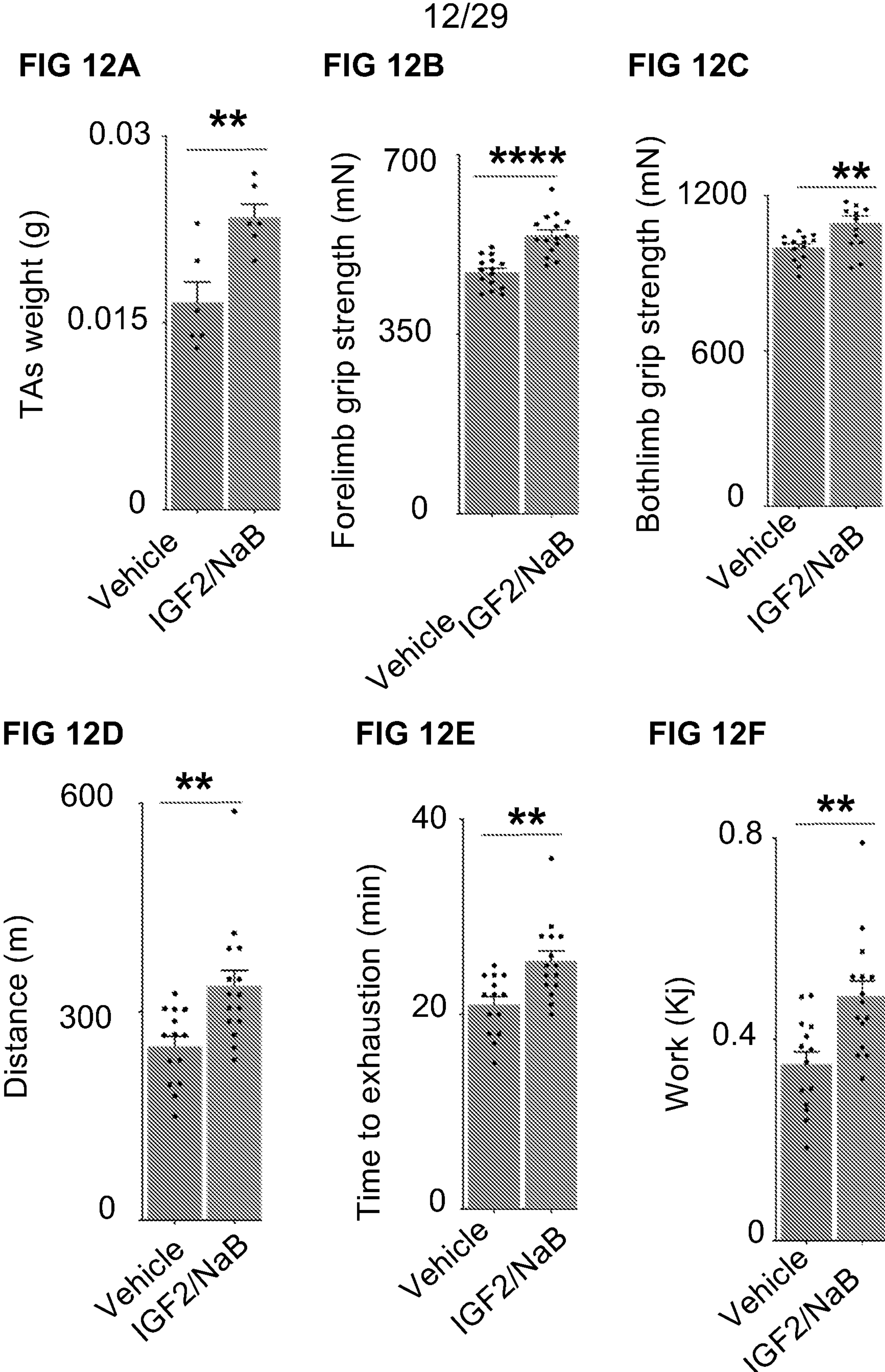


FIG 12A
**
0.03
0.015
0
TAs weight (g)
Vehicle
IGF2/NaB
FIG 12B
****
700
350
0
Forelimb grip strength (mN)
Vehicle
IGF2/NaB
FIG 12C
**
1200
600
0
Bothlimb grip strength (mN)
Vehicle
IGF2/NaB
FIG 12D
**
600
300
0
Distance (m)
Vehicle
IGF2/NaB
FIG 12E
**
40
20
0
Time to exhaustion (min)
Vehicle
IGF2/NaB
FIG 12F
**
0.8
0.4
0
Work (Kj)
Vehicle
IGF2/NaB

FIG 13A

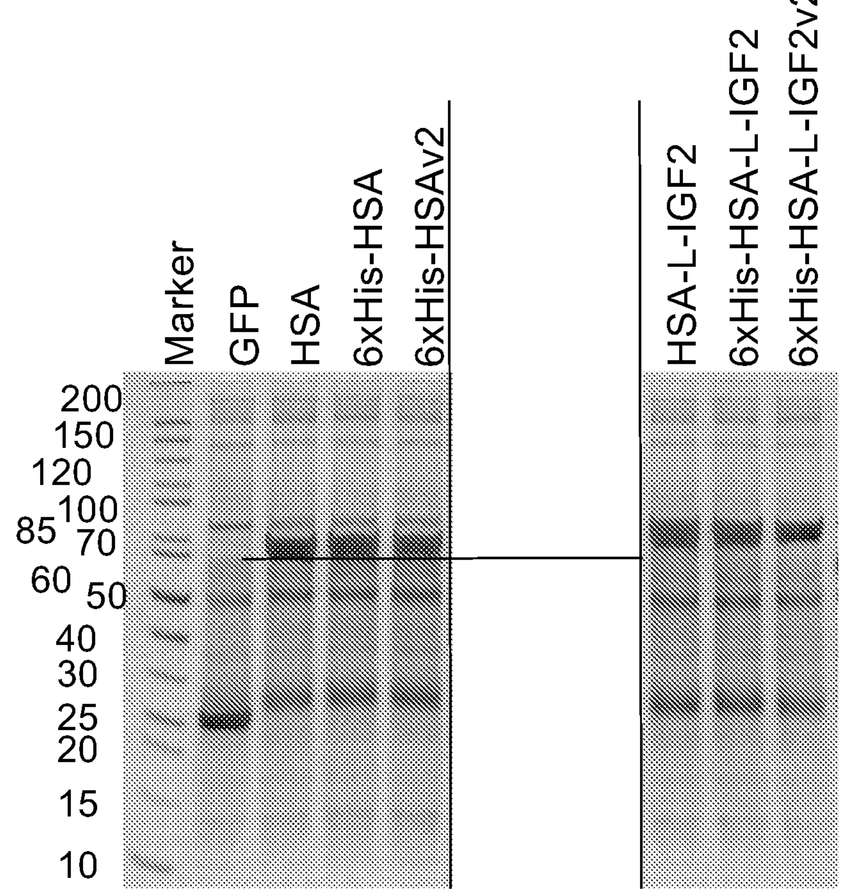


FIG 13B

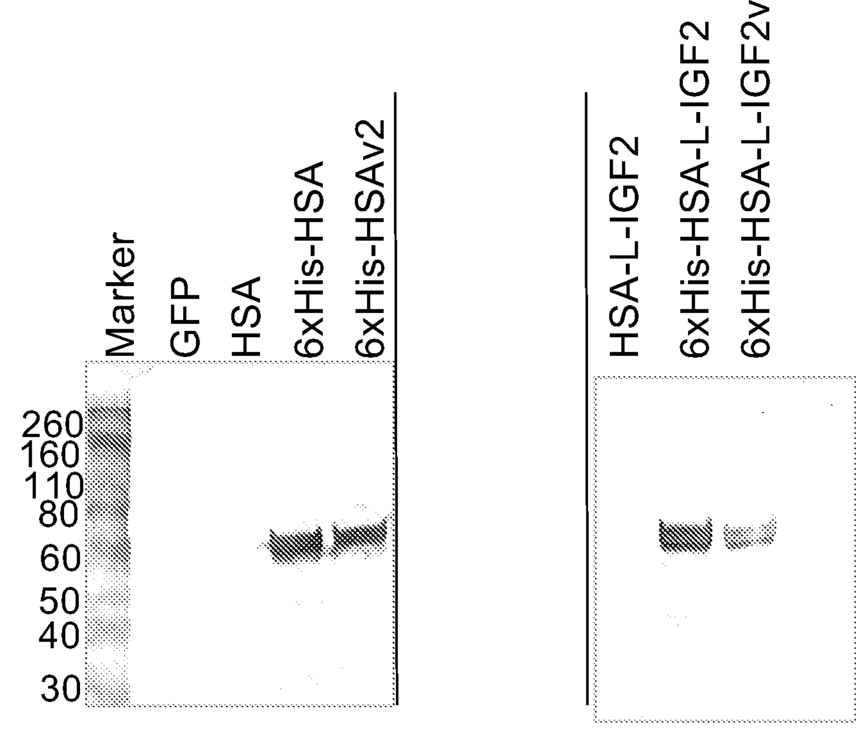


FIG 14

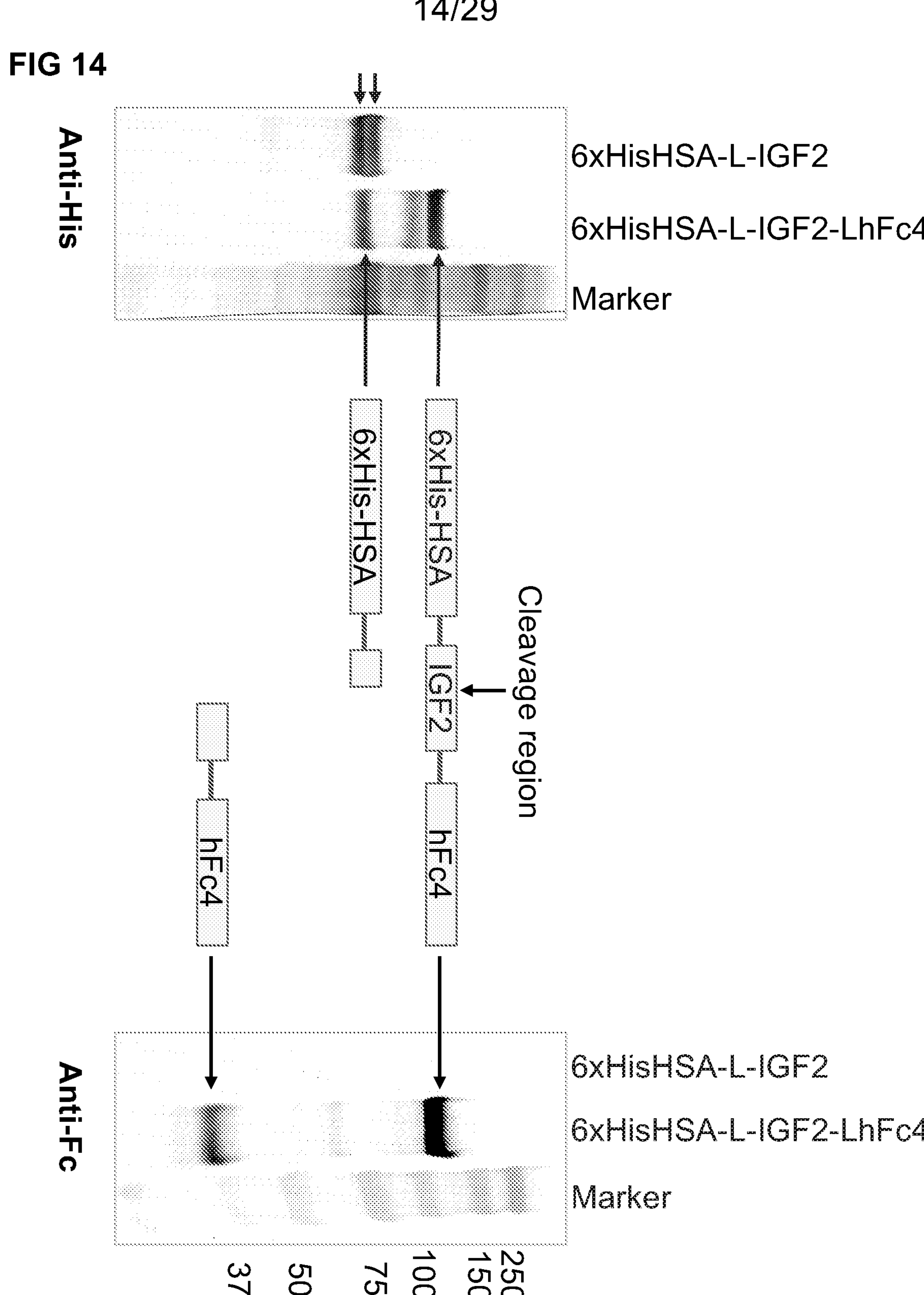


FIG 15A

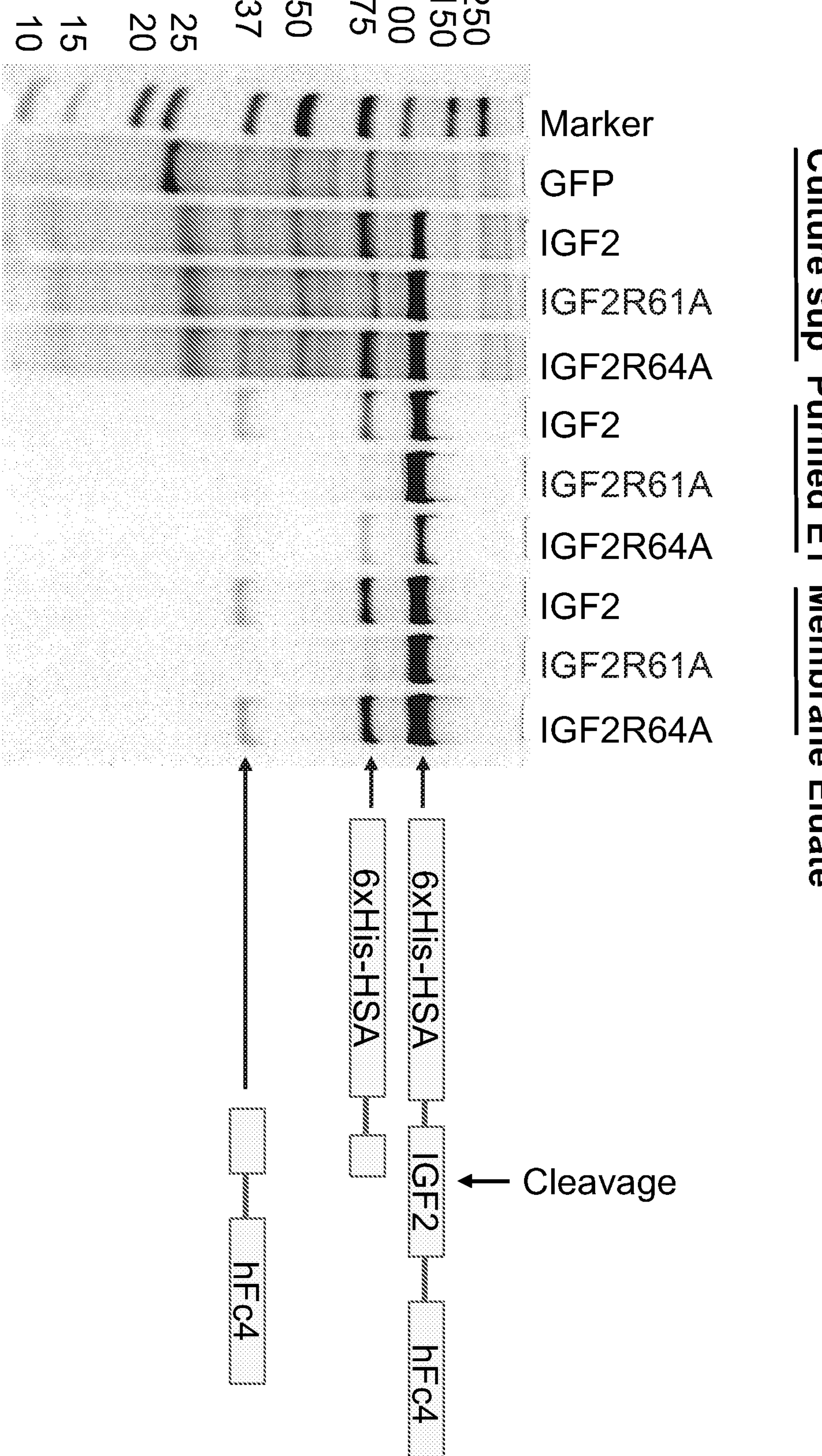


FIG 15B

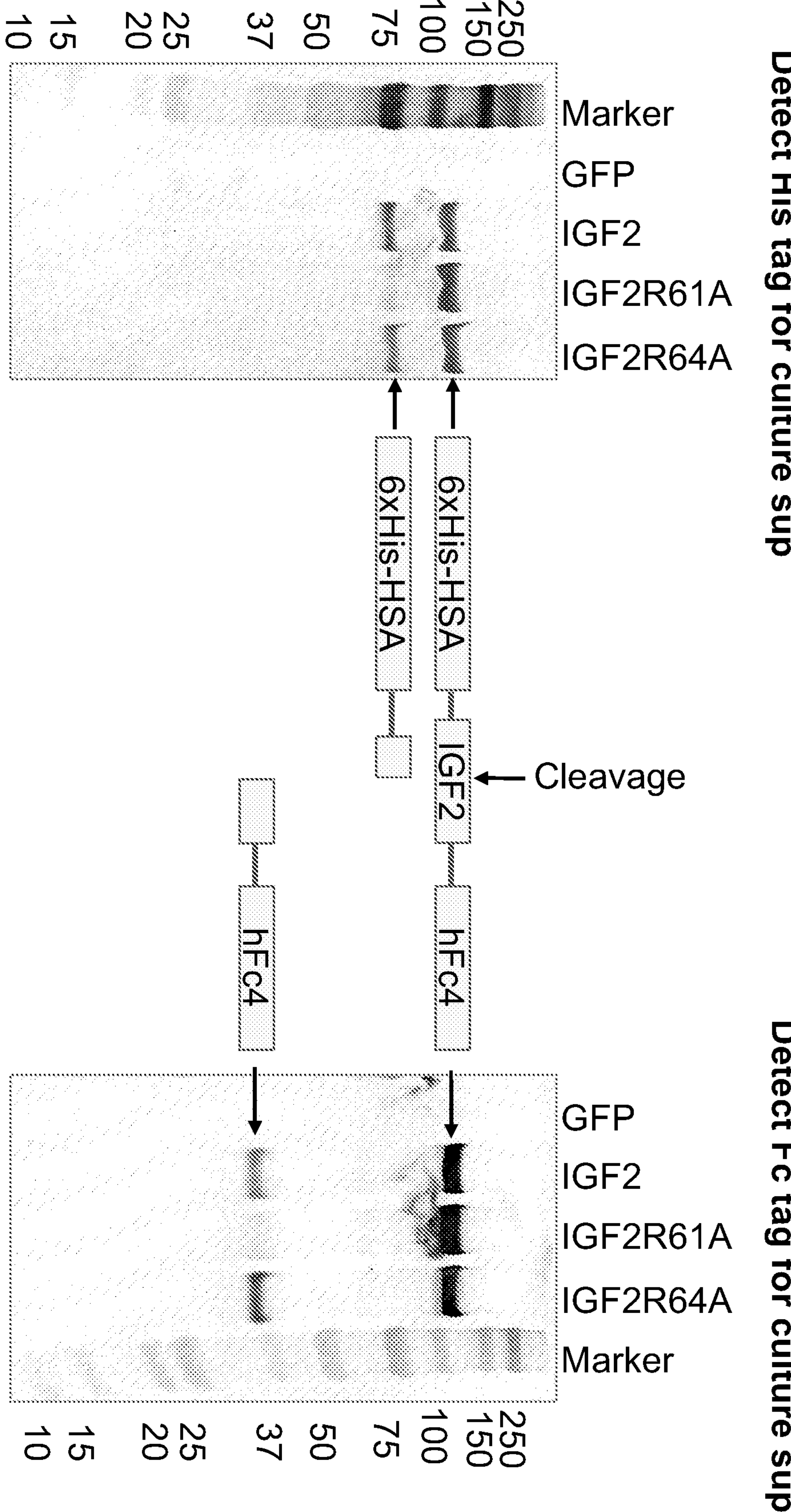


FIG 16A

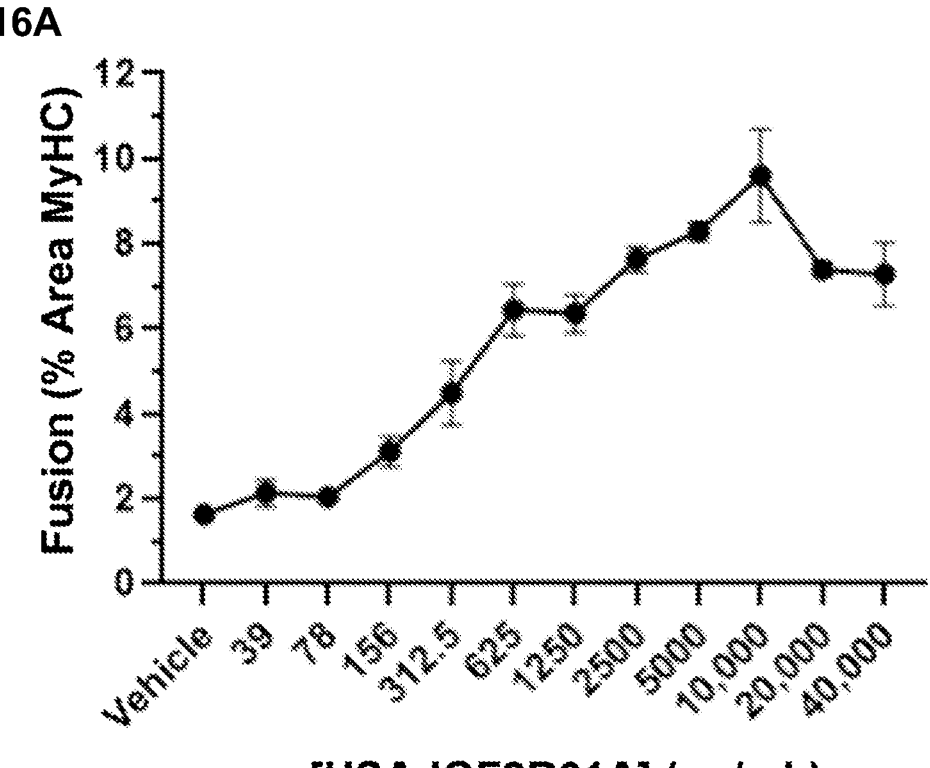


FIG 16B

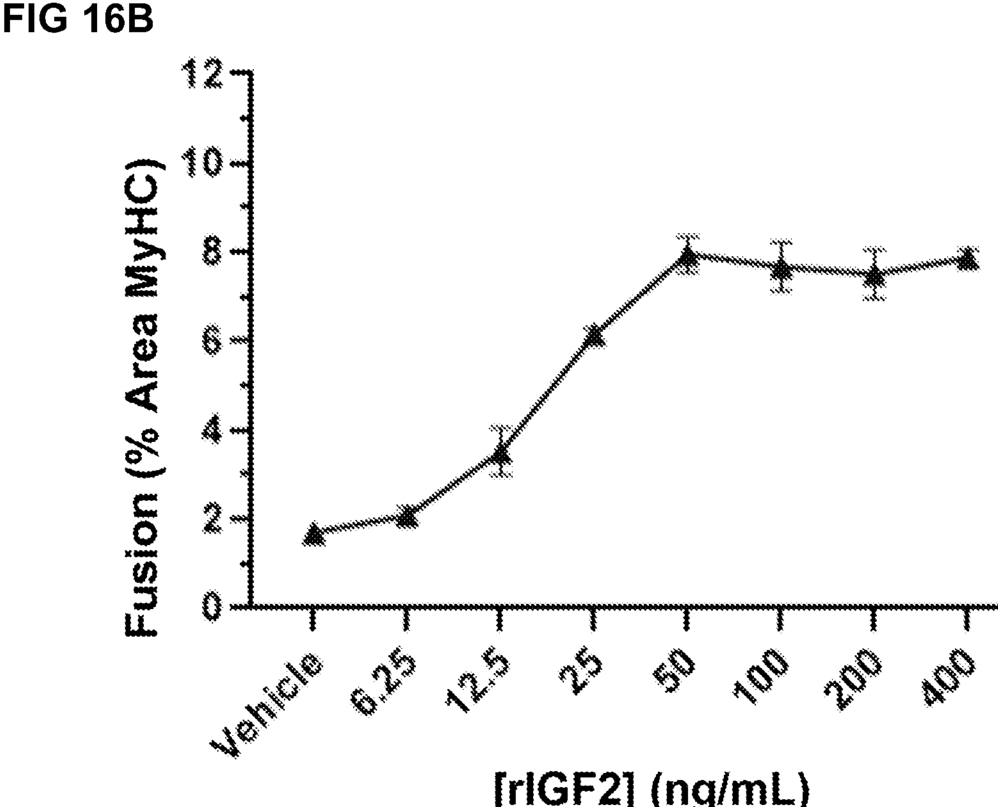


FIG 17A

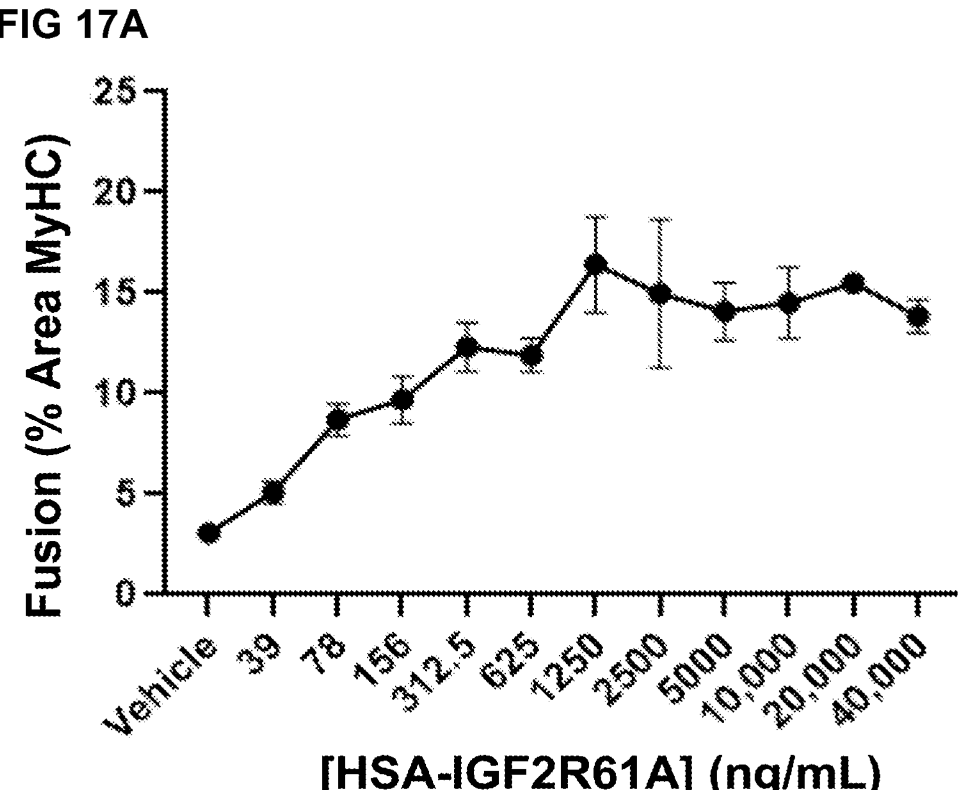


FIG 17B

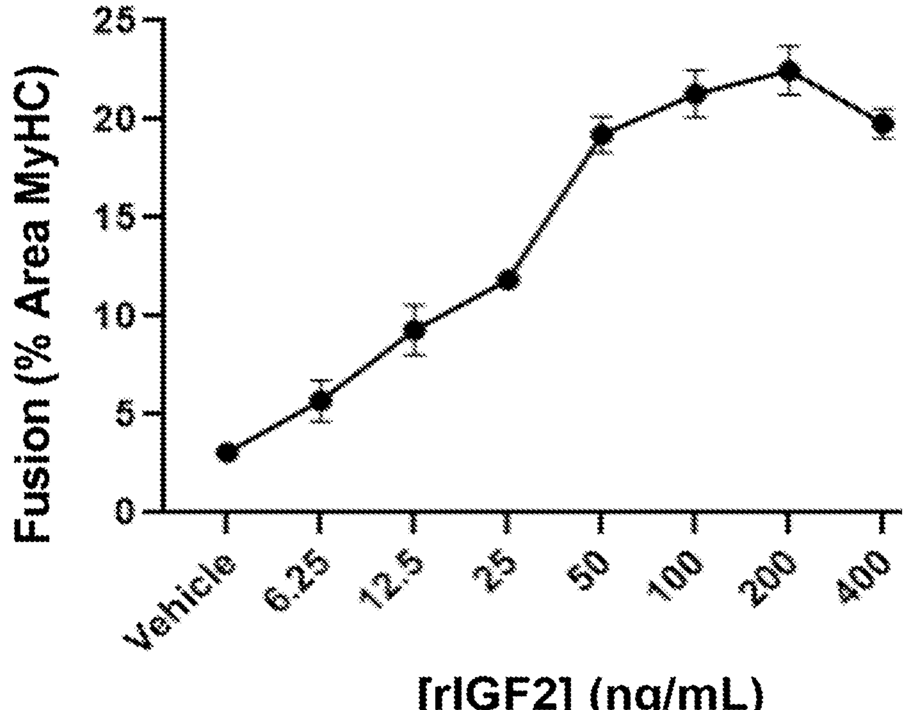


FIG 18A

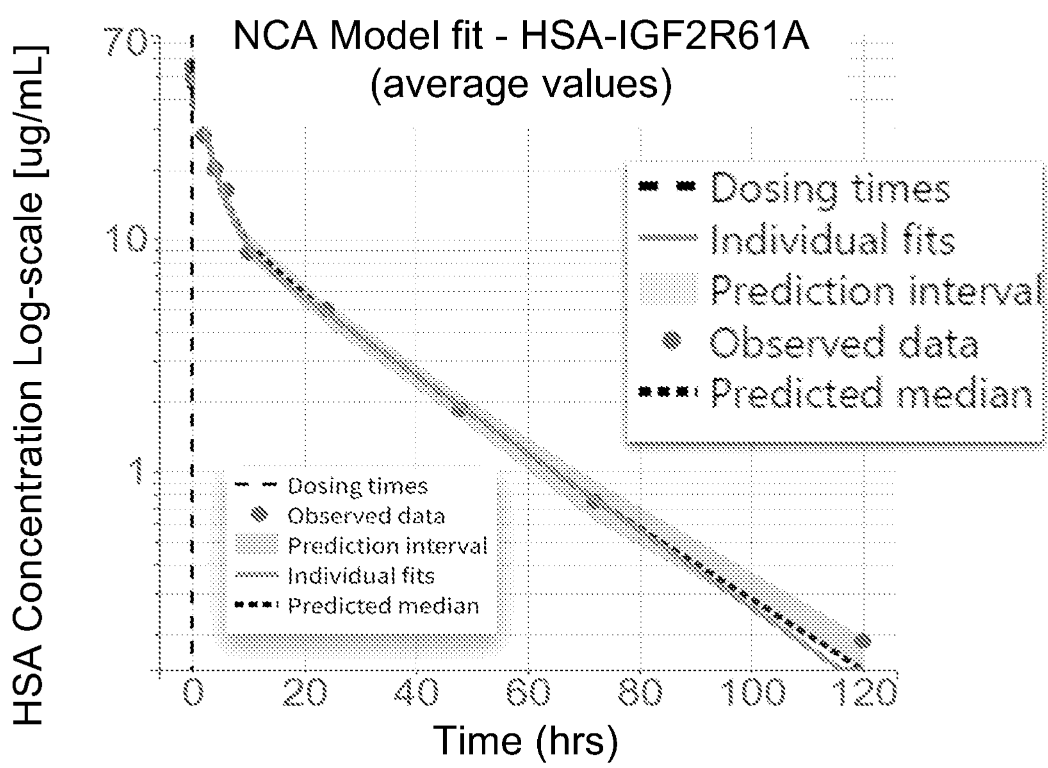


FIG 18B

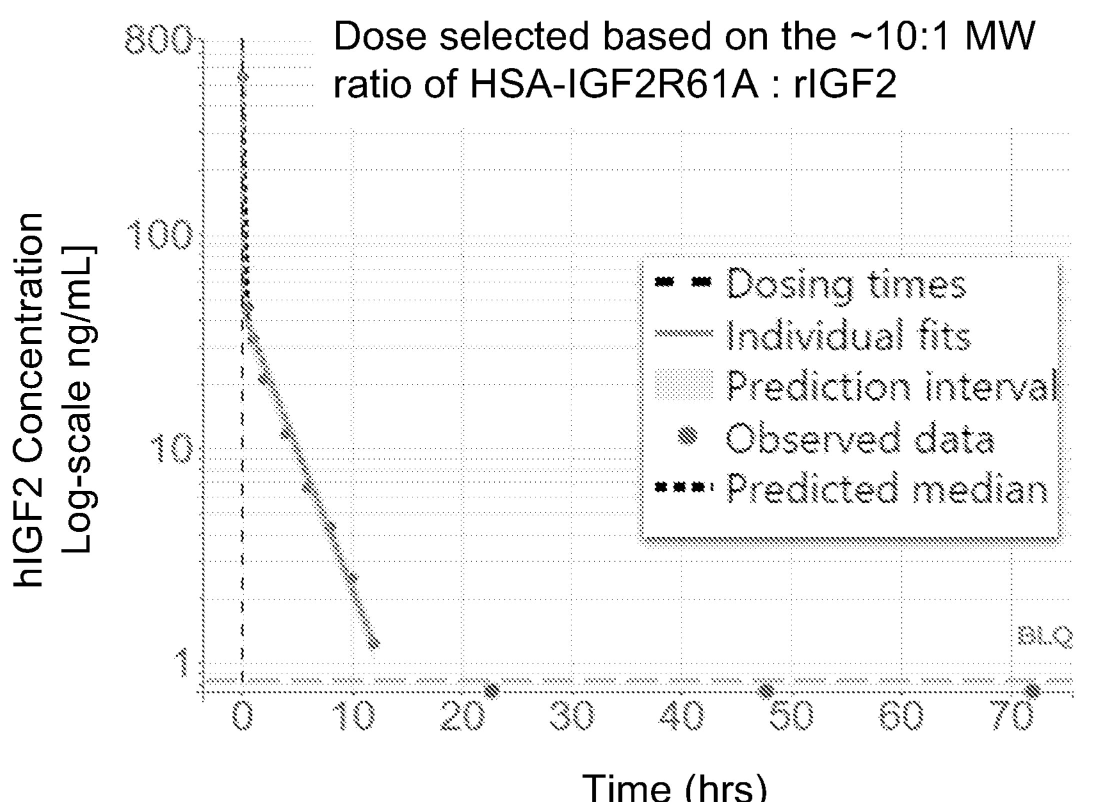
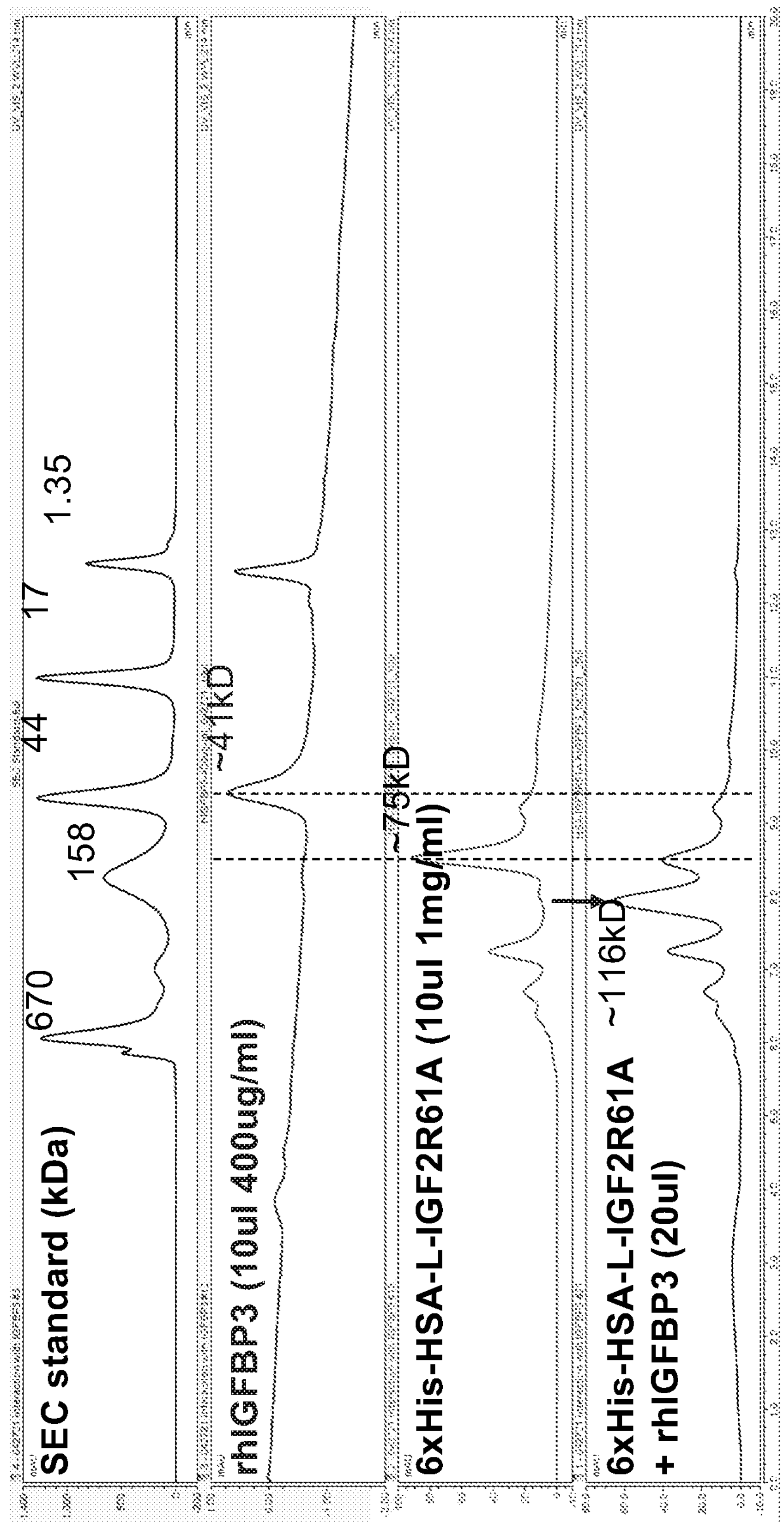


FIG 19



Log Dose Response

Fusion (% Area MyHC)

0
5
10
15
20
25

10
100
1,000
10,000
100,000

[IGF2] (ng/mL)

HSA-IGF2
HSA-IGF2R61A
HSA-IGF2R61A (SEC purification)

FIG 21A

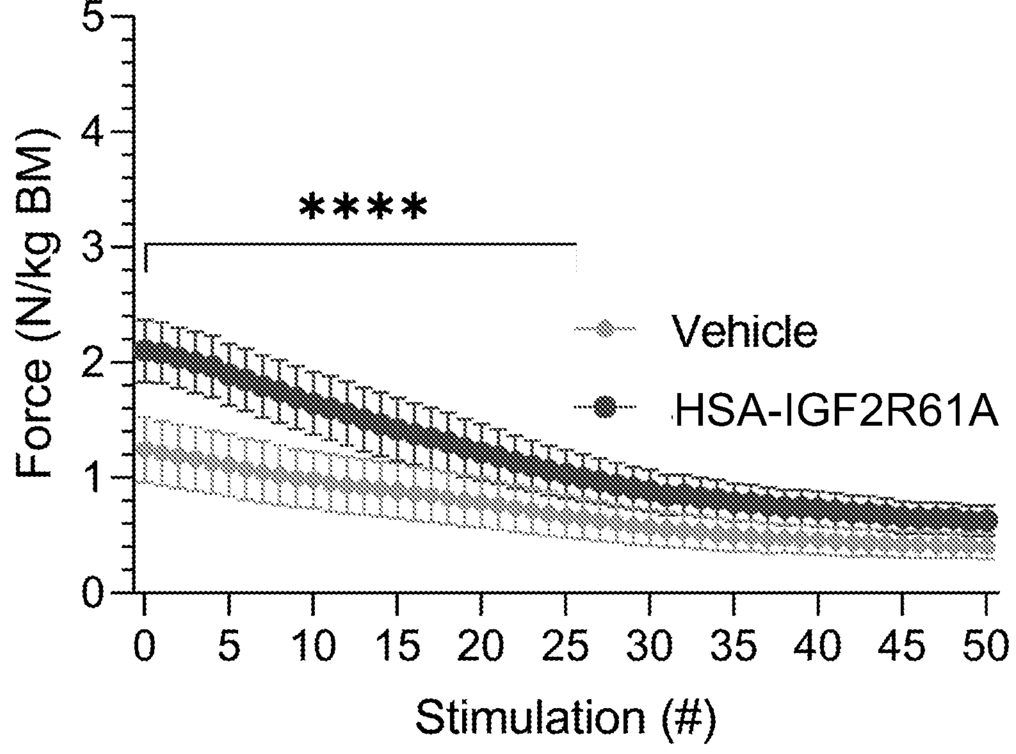


FIG 21B

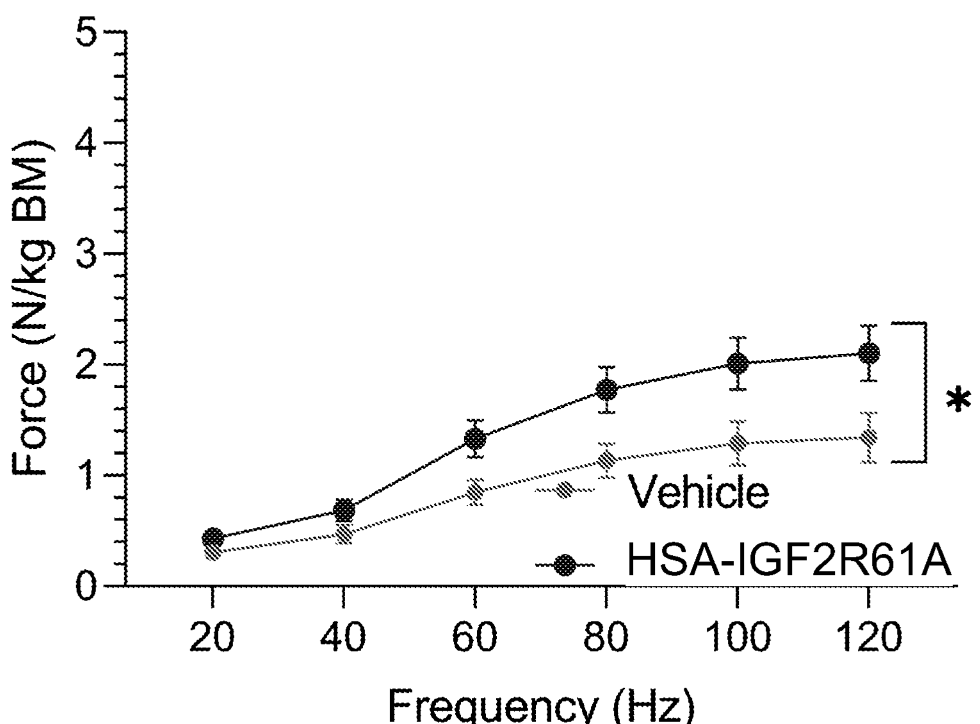


FIG 21C

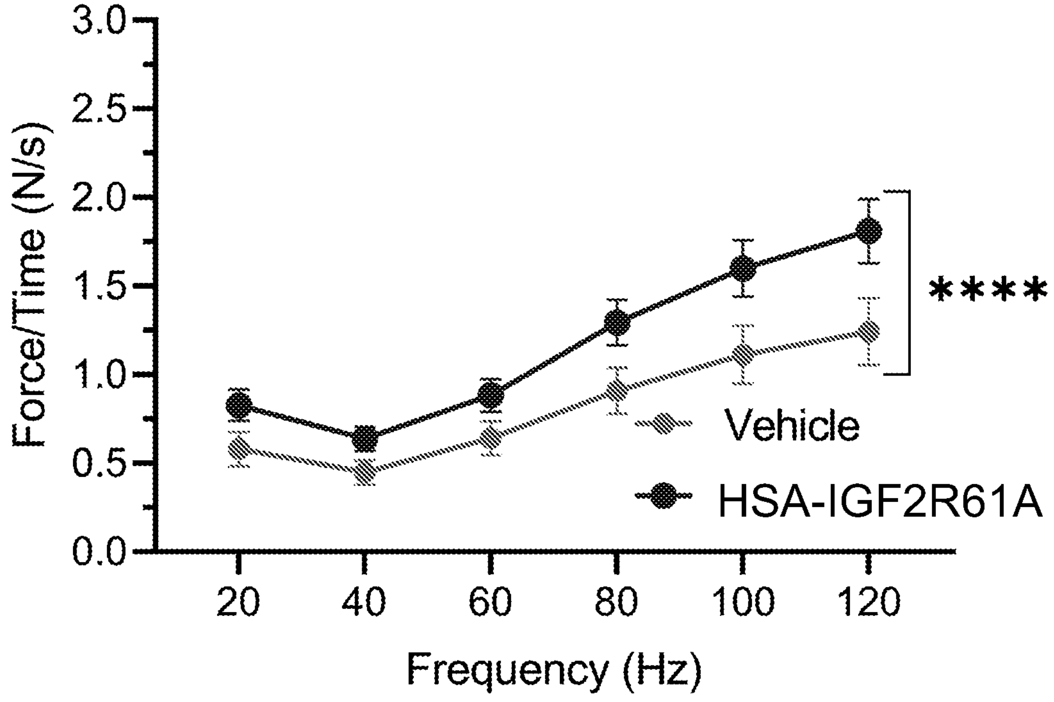


FIG 21D

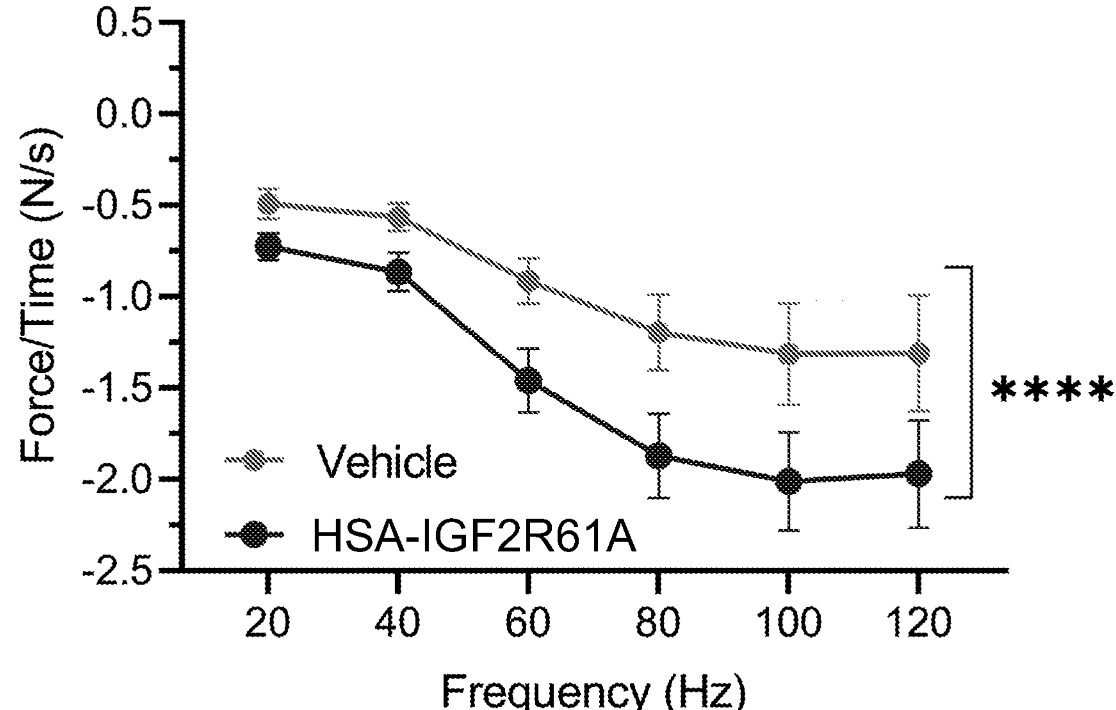


FIG 21E

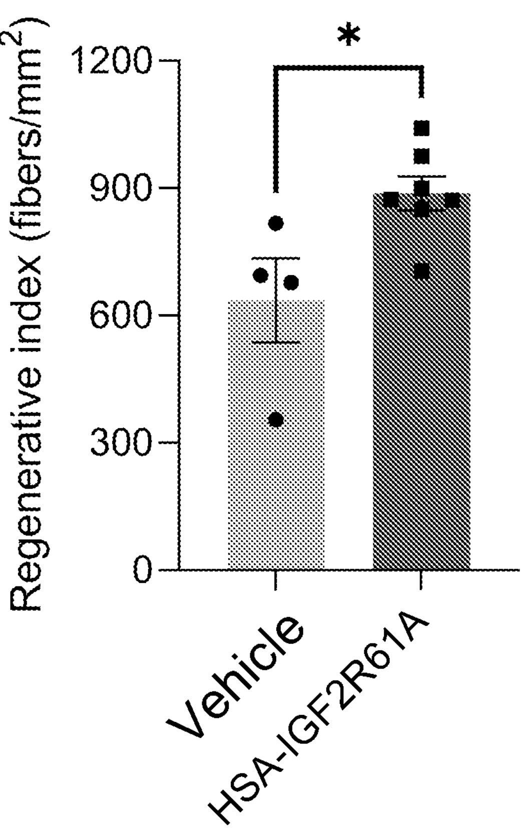
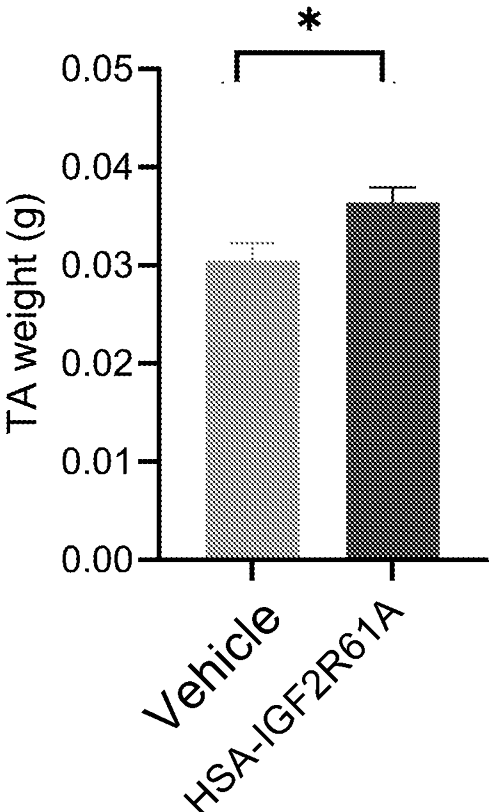


FIG 21F



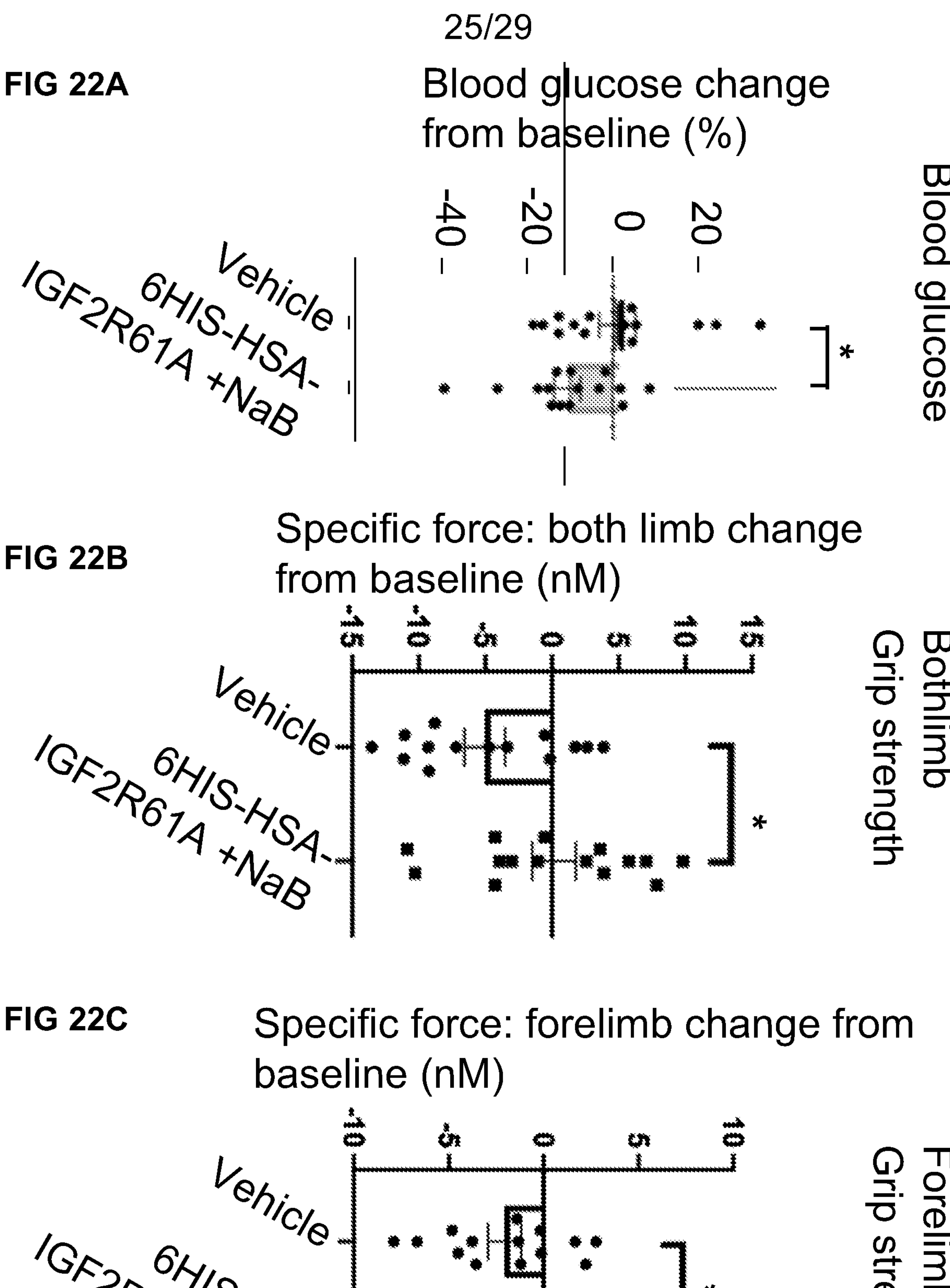


FIG 22A
Blood glucose change from baseline (%)
Blood glucose
-40
-20
0
20
Vehicle
6HIS-HSA-IGF2R61A +NaB
*
FIG 22B
Specific force: both limb change from baseline (nM)
Bothlimb Grip strength
-15
-10
-5
0
5
10
15
Vehicle
6HIS-HSA-IGF2R61A +NaB
*
FIG 22C
Specific force: forelimb change from baseline (nM)
Forelimb Grip strength
-10
-5
0
5
10
Vehicle
6HIS-HSA-IGF2R61A +NaB
**

FIG 22D

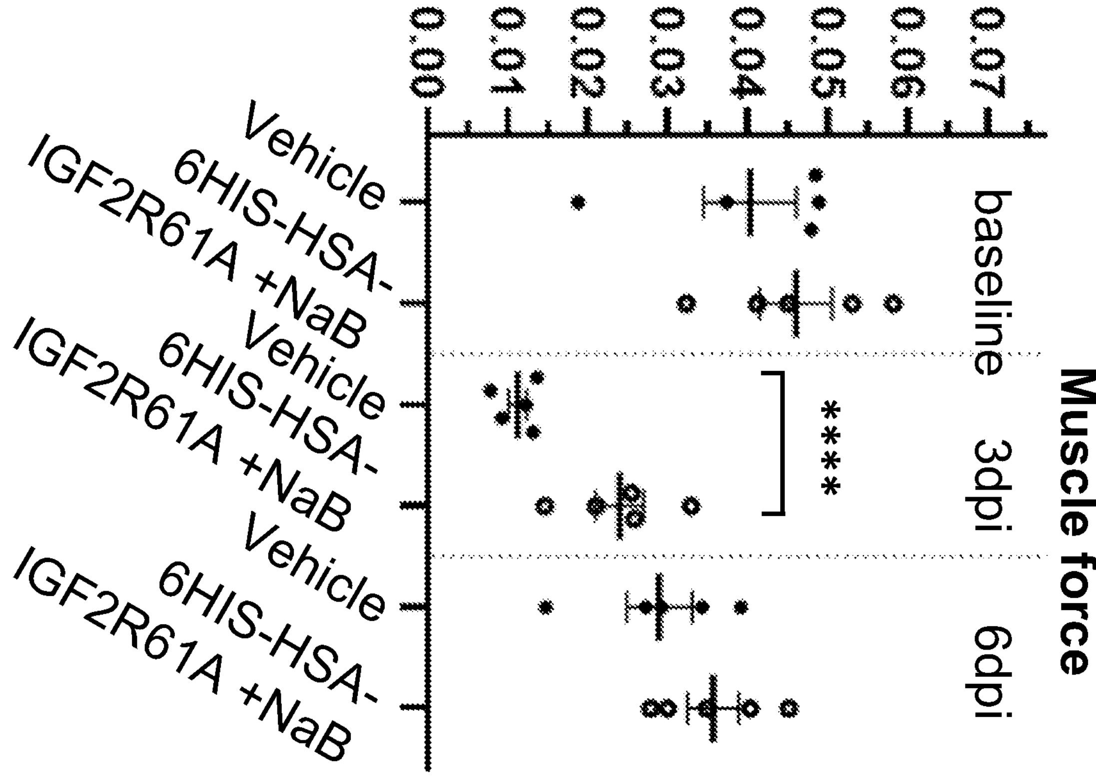


FIG 22E

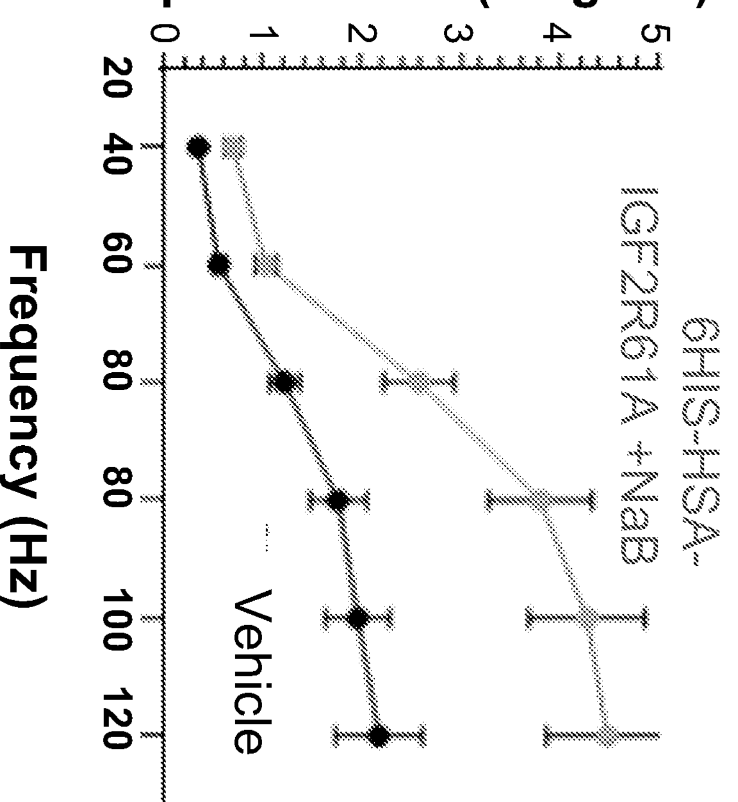


FIG 23A

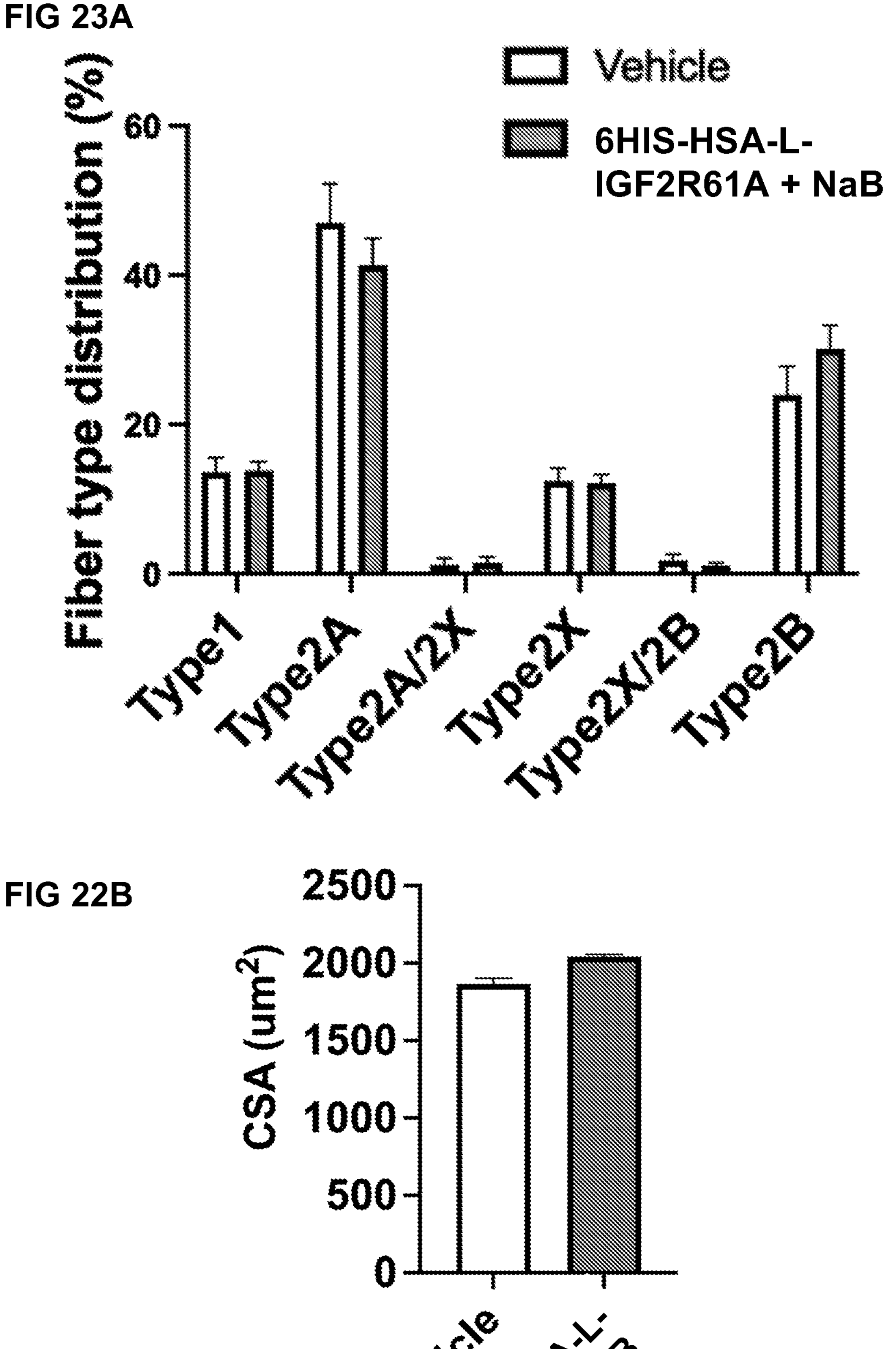


FIG 22B

FIG 24A

FIG 24B

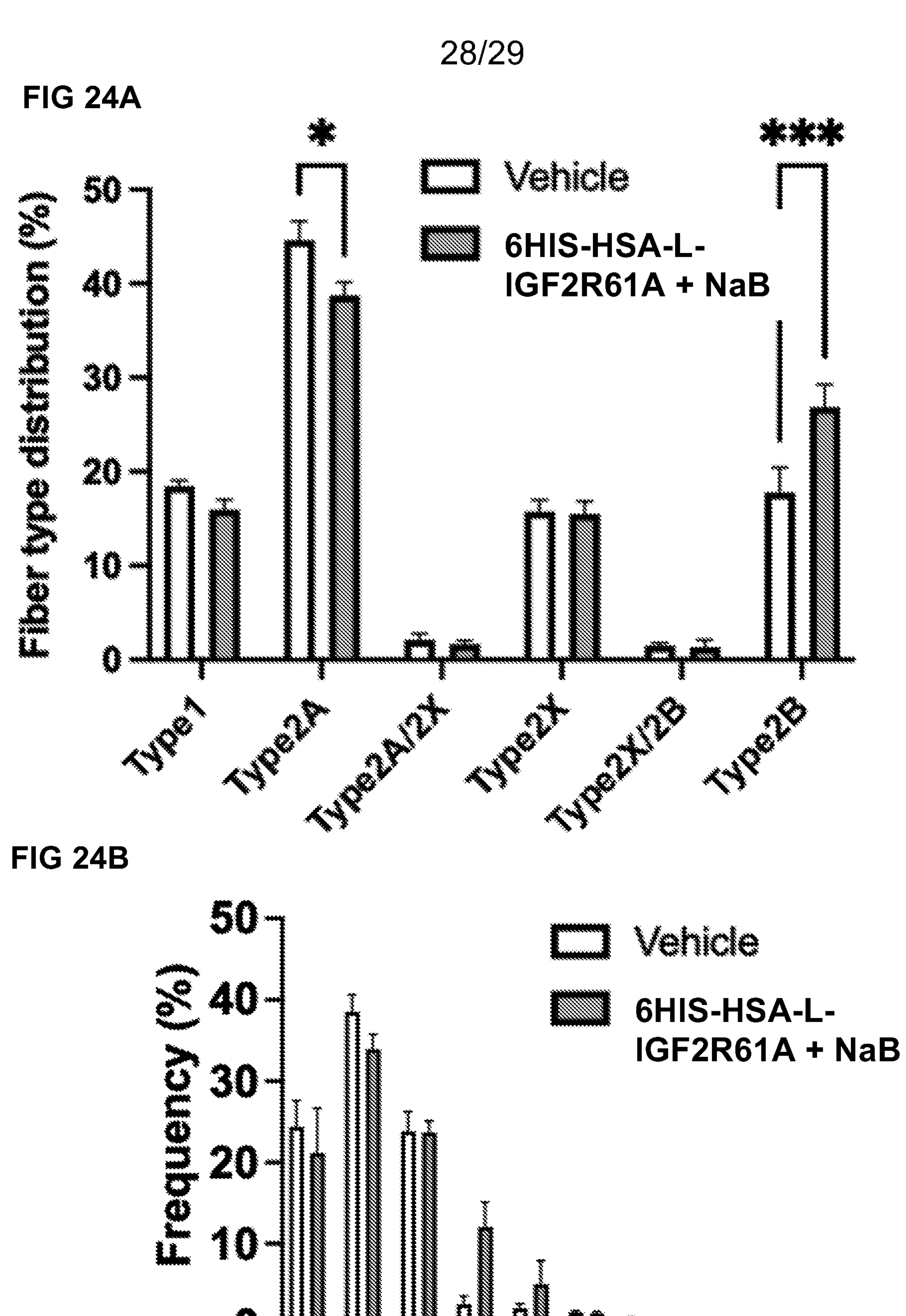


FIG 24C

% Twitch from baseline

% Change

100

50

0

-50

Vehicle

6HIS-HSA-L-IGF2R61A + NaB

FIG 24D

Novel Object Recognition

DI(%)

60

40

20

0

-20

-40

-60

Vehicle

6HIS-HSA-L-IGF2R61A + NaB