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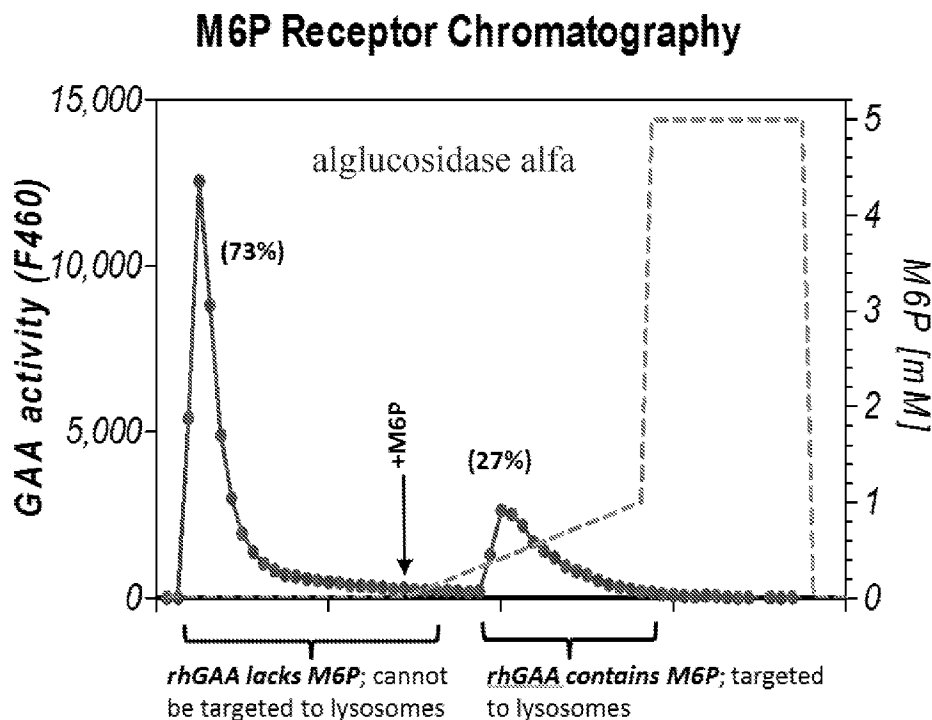


FIG. 1

(57) Abrégé/Abstract:

Provided herein are improved gene therapy vectors and methods of use, in some embodiments, comprising sequences for improved expression and cellular targeting of a therapeutic protein.

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(54) Title: GENE THERAPY CONSTRUCTS AND METHODS OF USE

M6P Receptor Chromatography

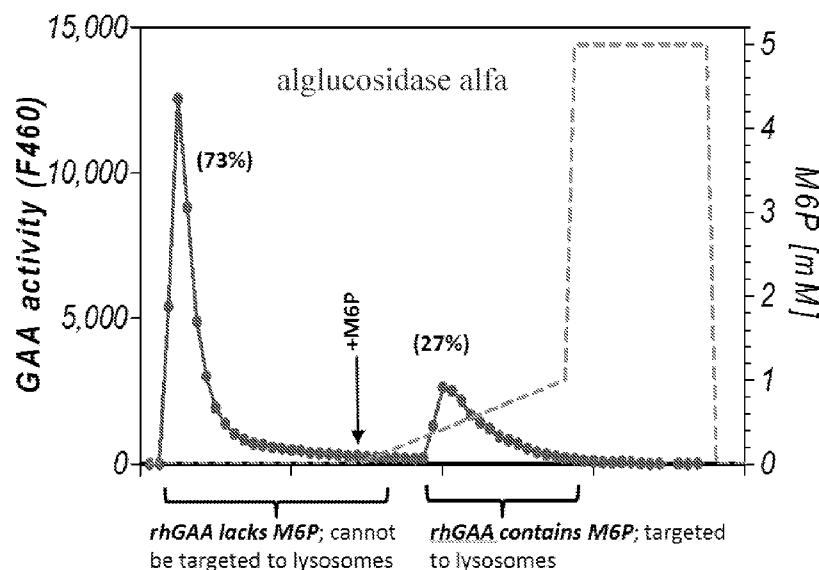


FIG. 1

(57) Abstract: Provided herein are improved gene therapy vectors and methods of use, in some embodiments, comprising sequences for improved expression and cellular targeting of a therapeutic protein.

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GENE THERAPY CONSTRUCTS AND METHODS OF USE

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/664,741, filed April 30, 2018; U.S. Provisional Application No. 62/688,640, filed June 22, 2018; and U.S. Provisional Application No. 62/744,068, filed October 10, 2018, each of which application is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Genetic disorders arise via heritable or de novo mutations occurring in gene coding regions of the genome. In some cases, such genetic disorders are treated by administration of a protein encoded by the gene mutated in the individual having the genetic disorder. Such treatment has challenges however, as administration of the protein does not always result in the protein reaching the organs, cells, or organelle where it is needed. Furthermore, this treatment also often requires biweekly infusions, which are not needed with gene therapy, where a single treatment can offer lasting relief. Therefore, gene therapy has the potential to offer improved results over currently available treatments for genetic disorders.

SUMMARY

[0003] Provided herein are compositions and methods for treatment of genetic disorders using gene therapy. Also provided herein are gene therapy vector components and methods to be used in gene therapy for improving protein expression and increasing cellular uptake or delivery and intracellular or sub-cellular targeting of therapeutic proteins provided by gene therapy vectors.

[0004] In certain aspects, there are provided gene therapy vectors, for example, gene therapy vectors comprising a nucleic acid construct comprising, in 5' to 3' order: (a) a translation initiation sequence, and (b) a nucleic acid sequence encoding a therapeutic protein. In some embodiments, the translation initiation sequence comprises a Kozak sequence. In some embodiments, the translation initiation sequence and the nucleic acid sequence encoding the therapeutic protein may overlap, such that the last three nucleotides of the translation initiation sequence are also the start codon for the therapeutic protein. In some embodiments, the Kozak sequence comprises the sequence AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide. In some embodiments, X₁ comprises A. In some embodiments, X₂ comprises G. In some embodiments, the Kozak sequence comprises a nucleic acid sequence at least 85% identical to AAGATGA (SEQ ID NO: 29). In some embodiments, the Kozak sequence differs from the sequence of AAGATGA (SEQ ID NO: 29) by one or two nucleotides. In some embodiments, the Kozak sequence comprises AAGATGA (SEQ ID NO: 29). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to GCAAGATG (SEQ ID NO: 44), wherein the the last three nucleotides (ATG) are also the start codon for the therapeutic protein. In some embodiments the Kozak sequence differs from the sequence of GCAAGATG (SEQ ID NO: 44) by

one or two nucleotides. In some embodiments, the Kozak sequence comprises GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to CACCATG (SEQ ID NO: 47). In some embodiments the Kozak sequence differs from the sequence of CACCATG (SEQ ID NO: 47) by one or two nucleotides. In some embodiments, the Kozak sequence comprises CACCATG (SEQ ID NO: 47). In some embodiments, the nucleic acid construct further comprises a nucleic acid sequence encoding a signal peptide capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide. In some embodiments, the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide. In some embodiments, the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the signal peptide differs from a sequence selected from the group consisting of SEQ ID Nos: 13-17 by 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid. In some embodiments, the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32. In some embodiments, the signal peptide differs from the sequence of SEQ ID NO: 32 by 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid. In some embodiments, the Gaussia signal peptide comprises an amino acid sequence of SEQ ID NO: 32. In some embodiments, the nucleic acid construct further comprises an internal ribosomal entry sequence (IRES). In some embodiments, the IRES is a cricket paralysis virus (CrPV) IRES. In some embodiments, the IRES comprises a nucleic acid sequence at least 90% identical to SEQ ID NO: 12. In some embodiments, the IRES comprises SEQ ID NO: 12.

[0005] In additional aspects, there are provided gene therapy vectors comprising a nucleic acid construct comprising, in 5' to 3' order: (a) a nucleic acid sequence encoding a signal peptide, and (b) a nucleic acid sequence encoding a therapeutic protein, wherein the signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide. In some embodiments, the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide. In some embodiments, the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the signal peptide differs from a sequence selected from the group consisting of SEQ ID Nos: 13-17 by 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid. In some embodiments, the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the signal peptide comprises a Gaussia signal peptide. In some embodiments, the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32. In some embodiments, the signal peptide differs from the sequence of SEQ ID NO: 32 by 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid. In some embodiments, the Gaussia signal peptide comprises SEQ ID NO: 32. In some embodiments, the nucleic acid construct

further comprises a translation initiation sequence. In some embodiments, the translation initiation sequence comprises a Kozak sequence comprising AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide. In some embodiments, X₁ comprises A. In some embodiments, X₂ comprises G. In some embodiments, the Kozak sequence comprises a nucleic acid sequence at least 85% identical to AAGATGA (SEQ ID NO: 29). In some embodiments, the Kozak sequence differs from the sequence of AAGATGA (SEQ ID NO: 29) by one or two nucleotides. In some embodiments, the Kozak sequence comprises AAGATGA (SEQ ID NO: 29). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence differs from the sequence of GCAAGATG (SEQ ID NO: 44) by one or two nucleotides. In some embodiments, the Kozak sequence comprises GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to CACCATG (SEQ ID NO: 47). In some embodiments the Kozak sequence differs from the sequence of CACCATG (SEQ ID NO: 47) by one or two nucleotides. In some embodiments, the Kozak sequence comprises CACCATG (SEQ ID NO: 47). In some embodiments, the nucleic acid construct further comprises an internal ribosomal entry sequence (IRES). In some embodiments, the IRES comprises an IRES selected from the group consisting of a cricket paralysis virus (CrPV) IRES, a picornavirus IRES, an Aphthovirus IRES, a Kaposi's sarcoma-associated herpesvirus IRES, a Hepatitis A IRES, a Hepatitis C IRES, a Pestivirus IRES, a Cripavirus IRES, a Rhopalosiphum padi virus IRES, and a Merek's disease virus IRES. In some embodiments, the IRES comprises a nucleic acid sequence at least 90% identical to SEQ ID NO: 12. In some embodiments, the IRES comprises SEQ ID NO: 12.

[0006] In further aspects, there are provided gene therapy vectors comprising a nucleic acid construct comprising, in 5' to 3' order: (a) an internal ribosomal entry sequence (IRES), and (b) a nucleic acid sequence encoding a therapeutic protein. In some embodiments, the IRES comprises an IRES selected from the group consisting of a cricket paralysis virus (CrPV) IRES, a picornavirus IRES, an Aphthovirus IRES, a Kaposi's sarcoma-associated herpesvirus IRES, a Hepatitis A IRES, a Hepatitis C IRES, a Pestivirus IRES, a Cripavirus IRES, a Rhopalosiphum padi virus IRES, and a Merek's disease virus IRES. In some embodiments, the IRES is a cricket paralysis virus (CrPV) IRES. In some embodiments, the IRES comprises a nucleic acid sequence at least 90% identical to SEQ ID NO: 12. In some embodiments, the IRES comprises SEQ ID NO: 12. In some embodiments, the nucleic acid construct further comprises a translation initiation sequence. In some embodiments, the translation initiation sequence comprises a Kozak sequence comprising AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide. In some embodiments, X₁ comprises A. In some embodiments, X₂ comprises G. In some embodiments, the Kozak sequence comprises a nucleic acid sequence at least 90% identical to AAGATGA (SEQ ID NO: 29). In some embodiments, the Kozak sequence comprises AAGATGA (SEQ ID NO: 29). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence differs from the

sequence of GCAAGATG (SEQ ID NO: 44) by one or two nucleotides. In some embodiments, the Kozak sequence comprises GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to CACCATG (SEQ ID NO: 47). In some embodiments the Kozak sequence differs from the sequence of CACCATG (SEQ ID NO: 47) by one or two nucleotides. In some embodiments, the Kozak sequence comprises CACCATG (SEQ ID NO: 47). In some embodiments, the nucleic acid construct further comprises a signal nucleic acid sequence encoding a signal peptide capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide. In some embodiments, the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide. In some embodiments, the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32. In some embodiments, the Gaussia signal peptide comprises SEQ ID NO: 32.

[0007] In some embodiments, any of the the nucleic acid constructs provided herein further comprise a nucleic acid sequence encoding a peptide that selectively binds to the CI-MPR with high affinity, wherein the therapeutic protein and the peptide that selectively binds to the CI-MPR are expressed as a fusion protein. In some embodiments, the nucleic acid construct further comprises a sequence encoding a linker peptide between the nucleic acid encoding the peptide that selectively binds to the CI-MPR nucleotide sequence and the nucleic acid sequence encoding the therapeutic protein. In some embodiments, the sequence of the linker peptide may overlap with the sequence of the therapeutic peptide or the sequence of the peptide that selectively binds to the CI-MPR, or both. In some embodiments, the peptide that binds to CI-MPR with high affinity is a variant IGF2 peptide (vIGF2). In some embodiments, the vIGF2 peptide facilitates uptake into the cells. In some embodiments, the vIGF2 peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-11. In some embodiments, the vIGF2 peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-11. In some embodiments, the vIGF2 nucleotide sequence is 5' to the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the vIGF2 nucleotide sequence is 3' to the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the nucleic acid construct further comprises a sequence encoding a linker peptide between the vIGF2 nucleotide sequence and the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the linker peptide consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 18-21, SEQ ID NO: 33 or SEQ ID NO: 37. In some embodiments, the linker peptide comprises SEQ ID NO: 18-21, SEQ ID NO: 33 or SEQ ID NO: 37. In some embodiments, the therapeutic protein is associated with a lysosomal storage disorder. In some

embodiments, the therapeutic protein is a lysosomal enzyme or enzymatically active fragment thereof. In some embodiments, the therapeutic protein is selected from the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetyl-galactosamine-6-sulfatase, glycosaminoglycan N-acetyl-galactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is an alpha-galactosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is an alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is a palmitoyl protein thioesterase (PPT) – including palmitoyl protein thioesterase 1 and 2 (PPT1 and PPT2 respectively). In some embodiments, the therapeutic protein is palmitoyl protein thioesterase 1. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the therapeutic protein is associated with a genetic disorder selected from the group consisting of CDKL5 deficiency disorder, cystic fibrosis, alpha- and beta-thalassemias, sickle cell anemia, Marfan syndrome, fragile X syndrome, Huntington's disease, hemochromatosis, Congenital Deafness (nonsyndromic), Tay-Sachs, Familial hypercholesterolemia, Duchenne muscular dystrophy, Stargardt disease, Usher syndrome, choroideremia, achromatopsia, X-linked retinoschisis, hemophilia, Wiskott-Aldrich syndrome, X-linked chronic granulomatous disease, aromatic L-amino acid decarboxylase deficiency, recessive dystrophic epidermolysis bullosa, alpha 1 antitrypsin deficiency, Hutchinson-Gilford progeria syndrome (HGPS), Noonan syndrome, X-linked severe combined immunodeficiency (X-SCID). In some embodiments, the therapeutic protein is selected from the group consisting of CDKL5, Connexin 26, hexosaminidase A, LDL receptor, Dystrophin, CFTR, beta-globulin, HFE, Huntington, ABCA4, myosin VIIA (MYO7A), Rab escort protein -1 (REP1), cyclic nucleotide gated channel beta 3 (CNGB3), retinoschisin 1 (RS1), hemoglobin subunit beta (HBB), Factor IX, WAS, cytochrome B-245 beta chain, dopa decarboxylase (DDC), collagen type VII alpha 1 chain (COL7A1), serpin family A member 1 (SERPINA1), LMNA, PTPN11, SOS1, RAF1, KRAS, and IL2 receptor γ gene. In some embodiments, the therapeutic protein is capable of replacing a defective or deficient protein associated with a genetic disorder in a subject having the genetic disorder. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type

III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolysaccharidosis type I, mucopolysaccharidosis type II, mucopolysaccharidosis type III, mucopolysaccharidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteaux-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, and Schindler disease type II. In some embodiments, the lysosomal storage disorder is selected from the group consisting of activator deficiency, GM2-gangliosidosis; GM2-gangliosidosis, AB variant; alpha-mannosidosis (type 2, moderate form; type 3, neonatal, severe); beta-mannosidosis; lysosomal acid lipase deficiency; cystinosis (late-onset juvenile or adolescent nephropathic type; infantile nephropathic); Chanarin-Dorfman syndrome; neutral lipid storage disease with myopathy; NLSMD; Danon disease; Fabry disease; Fabry disease type II, late-onset; Farber disease; Farber lipogranulomatosis; fucosidosis; galactosialidosis (combined neuraminidase & beta-galactosidase deficiency); Gaucher disease ; type II Gaucher disease ; type III Gaucher disease; type IIIC Gaucher disease; Gaucher disease, atypical, due to saposin C deficiency; GM1-gangliosidosis (late-infantile/juvenile GM1-gangliosidosis; adult/chronic GM1-gangliosidosis); Globoid cell leukodystrophy, Krabbe disease (Late infantile onset; Juvenile Onset; Adult Onset); Krabbe disease, atypical, due to saposin A deficiency; Metachromatic Leukodystrophy (juvenile; adult); partial cerebroside sulfate deficiency; pseudoarylsulfatase A deficiency; metachromatic leukodystrophy due to saposin B deficiency; Mucopolysaccharidoses disorders: MPS I, Hurler syndrome; MPS I, Hurler-Scheie syndrome; MPS I, Scheie syndrome ; MPS II, Hunter syndrome ; MPS II, Hunter syndrome ; Sanfilippo syndrome Type A / MPS IIIA ; Sanfilippo syndrome Type B / MPS IIIB ; Sanfilippo syndrome Type C / MPS IIIC ; Sanfilippo syndrome Type D / MPS IIID ; Morquio syndrome, type A / MPS IVA ; Morquio syndrome, type B / MPS IVB ; MPS IX hyaluronidase deficiency ; MPS VI Maroteaux-Lamy syndrome ; MPS VII Sly syndrome; mucopolysaccharidosis I, sialidosis type II; I-cell disease, Leroy disease, mucopolysaccharidosis II; Pseudo-Hurler polydystrophy / mucopolysaccharidosis type III; mucopolysaccharidosis IIIC / ML III GAMMA; mucopolysaccharidosis type IV; multiple sulfatase deficiency; Niemann-Pick disease (type B; type C1 / chronic neuronopathic form; type C2; type D / Nova Scotian type); Neuronal Ceroid Lipofuscinoses: CLN6 disease - Atypical Late Infantile, Late-Onset variant, Early Juvenile; Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease; Finnish Variant Late Infantile CLN5; Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease; Kufs/Adult-onset NCL/CLN4 disease (type B); Northern Epilepsy/variant late infantile CLN8; Santavuori-Haltia/Infantile CLN1/PPT disease; Pompe disease (glycogen storage disease type II); late-onset Pompe disease; Pycnodysostosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 Gangliosidosis; Schindler disease (type III / intermediate, variable); Kanzaki disease; Salla disease; infantile free sialic acid storage disease (ISSD); spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME); Tay-Sachs disease / GM2 gangliosidosis; juvenile-onset Tay-Sachs disease; late-onset Tay-Sachs disease; Christianson syndrome; Lowe

oculocerebrorenal syndrome; Charcot-Marie-Tooth type 4J, CMT4J; Yunis-Varon syndrome; bilateral temporooccipital polymicrogyria (BTOP); X-linked hypercalciuric nephrolithiasis, Dent-1; and Dent disease 2, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and neuronal ceroid lipofuscinosis. In some embodiments, the genetic disorder is Pompe disease. In some embodiments, the genetic disorder is neuronal ceroid lipofuscinosis. In some embodiments, the neuronal ceroid lipofuscinosis is selected from the group consisting of Infantile NCL (Santavuori-Haltia disease), Late Infantile NCL (Jansky-Bielschowsky disease), Batten disease, Adult NCL (Kufs disease), Finnish Late Infantile NCL, Variant Late Infantile NCL, CLN7, CLN8, Turkish Late Infantile NCL, NCL type 9, and CLN10. In some embodiments, the gene therapy vector is a viral vector. In some embodiments, the viral vector is an adeno-associated virus vector, a retrovirus vector, a lentivirus vector, a pox virus vector, a vaccinia virus vector, an adenovirus vector, or a herpes virus vector. In some embodiments, the viral vector is an AAV vector. In some embodiments, the AAV vector comprises inverted terminal repeats (ITRs). In some embodiments, the AAV vector is selected from the group consisting of an AAV1 vector, an AAV2 vector, an AAV3 vector, an AAV4 vector, an AAV5 vector, an AAV6 vector, an AAV7 vector, an AAV8 vector, an AAV9 vector, an AAVrhS vector, an AAVrh10 vector, an AAVrh33 vector, an AAVrh34 vector, an AAVrh74 vector, an AAV Anc80 vector, an AAVPHP.B vector, an AAVhu68 vector, and an AAV-DJ vector.

[0008] In certain aspects, there are provided gene therapy vectors, such as gene therapy vectors comprising: (a) a nucleic acid sequence encoding a therapeutic protein, and (b) a nucleic acid sequence encoding a peptide that binds to the CI-MPR with high affinity. In some embodiments, the peptide is a variant IGF2 (vIGF2) peptide. In some embodiments, the vIGF2 peptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 and having at least one substitution at one or more positions selected from the group consisting of positions 6, 26, 27, 43, 48, 49, 50, 54, 55, and 65 of SEQ ID NO: 1. In some embodiments, the at least one substitution is selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R, and K65R of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises at least two substitutions at two or more positions selected from the group consisting of positions 6, 26, 27, 43, 48, 49, 50, 54, and 55 of SEQ ID NO: 1. In some embodiments, the at least two substitutions are selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, and L55R of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion at position 1 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-2 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-3 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-4 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-4 of SEQ ID NO: 1 and substitutions of E6R, Y27L, and K65R. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of

positions 1-4 of SEQ ID NO:1 and substitutions of E6R and Y27L. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-5 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion at positions 1-6 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion at positions 1-7 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide has decreased or no affinity for the insulin receptor and IGF1R as compared to native IGF2 peptide. In some embodiments, the vIGF2 peptide is capable of facilitating uptake of the therapeutic protein into a cell. In some embodiments, the vIGF2 peptide is capable of facilitating uptake of the therapeutic protein into a lysosome. In some embodiments, the therapeutic protein is capable of replacing a defective or deficient protein associated with a genetic disorder in a subject having the genetic disorder. In some embodiments, the therapeutic protein is a lysosomal enzyme or enzymatically active fragment thereof. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, and Schindler disease type II. In some embodiments, the lysosomal storage disorder is selected from the group consisting of activator deficiency, GM2-gangliosidosis; GM2-gangliosidosis, AB variant; alpha-mannosidosis (type 2, moderate form; type 3, neonatal, severe); beta-mannosidosis; lysosomal acid lipase deficiency; cystinosis (late-onset juvenile or adolescent nephropathic type; infantile nephropathic); Chanarin-Dorfman syndrome; neutral lipid storage disease with myopathy; NLSDM; Danon disease; Fabry disease; Fabry disease type II, late-onset; Farber disease; Farber lipogranulomatosis; fucosidosis; galactosialidosis (combined neuraminidase & beta-galactosidase deficiency); Gaucher disease ; type II Gaucher disease ; type III Gaucher disease; type IIIC Gaucher disease; Gaucher disease, atypical, due to saposin C deficiency; GM1-gangliosidosis (late-infantile/juvenile GM1-gangliosidosis; adult/chronic GM1-gangliosidosis); Globoid cell leukodystrophy, Krabbe disease (Late infantile onset; Juvenile Onset; Adult Onset); Krabbe disease, atypical, due to saposin A deficiency; Metachromatic Leukodystrophy (juvenile; adult); partial cerebroside sulfate deficiency; pseudoarylsulfatase A deficiency; metachromatic leukodystrophy due to saposin B deficiency; Mucopolysaccharidoses disorders: MPS I, Hurler syndrome; MPS I, Hurler-Scheie syndrome; MPS I, Scheie syndrome ; MPS II, Hunter syndrome ; MPS II, Hunter syndrome ; Sanfilippo syndrome Type A / MPS IIIA ; Sanfilippo syndrome Type B / MPS IIIB ; Sanfilippo syndrome Type C / MPS IIIC ; Sanfilippo syndrome Type D / MPS IIID ; Morquio syndrome, type A / MPS IVA ; Morquio syndrome, type B / MPS IVB ; MPS IX hyaluronidase deficiency ; MPS VI Maroteaux-Lamy syndrome ;

MPS VII Sly syndrome; mucopolipidosis I, sialidosis type II; I-cell disease, Leroy disease, mucopolipidosis II; Pseudo-Hurler polydystrophy / mucopolipidosis type III; mucopolipidosis IIIC / ML III GAMMA; mucopolipidosis type IV; multiple sulfatase deficiency; Niemann-Pick disease (type B; type C1 / chronic neuronopathic form; type C2; type D / Nova Scotian type); Neuronal Ceroid Lipofuscinoses: CLN6 disease - Atypical Late Infantile, Late-Onset variant, Early Juvenile; Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease; Finnish Variant Late Infantile CLN5; Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease; Kufs/Adult-onset NCL/CLN4 disease (type B); Northern Epilepsy/variant late infantile CLN8; Santavuori-Haltia/Infantile CLN1/PPT disease; Pompe disease (glycogen storage disease type II); late-onset Pompe disease; Pycnodysostosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 Gangliosidosis; Schindler disease (type III / intermediate, variable); Kanzaki disease; Salla disease; infantile free sialic acid storage disease (ISSD); spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME); Tay-Sachs disease / GM2 gangliosidosis; juvenile-onset Tay-Sachs disease; late-onset Tay-Sachs disease; Christianson syndrome; Lowe oculocerebrorenal syndrome; Charcot-Marie-Tooth type 4J, CMT4J; Yunis-Varon syndrome; bilateral temporooccipital polymicrogyria (BTOP); X-linked hypercalciuric nephrolithiasis, Dent-1; and Dent disease 2, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), and neuronal ceroid lipofuscinosis. In some embodiments, the genetic disorder is Pompe disease. In some embodiments, the genetic disorder is neuronal ceroid lipofuscinosis. In some embodiments, the neuronal ceroid lipofuscinosis is selected from the group consisting of Infantile NCL (Santavuori-Haltia disease), Late Infantile NCL (Jansky-Bielschowsky disease), Batten disease, Adult NCL (Kufs disease), Finnish Late Infantile NCL, Variant Late Infantile NCL, CLN7, CLN8, Turkish Late Infantile NCL, NCL type 9, and CLN10. In some embodiments, the therapeutic protein is a soluble lysosomal enzyme. In some embodiments, the therapeutic protein comprises an enzyme selected from the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is an alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the palmitoyl protein thioesterase is palmitoyl protein

thioesterase 1 (PPT1) or 2 (PPT2). In some embodiments, the palmitoyl protein thioesterase is palmitoyl protein thioesterase 1. In some embodiments, the nucleic acid construct further comprises a translation initiation sequence. In some embodiments, the translation initiation sequence comprises a Kozak sequence. In some embodiments, the Kozak sequence comprises the sequence AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide. In some embodiments, X₁ comprises A. In some embodiments, X₂ comprises G. In some embodiments, the Kozak sequence comprises a nucleic acid sequence at least 90% identical to AAGATGA (SEQ ID NO: 29). In some embodiments, the Kozak sequence comprises AAGATGA (SEQ ID NO: 29). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence differs from the sequence of GCAAGATG (SEQ ID NO: 44) by one or two nucleotides. In some embodiments, the Kozak sequence comprises GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to CACCATG (SEQ ID NO: 47). In some embodiments the Kozak sequence differs from the sequence of CACCATG (SEQ ID NO: 47) by one or two nucleotides. In some embodiments, the Kozak sequence comprises CACCATG (SEQ ID NO: 47). In some embodiments, the nucleic acid construct further comprises a nucleic acid sequence encoding a signal peptide wherein the signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide. In some embodiments, the nucleic acid construct further comprises a nucleic acid sequence encoding a signal peptide wherein the signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein with the natural signal peptide. In some embodiments, the nucleic acid construct comprises a nucleic acid sequence encoding a non-native signal peptide, wherein the non-native signal peptide is capable of increasing secretion of the therapeutic protein as compared to the native signal peptide for the therapeutic protein. In some embodiments, the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide. In some embodiments, the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the signal peptide comprises a Gaussia signal peptide. In some embodiments, the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32. In some embodiments, the Gaussia signal peptide comprises SEQ ID NO: 32. In some embodiments, the vIGF2 nucleic acid sequence is 5' to the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the vIGF2 nucleic acid sequence is 3' to the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the nucleic acid construct further comprises a sequence encoding a linker peptide between the vIGF2 nucleotide sequence and the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker

peptide comprises SEQ ID NO: 18-21 or SEQ ID NO: 33. In some embodiments, the gene therapy vector is a virus vector. In some embodiments, the virus vector is an adenovirus vector, an adeno-associated virus (AAV) vector, a retrovirus vector, a lentivirus vector, or a herpes virus vector. In some embodiments, the virus vector is an AAV vector. In some embodiments, the AAV vector comprises inverted terminal repeats (ITRs). In some embodiments, the AAV vector is selected from the group consisting of an AAV1 vector, an AAV2 vector, an AAV3 vector, an AAV4 vector, an AAV5 vector, an AAV6 vector, an AAV7 vector, an AAV8 vector, an AAV9 vector, an AAVrhS vector, an AAVrh10 vector, an AAVrh33 vector, an AAVrh34 vector, an AAVrh74 vector, an AAV Anc80 vector, an AAVPHP.B vector, an AAVhu68 vector, and an AAV-DJ vector.

[0009] A gene therapy vector comprising a nucleic acid construct comprising: (a) a nucleic acid sequence encoding a therapeutic protein, and (b) a nucleic acid sequence encoding a peptide that increases endocytosis of the therapeutic protein. In some embodiments, the peptide that increases endocytosis of the therapeutic protein is a peptide that binds to the CI-MPR. In some embodiments, the peptide is a variant IGF2 (vIGF2) peptide, a HIRMaB, or a TfRMaB or other cell targeting peptide or protein. In some embodiments, the peptide is vIGF2. In some embodiments, the vIGF2 peptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 and having at least one substitution at one or more positions selected from the group consisting of positions 6, 26, 27, 43, 48, 49, 50, 54, 55, and 65 of SEQ ID NO: 1. In some embodiments, the at least one substitution is selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R, and K65R of SEQ ID NO: 1. In some embodiments, the at least one substitution is selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, and L55R of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises at least two substitutions at two or more positions selected from the group consisting of positions 6, 26, 27, 43, 48, 49, 50, 54, and 55 of SEQ ID NO: 1. In some embodiments, the at least two substitutions are selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, and L55R of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion at position 1 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion at positions 1-6 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-2 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-3 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-4 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-4 of SEQ ID NO: 1 and a substitution of E6R, Y27L, and K65R. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-4 of SEQ ID NO: 1 and a substitution of E6R and Y27L. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-5 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion of positions 1-6 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises

an N-terminal deletion at positions 1-7 of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide has increased specificity for the cation-independent M6P receptor (CI-MPR) as compared to native IGF2 peptide. In some embodiments, the vIGF2 peptide is capable of facilitating uptake of the therapeutic protein into a lysosome in a cell. In some embodiments, the therapeutic protein is capable of replacing a defective or deficient protein associated with a genetic disorder in a subject having the genetic disorder. In some embodiments, the therapeutic protein is a lysosomal enzyme or enzymatically active fragment thereof. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteaux-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, and Schindler disease type II. In some embodiments, the lysosomal storage disorder is selected from the group consisting of activator deficiency, GM2-gangliosidosis; GM2-gangliosidosis, AB variant; alpha-mannosidosis (type 2, moderate form; type 3, neonatal, severe); beta-mannosidosis; lysosomal acid lipase deficiency; cystinosis (late-onset juvenile or adolescent nephropathic type; infantile nephropathic); Chanarin-Dorfman syndrome; neutral lipid storage disease with myopathy; NLSMD; Danon disease; Fabry disease; Fabry disease type II, late-onset; Farber disease; Farber lipogranulomatosis; fucosidosis; galactosialidosis (combined neuraminidase & beta-galactosidase deficiency); Gaucher disease ; type II Gaucher disease ; type III Gaucher disease; type IIIC Gaucher disease; Gaucher disease, atypical, due to saposin C deficiency; GM1-gangliosidosis (late-infantile/juvenile GM1-gangliosidosis; adult/chronic GM1-gangliosidosis); Globoid cell leukodystrophy, Krabbe disease (Late infantile onset; Juvenile Onset; Adult Onset); Krabbe disease, atypical, due to saposin A deficiency; Metachromatic Leukodystrophy (juvenile; adult); partial cerebroside sulfate deficiency; pseudoarylsulfatase A deficiency; metachromatic leukodystrophy due to saposin B deficiency; Mucopolysaccharidoses disorders: MPS I, Hurler syndrome; MPS I, Hurler-Scheie syndrome; MPS I, Scheie syndrome ; MPS II, Hunter syndrome ; MPS II, Hunter syndrome ; Sanfilippo syndrome Type A / MPS IIIA ; Sanfilippo syndrome Type B / MPS IIIB ; Sanfilippo syndrome Type C / MPS IIIC ; Sanfilippo syndrome Type D / MPS IIID ; Morquio syndrome, type A / MPS IVA ; Morquio syndrome, type B / MPS IVB ; MPS IX hyaluronidase deficiency ; MPS VI Maroteaux-Lamy syndrome ; MPS VII Sly syndrome; mucopolipidosis I, sialidosis type II; I-cell disease, Leroy disease, mucopolipidosis II; Pseudo-Hurler polydystrophy / mucopolipidosis type III; mucopolipidosis IIIC / ML III GAMMA; mucopolipidosis type IV; multiple sulfatase deficiency; Niemann-Pick disease (type B; type C1 / chronic neuronopathic form; type C2; type D / Nova Scotian type); Neuronal Ceroid Lipofuscinoses: CLN6 disease - Atypical Late

Infantile, Late-Onset variant, Early Juvenile; Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease; Finnish Variant Late Infantile CLN5; Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease; Kufs/Adult-onset NCL/CLN4 disease (type B); Northern Epilepsy/variant late infantile CLN8; Santavuori-Haltia/Infantile CLN1/PPT disease; Pompe disease (glycogen storage disease type II); late-onset Pompe disease; Pycnodysostosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 Gangliosidosis; Schindler disease (type III / intermediate, variable); Kanzaki disease; Salla disease; infantile free sialic acid storage disease (ISSD); spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME); Tay-Sachs disease / GM2 gangliosidosis; juvenile-onset Tay-Sachs disease; late-onset Tay-Sachs disease; Christianson syndrome; Lowe oculocerebrorenal syndrome; Charcot-Marie-Tooth type 4J, CMT4J; Yunis-Varon syndrome; bilateral temporooccipital polymicrogyria (BTOP); X-linked hypercalciuric nephrolithiasis, Dent-1; and Dent disease 2, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), and neuronal ceroid lipofuscinosis. In some embodiments, the genetic disorder is Pompe disease. In some embodiments, the genetic disorder is neuronal ceroid lipofuscinosis. In some embodiments, the neuronal ceroid lipofuscinosis is selected from the group consisting of Infantile NCL (Santavuori-Haltia disease), Late Infantile NCL (Jansky-Bielschowsky disease), Batten disease, Adult NCL (Kufs disease), Finnish Late Infantile NCL, Variant Late Infantile NCL, CLN7, CLN8, Turkish Late Infantile NCL, NCL type 9, and CLN10. In some embodiments, the therapeutic protein is a soluble lysosomal enzyme or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises an enzyme selected from the group consisting of alpha-galactosidase (A or B), beta-galactosidase, beta-hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), beta-glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl-alpha-D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, beta-glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is an alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl-alpha-D-glucosaminidase (NAGLU). In some embodiments, the palmitoyl protein thioesterase is palmitoyl protein thioesterase 1 (PPT1) or 2 (PPT2). In some embodiments, the palmitoyl protein thioesterase is palmitoyl protein thioesterase 1. In some embodiments, the nucleic acid construct further comprises a translation initiation sequence. In some embodiments, the translation initiation sequence comprises a Kozak sequence. In some embodiments, the

Kozak sequence comprises the sequence AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide. In some embodiments, X₁ comprises A. In some embodiments, X₂ comprises G. In some embodiments, the Kozak sequence comprises a nucleic acid sequence at least 90% identical to AAGATGA (SEQ ID NO: 29). In some embodiments, the Kozak sequence comprises AAGATGA (SEQ ID NO: 29). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence differs from the sequence of GCAAGATG (SEQ ID NO: 44) by one or two nucleotides. In some embodiments, the Kozak sequence comprises GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to CACCATG (SEQ ID NO: 47). In some embodiments the Kozak sequence differs from the sequence of CACCATG (SEQ ID NO: 47) by one or two nucleotides. In some embodiments, the Kozak sequence comprises CACCATG (SEQ ID NO: 47). In some embodiments, the nucleic acid construct further comprises a signal nucleic acid sequence encoding a signal peptide wherein the signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide. In some embodiments, the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide. In some embodiments, the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the signal peptide comprises a Gaussia signal peptide. In some embodiments, the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32. In some embodiments, the Gaussia signal peptide comprises SEQ ID NO: 32. In some embodiments, the vIGF2 nucleic acid sequence is 5' to the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the vIGF2 nucleic acid sequence is 3' to the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the nucleic acid construct further comprises a linker sequence encoding a linker peptide between the vIGF2 nucleotide sequence and the nucleic acid sequence encoding a therapeutic protein. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker peptide comprises SEQ ID NO: 18-21 or SEQ ID NO: 33. In some embodiments, the gene therapy vector is a virus vector. In some embodiments, the virus vector is an adenovirus vector, an adeno-associated virus (AAV) vector, a retrovirus vector, a lentivirus vector, or a herpes virus vector. In some embodiments, the virus vector is an AAV vector. In some embodiments, the AAV vector comprises inverted terminal repeats (ITRs). In some embodiments, the AAV vector is selected from the group consisting of an AAV1 vector, an AAV2 vector, an AAV3 vector, an AAV4 vector, an AAV5 vector, an AAV6 vector, an AAV7 vector, an AAV8 vector, an AAV9 vector, an AAVrhS vector, an AAVrh10 vector, an AAVrh33 vector, an AAVrh34 vector, an AAVrh74 vector, an AAV Anc80 vector, an AAVPHP.B vector, an AAVhu68 vector, and an AAV-DJ vector.

[0010] In additional aspects, there are provided pharmaceutical composition comprising (i) a therapeutically effective amount of any one of the gene therapy vectors herein and (ii) a pharmaceutically acceptable carrier or excipient. In some embodiments, the carrier or excipient comprises a non-ionic, low-osmolar compound, a buffer, a polymer, a salt, or a combination thereof.

[0011] In further aspects, there are provided methods for treating a genetic disorder comprising administering to a subject in need thereof anyone of the gene therapy vectors provided herein or the any one of the pharmaceutical compositions provided herein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteaux-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, and Schindler disease type II. In some embodiments, the lysosomal storage disorder is selected from the group consisting of activator deficiency, GM2-gangliosidosis; GM2-gangliosidosis, AB variant; alpha-mannosidosis (type 2, moderate form; type 3, neonatal, severe); beta-mannosidosis; lysosomal acid lipase deficiency; cystinosis (late-onset juvenile or adolescent nephropathic type; infantile nephropathic); Chananin-Dorfman syndrome; neutral lipid storage disease with myopathy; NLSMD; Danon disease; Fabry disease; Fabry disease type II, late-onset; Farber disease; Farber lipogranulomatosis; fucosidosis; galactosialidosis (combined neuraminidase & beta-galactosidase deficiency); Gaucher disease ; type II Gaucher disease ; type III Gaucher disease; type IIIC Gaucher disease; Gaucher disease, atypical, due to saposin C deficiency; GM1-gangliosidosis (late-infantile/juvenile GM1-gangliosidosis; adult/chronic GM1-gangliosidosis); Globoid cell leukodystrophy, Krabbe disease (Late infantile onset; Juvenile Onset; Adult Onset); Krabbe disease, atypical, due to saposin A deficiency; Metachromatic Leukodystrophy (juvenile; adult); partial cerebroside sulfate deficiency; pseudoarylsulfatase A deficiency; metachromatic leukodystrophy due to saposin B deficiency; Mucopolysaccharidoses disorders: MPS I, Hurler syndrome; MPS I, Hurler-Scheie syndrome; MPS I, Scheie syndrome ; MPS II, Hunter syndrome ; MPS II, Hunter syndrome ; Sanfilippo syndrome Type A / MPS IIIA ; Sanfilippo syndrome Type B / MPS IIIB ; Sanfilippo syndrome Type C / MPS IIIC ; Sanfilippo syndrome Type D / MPS IIID ; Morquio syndrome, type A / MPS IVA ; Morquio syndrome, type B / MPS IVB ; MPS IX hyaluronidase deficiency ; MPS VI Maroteaux-Lamy syndrome ; MPS VII Sly syndrome; mucopolidosis I, sialidosis type II; I-cell disease, Leroy disease, mucopolidosis II; Pseudo-Hurler polydystrophy / mucopolidosis type III; mucopolidosis IIIC / ML III GAMMA; mucopolidosis type IV; multiple sulfatase deficiency; Niemann-Pick disease (type B; type C1 / chronic neuronopathic form; type C2; type D / Nova Scotian type); Neuronal Ceroid

Lipofuscinoses: CLN6 disease - Atypical Late Infantile, Late-Onset variant, Early Juvenile; Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease; Finnish Variant Late Infantile CLN5; Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease; Kufs/Adult-onset NCL/CLN4 disease (type B); Northern Epilepsy/variant late infantile CLN8; Santavuori-Haltia/Infantile CLN1/PPT disease; Pompe disease (glycogen storage disease type II); late-onset Pompe disease; Pycnodysostosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 Gangliosidosis; Schindler disease (type III / intermediate, variable); Kanzaki disease; Salla disease; infantile free sialic acid storage disease (ISSD); spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME); Tay-Sachs disease / GM2 gangliosidosis; juvenile-onset Tay-Sachs disease; late-onset Tay-Sachs disease; Christianson syndrome; Lowe oculocerebrorenal syndrome; Charcot-Marie-Tooth type 4J, CMT4J; Yunis-Varon syndrome; bilateral temporooccipital polymicrogyria (BTOP); X-linked hypercalciuric nephrolithiasis, Dent-1; and Dent disease 2, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), CDKL5 deficiency disorder, and neuronal ceroid lipofuscinosis. In some embodiments, the genetic disorder is Pompe disease. In some embodiments, the genetic disorder is neuronal ceroid lipofuscinosis. In some embodiments, the neuronal ceroid lipofuscinosis is selected from the group consisting of Infantile NCL (Santavuori-Haltia disease), Late Infantile NCL (Jansky-Bielschowsky disease), Batten disease, Adult NCL (Kufs disease), Finnish Late Infantile NCL, Variant Late Infantile NCL, CLN7, CLN8, Turkish Late Infantile NCL, NCL type 9, and CLN10. In some embodiments, the administering is performed intrathecally, intraocularly, intravitreally, retinally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, intracerebroventricularly, intraparenchymally, ocularly, subcutaneously, or a combination thereof. In some embodiments, the administering is performed intrathecally. In some embodiments, the administering is performed intraocularly, intravitreally, or retinally.

[0012] In additional aspects, there are provided pharmaceutical compositions comprising any one of the gene therapy vectors herein and a pharmaceutically acceptable carrier or excipient for use in treating a genetic disorder. In further aspects, there are provided pharmaceutical compositions comprising any one of the gene therapy vectors herein and a pharmaceutically acceptable carrier or excipient for use in preparation of a medicament for treatment of a genetic disorder. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, and Schindler

disease type II. In some embodiments, the lysosomal storage disorder is selected from the group consisting of activator deficiency, GM2-gangliosidosis; GM2-gangliosidosis, AB variant; alpha-mannosidosis (type 2, moderate form; type 3, neonatal, severe); beta-mannosidosis; lysosomal acid lipase deficiency; cystinosis (late-onset juvenile or adolescent nephropathic type; infantile nephropathic); Chanarin-Dorfman syndrome; neutral lipid storage disease with myopathy; NLSDM; Danon disease; Fabry disease; Fabry disease type II, late-onset; Farber disease; Farber lipogranulomatosis; fucosidosis; galactosialidosis (combined neuraminidase & beta-galactosidase deficiency); Gaucher disease ; type II Gaucher disease ; type III Gaucher disease; type IIIC Gaucher disease; Gaucher disease, atypical, due to saposin C deficiency; GM1-gangliosidosis (late-infantile/juvenile GM1-gangliosidosis; adult/chronic GM1-gangliosidosis); Globoid cell leukodystrophy, Krabbe disease (Late infantile onset; Juvenile Onset; Adult Onset); Krabbe disease, atypical, due to saposin A deficiency; Metachromatic Leukodystrophy (juvenile; adult); partial cerebroside sulfate deficiency; pseudoarylsulfatase A deficiency; metachromatic leukodystrophy due to saposin B deficiency; Mucopolysaccharidoses disorders: MPS I, Hurler syndrome; MPS I, Hurler-Scheie syndrome; MPS I, Scheie syndrome ; MPS II, Hunter syndrome ; MPS II, Hunter syndrome ; Sanfilippo syndrome Type A / MPS IIIA ; Sanfilippo syndrome Type B / MPS IIIB ; Sanfilippo syndrome Type C / MPS IIIC ; Sanfilippo syndrome Type D / MPS IIID ; Morquio syndrome, type A / MPS IVA ; Morquio syndrome, type B / MPS IVB ; MPS IX hyaluronidase deficiency ; MPS VI Maroteaux-Lamy syndrome ; MPS VII Sly syndrome; mucopolipidosis I, sialidosis type II; I-cell disease, Leroy disease, mucopolipidosis II; Pseudo-Hurler polydystrophy / mucopolipidosis type III; mucopolipidosis IIIC / ML III GAMMA; mucopolipidosis type IV; multiple sulfatase deficiency; Niemann-Pick disease (type B; type C1 / chronic neuronopathic form; type C2; type D / Nova Scotian type); Neuronal Ceroid Lipofuscinoses: CLN6 disease - Atypical Late Infantile, Late-Onset variant, Early Juvenile; Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease; Finnish Variant Late Infantile CLN5; Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease; Kufs/Adult-onset NCL/CLN4 disease (type B); Northern Epilepsy/variant late infantile CLN8; Santavuori-Haltia/Infantile CLN1/PPT disease; Pompe disease (glycogen storage disease type II); late-onset Pompe disease; Pycnodysostosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 Gangliosidosis; Schindler disease (type III / intermediate, variable); Kanzaki disease; Salla disease; infantile free sialic acid storage disease (ISSD); spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME); Tay-Sachs disease / GM2 gangliosidosis; juvenile-onset Tay-Sachs disease; late-onset Tay-Sachs disease; Christianson syndrome; Lowe oculocerebrorenal syndrome; Charcot-Marie-Tooth type 4J, CMT4J; Yunis-Varon syndrome; bilateral temporooccipital polymicrogyria (BTOP); X-linked hypercalciuric nephrolithiasis, Dent-1; and Dent disease 2, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), CDKL5 deficiency disorder, and neuronal ceroid lipofuscinosis. In some embodiments, the genetic disorder is Pompe disease. In some embodiments, the genetic disorder is neuronal ceroid lipofuscinosis. In some embodiments, the neuronal ceroid

lipofuscinosis is selected from the group consisting of Infantile NCL (Santavuori-Haltia disease), Late Infantile NCL (Jansky-Bielschowsky disease), Batten disease, Adult NCL (Kufs disease), Finnish Late Infantile NCL, Variant Late Infantile NCL, CLN7, CLN8, Turkish Late Infantile NCL, NCL type 9, and CLN10. In some embodiments, the composition is formulated for administration intrathecally, intraocularly, intravitreally, retinally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, ocularly, or subcutaneously. In some embodiments, the composition is formulated for administration intrathecally. In some embodiments, the composition is formulated for administration intrathecally for treating a neurodegenerative disorder. In some embodiments, the composition is formulated for administration ocularly, intravitreally, or retinally.

[0013] Provided herein are gene therapy vectors comprising a nucleic acid construct encoding a polypeptide comprising: (a) a therapeutic protein; (b) a peptide that binds to the cation-independent mannose 6-phosphate (M6P) receptor (CI-MPR) with high affinity; and (c) a linker between the therapeutic protein and the peptide that binds CI-MPR. In some embodiments, the peptide is a variant IGF2 (vIGF2) peptide. In some embodiments, the vIGF2 peptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 and having at least one substitution at one or more positions selected from the group consisting of positions 6, 26, 27, 43, 48, 49, 50, 54, 55, and 65 of SEQ ID NO: 1. In some embodiments, the at least one substitution is selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R, and K65R of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises at least two substitutions at two or more positions selected from the group consisting of positions 6, 26, 27, 43, 48, 49, 50, 54, 55, 65 of SEQ ID NO: 1. In some embodiments, the at least two substitutions are selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R, K65R of SEQ ID NO: 1. In some embodiments, the vIGF2 peptide comprises an N-terminal deletion at positions 1-4 of SEQ ID NO: 1. In some embodiments, wherein the vIGF2 peptide has decreased affinity for insulin receptor and IGF1R as compared to native IGF2 peptide. In some embodiments, the vIGF2 peptide is capable of facilitating uptake of the therapeutic protein into a cell. In some embodiments, the vIGF2 peptide is capable of facilitating uptake of the therapeutic protein into a lysosome. In some embodiments, the therapeutic protein is capable of replacing a defective or deficient protein associated with a genetic disorder in a subject having the genetic disorder. In some embodiments, genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease

type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), , and neuronal ceroid lipofuscinosis. In some embodiments, the genetic disorder is Pompe disease. In some embodiments, the genetic disorder is a CLN1 disease. In some embodiments, the therapeutic protein comprises a soluble lysosomal enzyme or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises a lysosomal enzyme or an enzymatically active fragment thereof, wherein the lysosomal enzyme is selected from the group consisting of alpha-galactosidase A, β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, glycosaminoglycan alpha-L-iduronohydrolase, iduronate-2-sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, palmitoyl protein thioesterases, cyclin dependent kinase like 5, and alpha-glucosidase. In some embodiments, the therapeutic protein is alpha-glucosidase or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is a palmitoyl protein thioesterase or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is palmitoyl protein thioesterase-1 or an enzymatically active fragment thereof. In some embodiments, the nucleic acid construct further comprises a translation initiation sequence. In some embodiments, the translation initiation sequence comprises a Kozak sequence. In some embodiments, the nucleic acid construct further comprises a nucleic acid sequence encoding a signal peptide wherein the signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide. In some embodiments, the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide. In some embodiments, the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17. In some embodiments, the vIGF2 peptide comprises the sequence of SEQ ID NO:31. In some embodiments, the construct comprises SEQ ID NO:36. In some embodiments, the polypeptide comprises SEQ ID NO:23. In some embodiments, the construct comprises SEQ ID NO:38. In some embodiments, the vIGF2 at the N-terminus of the polypeptide. In some embodiments, the vIGF2 is at the C-terminus of the polypeptide. In some embodiments, the linker peptide comprises SEQ ID NO: 18-21 or SEQ ID NO: 33. In some embodiments, the gene therapy vector is a virus vector selected from the group consisting of an adenovirus vector, an adeno-associated virus (AAV) vector, a retrovirus vector, a lentivirus vector, a pox virus vector, a vaccinia virus vector, an adenovirus vector, and a herpes virus vector.

[0014] In certain aspects, there are provided fusion proteins, such as fusion proteins comprising a variant IGF2 peptide and a therapeutic protein. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37, also referred to herein as “2GS”), or GGSGSGSTS (SEQ ID NO: 33). In some embodiments, the fusion

protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein is encoded by a nucleic acid comprising a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6) , or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the fusion protein is encoded by a nucleic acid comprising a Kozak sequence.

[0015] In additional aspects, there are provided fusion proteins comprising a signal peptide and a therapeutic protein, wherein the signal peptide is removed after translation upon secretion from the cell. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18) , GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the fusion protein is encoded by a nucleic acid comprising a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-

mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the fusion protein is encoded by a nucleic acid comprising a Kozak sequence.

[0016] In further aspects, there are provided nucleic acid sequences encoding fusion proteins comprising a therapeutic protein, wherein the fusion protein is encoded by a nucleic acid comprising a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the fusion protein is encoded by a nucleic acid comprising a Kozak sequence.

[0017] In additional aspects, there are provided fusion proteins comprising a therapeutic protein, wherein the fusion protein is encoded by a nucleic acid comprising a Kozak sequence. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18),

GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), or GGGSGGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the fusion protein is encoded by a nucleic acid comprising a cricket paralysis virus internal ribosome entry sequence (CrPV IRES).

[0018] In additional aspects, there are provided nucleic acids encoding a fusion protein, such as nucleic acids encoding a fusion protein comprising a variant IGF2 peptide and a therapeutic protein. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGGSGGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-

acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus.

[0019] In further aspects, there are provided nucleic acids encoding a fusion protein comprising a signal peptide and a therapeutic protein. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGSGSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus.

[0020] In additional aspects, there are provided nucleic acids encoding a fusion protein comprising a therapeutic protein, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus.

[0021] In further aspects, there are provided nucleic acids encoding a fusion protein comprising a therapeutic protein, wherein the nucleic acid further comprises a Kozak sequence. In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein

(BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus.

[0022] In additional aspects, there are provided compositions comprising (a) a nucleic acid encoding a fusion protein comprising a variant IGF2 peptide and a therapeutic protein; and (b) a buffer or excipient suitable for gene therapy. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGSGSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase,

phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein.

[0023] In additional aspects, there are provided compositions comprising (a) a nucleic acid encoding a fusion protein comprising a signal peptide and a therapeutic protein; and (b) a buffer or excipient suitable for gene therapy. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an

enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein.

[0024] In further aspects, there are provided compositions comprising (a) a nucleic acid encoding a fusion protein comprising a therapeutic protein; and (b) a buffer or excipient suitable for gene therapy, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetyl-galactosamine-6-sulfatase, glycosaminoglycan N-acetyl-galactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6) , or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some

embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein.

[0025] In further aspects, there are provided compositions comprising (a) a nucleic acid encoding a fusion protein comprising a therapeutic protein; and (b) a buffer or excipient suitable for gene therapy, wherein the nucleic acid further comprises a Kozak sequence. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetylneuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or

excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein.

[0026] In additional aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a composition comprising (a) a nucleic acid encoding a fusion protein comprising a variant IGF2 peptide and a therapeutic protein; and (b) a buffer or excipient suitable for gene therapy. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGSGSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetyl-galactosamine-6-sulfatase, glycosaminoglycan N-acetyl-galactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an

HIV coat protein, and an influenza virus coat protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, cells from the individual are treated ex vivo and administered to the individual after ex vivo treatment.

[0027] In additional aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a composition comprising (a) a nucleic acid encoding a fusion protein comprising a signal peptide and a therapeutic protein; and (b) a buffer or excipient suitable for gene therapy. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some

embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, cells from the individual are treated ex vivo and administered to the individual after ex vivo treatment.

[0028] In further aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a composition comprising (a) a nucleic acid encoding a fusion protein comprising a therapeutic protein and a targeting peptide; and (b) a buffer or excipient suitable for gene therapy, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGSGSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase,

formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, cells from the individual are treated ex vivo and administered to the individual after ex vivo treatment.

[0029] In additional aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a composition comprising (a) a nucleic acid encoding a fusion protein comprising a therapeutic protein; and (b) a buffer or excipient suitable for gene therapy, wherein the nucleic acid further comprises a Kozak sequence. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the

fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetyl-galactosamine-6-sulfatase, glycosaminoglycan N-acetyl-galactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, cells from the individual are treated ex vivo and administered to the individual after ex vivo treatment.

[0030] In further aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a cell comprising a nucleic acid encoding a fusion protein comprising a variant IGF2 peptide and a therapeutic protein. In some embodiments, the fusion protein further comprises a

linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGSGSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6) , or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease,

Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, the cells are derived from the individual.

[0031] In additional aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a cell comprising a nucleic acid encoding a fusion protein comprising a signal peptide and a therapeutic protein. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6) , or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat

protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteaux-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, the cells are derived from the individual.

[0032] In additional aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a cell comprising a nucleic acid encoding a fusion protein comprising a therapeutic protein, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the nucleic acid further comprises a Kozak sequence. In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGS GSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N-acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments,

the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy comprises a liposome, a nanoparticle, or a cell-penetrating peptide. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, the cells are derived from the individual.

[0033] In further aspects, there are provided methods of treating a genetic disorder in an individual comprising administering a cell comprising a nucleic acid encoding a fusion protein comprising a therapeutic protein, wherein the nucleic acid further comprises a Kozak sequence. In some embodiments, the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES). In some embodiments, the fusion protein further comprises a linker. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 7, 8, 9, 10, 11, 12 or 13 amino acids. In some embodiments, the linker comprises an amino acid sequence of GGGGSGGGG (SEQ ID NO: 18), GGGGS (SEQ ID NO: 19), GGGSGGGGS (SEQ ID NO: 20), GGGGSGGGGS (SEQ ID NO: 21), GGGGSGGGGS (SEQ ID NO: 37), or GGSGSGSTS (SEQ ID NO: 33). In some embodiments, the fusion protein further comprises a signal peptide. In some embodiments, the signal peptide comprises a binding immunoglobulin protein (BiP) signal peptide. In some embodiments, the fusion protein further comprises a variant IGF2 peptide. In some embodiments, the therapeutic protein comprises at least one enzyme of the group consisting of alpha-galactosidase (A or B), beta-galactosidase, beta-hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), beta-glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl-alpha-D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-

acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6) , or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein comprises alpha-glucosidase, or an enzymatically active fragment thereof. In some embodiments, the therapeutic protein is N-acetyl- α -D-glucosaminidase (NAGLU). In some embodiments, the nucleic acid further comprises a promoter. In some embodiments, the nucleic acid is comprised within a viral vector. In some embodiments, the viral vector comprises a retrovirus, an adenovirus, an adeno associated virus, a lentivirus, or a herpes virus. In some embodiments, the buffer or excipient suitable for gene therapy. In some embodiments, the buffer or excipient suitable for gene therapy comprises a viral coat protein. In some embodiments, the viral coat protein is selected from the group consisting of a vesicular stomatitis virus coat protein, an adenovirus coat protein, an adeno-associated virus coat protein, a murine leukemia virus coat protein, an HIV coat protein, and an influenza virus coat protein. In some embodiments, the genetic disorder is a lysosomal storage disorder. In some embodiments, the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and chronic granulomatous disease (CGD). In some embodiments, the cells are derived from the individual.

[0034] Further provided herein is a fusion protein comprising a native signal peptide, an ER proteolytic cleavage domain, a variant IGF2 peptide, and an alpha-glucosidase lacking its native signal peptide, wherein the fusion protein is encoded by a nucleic acid comprising a Kozak sequence.

[0035] Additionally provided herein is a fusion protein comprising a binding immunoglobulin protein (BiP) signal peptide, a variant IGF2 peptide, and an alpha-glucosidase, wherein the fusion protein is encoded by a nucleic acid comprising a Kozak sequence.

[0036] Additionally provided herein is a fusion protein comprising a binding immunoglobulin protein (BiP) signal peptide, a variant IGF2 peptide, and an alpha-glucosidase lacking its native signal peptide, wherein the fusion protein is encoded by a nucleic acid comprising a cricket paralysis virus internal ribosome entry sequence (CrPV IRES).

[0037] Additionally provided herein is a nucleic acid encoding a fusion protein comprising a native signal peptide, an ER proteolytic cleavage domain, a variant IGF2 peptide, and an alpha-glucosidase lacking its native signal peptide.

[0038] Additionally provided herein is a nucleic acid encoding a fusion protein comprising a binding immunoglobulin protein (BiP) signal peptide, a variant IGF2 peptide and an alpha-glucosidase lacking its native signal peptide, wherein the nucleic acid further comprises a Kozak sequence.

[0039] Additionally provided herein is a nucleic acid encoding a fusion protein comprising a variant IGF2 peptide and an alpha-glucosidase, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES).

[0040] Additionally provided herein is a composition comprising (a) a nucleic acid encoding a fusion protein comprising a native signal peptide, an ER proteolytic cleavage domain, a variant IGF2 peptide, and an alpha-glucosidase lacking its native signal peptide; and (b) a buffer or excipient suitable for gene therapy.

[0041] Additionally provided herein is a composition comprising (a) a nucleic acid encoding a fusion protein comprising a binding immunoglobulin protein (BiP) signal peptide, a variant IGF2 peptide and an alpha-glucosidase lacking its native signal peptide, wherein the nucleic acid further comprises a Kozak sequence; and (b) a buffer or excipient suitable for gene therapy.

[0042] Additionally provided herein is a composition comprising (a) a nucleic acid encoding a fusion protein comprising a variant IGF2 peptide and an alpha-glucosidase, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES); and (b) a buffer or excipient suitable for gene therapy.

[0043] Additionally provided herein is a method of treating Pompe disease in an individual comprising administering a composition comprising (a) a nucleic acid encoding a fusion protein comprising a native signal peptide, an ER proteolytic cleavage domain, a variant IGF2 peptide, and an alpha-glucosidase lacking its native signal peptide; and (b) a buffer or excipient suitable for gene therapy.

[0044] Additionally provided herein is a method of treating Pompe disease in an individual comprising administering a composition comprising (a) a nucleic acid encoding a fusion protein comprising a binding immunoglobulin protein (BiP) signal peptide, a variant IGF2 peptide and an alpha-glucosidase lacking its native signal peptide, wherein the nucleic acid further comprises a Kozak sequence; and (b) a buffer or excipient suitable for gene therapy.

[0045] Additionally provided herein is a method of treating Pompe disease in an individual comprising administering a composition comprising (a) a nucleic acid encoding a fusion protein comprising a variant IGF2 peptide and an alpha-glucosidase, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES); and (b) a buffer or excipient suitable for gene therapy.

[0046] Additionally provided herein is a method of treating Pompe disease in an individual comprising administering a cell comprising a nucleic acid encoding a fusion protein comprising a native signal peptide, an ER proteolytic cleavage domain, a variant IGF2 peptide, and an alpha-glucosidase lacking its native signal peptide.

[0047] Additionally provided herein is a method of treating Pompe disease in an individual comprising administering a cell comprising a nucleic acid encoding a fusion protein comprising a binding immunoglobulin protein (BiP) signal peptide, a variant IGF2 peptide and an alpha-glucosidase, or an enzymatically active fragment thereof, wherein the nucleic acid further comprises a Kozak sequence.

[0048] Additionally provided herein is a method of treating Pompe disease in an individual comprising administering a cell comprising a nucleic acid encoding a fusion protein comprising a variant IGF2 peptide and an alpha-glucosidase, wherein the nucleic acid further comprises a cricket paralysis virus internal ribosome entry sequence (CrPV IRES).

INCORPORATION BY REFERENCE

[0049] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] The patent application file contains at least one drawing executed in color. Copies of this patent application with color drawing(s) will be provided by the Office upon request and payment of the necessary fee. An understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0051] FIG. 1 shows GAA activity of alglucosidase-alfa rhGAA with and without M6P. FIG. 1 shows the proportion of commercial ERT that is able to bind to the CI-MPR. The first peak is the rhGAA that lack any M6P containing glycans and thus unable to be taken up and delivered to the lysosome. The second peak is the fraction that contains at least one phosphorylated glycan and has the potential to be taken up by the cell and delivered to the lysosome for hydrolysis of glycogen.

[0052] FIG. 2 shows structure of the CI-MPR including the different binding domains for the IGF2 and for mono- and bis-phosphorylated oligosaccharides.

[0053] FIG. 3 shows the sequence and structure of the mature, human IGF2 peptide. Site specific amino acid substitutions are proposed to influence binding of other receptors.

[0054] FIG. 4 shows binding of the wild-type IGF2 (wtIGF2) peptide to CI-MPR as measured by surface plasmon resonance

[0055] FIG. 5 shows binding of the variant IGF2 (vIGF2) peptide binding to CI-MPR as measured by surface plasmon resonance.

[0056] FIG. 6 shows benefit of adding vIGF2 to aluglucisidase alfa to increase the binding to the IGF2/CI-MPR.

[0057] FIG. 7 shows the benefit of adding a vIGF2 to recombinant human N-acetyl- α -D-glucosaminidase (rhNAGLU) to increase the binding to the IGF2/CI-MPR.

[0058] FIG. 8 shows binding of wildtype human IGF2 to insulin receptor.

[0059] FIG. 9 shows no detectable binding of vIGF2 to insulin receptor.

[0060] FIG. 10 shows binding of wildtype IGF2 to insulin-like growth factor 1 receptor.

[0061] FIG. 11 shows decreased binding of vIGF2 peptide to insulin-like growth factor 1 receptor, as compared to wildtype IGF2.

[0062] FIG. 12 shows two examples of gene therapy expression cassettes encoding Natural hGAA and Engineered hGAA. Natural hGAA has poor phosphorylation leading to poor CIMPR binding and cellular uptake. Engineered hGAA has element added for improved CIMPR binding (vIGF2), a 2GS linker that reduces steric hinderance of the vIGF2-GAA protein with CIMPR, and a BiP signal peptide to improve secretion.

[0063] FIG. 13 shows a Western blot of PPT1 from cells expressing recombinant human PPT1 (PPT1-1), recombinant human PPT1 having a vIGF2 targeting domain (PPT1-2) and recombinant human PPT1 having a vIGF2 targeting domain and a BiP signal sequence (PPT1-29).

[0064] FIG. 14 shows binding of PPT1 constructs to CI-MPR.

[0065] FIG. 15 shows GAA activity in conditioned media of CHO cells expressing engineered or natural hGAA.

[0066] FIG. 16 shows the study design of a 4 week mouse study of gene therapy in a GAA knockout mouse.

[0067] FIG. 17 shows GAA plasma activity in untreated wild type ("Normal") mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0068] FIG. 18 shows GAA levels measured in untreated wild type ("Normal") mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0069] FIG. 19 shows cell surface receptor binding of rhGAA from plasma samples obtained from treated mice as indicated.

[0070] FIG. 20 shows GAA activity, and quad glycogen histopathology score for tibialis anterior of untreated wild type ("Normal") mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0071] FIG. 21 shows glycogen PAS of tibialis anterior from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0072] FIG. 22 shows hGAA immunohistochemistry of tibialis anterior from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0073] FIG. 23 shows brain GAA activity, brain glycogen, and spinal cord glycogen histopathology scoring for brain and spinal cord from untreated wild type (“Normal”) mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0074] FIG. 24 shows glycogen PAS of brain from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0075] FIG. 25 shows hGAA immunohistochemistry of brainstem and choroid plexus from untreated wild type (“Normal”) mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0076] FIG. 26 shows glycogen PAS of spinal cord from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0077] FIG. 27 shows hGAA immunohistochemistry of spinal cord from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0078] FIG. 28 shows quadriceps GAA activity and glycogen histopathology scoring from untreated wild type (“Normal”) mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0079] FIG. 29 shows glycogen luxol/PAS for quadriceps from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0080] FIG. 30 shows hGAA immunohistochemistry of quadriceps from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0081] FIG. 31 shows triceps GAA activity and histopathology scoring for untreated wild type (“Normal”) mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0082] FIG. 32 shows glycogen luxol/PAS of triceps from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

[0083] FIG. 33 shows hGAA immunohistochemistry of triceps from untreated wild type mice or GAA knockout mice treated with gene therapy vectors or vehicle as indicated.

DETAILED DESCRIPTION

[0084] Gene therapy for single gene genetic disorders presents a potential one-time treatment for diseases and disorders, some of which have devastating symptoms that can appear early in life and sometimes lead to life-long disability. Neurologic genetic disorders, such as lysosomal storage disorders, are often treated with enzyme replacement therapies which administer to the patient a therapeutic protein that is an active form of the protein that is defective or deficient in the disease or disorder state. However, there are challenges for current therapies, including frequent treatments, development of an immune response to the therapeutic protein, and difficulty targeting the therapeutic protein to the affected tissue, cell, or subcellular compartment. Gene therapy offers advantages including a reduced number of treatments and long lasting efficacy.

[0085] Provided herein are components for gene therapy vectors that offer improvements to gene therapy, such as providing more therapeutic protein where it is needed, thus improving treatment efficacy. Such challenges are addressed herein by improving expression and cellular uptake or delivery and intracellular or subcellular targeting of therapeutic proteins. Specific tools or components provided herein include but are not limited to signal peptides (e.g., binding immunoglobulin protein (BiP) and Gaussia signal peptides) for increasing secretion and peptides that increase endocytosis of the therapeutic protein (e.g., peptides that bind to the CI-MPR with high affinity for increasing cellular uptake and lysosomal delivery). Such peptides are fused to therapeutic proteins encoded by gene therapy vectors. In some embodiments, the peptides are IGF2 (Insulin Like growth factor 2) peptides or variants thereof. Gene therapy vectors provided herein are contemplated to comprise, in some embodiments, a nucleic acid encoding a therapeutic protein fused to a peptide that bind to the CI-MPR with high affinity for optimizing efficacy of gene therapy.

[0086] Gene therapy constructs for enzyme replacement gene therapy were designed. A translation initiation sequence, including, but not limited to a Kozak sequence or an IRES sequence, such as CrPV IRES, located at the 5' end of the construct, followed by a nucleic acid encoding a signal peptide selected from one or more of a GAA signal peptide, a nucleic acid encoding an anti-trypsin inhibitor, and a nucleic acid encoding BiP sequence. These are followed by a nucleic acid encoding a cell targeting domain which can be a vIGF-2, a HIRMab, or a TfRMab or other cell targeting peptide or protein. The gene therapy construct further comprises a nucleic acid encoding a linker and a nucleic acid encoding a corrective enzyme or enzymatically active fragment thereof, wherein the linker connects the cell targeting domain to the corrective enzyme, or enzymatically active fragment thereof. Suitable corrective enzymes include but are not limited to alpha-glucosidase (GAA), alpha-galactosidase (GLA), iduronidase (IDUA), iduronate-2-sulfatase (IDS), PPT1, or enzymatically active fragments thereof, and other enzymes found deficient in an individual.

Intracellular targeting of therapeutic proteins

[0087] N-linked carbohydrates of most lysosomal proteins are modified to contain a specialized carbohydrate structure called mannose 6-phosphate (M6P). M6P is the biological signal that enables transport of lysosomal proteins to lysosomes via membrane-bound M6P receptors. Enzyme replacement therapies for lysosomal storage disorders utilize M6P receptors for uptake and delivery of therapeutic proteins to lysosomes. Certain therapeutics do not utilize M6P receptors including Cerezyme® and other versions of recombinant human GCase, utilize the mannose receptor that is able to bind terminal mannose on protein glycans and deliver to the lysosome. A problem facing certain enzyme replacement therapeutics is there are low amounts of M6P present on the enzyme therapeutic which necessitate higher doses to reach therapeutic efficacy. This leads to substantially longer infusion times, higher probability of developing immune responses to the therapeutic, and higher drug demand, requiring increased protein manufacturing resulting in increased costs.

[0088] The CI-MPR captures M6P-containing lysosomal enzymes from circulation. The receptor has distinct binding domains for M6P and insulin-like growth factor (domains 1-3 and 7-9, see FIG. 2) and therefore is also known as the IGF2/Mannose-6-phosphate receptor or IGF2/CI-MPR. This receptor can be utilized for targeting M6P- or IGF2- or IGF2 variant- containing enzyme replacement therapeutics. Binding affinity of this receptor for these ligands including insulin-like growth factor is provided in Table 1. Notably, IGF2 peptide has a higher binding affinity for CI-MPR than mono- or bis-phosphorylated oligosaccharides.

Table 1: Ligands for CI-MPR	
Ligand	Binding Affinity (Apparent K_d; nM)
IGF2	0.03-0.2
[Leu27]IGF2	0.05
Bis-M6P	2
Beta-galactosidase	20
Pentamannose-M6P	6,000
Free M6P	7,000

Therapeutic Fusion Proteins for Gene Therapy

[0089] Therapeutic fusion proteins produced from gene therapy vectors are provided herein. In some embodiments the fusion protein is secreted by cells transduced with the gene therapy vector encoding the fusion protein. In some embodiments, the transduced cells are within a tissue or organ (e.g., liver). Once secreted from a cell, the fusion protein is transported through a patient's vascular system and reaches the tissue of interest. In some embodiments, the therapeutic fusion protein is engineered to have improved secretion. In some embodiments, the fusion protein comprises a signal peptide for improving the secretion level as compared to the corresponding therapeutic protein or a fusion protein comprising the therapeutic protein but lacking a signal peptide.

[0090] The provided gene therapy vectors are, in some embodiments, engineered to address issues with gene therapy with regard to delivery of the therapeutic protein. For example, in some instances gene therapy may not achieve the intended treatment by merely generating a sufficient amount of a therapeutic protein in the body of the patient if an insufficient amount of the therapeutic protein is delivered *into* the cells in need of the therapeutic protein, due to, for example, physical and/or biological barriers that impede distribution of the therapeutic protein to the site where needed. As such, even if a gene therapy is capable of flooding blood or a tissue, to a point of saturation, with a high concentration of a therapeutic protein, the gene therapy may not be sufficiently therapeutic. Additionally, non-productive clearance pathways may remove the vast majority of the therapeutic protein. Even if the therapeutic protein is transported out of the vasculature to the interstitial space within the tissue (e.g., muscle fibers), adequate therapeutic effects are not assured. For effective treatment of lysosomal storage disorders, a

therapeutically effective amount of the therapeutic protein must undergo cellular endocytosis and lysosomal delivery to result in a meaningful efficacy. The present disclosure addresses these issues by providing gene therapy vectors encoding fusion proteins comprising a peptide that enables endocytosis of the therapeutic protein into a target cell for treatment resulting in efficacious treatment. In some embodiments, the peptide that enables endocytosis is a peptide that binds the CI-MPR. In some embodiments, the peptide that binds the CI-MPR is a vIGF2 peptide.

[0091] Provided herein are gene therapy vectors encoding fusion proteins comprising a peptide that enables endocytosis the therapeutic protein into a target cell for treatment. In some embodiments, the gene therapy vectors encode fusion proteins comprising a therapeutic protein and a peptide that binds the CI-MPR. Such fusion proteins when expressed from a gene therapy vector target therapeutic proteins, such as enzyme replacement therapeutics, to the cells where they are needed, increase delivery into or cellular uptake by such cells and target the therapeutic protein to a subcellular location (e.g., a lysosome). In some embodiments, the peptide is an IGF2 peptide or variant thereof, which can target a therapeutic protein to the lysosome. Fusion proteins herein also, in some embodiments, further comprise a signal peptide that increases secretion, such as a BiP signal peptide or a Gaussia signal peptide. In some embodiments, fusion proteins comprise a linker sequence. In some embodiments, nucleic acids encoding fusion proteins herein, comprise internal ribosomal entry sequences.

[0092] Therapeutic proteins for gene therapy comprising a vIGF2 peptide are provided herein. Exemplary proteins are provided in Table 2 below.

Table 2: Amino Acid Sequences		SEQ ID NO
Natural hGAA	MGVRHPPCSHRLAVCALVSLATAALLGHILLHDFLLVPRELSGSSPVLEE THPAHQQGASRPGPRDAQAHPGRPRAVPTQCDVPPNSRFDCAPDKAITQE QCEARGCCYIPAKQGLQGAQMGPWCFFPPSYPSYKLENLSSSEMGYTAT LTRTTPTFFPKDILTLRLDVMETENRLHFTIKDPANRRYEVPLETPHVHSR APSPLYSVEFSEEPFGVIVRRQLDGRVLLNTTVAPLFFADQFLQLSTSLPSQ YITGLAEHLSPLMLSTSWTRITLWNRDLAPTPGANLYGSHPFYLALEDGGS AHGVFLLNSNAMDVVLQPSPALSWRSTGGILDVYIFLGPEPKSVVQQYLD VVGYPFMPPYWGLGFHLCRWGYSSTAITRQVVENMTRAHFPLDVQWND LDYMDSRDFTFNKDGRDFPAMVQELHQGRRYMMIVDPAISSSGPAGS YRPYDEGLRRGVFITNETGQPLIGKVWPGSTAFPDTNPTALAWWEDMVA EFHDQVPFDGMWIDMNEPSNFIRGSEDGCPNNELENPPYVPGVVGGTLQA ATICASSHQFLSTHYNLHNLGLTEAIAASHRALVKARGTRPFVISRSTFAGH GRYAGHWTGDVWSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSE ELCVRWTQLGAFYPFMRNHNSLLSLPQEPYSFSEPAQQAMRKALTLRYAL LPHLYTLFHQAHVAGETVARPLFLEFPKDSSTWTVDHQLLWGEALLITPVL QAGKAEVTGYFPLGTWYDLQTVPEALGSLPPPPAAPREPAIHSEGQWVT	22

	LPAPLDTINVHLRAGYIIP LQGPGLT TTESRQQPMALAVALT KGGGEARGEL FWDDGESLEVLERGAYTQVIFLARNNTIVNELVRVTSEGAGLQLQKVTVL GVATAPQQVLSNGVPVSNFTYSPDTKVLDICVSLLMGEQFLVSWC	
Engineered hGAA (BiP- vIGF2-GAA)	MKLSLVAAMLLLLSAARASRTL CGGELVDTLQFVCGDRGFLFSRPASRVS RRSRGIVEECCFRSCDLALLE TYCATPARSEGGGSGGGGSRPGPRDAQAH PGRPRAVPTQCDVPPNSRFD CAPDKAITQE QCEARGCCYIPAKQGLQGAQ MGQPWCFFPPSYPSYKLENLSSEM GYTATLTRTTPTFFPKDILTLRLDVM METENRLHFTIKDPANRRYEVPLETPHVHSRAPSPLYSVEFSEEPFGVIVRR QLDGRVLLNTTVAPLFFADQFLQLSTSLPSQYITGLAEHLSPLMLSTSWTRI TLWNRDLAPTPGANLYGSHPFYLALEDG GSAHGVFLLNSNAMDVVLQPS PALSWRSTGGILDVYIFLGPEPKSVVQQYLDVVGYPFMPPYWGLGFHLCR WGYSSAITRQVVENMTRAHFPLDVQWNDLDY MDSRRDFTFNKDGFRDF PAMVQELHQGGRRYMMIVDPAISSSGPAGSYR PYDEGLRRGVFITNETGQ PLIGKVWPGSTAFPDTNPTALAWWEDMVAEFHDQVPFDGMWIDMNEPS NFIRGSEDGCPNNELENPPYVPGVVGGTLQAATICASSHQFLSTHYNLHNL YGLTEAIAASHRALVKARGTRPFVISRSTFAGHGRYAGHWTGDVWSSWEQ LASSVPEILQFNLLGVPLVGADVCGFLGNTSEELCVRWTQLGAFYPFMRN HNSLLSLPQEPYSFSEPAQQAMRKALTRYALLPHLYTLFHQAHVAGETV ARPLFLEFPKDSSTWTVDHQLLWGEALLITPVLQAGKAEVTGYFPLGTWY DLQTVPVEALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINVHLRAGYIIP LQGPGLT TTESRQQPMALAVALT KGGGEARGELFWDDGESLEVLERGAYT QVIFLARNNTIVNELVRVTSEGAGLQLQKVTVLGVATAPQQVLSNGVPVS NFTYSPDTKVLDICVSLLMGEQFLVSWC	23
hGAA Δ1-60	SRPGPRDAQAH PGRPRAVPTQCDVPPNSRFD CAPDKAITQE QCEARGCCYI PAKQGLQGAQMGQPWCFFPPSYPSYKLENLSSEM GYTATLTRTTPTFFPK DILTLRLDVM METENRLHFTIKDPANRRYEVPLETPHVHSRAPSPLYSVEFS EFPFGVIVRRQLDGRVLLNTTVAPLFFADQFLQLSTSLPSQYITGLAEHLSPL LMLSTSWTRITLWNRDLAPTPGANLYGSHPFYLALEDG GSAHGVFLLNSN AMDVVLQPS PALSWRSTGGILDVYIFLGPEPKSVVQQYLDVVGYPFMPPY WGLGFHLCRWGYSSAITRQVVENMTRAHFPLDVQWNDLDY MDSRRDF TFNKDGFRDFPAMVQELHQGGRRYMMIVDPAISSSGPAGSYR PYDEGLRR GVFITNETGQPLIGKVWPGSTAFPDTNPTALAWWEDMVAEFHDQVPFDG MWIDMNEPSNFIRGSEDGCPNNELENPPYVPGVVGGTLQAATICASSHQFL STHYNLHNL YGLTEAIAASHRALVKARGTRPFVISRSTFAGHGRYAGHWTG DVWSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSEELCVRWTQLG AFYPFMRNHNSLLSLPQEPYSFSEPAQQAMRKALTRYALLPHLYTLFHQ	46

	AHVAGETVARPLFLEFPKDSSTWTVVDHQLLWGEALLITPVLQAGKAEVTG YFPLGTWYDLQTVPVEALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINV HLRAGYIIPQLQGPGLTTTESRQQPMALAVALTGKGGEARGELFWDDGESLE VLERGAYTQVIFLARNTIVNELVRVTSEGAGLQLQKVTVLGVATAPQQV LSNGVPVSNFTYSPDTKVLDCVSLLMGEQFLVSWC	
wt-PPT1	MASPGCLWLLAVALLPWTCASRALQHLDPPAPLPLVIWHGMGDSCCNPL SMGAIKKMVEKKIPGIYVLSLEIGKTLMEDVENSFFLVNSQVTTVCQALA KDPKLQQGYNAMGFSQGGQFLRAVAQRCPSPPMINLISVGGQHQQGVFGLP RCPGESSHICDFIRKTLNAGAYSKVVQERLVQAEYWHDPIKEDVYRNHSIF LADINQERGINESYKKNLMALKKFVMVKFLNDSIVDPVDSEWFGFYRSGQ AKETIPLQETSLYTQDRLGLKEMDNAGQLVFLATEGDHLQLSEEWFYAHII PFLG	24
PPT1-2 (vIGF2- PPT1)	MASPGCLWLLAVALLPWTCASRALQHLSRTLCCGGELVDTLQFVCGDRGF LFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPARSEGGGGSGGGGS RPRAVPTQDPPAPLPLVIWHGMGDSCCNPLSMGAIKKMVEKKIPGIYVLSL EIGKTLMEDVENSFFLVNSQVTTVCQALAKDPKLQQGYNAMGFSQGGQ FLRAVAQRCPSPPMINLISVGGQHQQGVFGLPRCPGESSHICDFIRKTLNAGA YSKVVQERLVQAEYWHDPIKEDVYRNHSIFLADINQERGINESYKKNLMA LKKFVMVKFLNDSIVDPVDSEWFGFYRSGQAKETIPLQETSLYTQDRLGLK EMDNAGQLVFLATEGDHLQLSEEWFYAHIIIPFLG	25
PPT1-29 (BiP2aa- vIGF2- PPT1)	MKLSLVAAML LLLWVALLLLSAARAAASRTLCCGGELVDTLQFVCGDRGF LFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPARSEGGGGSGGGGS RPRAVPTQDPPAPLPLVIWHGMGDSCCNPLSMGAIKKMVEKKIPGIYVLSL EIGKTLMEDVENSFFLVNSQVTTVCQALAKDPKLQQGYNAMGFSQGGQ FLRAVAQRCPSPPMINLISVGGQHQQGVFGLPRCPGESSHICDFIRKTLNAGA YSKVVQERLVQAEYWHDPIKEDVYRNHSIFLADINQERGINESYKKNLMA LKKFVMVKFLNDSIVDPVDSEWFGFYRSGQAKETIPLQETSLYTQDRLGLK EMDNAGQLVFLATEGDHLQLSEEWFYAHIIIPFLG	26

[0093] Components of fusion proteins provided herein are further described below.

Peptides that bind CI-MPR (e.g., vIGF2 Peptides)

[0094] Provided herein are peptides that bind CI-MPR. Fusion proteins comprising such peptides and a therapeutic protein, when expressed from a gene therapy vector, target the therapeutic protein to the cells where it is needed, increase cellular uptake by such cells and target the therapeutic protein to a subcellular location (e.g., a lysosome). In some embodiments, the peptide is fused to the N-terminus of the therapeutic peptide. In some embodiments, the peptide is fused to the C-terminus of the therapeutic

protein. In some embodiments, the peptide is a vIGF2 peptide. Some vIGF2 peptides maintain high affinity binding to CI-MPR while their affinity for IGF1 receptor, insulin receptor, and IGF binding proteins (IGFBP) is decreased or eliminated. Thus, some variant IGF2 peptides are substantially more selective and have reduced safety risks compared to wt IGF2. vIGF2 peptides herein include those having the amino acid sequence of SEQ ID NO: 31. Variant IGF2 peptides further include those with variant amino acids at positions 6, 26, 27, 43, 48, 49, 50, 54, 55, or 65 compared to wt IGF2 (SEQ ID NO: 1). In some embodiments, the vIGF2 peptide has a sequence having one or more substitutions from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R, and K65R. In some embodiments, the vIGF2 peptide has a sequence having a substitution of E6R. In some embodiments, the vIGF2 peptide has a sequence having a substitution of F26S. In some embodiments, the vIGF2 peptide has a sequence having a substitution of Y27L. In some embodiments, the vIGF2 peptide has a sequence having a substitution of V43L. In some embodiments, the vIGF2 peptide has a sequence having a substitution of F48T. In some embodiments, the vIGF2 peptide has a sequence having a substitution of R49S. In some embodiments, the vIGF2 peptide has a sequence having a substitution of S50I. In some embodiments, the vIGF2 peptide has a sequence having a substitution of A54R. In some embodiments, the vIGF2 peptide has a sequence having a substitution of L55R. In some embodiments, the vIGF2 peptide has a sequence having a substitution of K65R. In some embodiments, the vIGF2 peptide has a sequence having a substitution of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, and L55R. In some embodiments, the vIGF2 peptide has an N-terminal deletion. In some embodiments, the vIGF2 peptide has an N-terminal deletion of one amino acid. In some embodiments, the vIGF2 peptide has an N-terminal deletion of two amino acids. In some embodiments, the vIGF2 peptide has an N-terminal deletion of three amino acids. In some embodiments, the vIGF2 peptide has an N-terminal deletion of four amino acids. In some embodiments, the vIGF2 peptide has an N-terminal deletion of four amino acids and a substitution of E6R, Y27L, and K65R. In some embodiments, the vIGF2 peptide has an N-terminal deletion of four amino acids and a substitution of E6R and Y27L. In some embodiments, the vIGF2 peptide has an N-terminal deletion of five amino acids. In some embodiments, the vIGF2 peptide has an N-terminal deletion of six amino acids. In some embodiments, the vIGF2 peptide has an N-terminal deletion of seven amino acids. In some embodiments, the vIGF2 peptide has an N-terminal deletion of seven amino acids and a substitution of Y27L and K65R.

Table 3: IGF2 Amino Acid Sequences (variant residues are underlined)		
Peptide	Sequence	SEQ ID NO
Wildtype	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIVECCFRSCDLALLE TYCATPAKSE	1
F26S	AYRPSETLCGGELVDTLQFVCGDRGS <u>Y</u> FSRPASR	2

	VSRRSRGIVEECCFRSCDLALLE ^T YCATPAKSE	
Y27L	AYRPSETLCGGELVDTLQFVCGDRGF ^L FSRPASRV SRRSRGIVEECCFRSCDLALLE ^T YCATPAKSE	3
V43L	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIL ^E ECCFRSCDLALLE ^T YCATPAKSE	4
F48T	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIVEECC ^T RSCDLALLE ^T YCATPAKSE	5
R49S	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIVEECCF ^S SCDLALLE ^T YCATPAKSE	6
S50I	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIVEECCFR ^I CDLALLE ^T YCATPAKSE	7
A54R	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIVEECCFRSCDL ^R LLE ^T YCATPAKSE	8
L55R	AYRPSETLCGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIVEECCFRSCDLA ^R LLE ^T YCATPAKSE	9
F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R	AYRPSETLCGGELVDTLQFVCGDRGS ^L FSRPASRV SRRSRGIL ^E ECC ^T S ^I CDL ^R R ^R LLE ^T YCATPAKSE	10
Δ1-6, Y27L, K65R	TLGGELVDTLQFVCGDRGF ^L FSRPASRVSRRSRG IVEECCFRSCDLALLE ^T YCATP ^A RSE	11
Δ1-7, Y27L, K65R	LCGGELVDTLQFVCGDRGF ^L FSRPASRVSRRSRGI VEECCFRSCDLALLE ^T YCATP ^A RSE	30
Δ1-4, E6R, Y27L, K65R	S ^R TLGGELVDTLQFVCGDRGF ^L FSRPASRVSRRS RGIVEECCFRSCDLALLE ^T YCATP ^A RSE	31
Δ1-4, E6R, Y27L	S ^R TLGGELVDTLQFVCGDRGF ^L FSRPASRVSRRS RGIVEECCFRSCDLALLE ^T YCATPAKSE	34
E6R	AYRPS ^R TLGGELVDTLQFVCGDRGFYFSRPASR VSRRSRGIVEECCFRSCDLALLE ^T YCATPAKSE	35

Table 4: IGF2 DNA Coding Sequences		
Peptide	DNA Sequence	SEQ ID NO
Mature WT IGF2	GCTTACCGCCCCAGTGAGACCCTGTGCGGCGGG GAGCTGGTGGACACCCTCCAGTTCGTCTGTGGG GACCGCGGCTTCTACTTCAGCAGGCCCGCAAGC CGTGTGAGCCGTCGCAGCCGTGGCATCGTTGAG	48

	GAGTGCTGTTTCCGCAGCTGTGACCTGGCCCTC CTGGAGACGTACTGTGCTACCCCCGCCAAGTCC GAG	
vIGF2 Δ1-4, E6R, Y27L, K65R	TCTAGAACACTGTGCGGAGGGGAGCTTGTAGAC ACTCTTCAGTTCGTGTGTGGAGATCGCGGGTTC CTCTTCTCTCGCCCCGCTTCCAGAGTTTCACGGA GGTCTAGGGGTATAGTAGAGGAGTGTGTTTCA GGTCCTGTGACTTGGCGCTCCTCGAGACCTATT GCGCGACGCCAGCCAGGTCCGAA	36

Internal Ribosomal Entry Sequences

[0095] Provided herein are gene therapy constructs useful in treating a disorder further comprising an internal ribosome entry sequence (IRES) for increasing gene expression by bypassing the bottleneck of translation initiation. Suitable internal ribosomal entry sequences for optimizing expression for gene therapy include but are not limited to a cricket paralysis virus (CrPV) IRES, a picornavirus IRES, an Aphthovirus IRES, a Kaposi's sarcoma-associated herpesvirus IRES, a Hepatitis A IRES, a Hepatitis C IRES, a Pestivirus IRES, a Cripavirus IRES, a Rhopalosiphum padi virus IRES, a Merek's disease virus IRES, and other suitable IRES sequences. In some embodiments, the gene therapy construct comprises a CrPV IRES. In some embodiments, the CrPV IRES has a nucleic acid sequence of
AAAAATGTGATCTTGCTTGTAATACAATTTTGAGAGGTTAATAAATTACAAGTAGTGCTATT
TTTGTATTTAGGTTAGCTATTTAGCTTTACGTTCCAGGATGCCTAGTGGCAGCCCCACAATAT
CCAGGAAGCCCTCTCTGCGGTTTTTCAGATTAGGTAGTCGAAAAACCTAAGAAATTTACCTG
CT (SEQ ID NO: 12). In some embodiments, the CrPV IRES sequence is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 12.

Signal Peptides

[0096] Gene therapy constructs provided herein, in some embodiments, further comprise a signal peptide, which improves secretion of the therapeutic protein from the cell transduced with the gene therapy construct. The signal peptide in some embodiments improves protein processing of therapeutic proteins, and facilitates translocation of the nascent polypeptide-ribosome complex to the ER and ensuring proper co-translational and post-translational modifications. In some embodiments, the signal peptide is located (i) in an upstream position of the signal translation initiation sequence, (ii) in between the translation initiation sequence and the therapeutic protein, or (iii) a downstream position of the therapeutic protein. Signal peptides useful in gene therapy constructs include but are not limited to binding immunoglobulin protein (BiP) signal peptide from the family of HSP70 proteins (e.g., HSPA5, heat shock protein family A member 5) and Gaussia signal peptides, and variants thereof. These signal peptides have

ultrahigh affinity to the signal recognition particle. Examples of BiP and Gaussia amino acid sequences are provided in Table 5 below. In some embodiments, the signal peptide has an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of SEQ ID Nos: 13-17. In some embodiments, the signal peptide differs from a sequence selected from the group consisting of SEQ ID Nos: 13-17 by 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid.

Table 5: Signal Peptide Sequences		
Signal Peptide	Amino Acid Sequence	SEQ ID NO:
Native human BiP	MKLSLVAAML LLLSAARA	13
Modified BiP-1	MKLSLVAAML LLLSLVAAML LLLSAARA	14
Modified BiP-2	MKLSLVAAML LLLWVALL LLSAARA	15
Modified BiP-3	MKLSLVAAML LLLSLVALL LLSAARA	16
Modified BiP-4	MKLSLVAAML LLLALVALL LLSAARA	17
Gaussia	MGVKVLFALICIAVAEA	32

[0097] The BiP signal peptide-signal recognition particle (SRP) interaction facilitates translocation to the ER. This interaction is illustrated in **FIG 20**.

[0098] The Gaussia signal peptide is derived from the luciferase from *Gaussia princeps* and directs increased protein synthesis and secretion of therapeutic proteins fused to this signal peptide. In some embodiments, the Gaussia signal peptide has an amino acid sequence that is at least 90% identical to SEQ ID NO: 32. In some embodiments, the signal peptide differs from SEQ ID NO: 32 by 5 or fewer, 4 or fewer, 3 or fewer, 2 or fewer, or 1 amino acid.

Linker

[0099] Gene therapy constructs provided herein, in some embodiments, comprise a linker between the targeting peptide and the therapeutic protein. Such linkers, in some embodiments, maintain correct spacing and mitigate steric clash between the vIGF2 peptide and the therapeutic protein. Linkers, in some embodiments, comprise repeated glycine residues, repeated glycine-serine residues, and combinations thereof. In some embodiments, the linker consists of 5-20 amino acids, 5-15 amino acids, 5-10 amino acids, 8-12 amino acids, or about 5, 6, 7, 8, 9, 10, 11, 12 or 13 amino acids. Suitable linkers for gene therapy constructs herein include but are not limited to those provided in Table 6 below.

Table 6: Linker Sequences	
Sequence	SEQ ID NO:
GGGGS GGGG	18
GGGGS	19
GGGS GGGGS	20
GGGGS GGGGS	21
GGSGSGSTS	33

GGGGS GGGGS	37
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Translation Initiation Sequence

[00100] Gene therapy constructs provided herein comprise a nucleic acid having a translation initiation sequence, such as a Kozak sequence which aids in initiation of translation of the mRNA. Kozak sequences contemplated herein have a consensus sequence of (gcc)RccATGG (SEQ ID NO: 27) where a lowercase letter denotes the most common base at the position and the base varies, uppercase letters indicate highly conserved bases that only vary rarely change. R indicates that a purine (adenine or guanine) is always observed at that position. The sequence in parentheses (gcc) is of uncertain significance. In some embodiments, the Kozak sequence comprises the sequence AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide. In some embodiments, X₁ comprises A. In some embodiments, X₂ comprises G. In some embodiments, the Kozak sequence comprises a nucleic acid sequence at least 85% identical to AAGATGA (SEQ ID NO: 29). In some embodiments, the Kozak sequence differs from the sequence of AAGATGA (SEQ ID NO: 29) by one or two nucleotides. In some embodiments, Kozak sequences provided herein have a sequence of AAGATGA (SEQ ID NO: 29). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence differs from the sequence of GCAAGATG (SEQ ID NO: 44) by one or two nucleotides. In some embodiments, the Kozak sequence comprises GCAAGATG (SEQ ID NO: 44). In some embodiments the Kozak sequence comprises a nucleic acid sequence at least 85% identical to CACCATG (SEQ ID NO: 47). In some embodiments the Kozak sequence differs from the sequence of CACCATG (SEQ ID NO: 47) by one or two nucleotides. In some embodiments, the Kozak sequence comprises CACCATG (SEQ ID NO: 47).

Therapeutic Protein

[00101] Gene therapy constructs provided herein comprise a nucleic acid encoding a therapeutic protein for treating a genetic disorder due to a genetic defect in an individual resulting in an absent or defective protein. The therapeutic protein expressed from the gene therapy construct replaces the absent or defective protein. Therapeutic proteins, therefore, are chosen based on the genetic defect in need of treatment in an individual. In some embodiments, the therapeutic protein is a structural protein. In some embodiments, the therapeutic protein is an enzyme. In some embodiments, the therapeutic protein is a regulatory protein. In some embodiments, the therapeutic protein is a receptor. In some embodiments, the therapeutic protein is a peptide hormone. In some embodiments, the therapeutic protein is a cytokine or a chemokine.

[00102] In some embodiments, gene therapy constructs herein encode an enzyme, such as an enzyme having a genetic defect in an individual with a lysosomal storage disorder. In some embodiments, gene therapy constructs encode a lysosomal enzyme, such as a glycosidase, a protease, or a sulfatase. In some embodiments, enzymes encoded by gene therapy constructs provided herein include but are not limited to

α -D-mannosidase; N-aspartyl- β -glucosaminidase; β -galactosidase; ceramidase; fucosidase; galactocerebrosidase; arylsulfatase A; N-acetylglucosamine-1-phosphotransferase; iduronate sulfatase; N-acetylglucosaminidase; acetyl-CoA: α -glucosaminide acetyltransferase; N-acetylglucosamine 6-sulfatase; β -glucuronidase; hyaluronidase; sialidase; sulfatase; sphingomyelinase; acid β -mannosidase; cathepsin K; 3-hexosaminidase A; β -hexosaminidase B; α -N-acetylgalactosaminidase; sialin; hexosaminidase A; beta-glucosidase; α -iduronidase; α -galactosidase A; β -glucocerebrosidase; lysosomal acid lipase; glycosaminoglycan alpha-L-iduronohydrolase; iduronate-2-sulfatase; N-acetylgalactosamine-6-sulfatase; glycosaminoglycan N-acetylgalactosamine 4-sulfatase; alpha-glucosidase; heparan sulfamidase; gp-91 subunit of NADPH oxidase; adenosine deaminase; cyclin dependent kinase like 5; and palmitoyl protein thioesterase 1. In some embodiments, enzymes encoded by gene therapy constructs provided herein comprise alpha-glucosidase. In some embodiments, the therapeutic protein is associated with a genetic disorder selected from the group consisting of CDKL5 deficiency disorder, cystic fibrosis, alpha- and beta-thalassemias, sickle cell anemia, Marfan syndrome, fragile X syndrome, Huntington's disease, hemochromatosis, Congenital Deafness (nonsyndromic), Tay-Sachs, Familial hypercholesterolemia, Duchenne muscular dystrophy, Stargardt disease, Usher syndrome, choroideremia, achromatopsia, X-linked retinoschisis, hemophilia, Wiskott-Aldrich syndrome, X-linked chronic granulomatous disease, aromatic L-amino acid decarboxylase deficiency, recessive dystrophic epidermolysis bullosa, alpha 1 antitrypsin deficiency, Hutchinson-Gilford progeria syndrome (HGPS), Noonan syndrome, X-linked severe combined immunodeficiency (X-SCID). In some embodiments, the therapeutic protein is selected from the group consisting of CDKL5, Connexin 26, hexosaminidase A, LDL receptor, Dystrophin, CFTR, beta-globulin, HFE, Huntington, ABCA4, myosin VIIA (MYO7A), Rab escort protein -1 (REP1), cyclic nucleotide gated channel beta 3 (CNGB3), retinoschisin 1 (RS1), hemoglobin subunit beta (HBB), Factor IX, WAS, cytochrome B-245 beta chain, dopa decarboxylase (DDC), collagen type VII alpha 1 chain (COL7A1), serpin family A member 1 (SERPINA1), LMNA, PTPN11, SOS1, RAF1, KRAS, and IL2 receptor γ gene.

[00103] *Gene Therapy Vector Examples*

Gene Therapy Vectors and Compositions

[00104] Provided herein are gene therapy vectors in which a nucleic acid, such as a DNA, encoding a therapeutic fusion protein, such as a vIGF2 fusion, optionally having a signal peptide. The gene therapy vector optionally comprises an internal ribosomal entry sequence. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral and adeno-associated viral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they are capable of transducing non-proliferating cells, such as hepatocytes and neurons. They also have the added advantage of low immunogenicity.

[00105] Exemplary gene therapy vectors herein encode therapeutic proteins and therapeutic fusion proteins comprising a vIGF2 peptide. Nucleic acids encoding exemplary fusion protein amino acid sequences are provided in Table 7 below.

Table 7: DNA Sequences		
Construct	DNA Sequence	SEQ ID NO
Kozak-hGAA (Natural GAA)	GCAAGATGGGAGTGAGGCACCCGCCCTGCTCCACCGGCTCCTGGCCG TCTGCGCCCTCGTGTCCTTGGCAACCGCTGCACTCCTGGGGCACATCCT ACTCCATGATTTCTGCTGGTTCCCCGAGAGCTGAGTGGCTCCTCCCCA GTCCTGGAGGAGACTCACCCAGCTCACCAGCAGGGAGCCAGTAGACCA GGGCCCCGGGATGCCCAGGCACACCCCGGCCGTCCCAGAGCAGTGCCC ACACAGTGCGACGTCCCCCCCCAACAGCCGCTTCGATTGCGCCCCTGAC AAGGCCATCACCCAGGAACAGTGCGAGGCCCGCGGCTGTTGCTACATC CCTGCAAAGCAGGGGCTGCAGGGAGCCCAGATGGGGCAGCCCTGGTGC TTCTTCCACCCAGCTACCCAGCTACAAGCTGGAGAACCTGAGCTCCT CTGAAATGGGCTACACGGCCACCCTGACCCGTACCACCCCCACCTTCTT CCCCAAGGACATCCTGACCCTGCGGCTGGACGTGATGATGGAGACTGA GAACCGCCTCCACTTCACGATCAAAGATCCAGCTAACAGGCGCTACGA GGTGCCCTTGGAGACCCCGCATGTCCACAGCCGGGCACCGTCCCCACT CTACAGCGTGGAGTTCTCCGAGGAGCCCTTCGGGGTGATCGTGCGCCG GCAGCTGGACGGCCGCGTGCTGCTGAACACGACGGTGGCGCCCCTGTT CTTTGCGGACCAGTTCTTCAGCTGTCCACCTCGCTGCCCTCGCAGTAT ATCACAGGCCTCGCCGAGCACCTCAGTCCCCTGATGCTCAGCACCAGCT GGACCAGGATCACCTGTGGAACCGGGACCTTGCGCCACGCCCCGGTG CGAACCTCTACGGGTCTCACCTTTCTACCTGGCGCTGGAGGACGGCGG GTCGGCACACGGGGTGTTCCTGCTAAACAGCAATGCCATGGATGTGGT CCTGCAGCCGAGCCCTGCCCTTAGCTGGAGGTCGACAGGTGGGATCCT GGATGTCTACATCTTCTGGGCCAGAGCCCAAGAGCGTGGTGCAGCA GTACCTGGACGTTGTGGGATACCCGTTTCATGCCGCCATACTGGGGCCTG GGCTTCCACCTGTGCCGCTGGGGCTACTCCTCCACCGCTATCACCCGCC AGGTGGTGGAGAACATGACCAGGGCCCACTTCCCCCTGGACGTCCAGT GGAACGACCTGGACTACATGGACTCCCGGAGGGACTTCACGTTCAACA AGGATGGCTTCCGGGACTTCCCGGCCATGGTGCAGGAGCTGCACCAGG GCGGCCGGCGCTACATGATGATCGTGGATCCTGCCATCAGCAGCTCGG GCCCTGCCGGGAGCTACAGGCCCTACGACGAGGGTCTGCGGAGGGGGG TTTTTCATCACCAACGAGACCGGCCAGCCGCTGATTGGGAAGGTATGGC	44

	CCGGGTCCACTGCCTTCCCCGACTTCACCAACCCACAGCCCTGGCCTG GTGGGAGGACATGGTGGCTGAGTTCCATGACCAGGTGCCCTTCGACGG CATGTGGATTGACATGAACGAGCCTTCCAACCTTCATCAGGGGCTCTGA GGACGGCTGCCCCAACAATGAGCTGGAGAACCCACCCTACGTGCCTGG GGTGGTTGGGGGGACCCTCCAGGCGGCCACCATCTGTGCCTCCAGCCA CCAGTTTCTCTCCACACACTACAACCTGCACAACCTCTACGGCCTGACC GAAGCCATCGCCTCCCACAGGGCGCTGGTGAAGGCTCGGGGGACACGC CCATTTGTGATCTCCCGCTCGACCTTTGCTGGCCACGGCCGATACGCCG GCCACTGGACGGGGGACGTGTGGAGCTCCTGGGAGCAGCTCGCCTCCT CCGTGCCAGAAATCCTGCAGTTTAACCTGCTGGGGGTGCCTCTGGTCGG GGCCGACGTCTGCGGCTTCCTGGGCAACACCTCAGAGGAGCTGTGTGT GCGCTGGACCCAGCTGGGGGCCTTCTACCCCTTCATGCGGAACCACAA CAGCCTGCTCAGTCTGCCCCAGGAGCCGTACAGCTTCAGCGAGCCGGC CCAGCAGGCCATGAGGAAGGCCCTCACCTGCGCTACGCACTCCTCCC CCACCTCTACACACTGTTCCACCAGGCCACGTGCGGGGGAGACCGT GGCCCGGCCCTCTTCCTGGAGTTCCTCAAGGACTCTAGCACCTGGACT GTGGACCACCAGCTCCTGTGGGGGGAGGCCCTGCTCATCACCCAGTG CTCCAGGCCGGGAAGGCCGAAGTGACTGGCTACTTCCCCTTGGGCACA TGGTACGACCTGCAGACGGTGCCAGTAGAGGCCCTTGGCAGCCTCCCA CCCCACCTGCAGCTCCCCGTGAGCCAGCCATCCACAGCGAGGGGCAG TGGGTGACGCTGCCGGCCCCCTGGACACCATCAACGTCCACCTCCGG GCTGGGTACATCATCCCCCTGCAGGGCCCTGGCCTCACAACCACAGAG TCCCGCCAGCAGCCCATGGCCCTGGCTGTGGCCCTGACCAAGGGTGGG GAGGCCCGAGGGGAGCTTTTCTGGGACGATGGAGAGAGCCTGGAAGTG CTGGAGCGAGGGGCCTACACACAGGTCATCTTCCTGGCCAGGAATAAC ACGATCGTGAATGAGCTGGTACGTGTGACCAGTGAGGGAGCTGGCCTG CAGCTGCAGAAGGTGACTGTCCTGGGCGTGGCCACGGCGCCCCAGCAG GTCCTCTCCAACGGTGTCCCTGTCTCCAACCTCACCTACAGCCCCGACA CCAAGGTCCTGGACATCTGTGTCTCGCTGTTGATGGGAGAGCAGTTTCT CGTCAGCTGGTGTTAG	
Kozak BiP- vIGF2-GAA (“Engineered hGAA”)	GCAAGATGAAGCTCTCCCTGGTGGCCGCGATGCTGCTGCTGCTCAGCG CGGCGCGGGCCTCTAGAACACTGTGCGGAGGGGAGCTTGTAGACACTC TTCAGTTCGTGTGTGGAGATCGCGGGTTCCTCTTCTCTCGCCCCGCTTCC AGAGTTTCACGGAGGTCTAGGGGTATAGTAGAGGAGTGTTGTTTCAGG TCCTGTGACTTGGCGCTCCTCGAGACCTATTGCGCGACGCCAGCCAGGT CCGAAGGGGGCGGTGGCTCAGGTGGTGGAGGTAGCAGACCAGGGCCC	38

CGGGATGCCCAGGCACACCCCGGCCGTCCCAGAGCAGTGCCCACACAG
 TGCACGTCCCCCAACAGCCGCTTCGATTGCGCCCCTGACAAGGCC
 ATCACCAGGAACAGTGCGAGGCCCGCGGCTGTTGCTACATCCCTGCA
 AAGCAGGGGCTGCAGGGAGCCCAGATGGGGCAGCCCTGGTGCTTCTTC
 CCACCCAGCTACCCAGCTACAAGCTGGAGAACCTGAGCTCCTCTGAA
 ATGGGCTACACGGCCACCCTGACCCGTACCACCCCCACCTTCTTCCCCA
 AGGACATCCTGACCCTGCGGCTGGACGTGATGATGGAGACTGAGAACC
 GCCTCCACTTCACGATCAAAGATCCAGCTAACAGGCGCTACGAGGTGC
 CCTTGAGACCCCGCATGTCCACAGCCGGGCACCGTCCCCACTCTACA
 GCGTGGAGTTCTCCGAGGAGCCCTTCGGGGTGATCGTGCGCCGGCAGC
 TGGACGGCCGCGTGCTGCTGAACACGACGGTGGCGCCCCTGTTCTTTGC
 GGACCAGTTCCTTCAGCTGTCCACCTCGCTGCCCTCGCAGTATATCACN
 GGCCTCGCCGAGCACCTCAGTCCCCTGATGCTCAGCACCAGCTGGACC
 AGGATCACCTGTGGAACCGGGACCTTGCGCCACGCCCCGGTGCGAAC
 CTCTACGGGTCTCACCTTTCTACCTGGCGCTGGAGGACGGCGGGTCGG
 CACACGGGGTGTTCTGCTAAACAGCAATGCCATGGATGTGGTCTCTGC
 AGCCGAGCCCTGCCCTTAGCTGGAGGTGACAGGTGGGATCCTGGATG
 TCTACATCTTCCTGGGCCCAGAGCCCAAGAGCGTGGTGCAGCAGTACC
 TGGACGTTGTGGGATACCCGTTTCATGCCGCCATACTGGGGCCTGGGCTT
 CCACCTGTGCCGCTGGGGCTACTCCTCCACCGCTATCACCCGCCAGGTG
 GTGGAGAACATGACCAGGGGCCACTTCCCCCTGGACGTCCAGTGGAAC
 GACCTGGACTACATGGACTCCCGGAGGGACTTCACGTTCAACAAGGAT
 GGCTTCCGGGACTTCCCGGCCATGGTGCAGGAGCTGCACCAGGGCGGC
 CGGCGCTACATGATGATCGTGGATCCTGCCATCAGCAGCTCGGGCCCT
 GCCGGGAGCTACAGGCCCTACGACGAGGGTCTGCGGAGGGGGGTTTTTC
 ATCACCAACGAGACCGGCCAGCCGCTGATTGGGAAGGTATGGCCCGGG
 TCCACTGCCTTCCCCGACTTCACCAACCCACAGCCCTGGCCTGGTGGG
 AGGACATGGTGGCTGAGTTCCATGACCAGGTGCCCTTCGACGGCATGT
 GGATTGACATGAACGAGCCTTCCAACCTTCATCAGGGGCTCTGAGGACG
 GCTGCCCCAACAAATGAGCTGGAGAACCACCCCTACGTGCCTGGGGTG
 TTGGGGGGACCCTCCAGGCGGCCACCATCTGTGCCTCCAGCCACCAGTT
 TCTCTCCACACACTACAACCTGCACAACCTCTACGGCCTGACCGAAGCC
 ATCGCCTCCCACAGGGCGCTGGTGAAGGCTCGGGGGACACGCCCATTT
 GTGATCTCCCGCTCGACCTTTGCTGGCCACGGCCGATACGCCGGCCACT
 GGACGGGGGACGTGTGGAGCTCCTGGGAGCAGCTCGCCTCCTCCGTGC
 CAGAAATCCTGCAGTTTAACCTGCTGGGGGTGCCTCTGGTCGGGGCCG

	ACGTCTGCGGCTTCCTGGGCAACACCTCAGAGGAGCTGTGTGTGCGCT GGACCCAGCTGGGGGCTTCTACCCCTTCATGCGGAACCAACAACAGCC TGCTCAGTCTGCCCCAGGAGCCGTACAGCTTCAGCGAGCCGGCCCAGC AGGCCATGAGGAAGGCCCTCACCTGCGCTACGCACTCCTCCCCACCT CTACACACTGTTCCACCAGGCCACGTCGCGGGGGAGACCGTGGCCCG GCCCCCTTCTCCTGGAGTTCCCCAAGGACTCTAGCACCTGGACTGTGGAC CACCAGCTCCTGTGGGGGGAGGCCCTGCTCATCACCCAGTGCTCCAG GCCGGGAAGGCCGAAGTGACTGGCTACTTCCCCTTGGGCACATGGTAC GACCTGCAGACGGTGCCAGTAGAGGCCCTTGGCAGCCTCCCACCCCA CCTGCAGCTCCCCGTGAGCCAGCCATCCACAGCGAGGGGCAGTGGGTG ACGCTGCCGGCCCCCTGGACACCATCAACGTCCACCTCCGGGCTGGG TACATCATCCCCCTGCAGGGCCCTGGCCTCACAACCACAGAGTCCCGCC AGCAGCCCATGGCCCTGGCTGTGGCCCTGACCAAGGGTGGGGAGGCC GAGGGGAGCTGTTCTGGGACGATGGAGAGAGCCTGGAAGTGCTGGAG CGAGGGGCCTACACACAGGTCATCTTCTGGCCAGGAATAACACGATC GTGAATGAGCTGGTACGTGTGACCAGTGAGGGAGCTGGCCTGCAGCTG CAGAAGGTGACTGTCCTGGGCGTGGCCACGGCGCCCCAGCAGGTCCTC TCCAACGGTGTCCTGTCTCCAACCTTACCTACAGCCCCGACACCAAGG TCCTGGACATCTGTGTCTCGCTGTTGATGGGAGAGCAGTTTCTCGTCAG CTGGTGTTAG	
Cricket Paralysis Virus IRES (underlined)- BiP-vIGF2- GAA	<u>AAAAATGTGATCTTGCTTGTAATAACAATTTTGAGAGGTTAATAAATTACAAGT</u> <u>AGTGCTATTTTTGTATTTAGGTTAGCTATTTAGCTTTACGTTCCAGGATGCCTA</u> <u>GTGGCAGCCCCACAATATCCAGGAAGCCCTCTCTGCGGTTTTTCAGATTAGG</u> <u>TAGTCGAAAAACCTAAGAAATTTACCTGCTATGAAGCTCTCCCTGGTGGCC</u> GCGATGCTGCTGCTGCTCAGCGCGGCGCGGGCCTCTAGAACACTGTGC GGAGGGGAGCTTGTTAGACACTCTTCAGTTCGTGTGTGGAGATCGCGGG TTCTCTTCTCTCGCCCCGCTTCCAGAGTTTCACGGAGGTCTAGGGGTA TAGTAGAGGAGTGTTGTTTCAGGTCTGTGACTTGGCGCTCCTCGAGAC CTATTGCGCGACGCCAGCCAGGTCCGAAGGGGGCGGTGGCTCAGGTGG TGGAGGTAGCAGACCAGGGCCCCGGGATGCCAGGCACACCCCGGCCG TCCCAGAGCAGTGCCACACAGTGCGACGTCCCCCAACAGCCGCTT CGATTGCGCCCCTGACAAGGCCATCACCCAGGAACAGTGCGAGGCCCG CGGCTGTTGCTACATCCCTGCAAAGCAGGGGCTGCAGGGAGCCCAGAT GGGGCAGCCCTGGTGCTTCTTCCCACCCAGCTACCCAGCTACAAGCTG GAGAACCTGAGCTCCTCTGAAATGGGCTACACGGCCACCCTGACCCGT ACCACCCCACTTCTTCCCCAAGGACATCCTGACCCTGCGGCTGGACG	39

TGATGATGGAGACTGAGAACCGCCTCCACTTCACGATCAAAGATCCAG
 CTAACAGGCGCTACGAGGTGCCCTTGGAGACCCCGCATGTCCACAGCC
 GGGCACCGTCCCCACTCTACAGCGTGGAGTTCTCCGAGGAGCCCTTCG
 GGGTGATCGTGCGCCGGCAGCTGGACGGCCGCGTGCTGCTGAACACGA
 CGGTGGCGCCCCTGTTCTTTGCGGACCAGTTCCTTCAGCTGTCCACCTC
 GCTGCCCTCGCAGTATATCACAGGCCTCGCCGAGCACCTCAGTCCCCTG
 ATGCTCAGCACCAGCTGGACCAGGATCACCTGTGGAACCGGGACCTT
 GCGCCACGCCCCGGTGCGAACCTCTACGGGTCTCACCTTTTCTACCTGG
 CGCTGGAGGACGGCGGGTTCGGCACACGGGGTGTTCTGCTAAACAGCA
 ATGCCATGGATGTGGTCCTGCAGCCGAGCCCTGCCCTTAGCTGGAGGTC
 GACAGGTGGGATCCTGGATGTCTACATCTTCCTGGGCCCAGAGCCCAA
 GAGCGTGGTGCAGCAGTACCTGGACGTTGTGGGATACCCGTTTCATGCC
 GCCATACTGGGGCCTGGGCTTCCACCTGTGCCGCTGGGGCTACTCCTCC
 ACCGCTATCACCCGCCAGGTGGTGGAGAACATGACCAGGGCCCCTTC
 CCCCTGGACGTCCAGTGGAACGACCTGGACTACATGGACTCCCGGAGG
 GACTTCACGTTCAACAAGGATGGCTTCCGGGACTTCCCGGCCATGGTGC
 AGGAGCTGCACCAGGGCGGCCGGCGCTACATGATGATCGTGGATCCTG
 CCATCAGCAGCTCGGGCCCTGCCGGGAGCTACAGGCCCTACGACGAGG
 GTCTGCGGAGGGGGGTTTTTCATCACCAACGAGACCGGCCAGCCGCTGA
 TTGGGAAGGTATGGCCCGGGTCCACTGCCTTCCCCGACTTCACCAACCC
 CACAGCCCTGGCCTGGTGGGAGGACATGGTGGCTGAGTTCCATGACCA
 GGTGCCCTTCGACGGCATGTGGATTGACATGAACGAGCCTTCCAACCTC
 ATCAGGGGCTCTGAGGACGGCTGCCCCAACAAATGAGCTGGAGAACCCA
 CCCTACGTGCCTGGGGTGGTTGGGGGGACCCTCCAGGCGGCCACCATC
 TGTGCCTCCAGCCACCAGTTTCTCTCCACACACTACAACCTGCACAACC
 TCTACGGCCTGACCGAAGCCATCGCCTCCCACAGGGCGCTGGTGAAGG
 CTCGGGGGACACGCCCATTGTGATCTCCCGCTCGACCTTTGCTGGCCA
 CGGCCGATACGCCGGCCACTGGACGGGGGACGTGTGGAGCTCCTGGGA
 GCAGCTCGCCTCCTCCGTGCCAGAAATCCTGCAGTTTAACCTGCTGGGG
 GTGCCTCTGGTCGGGGCCGACGTCTGCGGCTTCCTGGGCAACACCTCAG
 AGGAGCTGTGTGTGCGCTGGACCCAGCTGGGGGCCTTCTACCCCTTCAT
 GCGGAACCACAACAGCCTGCTCAGTCTGCCCCAGGAGCCGTACAGCTT
 CAGCGAGCCGGCCCAGCAGGCCATGAGGAAGGCCCTCACCTGCGCTA
 CGCACTCCTCCCCACCTCTACACACTGTTCCACCAGGCCACGTCGCG
 GGGGAGACCGTGGCCCGGCCCTCTTCCTGGAGTTCCCAAGGACTCT
 AGCACCTGGACTGTGGACCACCAGCTCCTGTGGGGGGAGGCCCTGCTC

	ATCACCCCAGTGCTCCAGGCCGGGAAGGCCGAAGTGACTGGCTACTTC CCCTTGGGCACATGGTACGACCTGCAGACGGTGCCAGTAGAGGCCCTT GGCAGCCTCCCACCCCCACCTGCAGCTCCCCGTGAGCCAGCCATCCAC AGCGAGGGGCAGTGGGTGACGCTGCCGGCCCCCCTGGACACCATCAAC GTCCACCTCCGGGCTGGGTACATCATCCCCCTGCAGGGCCCTGGCCTCA CAACCACAGAGTCCCGCCAGCAGCCCATGGCCCTGGCTGTGGCCCTGA CCAAGGGTGGGGAGGCCCGAGGGGAGCTGTTCTGGGACGATGGAGAG AGCCTGGAAGTGCTGGAGCGAGGGGCCTACACACAGGTCATCTTCCTG GCCAGGAATAACACGATCGTGAATGAGCTGGTACGTGTGACCAGTGAG GGAGCTGGCCTGCAGCTGCAGAAGGTGACTGTCCTGGGCGTGGCCACG GCGCCCCAGCAGGTCCTCTCCAACGGTGTCCCTGTCTCCAACCTCACCT ACAGCCCCGACACCAAGGTCCTGGACATCTGTGTCTCGCTGTTGATGGG AGAGCAGTTTCTCGTCAGCTGGTGTTAG	
wt-PPT1 IDT codon optimized	ATGGCATCACCGGGTTGCCTCTGGTTGTTGGCCGTTGCGTTGCTTCCGT GGACATGTGCATCAAGAGCTCTTCAACATCTGGATCCCCCAGCTCCCCT GCCGCTCGTAATCTGGCACGGGATGGGGGATTCATGTTGTAACCCGTTG TCAATGGGCGCGATAAAAAAGATGGTTGAAAAGAAGATTCCAGGCATC TACGTTCTGTCCCTGGAAATCGGTAAGACACTGATGGAAGACGTGGAG AACTCCTTCTTTCTCAACGTCAATAGTCAGGTCACTACCGTCTGTCAAG CATTGGCAAAGGACCCTAAACTTCAGCAGGGGTACAATGCGATGGGGT TTAGCCAGGGCGGACAGTTTCTTAGAGCCGTCGCACAGCGCTGTCCATC TCCCCCGATGATTAACCTTATATCTGTGCGGGGACAACACCAGGGTGTT TTTGGTCTTCCTCGCTGTCCTGGTGAAAGCTCCCACATCTGTGATTTTCA ACGCAAAACGTTGAACGCAGGAGCTTATAGTAAAGTCGTCCAAGAACG GCTTGTTCAAGCGGAGTATTGGCATGACCCAATAAAAGAAGACGTTTA TAGGAATCACTCTATCTTCTTGGCCGATATCAACCAAGAACGCGGAATC AACGAAAGCTACAAAAAGAATCTTATGGCTCTCAAGAAATTTGTTATG GTGAAATTCTTAATGACTCTATAGTAGATCCTGTGATTGAGAATGGT TCGGGTTCTACAGGTCTGGCCAGGCGAAGGAGACTATCCCCTCCAAG AAACGTCTCTCTATACACAAGACAGACTCGGACTGAAAGAGATGGATA ATGCGGGCCAGTTGGTCTTCTTGGCTACGGAAGGCGATCATCTCCAAC CTCCGAAGAGTGGTTCTATGCCCATATAATCCCGTTCCTGGGCTAA	40
PPT1-2 (wt- vIGF2-PPT1; Codon	ATGGCATCCCCCGGATGTTTGTGGCTGCTGGCGGTTGCGCTTCTGCCAT GGACGTGCGCCTCCCGAGCCCTCCAACACCTGTCCAGGACACTTTGCG GCGGAGAGTTGGTCGATACGCTTCAATTCGTGTGTGGGGATAGAGGCT	41

optimized by IDT codon optimization tool)	TCCTTTTTTCTCGGCCCGCTAGCCGCGTGTCCCGAAGGTCCCGGGGTAT CGTTGAGGAATGCTGTTTCCGGTCCTGCGATCTTGCACTGTTGGAGACA TACTGTGCTACGCCTGCGAGAAGCGAGGGTGGAGGGGGTTCTGGAGGT GGAGGGAGCCGGCCTCGGGCGGTTCCCACCCAGGATCCTCCAGCTCCT CTGCCTCTGGTCATCTGGCATGGGATGGGGGACTCATGTTGTAACCCGC TGAGTATGGGGGCAATTAATAAATAATGGTTGAAAAGAAAATTCCAGGTA TTTATGTCCTCTCTCTTGAAATCGGTAAGACACTTATGGAGGATGTGGA AAACTCCTTTTTTCCTTAATGTCAATTCTCAGGTCACAACAGTTTGTGAG GCTCTGGCGAAGGATCCTAAGCTGCAGCAAGGCTACAACGCCATGGGT TTTTCCCAGGGAGGCCAATTTCTCAGAGCGGTAGCTCAGCGATGTCCAT CACCACCGATGATAAATCTGATCAGTGTGCGCGGACAACACCAGGGAG TTTTCGGGCTGCCCAGGTGTCCGGGGGAATCTAGTCACATATGTGACTT CATTCGCAAGACCCTTAACGCCGGCGCTTACTCAAAGGTGGTTCAAGA ACGGCTTGTGCAGGCTGAATACTGGCACGATCCCATCAAGGAAGATGT ATATAGGAACCACAGTATCTTTCTGGCAGACATAAATCAGGAAAGGGG TATTAACGAAAGCTACAAGAAAAATCTCATGGCCCTGAAGAAATTTGT AATGGTTAAGTTTTTTGAACGATTCTATAGTAGATCCTGTTGACTCCGAG TGGTTCGGGTTCTATCGATCTGGTCAAGCCAAGGAGACGATTCCGCTTC AGGAAACTTCACTGTACACACAGGATCGGCTGGGACTCAAGGAGATGG ACAATGCGGGCCAGTTGGTGTCTTCTGGCTACAGAGGGAGACCATCTCC AGTTGAGTGAAGAATGGTTCTATGCACATATTATCCCATTCCTCGGCTA A	
PPT1-29 (BiP2aa- vIGF2-PPT1; native human sequence)	ATGAAGCTCTCCCTGGTGGCCGCGATGCTGCTGCTGCTCTGGGTGGCAC TGCTGCTGCTCAGCGCGGCGAGGGCCGCGCGAGTCGCACGTTGTGTG GAGGTGAACTCGTCGACACCCTTCAGTTTCGTATGTGGAGATCGCGGTTT CCTCTTCTCACGCCAGCTTCCAGAGTTTCCCGAAGATCACGAGGAATA GTTGAGGAGTGCTGTTTTTCGGTCTTGTGATCTGGCTCTCCTCGAGACTT ATTGTGCTACGCCGGCCCGCTCTGAAGGAGGTGGTGGCAGTGGAGGAG GAGGGAGTCGGCCTAGGGCAGTCCCAACCCAGGACCCGCGGCGCCGC TGCCGTTGGTGATCTGGCATGGGATGGGAGACAGCTGTTGCAATCCCTT AAGCATGGGTGCTATTAATAAATAATGGTGGAGAAGAAAATACCTGGAAT TTACGTCTTATCTTTAGAGATTGGGAAGACCCTGATGGAGGACGTGGA GAACAGCTTCTTCTTGAATGTCAATTCCCAAGTAACAACAGTGTGTCAG GCACTTGCTAAGGATCCTAATTTGCAGCAAGGCTACAATGCTATGGGA TTCTCCCAGGGAGGCCAATTTCTGAGGGCAGTGGCTCAGAGATGCCCTT	42

	CACCTCCCATGATCAATCTGATCTCGGTTGGGGGACAACATCAAGGTGT TTTTGGACTCCCTCGATGCCCAGGAGAGAGCTCTCACATCTGTGACTTC ATCCGAAAAACACTGAATGCTGGGGCGTACTCCAAAGTTGTTTCAGGAA CGCCTCGTGCAAGCCGAATACTGGCATGACCCCATAAAGGAGGATGTG TATCGCAACCACAGCATCTTCTTGGCAGATATAAATCAGGAGCGGGGT ATCAATGAGTCCTACAAGAAAAACCTGATGGCCCTGAAGAAGTTTGTG ATGGTGAAATTCCTCAATGATTCCATTGTGGACCCTGTAGATTCCGAGT GGTTTGGATTTTACAGAAGTGGCCAAGCCAAGGAAACCATTCCCTTAC AGGAGACCTCCCTGTACACACAGGACCGCCTGGGGCTAAAGGAAATGG ACAATGCAGGACAGCTAGTGTCTGGCTACAGAAGGGGACCATCTTC AGTTGTCTGAAGAATGGTTTTATGCCACATCATACCATTCTTGGATG A	
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[00106] In some embodiments, the vector comprising the nucleic acid encoding the desired therapeutic fusion protein, such as a vIGF2 fusion or a signal peptide fusion, optionally having an internal ribosomal entry sequence, provided herein is an adeno-associated viral vector (A5/35).

[00107] In some embodiments, the nucleic acid encoding the therapeutic fusion protein, such as a vIGF2 fusion, optionally having an internal ribosomal entry sequence, is cloned into a number of types of vectors. For example, in some embodiments, the nucleic acid is cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[00108] Further, the expression vector encoding the therapeutic fusion protein, such as a vIGF2 fusion or a signal peptide fusion, optionally having an internal ribosomal entry sequence, in some embodiments, is provided to a cell in the form of a viral vector. Viral vector technology is described, e.g., in Sambrook et al., 2012, *Molecular Cloning: A Laboratory Manual*, volumes 1-4, Cold Spring Harbor Press, NY), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[00109] Also provided herein are compositions and systems for gene transfer. A number of virally based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene, in some embodiments, is inserted into a vector and packaged in retroviral particles using suitable techniques. The recombinant virus is then isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are suitable for gene therapy. In some embodiments, adenovirus vectors are used. A number of adenovirus

vectors are suitable for gene therapy. In some embodiments, adeno-associated virus vectors are used. A number of adeno-associated viruses are suitable for gene therapy. In one embodiment, lentivirus vectors are used.

[00110] Gene therapy constructs provided herein comprise a vector (or gene therapy expression vector) into which the gene of interest is cloned or otherwise which includes the gene of interest in a manner such that the nucleotide sequences of the vector allow for the expression (constitutive or otherwise regulated in some manner) of the gene of interest. The vector constructs provided herein include any suitable gene expression vector that is capable of being delivered to a tissue of interest and which will provide for the expression of the gene of interest in the selected tissue of interest.

[00111] In some embodiments, the vector is an adeno-associated virus (AAV) vector because of the capacity of AAV vectors to cross the blood-brain barrier and transduction of neuronal tissue. In methods provided herein, AAV of any serotype is contemplated to be used. The serotype of the viral vector used in certain embodiments is selected from the group consisting of an AAV1 vector, an AAV2 vector, an AAV3 vector, an AAV4 vector, an AAV5 vector, an AAV6 vector, an AAV7 vector, an AAV8 vector, an AAV9 vector, an AAVrhS vector, an AAVrh10 vector, an AAVrh33 vector, an AAVrh34 vector, an AAVrh74 vector, an AAV Anc80 vector, an AAVPHP.B vector, an AAVhu68 vector, an AAV-DJ vector, and others suitable for gene therapy.

[00112] AAV vectors are DNA parvoviruses that are nonpathogenic for mammals. Briefly, AAV-based vectors have the rep and cap viral genes that account for 96% of the viral genome removed, leaving the two flanking 145 base pair inverted terminal repeats (ITRs) which are used to initiate viral DNA replication, packaging, and integration.

[00113] Further embodiments include use of other serotype capsids to create an AAV1 vector, an AAV2 vector, an AAV3 vector, an AAV4 vector, an AAV5 vector, an AAV6 vector, an AAV7 vector, an AAV8 vector, an AAV9 vector, an AAVrhS vector, an AAVrh10 vector, an AAVrh33 vector, an AAVrh34 vector, an AAVrh74 vector, an AAV Anc80 vector, an AAVPHP.B vector, an AAV-DJ vector, and others suitable for gene therapy. Optionally, the AAV viral capsid is AAV2/9, AAV9, AAVrhS, AAVrh10, AAVAnc80, or AAV PHP.B.

[00114] Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements is often increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements function either cooperatively or independently to activate transcription.

[00115] An example of a promoter that is capable of expressing a therapeutic fusion protein, such as a vIGF2 fusion or a signal peptide fusion, optionally having an internal ribosomal entry sequence, transgene in a mammalian T-cell is the EF1a promoter. The native EF1a promoter drives expression of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. The EF1a promoter has been extensively used in mammalian expression plasmids and has been shown to be effective in driving expression from transgenes cloned into a lentiviral vector (see, e.g., Milone et al., Mol. Ther. 17(8): 1453-1464 (2009)). Another example of a promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. However, other constitutive promoter sequences are sometimes also used, including, but not limited to the chicken β actin promoter, the P546 promoter, the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the elongation factor-1a promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, gene therapy vectors are not contemplated to be limited to the use of constitutive promoters. Inducible promoters are also contemplated here. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline-regulated promoter.

[00116] In order to assess the expression of a therapeutic fusion protein, such as a vIGF fusion or a signal peptide fusion, optionally having an internal ribosomal entry sequence, or portions thereof, the expression vector to be introduced into a cell often contains either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable marker is often carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes are sometimes flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

[00117] Methods and compositions for introducing and expressing genes into a cell are suitable for methods herein. In the context of an expression vector, the vector is readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector is transferred into a host cell by physical, chemical, or biological means.

[00118] Physical methods and compositions for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, gene gun,

electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are suitable for methods herein (see, e.g., Sambrook et al., 2012, *Molecular Cloning: A Laboratory Manual*, volumes 1-4, Cold Spring Harbor Press, NY). One method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

[00119] Chemical means and compositions for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, nucleic acid-lipid particles, and liposomes. An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle). Other methods of state-of-the-art targeted delivery of nucleic acids are available, such as delivery of polynucleotides with targeted nanoparticles or other suitable sub-micron sized delivery system.

[00120] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (in vitro, ex vivo or in vivo). In another aspect, the nucleic acid is associated with a lipid. The nucleic acid associated with a lipid, in some embodiments, is encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, in some embodiments, they are present in a bilayer structure, as micelles, or with a “collapsed” structure. Alternately, they are simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which are, in some embodiments, naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[00121] Lipids suitable for use are obtained from commercial sources. For example, in some embodiments, dimyristyl phosphatidylcholine (“DMPC”) is obtained from Sigma, St. Louis, Mo.; in some embodiments, dicetyl phosphate (“DCP”) is obtained from K & K Laboratories (Plainview, N.Y.); cholesterol (“Choi”), in some embodiments, is obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol (“DMPG”) and other lipids are often obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock solutions of lipids in chloroform or chloroform/methanol are often stored at about -20 °C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. “Liposome” is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes are often characterized as having

vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., 1991 *Glycobiology* 5: 505-10). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids, in some embodiments, assume a micellar structure or merely exist as non-uniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

[00122] Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the therapeutic fusion protein, such as a vIGF2 fusion or a signal peptide fusion, optionally having an internal ribosomal entry sequence, provided herein, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays are contemplated to be performed. Such assays include, for example, “molecular biological” assays suitable for methods herein, such as Southern and Northern blotting, RT-PCR and PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and western blots) or by assays described herein to identify agents falling within the scope herein.

[00123] The present disclosure further provides a vector comprising a therapeutic fusion protein, such as a vIGF2 fusion or a signal peptide fusion, optionally having an internal ribosomal entry sequence, encoding nucleic acid molecule. In one aspect, a therapeutic fusion protein vector is capable of being directly transduced into a cell. In one aspect, the vector is a cloning or expression vector, e.g., a vector including, but not limited to, one or more plasmids (e.g., expression plasmids, cloning vectors, minicircles, minivectors, double minute chromosomes), retroviral and lentiviral vector constructs. In one aspect, the vector is capable of expressing the vIGF2-therapeutic fusion protein construct in mammalian cells. In one aspect, the mammalian cell is a human cell.

Uses and Methods of Treatment

[00124] Also provided herein are methods of treating genetic disorders using gene therapy comprising administering to an individual a nucleic acid encoding a therapeutic fusion protein (such as a vIGF2 fusion or a signal peptide fusion or a signal peptide-vIGF2 fusion), optionally having an internal ribosomal entry sequence, disclosed herein. Genetic disorders suitable for treatment using methods herein comprise disorders in an individual caused by one or more mutations in the genome causing lack of expression or expression of a dysfunctional protein by the mutant gene.

[00125] Further provided herein are pharmaceutical compositions comprising a gene therapy vector, such as a gene therapy vector comprising a nucleic acid encoding a therapeutic fusion protein (such as a vIGF2 fusion or a signal peptide fusion or a signal peptide-vIGF2 fusion), optionally having an internal ribosomal entry sequence, disclosed herein and a pharmaceutically acceptable carrier or excipient for use in preparation of a medicament for treatment of a genetic disorder.

[00126] Genetic disorders suitable for treatment by methods herein include but are not limited to Achondroplasia, Alpha-1 Antitrypsin Deficiency, Antiphospholipid Syndrome, Autosomal Dominant Polycystic Kidney Disease, Charcot-Marie-Tooth, Colon cancer, Cri du chat, Crohn's Disease, Cystic fibrosis, Dercum Disease, Duane Syndrome, Duchenne Muscular Dystrophy, Factor V Leiden Thrombophilia, Familial Hypercholesterolemia, Familial Mediterranean Fever, Fragile X Syndrome, Gaucher Disease, Hemochromatosis, Hemophilia, Holoprosencephaly, Huntington's disease, Klinefelter syndrome, Marfan syndrome, Myotonic Dystrophy, Neurofibromatosis, Noonan Syndrome, Osteogenesis Imperfecta, Parkinson's disease, Phenylketonuria, Poland Anomaly, Porphyria, Progeria, Retinitis Pigmentosa, Severe Combined Immunodeficiency (SCID), Sickle cell disease, Spinal Muscular Atrophy, Tay-Sachs disease, Thalassemia, Trimethylaminuria, Turner Syndrome, Velocardiofacial Syndrome, WAGR Syndrome, or Wilson Disease. In some embodiments, the genetic disorder is selected from the group consisting of CDKL5 deficiency disorder, cystic fibrosis, alpha- and beta-thalassemias, sickle cell anemia, Marfan syndrome, fragile X syndrome, Huntington's disease, hemochromatosis, Congenital Deafness (nonsyndromic), Tay-Sachs, Familial hypercholesterolemia, Duchenne muscular dystrophy, Stargardt disease, Usher syndrome, choroideremia, achromatopsia, X-linked retinoschisis, hemophilia, Wiskott-Aldrich syndrome, X-linked chronic granulomatous disease, aromatic L-amino acid decarboxylase deficiency, recessive dystrophic epidermolysis bullosa, alpha 1 antitrypsin deficiency, Hutchinson-Gilford progeria syndrome (HGPS), Noonan syndrome, X-linked severe combined immunodeficiency (X-SCID).

[00127] In some embodiments, genetic disorders suitable for treatment using methods provided herein are lysosomal storage disorder. In some embodiments, lysosomal storage disorders are treated herein using gene therapy to deliver missing or defective enzymes to the patient. In some embodiments, methods herein deliver an enzyme fused to a vIGF2 or fused to a signal peptide to the patient in order to deliver the enzyme to the cell where it is needed. In some embodiments, the lysosomal storage disorders is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, and Schindler disease type II. In some embodiments, the lysosomal storage disorder is selected from the group consisting of activator deficiency, GM2-gangliosidosis; GM2-gangliosidosis, AB variant; alpha-mannosidosis (type 2, moderate form; type 3, neonatal, severe); beta-mannosidosis; aspartylglucosaminuria; lysosomal acid lipase deficiency; cystinosis (late-onset juvenile or adolescent nephropathic type; infantile nephropathic); Chanarin-Dorfman syndrome; neutral lipid storage disease

with myopathy; NLSDM; Danon disease; Fabry disease; Fabry disease type II, late-onset; Farber disease; Farber lipogranulomatosis; fucosidosis; galactosialidosis (combined neuraminidase & beta-galactosidase deficiency); Gaucher disease ; type II Gaucher disease ; type III Gaucher disease; type IIIC Gaucher disease; Gaucher disease, atypical, due to saposin C deficiency; GM1-gangliosidosis (late-infantile/juvenile GM1-gangliosidosis; adult/chronic GM1-gangliosidosis); Globoid cell leukodystrophy, Krabbe disease (Late infantile onset; Juvenile Onset; Adult Onset); Krabbe disease, atypical, due to saposin A deficiency; Metachromatic Leukodystrophy (juvenile; adult); partial cerebroside sulfate deficiency; pseudoarylsulfatase A deficiency; metachromatic leukodystrophy due to saposin B deficiency; Mucopolysaccharidoses disorders: MPS I, Hurler syndrome; MPS I, Hurler-Scheie syndrome; MPS I, Scheie syndrome ; MPS II, Hunter syndrome ; MPS II, Hunter syndrome ; Sanfilippo syndrome Type A / MPS IIIA ; Sanfilippo syndrome Type B / MPS IIIB ; Sanfilippo syndrome Type C / MPS IIIC ; Sanfilippo syndrome Type D / MPS IIID ; Morquio syndrome, type A / MPS IVA ; Morquio syndrome, type B / MPS IVB ; MPS IX hyaluronidase deficiency ; MPS VI Maroteaux-Lamy syndrome ; MPS VII Sly syndrome; mucopolipidosis I, sialidosis type II; I-cell disease, Leroy disease, mucopolipidosis II; Pseudo-Hurler polydystrophy / mucopolipidosis type III; mucopolipidosis IIIC / ML III GAMMA; mucopolipidosis type IV; multiple sulfatase deficiency; Niemann-Pick disease (type B; type C1 / chronic neuronopathic form; type C2; type D / Nova Scotian type); Neuronal Ceroid Lipofuscinoses: CLN6 disease - Atypical Late Infantile, Late-Onset variant, Early Juvenile; Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease; Finnish Variant Late Infantile CLN5; Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease; Kufs/Adult-onset NCL/CLN4 disease (type B); Northern Epilepsy/variant late infantile CLN8; Santavuori-Haltia/Infantile CLN1/PPT disease; Pompe disease (glycogen storage disease type II); late-onset Pompe disease; Pycnodysostosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 gangliosidosis; Sandhoff disease / GM2 Gangliosidosis; Schindler disease (type III / intermediate, variable); Kanzaki disease; Salla disease; infantile free sialic acid storage disease (ISSD); spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME); Tay-Sachs disease / GM2 gangliosidosis; juvenile-onset Tay-Sachs disease; late-onset Tay-Sachs disease; Christianson syndrome; Lowe oculocerebrorenal syndrome; Charcot-Marie-Tooth type 4J, CMT4J; Yunis-Varon syndrome; bilateral temporooccipital polymicrogyria (BTOP); X-linked hypercalciuric nephrolithiasis, Dent-1; and Dent disease 2. In some embodiments, the therapeutic protein is associated with a lysosomal storage disorder and the therapeutic protein is selected from the group consisting of GM2-activator protein ; α -mannosidase ; MAN2B1 ; lysosomal β -mannosidase ; glycosylasparaginase ; lysosomal acid lipase ; cystinosis ; CTNS ; PNPLA2 ; lysosome-associated membrane protein-2; α -galactosidase A; GLA; acid ceramidase; α -L-fucosidase; protective protein/cathepsin A; acid β -glucosidase; GBA ; PSAP; β -galactosidase-1; GLB1 ; galactosylceramide β -galactosidase ; GALC ; PSAP; arylsulfatase A; ARSA ; α -L-iduronidase; iduronate 2-sulfatase; heparan N-sulfatase; N- α -acetylglucosaminidase; heparan acetyl CoA: α -glucosaminide acetyltransferase; N-acetylglucosamine 6-sulfatase; galactosamine-6-sulfate

sulfatase; β -galactosidase; hyaluronidase; arylsulfatase B; β -glucuronidase; neuraminidase; NEU1; gamma subunit of N-acetylglucosamine-1-phosphotransferase; mucolipin-1; sulfatase-modifying factor-1; acid sphingomyelinase; SMPD1; NPC1; and NPC2.

[00128] In some embodiments, treatment via methods herein delivers a gene encoding a therapeutic protein to a cell in need of the therapeutic protein. In some embodiments, the treatment delivers the gene to all somatic cells in the individual. In some embodiments, the treatment replaces the defective gene in the targeted cells. In some embodiments, cells treated ex vivo to express the therapeutic protein are delivered to the individual.

[00129] Gene therapy for disorders disclosed herein provides superior treatment outcomes to conventional treatments, including enzyme replacement therapy, because it does not require long infusion treatments. In addition, it has reduced risk of the individual developing an immune response to the therapeutic protein, which is often experienced in individuals receiving enzyme replacement therapy.

Definitions

[00130] As used herein “ex vivo gene therapy” refers to methods where patient cells are genetically modified outside the subject, for example to express a therapeutic gene. Cells with the new genetic information are then returned to the subject from whom they were derived.

[00131] As used herein “in vivo gene therapy” refers to methods where a vector carrying the therapeutic gene(s) is directly administered to the subject.

[00132] As used herein “fusion protein” and “therapeutic fusion protein” are used interchangeably herein and refer to a therapeutic protein having at least one additional protein, peptide, or polypeptide, linked to it. In some instances, fusion proteins are a single protein molecule containing two or more proteins or fragments thereof, covalently linked via peptide bond within their respective peptide chains, without chemical linkers. In some embodiments, the fusion protein comprises a therapeutic protein and a signal peptide, a peptide that increases endocytosis of the fusion protein, or both. In some embodiments, the peptide that increases endocytosis is a peptide that binds CI-MPR.

[00133] As used herein “vector”, or “gene therapy vector”, used interchangeably herein, refers to gene therapy delivery vehicles, or carriers, that deliver therapeutic genes to cells. A gene therapy vector is any vector suitable for use in gene therapy, e.g., any vector suitable for the therapeutic delivery of nucleic acid polymers (encoding a polypeptide or a variant thereof) into target cells (e.g., sensory neurons) of a patient. In some embodiments, the gene therapy vector delivers the nucleic acid encoding a therapeutic protein or therapeutic fusion protein to a cell where the therapeutic protein or fusion is expressed and secreted from the cell. The vector may be of any type, for example it may be a plasmid vector or a minicircle DNA. Typically, the vector is a viral vector. These include both genetically disabled viruses such as adenovirus and nonviral vectors such as liposomes. The viral vector may for example be derived from an adeno-associated virus (AAV), a retrovirus, a lentivirus, a herpes simplex virus, or an adenovirus. AAV derived vectors. The vector may comprise an AAV genome or a derivative thereof.

[00134] “Construct” as used herein refers to a nucleic acid molecule or sequence that encodes a therapeutic protein or fusion protein and optionally comprises additional sequences such as a translation initiation sequence or IRES sequence.

[00135] As used herein “plasmid” refers to circular, double-stranded unit of DNA that replicates within a cell independently of the chromosomal DNA.

[00136] As used herein “promoter” refers to a site on DNA to which the enzyme RNA polymerase binds and initiates the transcription of DNA into RNA.

[00137] As used herein “somatic therapy” refers to methods where the manipulation of gene expression in cells that will be corrective to the patient but not inherited by the next generation. Somatic cells include all the non-reproductive cells in the human body

[00138] As used herein “somatic cells” refers to all body cells except the reproductive cells.

[00139] As used herein “tropism” refers to preference of a vector, such as a virus for a certain cell or tissue type. Various factors determine the ability of a vector to infect a particular cell. Viruses, for example, must bind to specific cell surface receptors to enter a cell. Viruses are typically unable to infect a cell if it does not express the necessary receptors.

[00140] The term “transduction” is used to refer to the administration/delivery of the nucleic acid encoding the therapeutic protein to a target cell either *in vivo* or *in vitro*, via a replication-deficient rAAV of the disclosure resulting in expression of a functional polypeptide by the recipient cell. Transduction of cells with a gene therapy vector such as a rAAV of the disclosure results in sustained expression of polypeptide or RNA encoded by the rAAV. The present disclosure thus provides methods of administering/delivering to a subject a gene therapy vector such as an rAAV encoding a therapeutic protein by an intrathecal, intraretinal, intraocular, intravitreous, intracerebroventricular, intraparechymal, or intravenous route, or any combination thereof. “Intrathecal” delivery refers to delivery into the space under the arachnoid membrane of the brain or spinal cord. In some embodiments, intrathecal administration is via intracisternal administration.

[00141] The terms “recipient”, “individual”, “subject”, “host”, and “patient”, are used interchangeably herein and in some cases, refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. “Mammal” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and laboratory, zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, mice, rats, rabbits, guinea pigs, monkeys etc. In some embodiments, the mammal is human.

[00142] As used herein, the terms “treatment,” “treating,” “ameliorating a symptom,” and the like, in some cases, refer to administering an agent, or carrying out a procedure, for the purposes of obtaining a therapeutic effect, including inhibiting, attenuating, reducing, preventing or altering at least one aspect or marker of a disorder, in a statistically significant manner or in a clinically significant manner. The term “ameliorate” or “treat” does not state or imply a cure for the underlying condition. “Treatment,” or “to

ameliorate” (and like) as used herein, may include treating a mammal, particularly in a human, and includes: (a) preventing the disorder or a symptom of a disorder from occurring in a subject which may be predisposed to the disorder but has not yet been diagnosed as having it (e.g., including disorders that may be associated with or caused by a primary disorder; (b) inhibiting the disorder, i.e., arresting its development; (c) relieving the disorder, i.e., causing regression of the disorder; and (d) improving at least one symptom of the disorder. Treating may refer to any indicia of success in the treatment or amelioration or prevention of a disorder, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the disorder condition more tolerable to the patient; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating. The treatment or amelioration of symptoms is based on one or more objective or subjective parameters; including the results of an examination by a physician. Accordingly, the term "treating" includes the administration of the compounds or agents of the present invention to prevent or delay, to alleviate, or to arrest or inhibit development of the symptoms or conditions associated with the disorder. The term "therapeutic effect" refers to the reduction, elimination, or prevention of the disorder, symptoms of the disorder, or side effects of the disorder in the subject.

[00143] The term “affinity” refers to the strength of binding between a molecule and its binding partner or receptor.

[00144] As used herein, the phrase “high affinity” refers to, for example, a therapeutic fusion containing such a peptide that binds CI-MPR which has an affinity to CI-MPR that is about 100 to 1,000 times or 500 to 1,000 times higher than that of the therapeutic protein without the peptide. In some embodiments, the affinity is at least 100, at least 500, or at least 1000 times higher than without the peptide. For example, where the therapeutic protein and CI-MPR are combined in relatively equal concentration, the peptide of high affinity will bind to the available CI-MPR so as to shift the equilibrium toward high concentration of the resulting complex.

[00145] “Secretion” as used herein refers to the release of a protein from a cell into, for example, the bloodstream to be carried to a tissue of interest or a site of action of the therapeutic protein. When a gene therapy product is secreted into the interstitial space of an organ, secretion can allow for cross-correction of neighboring cells.

[00146] “Delivery” as used herein means drug delivery. In some embodiments, the process of delivery means transporting a drug substance (e.g., therapeutic protein or fusion protein produced from a gene therapy vector) from outside of a cell (e.g., blood, tissue, or interstitial space) into a target cell for therapeutic activity of the drug substance.

[00147] “Engineering” or “protein engineering” as used here in refers to the manipulation of the structures of a protein by providing appropriate a nucleic acid sequence that encodes for the protein as to produce desired properties, or the synthesis of the protein with particular structures.

[00148] A "therapeutically effective amount" in some cases means the amount that, when administered to a subject for treating a disorder, is sufficient to effect treatment for that disorder.

[00149] As used herein, the term "about" a number refers to a range spanning that from 10% less than that number through 10% more than that number, and including values within the range such as the number itself.

[00150] As used herein, the term "comprising" an element or elements of a claim refers to those elements but does not preclude the inclusion of an additional element or elements.

EXAMPLES

[00151] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1: Binding of variant IGF2 peptide to CI-MPRReceptor

[00152] Surface plasmon resonance (SPR) experiments were conducted using Biacore to measure binding of wildtype and variant IGF2 (vIGF2) to the CI-MPR receptor. The wildtype, human mature IGF2 peptide (wt IGF2) has the sequence set forth in SEQ ID NO: 1. The vIGF2 sequence differs from wt IGF2 in that it lacks residues 1-4 and contains the following mutations: E6R, Y27L, and K65R. It has the amino sequence:

SRTLGGELVDTLQFVCGDRGFLFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPARSE

(SEQ ID NO: 31). vIGF2 also has an N-terminal linker with the sequence GGGGSGGGG (SEQ ID NO: 18). The combined sequence is

GGGGSGGGGSRTLGGELVDTLQFVCGDRGFLFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPARSE SEQ ID NO: 43).

FIG. 4 shows that as expected, the wildtype IGF2 peptide binds to the CI-MPRreceptor with high affinity (0.2 nM). **FIG. 5** shows that the variant IGF2 peptide (vIGF2) also binds to the CI-MPRreceptor with high affinity (0.5 nM). These data indicate that vIGF2 peptide has high affinity for the intended CI-MPRreceptor for targeting therapeutics to lysosomes.

[00153] SPR was utilized to measure peptide binding to the Insulin Receptor to assess potential side effects. Insulin binds the Insulin Receptor with high affinity (~8 nM; data not shown). Wildtype IGF2 and a vIGF2 were tested, where the vIGF2 had the sequence

SRTLGGELVDTLQFVCGDRGFLFSRPASRVSRRSRGIVEECCFRSCDLALLETYCATPARSE

(SEQ ID NO: 31) having an N-terminal linker with a sequence GGGGSGGGG (SEQ ID NO: 18). **FIG. 8**

shows that wildtype IGF2 also binds the Insulin Receptor with relatively high affinity (~100 nM). IGF2 peptide from Biomarin/Zystor IGF2-GAA fusion protein (BMN-701) also binds the Insulin Receptor with high affinity and was shown to cause hypoglycemia in clinical trials. **FIG. 9** shows no measurable binding of vIGF2 peptide to the insulin receptor. These data show that vIGF2 peptide confers a superior safety profile compared with wt IGF2 peptide fusions.

[00154] The same SPR binding analysis was utilized to characterize vIGF2 peptide interaction with the IGF1 Receptor. **FIG. 10** shows that the wildtype IGF2 peptide binds IGF1 receptor with relatively high affinity (~100 nM). **FIG. 11** shows no measurable binding of vIGF2 peptide to the IGF1 Receptor, showing an improved safety profile compared to wt IGF2.

Table 8: SPR Affinity Results		
Receptor	wt IGF2 Kd (nM)	vIGF2 Kd (nM)
CI-MPR	0.2	0.5
Insulin Receptor	100	No Binding Detected
IGF1 Receptor	100	No Binding Detected

Example 2: vIGF2 Converts Low Affinity Ligand to High Affinity ERT for CI-MPR

[00155] The vIGF2 peptide (SEQ ID NO: 31) with an N-terminal linker (SEQ ID NO: 18) was chemically coupled to alglucosidase-alfa, designated here as vIGF2- alglucosidase-alfa, to determine whether the vIGF2 peptide could improve affinity for CI-MPR. As shown in **FIG. 6**, binding affinities of alglucosidase-alfa and vIGF2- alglucosidase-alfa were directly compared using CI-MPR plate binding assays in 96-well ELISA plates coated with CI-MPR. Unbound enzyme was washed away prior to measuring bound enzyme activity. Varying concentrations of both enzyme preparations were used with or without free WT IGF2 peptide. vIGF2 substantially improved the affinity for CI-MPR. Further, binding of vIGF2-alglucosidase-alfa was blocked by free WT IGF2 indicating that binding was IGF2-dependent. (Data not shown.) Coupling of vIGF2 peptide did not impair GAA enzyme activity.

[00156] The vIGF2 was coupled to recombinant human N-acetyl- α -D-glucosaminidase (rhNAGLU). RhNAGLU, a lysosomal enzyme lacking M6P, to determine whether peptide can convert a non-ligand to high affinity ligand for CI-MPR. In this experiment, rhNAGLU and vIGF2-rhNAGLU were directly compared using CI-MPR plate binding assays, utilizing CI-MPR-coated plates. Unbound enzyme was washed away prior to measuring bound enzyme activity. Varying concentrations of both enzyme preparations were used with or without free vIGF2 peptide. As shown in **FIG. 7**, vIGF2-rhNAGLU has significantly higher affinity for CI-MPR than rhNAGLU lacking vIGF2. Further, vIGF2-rhNAGLU

binding was blocked by free vIGF2 peptide indicating that receptor binding was specific for IGF2 peptide. These results show that vIGF2 peptide can be utilized to improve drug targeting to lysosomes.

Example 3: Myoblast Uptake of vIGF2-GAA Fusion Proteins

[00157] vIGF2-GAA fusion proteins (same sequences as in Examples 1-2) were administered and L6 myoblast uptake of the enzyme was measured. FIG. 6 shows superior uptake of the vIGF2-rhGAA compared to rhGAA and M6P-GAA. Therefore, vIGF2 is effective at targeting GAA to the cells.

Example 4: Constructs for ERT Delivered by Gene Therapy

[00158] Two different constructs are illustrated in FIG. 12. In the top panel is a construct which contains a Kozak sequence and a nucleic acid encoding a recombinant human GAA with the native signal peptide (SEQ ID NO: 45), encoding “natural hGAA” (SEQ ID NO: 45). In the middle panel is the construct Kozak-BiP-vIGF2-2GS-GAA, encoding “engineered hGAA” (SEQ ID NO: 23). This construct is characterized by a Kozak sequence, a nucleic acid encoding BiP signal peptide, a nucleic acid encoding the vIGF2 peptide having the sequence set forth in SEQ ID NO: 31, and a nucleic acid encoding a 2GS linker (SEQ ID NO: 18) followed by a nucleic acid encoding a recombinant human GAA with the N-terminal 60 amino acids removed (SEQ ID NO: 46) to prevent premature processing and removal of the vIGF2.

Example 5: Enhanced Secretion of Gene Therapy Constructs

[00159] *Engineered hGAA has greater secretion and is able to interact with a cell surface receptor appropriate for cellular uptake and lysosomal targeting*

CHO expressing engineered hGAA, described in more detail below, or natural hGAA were cultured and conditioned media was collected for measurement of GAA activity. FIG. 15 shows the relative activity of engineered and natural hGAA showing that engineered hGAA has increased activity compared to natural hGAA, indicative of more efficient secretion of engineered hGAA.

Example 6: Analysis of PPT1 in conditioned media

[00160] *Cloning of PPT1 constructs*

PPT1 constructs were cloned into the pcDNA3.1 expression vector (ThermoFisher cat# V79020), which contains a CMV promoter. The tested constructs included PPT1-1 (WT-PPT1) (SEQ ID NO: 24); PPT1-2 (WT-vIGF2-PPT1) (SEQ ID NO: 25); PPT1-29 (BiP2aa-vIGF2-PPT1) (SEQ ID NO: 26).

PPT1 secretion & binding

[00161] The PPT1 constructs were transiently expressed in HEK293T cells for 3 days and the PPT1 secreted into the media. Secreted PPT1 was quantified by Western Blotting, and assayed for CI-MPR binding using established methods. Secreted PPT1 is shown in FIG. 13. CI-MPR binding is shown in FIG. 14.

[00162] Example 7: Testing Gene Therapy Vectors in an Animal Model of Pompe Disease**[00163]** *Pompe Gene Therapy: Preclinical Proof of Concept Study Design*

[00164] A preclinical study was conducted in GAA knockout (GAA KO) mice using a high dose for initial comparison of constructs. The constructs are shown in FIG. 12. Mice were treated with vehicle or one of two constructs, Natural – hGAA or Engineered - hGAA. Mice were administered 5e11 gc/mouse (approximately 2.5e13 gc/kg). GAA knockout mice were used at age 2 months. Normal (wildtype) mice were used as a control. The study design is outlined in FIG. 16.

[00165] *Pompe Gene Therapy: Plasma*

[00166] Plasma was collected from wild type (normal) mice or GAA KO mice treated with vehicle or a gene therapy vector as indicated and GAA activity and cell surface binding was measured. Data are summarized in FIG. 17, FIG. 27, and FIG. 19. Similar high GAA levels were seen in mice treated with gene therapy vectors (FIG. 17, FIG. 18). However, greater cell targeting receptor binding was observed with the engineered construct (FIG. 19).

[00167] *Pompe Gene Therapy: Quadriceps*

[00168] GAA activity, and glycogen storage/cytoplasmic vacuolization were assessed in normal (wild type) mice and treated GAA KO mice (FIG. 28). GAA activity in the quadriceps was about 20 fold higher than wild type. Glycogen PAS (FIG. 29) and immunohistochemistry (FIG. 30) were also assessed. Immunohistochemistry showed greater lysosomal targeting of engineered hGAA compared to wild type. Glycogen reduction was more consistent for engineered hGAA by PAS staining.

[00169] *Pompe Gene Therapy: Triceps*

[00170] GAA activity, and glycogen storage/cytoplasmic vacuolization were assessed in normal (wild type) mice and in treated GAA KO mice (FIG. 31). GAA activity was about 10-15 fold higher than wild type. Immunohistochemistry and glycogen PAS were also assessed (FIG. 32 and FIG. 33). Immunohistochemistry illustrated greater lysosomal targeting of engineered hGAA compared to wildtype GAA. Glycogen reduction was more consistent for engineered hGAA as measured by PAS staining.

[00171] *Pompe Gene Therapy: Tibialis Anterior (TA)*

[00172] GAA activity, and glycogen storage/cytoplasmic vacuolization were assessed in normal (wild type) and treated GAA KO mice (FIG. 20). GAA activity in the TA was about 15-20 fold higher than wild type. Immunohistochemistry and glycogen PAS were also assessed (FIG. 21 and FIG. 22). Immunohistochemistry illustrated greater lysosomal targeting of engineered hGAA compared to wildtype GAA. Glycogen levels were close to wildtype levels. Glycogen reduction was more consistent for engineered hGAA by PAS staining.

[00173] *Pompe Gene Therapy: Brain and Spinal Cord*

[00174] GAA activity, glycogen content, and glycogen storage/cytoplasmic vacuolization were assessed in normal (wild type) mice and treated GAA KO mice (FIG. 23). GAA activity in the brain was about 5 fold

lower than wildtype. Immunohistochemistry and glycogen PAS were also assessed (FIG. 24, FIG. 25, FIG. 26, FIG. 27). Immunohistochemistry indicated that there may be a direct transduction of some cells. However, little to no glycogen clearance was obtained with the natural construct. Glycogen levels were close to wild type levels for the engineered construct even though activity was only 20% of wild type. PAS staining in the spinal cord shows little to no glycogen clearance with the natural construct. Glycogen levels close to wild type for engineered construct was observed in the ventral horn including motor neurons. Immunohistochemistry demonstrated direct transduction in spinal cord neurons. Engineered hGAA produced by the choroid plexus and neuronal cells was able to reduce glycogen by cross correction in the spinal cord while little glycogen reduction was observed for natural hGAA.

[00175] *Conclusions*

[00176] Overall the data in this example demonstrated that the engineered gene therapy constructs have dramatically better uptake into tissues and glycogen reduction than the wildtype GAA used in conventional treatments, including effects in the brain and spinal cord.

EXAMPLE 8: Animal Study Protocols

[00177] AAVhu68 vectors were produced and titrated by the Penn Vector Core as described. (Lock, Alvira et al. 2010, "Rapid, simple, and versatile manufacturing of recombinant adeno-associated viral vectors at scale." Hum Gene Ther 21(10): 1259-1271).

[00178] Mus musculus, Pompe mice Gaa knock-out, in a C57BL/6/129 background founders were purchased at Jackson Labs (stock#004154, also known as 6neo mice).

[00179] Mice received 5×10^{11} GCs (approximately 2.5×10^{13} GC/kg) of AAVhu68.CAG.hGAA (comprising either natural hGAA (SEQ ID NO: 45) or engineered hGAA (SEQ ID NO: 38) in 0.1 mL via the lateral tail vein, were bled on Day 7 and Day 21 post vector dosing for serum isolation, and were terminally bled (for plasma isolation) and euthanized by exsanguination 28 days post injection. Tissues were promptly collected, starting with brain.

GAA Activity

[00180] Plasma was mixed with 5.6 mM 4-MU- α -glucopyranoside pH 4.0 and incubated for three hours at 37 °C. The reaction was stopped with 0.4 M sodium carbonate, pH 11.5. Relative fluorescence units, RFUs were measured using a Victor3 fluorimeter, ex 355 nm and emission at 460 nm. Activity in units of nmol/mL/hr was calculated by interpolation from a standard curve of 4-MU. Activity in individual tissue samples were further normalized based on total protein content in the homogenate.

GAA Signature Peptide by LC/MS

[00181] Plasma was precipitated in 100% methanol and centrifuged. Supernatants were discarded. The pellet was spiked with a stable isotope-labeled peptide unique to hGAA as an internal standard and resuspended with trypsin and incubated at 37 °C for one hour. The digestion was stopped with 10% formic acid. Tryptic peptides were separated by C-18 reverse phase chromatography and Identified and

quantified by ESI-mass spectroscopy. The total GAA concentration in plasma was calculated from the signature peptide concentration.

Cell surface Receptor Binding assay

[00182] A 96-well plate was coated with receptor, washed, and blocked with BSA. 28 day plasma from AAV treated mice was serially diluted to give a series of decreasing concentrations and incubated with coupled receptor. After incubation the plate was washed to remove any unbound hGAA and 4-MU- α -glucopyranoside added for one hour at 37 °C. The reaction was stopped with 1.0 M glycine, pH 10.5 and RFUs were read by a Spectramax fluorimeter; ex 370, emission 460. RFU's for each sample were converted to activity (nmol/mL/hr) by interpolation from a standard curve of 4-MU. Nonlinear regression was done using GraphPad Prism.

Histology

[00183] Tissues were formalin fixed and paraffin embedded. Muscle slides were stained with PAS; CNS slides with luxol fast blue/Periodic Acid-Schiff (PAS). A board certified veterinary pathologist (JH) blindly reviewed histological slides. A semi-quantitative estimation of the total percentage of cells with glycogen storage and cytoplasmic vacuolization was done on scanned slides. A score from 0 to 4 was attributed as described in table below.

Table 9: Histology Scoring	
	Storage/Vacuolization
0	0
1	1 to 9%
2	10 to 49%
3	50 to 74%
4	75 to 100%

Immuno-histochemistry (IHC)

We studied transgene expression and cellular localization from slides immunostained using an anti-human GAA antibody (Sigma HPA029126).

[00184] EXAMPLE 9: Histology-Tissue Processing- Protocols and Results in an Animal Model of Pompe Disease

[00185] All tissues were fixed in 10% NBF (neutral buffered formalin). The assays (PAS and IHC) are routinely used in the field.

[00186] PAS staining of quadriceps and triceps (FIG. 29 and FIG. 32) - Tissues were fixed in 10% NBF and embedded in paraffin. Sections were post-fixed in 1% periodic acid and stained with Schiff's reagent. Afterwards, sections were counterstained with hematoxylin. Glycogen appears as magenta aggregates (lysosomal bound) or diffused pink (cytosolic); nuclei are blue. Based on the images and assuming each is

representative of a group, the ranking order in terms of glycogen clearance is: Engineered hGAA > Natural hGAA. The Engineered hGAA construct produced more staining across the entire image compared to the rest, showing an improved endocytosis of GAA protein mediated through the binding of vIGF2 to CI-MPR.

[00187] PAS staining of spinal cord (FIG. 26) - Tissues were fixed in 10% NBF. Post-fixation in 1% periodic acid could have been done prior to or after paraffin embedding. Sections were stained with Schiff's reagent and counterstained likely with methylene blue. Glycogen appears as magenta aggregates (lysosomal bound); nerve fibers appear blue. The images focused on the ventral horn of the spinal cord and glycogen accumulation in the motor neurons. Engineered hGAA appeared most effective in glycogen reduction among the constructs.

[00188] GAA IHC (FIG. 22, FIG. 25, FIG. 27, FIG. 30, and FIG 35) - Tissues were fixed in 10% NBF and embedded in paraffin. Sections were incubated with an anti-GAA primary antibody, followed by a secondary antibody that recognizes the primary antibody and carries an enzyme tag --- HRP. Subsequently, an enzymatic reaction was carried out and a brown-colored precipitating product was formed. Sections were then counterstained with hematoxylin. The constructs showed GAA uptake into muscle fibers (FIG. 31). Engineered hGAA > Natural hGAA . The BiP-vIGF2 construct had more diffused staining across the entire image compared to the rest.

[00189] Compared to other vectors, engineered hGAA produced more GAA IHC signals with a punctum-like appearance inside the muscle fibers, showing a much more efficient lysosomal targeting (FIG. 22).

[00190] In all, engineered hGAA consistently demonstrated superiority in tissue uptake, lysosomal targeting, and glycogen reduction in various tissues among the constructs.

[00191] 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 described herein may be employed. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A gene therapy vector comprising a nucleic acid construct comprising:
 - (a) a nucleic acid sequence encoding a therapeutic protein, and
 - (b) a nucleic acid sequence encoding a peptide that binds to the cation-independent mannose 6-phosphate (M6P) receptor (CI-MPR) with high affinity.
2. The gene therapy vector of claim 1, wherein the peptide is a variant IGF2 (vIGF2) peptide.
3. The gene therapy vector of claim 2, wherein the vIGF2 peptide comprises an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 and having at least one substitution at one or more positions selected from the group consisting of positions 6, 26, 27, 43, 48, 49, 50, 54, 55, and 65 of SEQ ID NO: 1
4. The gene therapy vector of claim 3, wherein the at least one substitution is selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R, and K65R of SEQ ID NO: 1.
5. The gene therapy vector of claim 3 to 4, wherein the vIGF2 peptide comprises at least two substitutions at two or more positions selected from the group consisting of positions 26, 27, 43, 48, 49, 50, 54, and 55 of SEQ ID NO: 1.
6. The gene therapy vector of claim 5, wherein the at least two substitutions are selected from the group consisting of E6R, F26S, Y27L, V43L, F48T, R49S, S50I, A54R, L55R, and K65R of SEQ ID NO: 1.
7. The gene therapy vector of any one of claims 1 to 6, wherein the vIGF2 peptide comprises an N-terminal deletion at position 1 of SEQ ID NO: 1.
8. The gene therapy vector of claim 7, wherein the vIGF2 peptide comprises an N-terminal deletion at positions 1-4 of SEQ ID NO: 1.
9. The gene therapy vector of any one of claims 1 to 8, wherein the vIGF2 peptide has decreased or no affinity for the insulin receptor and IGFR1 as compared to native IGF2 peptide.
10. The gene therapy vector of claim 9, and wherein the vIGF2 peptide is capable of facilitating uptake of the therapeutic protein into a lysosome in a cell.
11. The gene therapy vector of any one of claims 1 to 10, wherein the therapeutic protein is capable of replacing a defective or deficient protein associated with a genetic disorder in a subject having the genetic disorder.
12. The gene therapy vector of claim 11, wherein genetic disorder is a lysosomal storage disorder.
13. The gene therapy vector of claim 11 or claim 12, wherein the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis

- type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), CDKL5 deficiency disorder, and neuronal ceroid lipofuscinosis.
14. The gene therapy vector of claim 11 or claim 12, wherein the genetic disorder is Pompe disease.
 15. The gene therapy vector of claim 11 or claim 12, wherein the genetic disorder is neuronal ceroid lipofuscinosis.
 16. The gene therapy vector of any one of claims 1 to 15, wherein the therapeutic protein comprises an enzyme selected from the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof.
 17. The gene therapy vector of claim 16, wherein the therapeutic protein is alpha-glucosidase, or an enzymatically active fragment thereof.
 18. The gene therapy vector of claim 16, wherein the therapeutic protein is palmitoyl protein thioesterases.
 19. The gene therapy vector of claim 1, wherein the nucleic acid construct further comprises a translation initiation sequence.
 20. The gene therapy vector of claim 19, wherein the translation initiation sequence comprises a Kozak sequence.
 21. The gene therapy vector of claim 20, wherein the Kozak sequence comprises the sequence AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide.
 22. The gene therapy vector of claim 21, wherein X₁ comprises A.
 23. The gene therapy vector of claim 21 or claim 22, wherein X₂, comprises G.

24. The gene therapy vector of claim 21 or claim 22, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to AAGATGA (SEQ ID NO: 29).
25. The gene therapy vector of any one of claims 20 to 24, wherein the Kozak sequence comprises AAGATGA (SEQ ID NO: 29).
26. The gene therapy vector of claim 20, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
27. The gene therapy vector of claim 20 or claim 26, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
28. The gene therapy vector of claim 1, wherein the nucleic acid construct further comprises a signal nucleic acid sequence encoding a signal peptide wherein the signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide.
29. The gene therapy vector of claim 28, wherein the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide.
30. The gene therapy vector of claim 29, wherein the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.
31. The gene therapy vector of claim 29, wherein the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.
32. The gene therapy vector of claim 28, wherein the signal peptide comprises a Gaussia signal peptide.
33. The gene therapy vector of claim 32, wherein the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32.
34. The gene therapy vector of claim 32, wherein the Gaussia signal peptide comprises SEQ ID NO: 32.
35. The gene therapy vector of any one of claims 1 to 34, wherein the vIGF2 nucleic acid sequence is 5' to the nucleic acid sequence encoding a therapeutic protein.
36. The gene therapy vector of any one of claims 1 to 34, wherein the vIGF2 nucleic acid sequence is 3' to the nucleic acid sequence encoding a therapeutic protein.
37. The gene therapy vector of any one of claims 1 to 36, wherein the nucleic acid construct further comprises a linker sequence encoding a linker peptide between the vIGF2 nucleotide sequence and the nucleic acid sequence encoding a therapeutic protein.
38. The gene therapy vector of claim 37, wherein the linker peptide comprises SEQ ID NO: 18-21, SEQ ID NO: 33, or SEQ ID NO: 37.

39. The gene therapy vector of any one of claims 1 to 38, wherein the gene therapy vector is a virus vector.
40. The gene therapy vector of claim 39, wherein the virus vector is an adenovirus vector, an adeno-associated virus (AAV) vector, a retrovirus vector, a lentivirus vector, a pox virus vector, a vaccinia virus vector, an adenovirus vector, or a herpes virus vector.
41. A pharmaceutical composition comprising a therapeutically effective amount of the gene therapy vector of any one of claims 1 to 40 and a pharmaceutically acceptable carrier or excipient.
42. The pharmaceutical composition of claim 41, wherein the excipient comprises a non-ionic, low-osmolar compound, a buffer, a polymer, a salt, or a combination thereof.
43. A method for treating a genetic disorder comprising administering to a subject in need thereof the gene therapy vector of any one of claims 1 to 40 or the pharmaceutical composition of claim 41 or claim 42.
44. The method of claim 43, wherein genetic disorder is a lysosomal storage disorder.
45. The method of claim 43 or claim 44, wherein the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), CDKL5 deficiency disorder, and neuronal ceroid lipofuscinosis.
46. The method of claim 43 or claim 44, wherein the genetic disorder is Pompe disease.
47. The method of claim 43 or claim 44, wherein the genetic disorder is neuronal ceroid lipofuscinosis.
48. The method of any one of claims 43 to 47, wherein the administering is performed intrathecally, intraocularly, intravitreally, retinally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, intracerebroventricularly, intraparenchymally, subcutaneously, or a combination thereof.
49. The method of any one of claims 43 to 47, wherein the administering is performed intrathecally.
50. A pharmaceutical composition comprising a gene therapy vector of any one of claims 1 to 40 and a pharmaceutically acceptable carrier or excipient for use in treating a genetic disorder.

51. A pharmaceutical composition comprising a gene therapy vector of any one of claims 1 to 40 and a pharmaceutically acceptable carrier or excipient for use in preparation of a medicament for treatment of a genetic disorder.
52. The pharmaceutical composition of claim 50 or claim 51, wherein the genetic disorder is a lysosomal storage disorder.
53. The pharmaceutical composition of any one of claims 50 to 52, wherein the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolidosis type I, mucopolidosis type II, mucopolidosis type III, mucopolidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), chronic granulomatous disease (CGD), and neuronal ceroid lipofuscinosis.
54. The pharmaceutical composition of any one of claims 50 to 53, wherein the genetic disorder is Pompe disease.
55. The pharmaceutical composition of any one of claims 50 to 53, wherein the genetic disorder is neuronal ceroid lipofuscinosis.
56. The pharmaceutical composition of any one of claims 50 to 55, wherein the composition is formulated for administration intrathecally, intraocularly, intravitreally, retinally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, or subcutaneously.
57. The pharmaceutical composition of any one of claims 50 to 56, wherein the composition is formulated for administration intrathecally.
58. A gene therapy vector comprising a nucleic acid construct comprising, in 5' to 3' order:
 - (a) a translation initiation sequence, and
 - (b) a nucleic acid sequence encoding a therapeutic protein.
58. The gene therapy vector of claim 58, wherein the translation initiation sequence comprises a Kozak sequence.
59. The gene therapy vector of claim 58, wherein the Kozak sequence comprises the sequence AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide.
60. The gene therapy vector of claim 59, wherein X₁ comprises A.
61. The gene therapy vector of claim 59 or claim 60, wherein X₂, comprises G.
62. The gene therapy vector of any one of claims 58 to 61, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to AAGATGA (SEQ ID NO: 29).

63. The gene therapy vector of any one of claims 58 to 62, wherein the Kozak sequence comprises AAGATGA (SEQ ID NO: 29).
64. The gene therapy vector of claim 58, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
65. The gene therapy vector of claim 58 or claim 64, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
66. The gene therapy vector of any one of claims 58 to 65, wherein the nucleic acid construct further comprises a nucleic acid sequence encoding a signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide.
67. The gene therapy vector of claim 66, wherein the signal peptide selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide.
68. The gene therapy vector of claim 67, wherein the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.
69. The gene therapy vector of claim 67, wherein the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.
70. The gene therapy vector of claim 67, wherein the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32.
71. The gene therapy vector of claim 67, wherein the Gaussia signal peptide comprises an amino acid sequence of SEQ ID NO: 32.
72. The gene therapy vector of any one of claims 58 to 71, wherein the nucleic acid construct further comprises an internal ribosomal entry sequence (IRES).
73. The gene therapy vector of claim 72, wherein the IRES is a cricket paralysis virus (CrPV) IRES.
74. The gene therapy vector of claim 72 or claim 73, wherein the IRES comprises a nucleic acid sequence at least 90% identical to SEQ ID NO: 12.
75. The gene therapy vector of claim 72 or claim 73, wherein the IRES comprises SEQ ID NO: 12.
76. A gene therapy vector comprising a nucleic acid construct comprising, in 5' to 3' order:
 - (a) a nucleic acid sequence encoding a signal peptide, and
 - (b) a nucleic acid sequence encoding a therapeutic protein, wherein the signal peptide is capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide.
77. The gene therapy vector of claim 76, wherein the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide.
78. The gene therapy vector of claim 77, wherein the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.

79. The gene therapy vector of claim 77, wherein the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.
80. The gene therapy vector of any one of claims 76 to 79, wherein the signal peptide comprises a Gaussia signal peptide.
81. The gene therapy vector of claim 80, wherein the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32.
82. The gene therapy vector of claim 80, wherein the Gaussia signal peptide comprises SEQ ID NO: 32.
83. The gene therapy vector of any one of claims 76 to 82, wherein the nucleic acid construct further comprises a translation initiation sequence.
84. The gene therapy vector of claim 83, wherein the translation initiation sequence comprises a Kozak sequence comprising AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂.
85. The gene therapy vector of claim 84, wherein X₁ comprises A.
86. The gene therapy vector of claim 84 or claim 85, wherein X₂ comprises G.
87. The gene therapy vector of any one of claims 84 to 86, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to AAGATGA (SEQ ID NO: 29).
88. The gene therapy vector of any one of claims 84 to 87, wherein the Kozak sequence comprises AAGATGA (SEQ ID NO: 29).
89. The gene therapy vector of claim 83, wherein the translation initiation sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
90. The gene therapy vector of claim 83 or claim 89, wherein the translation initiation sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
91. The gene therapy vector of any one of claims 76 to 90, wherein the nucleic acid construct further comprises an internal ribosomal entry sequence (IRES).
92. The gene therapy vector of claim 91, wherein the IRES comprises an IRES selected from the group consisting of a cricket paralysis virus (CrPV) IRES, a picornavirus IRES, an Aphthovirus IRES, a Kaposi's sarcoma-associated herpesvirus IRES, a Hepatitis A IRES, a Hepatitis C IRES, a Pestivirus IRES, a Cripavirus IRES, a Rhopalosiphum padi virus IRES, and a Merek's disease virus IRES.
93. The gene therapy vector of claim 91 or claim 92, wherein the IRES comprises a nucleic acid sequence at least 90% identical to SEQ ID NO: 12.
94. The gene therapy vector of any one of claims 91 to 93, wherein the IRES comprises SEQ ID NO: 12.
95. A gene therapy vector comprising a nucleic acid construct comprising, in 5' to 3' order:
 - (a) an internal ribosomal entry sequence (IRES), and
 - (b) a nucleic acid sequence encoding a therapeutic protein.

96. The gene therapy vector of claim 95, wherein the IRES comprises an IRES selected from the group consisting of a cricket paralysis virus (CrPV) IRES, a picornavirus IRES, an Aphthovirus IRES, a Kaposi's sarcoma-associated herpesvirus IRES, a Hepatitis A IRES, a Hepatitis C IRES, a Pestivirus IRES, a Cripavirus IRES, a Rhopalosiphum padi virus IRES, and a Merek's disease virus IRES.
97. The gene therapy vector of claim 96, wherein the IRES is a cricket paralysis virus (CrPV) IRES.
98. The gene therapy vector of claim 96 or claim 97, wherein the IRES comprises a nucleic acid sequence at least 90% identical to SEQ ID NO: 12.
99. The gene therapy vector of any one of claims 96 to 98, wherein the IRES comprises SEQ ID NO: 12.
100. The gene therapy vector of any one of claims 95 to 99, wherein the nucleic acid construct further comprises a translation initiation sequence.
101. The gene therapy vector of claim 100, wherein the translation initiation sequence comprises a Kozak sequence comprising AX₁X₂ATGA (SEQ ID NO: 28), wherein each of X₁ and X₂ is any nucleotide.
102. The gene therapy vector of claim 101, wherein X₁ comprises A.
103. The gene therapy vector of claim 101 or claim 102, wherein X₂ comprises G.
104. The gene therapy vector of any one of claims 101 to 103, wherein the Kozak sequence comprises a nucleic acid sequence at least 90% identical to AAGATGA (SEQ ID NO: 29).
105. The gene therapy vector of any one of claims 101 to 104, wherein the Kozak sequence comprises AAGATGA (SEQ ID NO: 29).
106. The gene therapy vector of claim 100, wherein the translation initiation sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
107. The gene therapy vector of claim 100 or claim 106, wherein the translation initiation sequence comprises a nucleic acid sequence at least 90% identical to GCAAGATG (SEQ ID NO: 44).
108. The gene therapy vector of any one of claims 95 to 107, wherein the nucleic acid construct further comprises a signal nucleic acid sequence encoding a signal peptide capable of increasing secretion of the therapeutic protein as compared to the therapeutic protein without the signal peptide.
109. The gene therapy vector of claim 106, wherein the signal peptide is selected from a binding immunoglobulin protein (BiP) signal peptide and a Gaussia signal peptide.
110. The gene therapy vector of claim 109, wherein the BiP signal peptide comprises an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.
111. The gene therapy vector of claim 109, wherein the BiP signal peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13-17.

112. The gene therapy vector of claim 109, wherein the Gaussia signal peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 32.
113. The gene therapy vector of claim 109, wherein the Gaussia signal peptide comprises SEQ ID NO: 32.
114. The gene therapy vector of any one of claims 58 to 113, wherein the nucleic acid construct further comprises a nucleic acid sequence encoding an IGF2 peptide.
115. The gene therapy vector of claim 114, wherein the IGF2 peptide is a vIGF2 peptide comprising an amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-11, SEQ ID NOs: 30-31, SEQ ID NO: 34, and SEQ ID NO: 35.
116. The gene therapy vector of claim 114, wherein the IGF2 peptide is a vIGF2 peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-11, SEQ ID NOs: 30-31, SEQ ID NO: 34, and SEQ ID NO: 35.
117. The gene therapy vector of any one of claims 114 to 116, wherein the vIGF2 nucleotide sequence is 5' to the nucleic acid sequence encoding a therapeutic protein.
118. The gene therapy vector of any one of claims 114 to 116, wherein the vIGF2 nucleotide sequence is 3' to the nucleic acid sequence encoding a therapeutic protein.
119. The gene therapy vector of any one of claims 114 to 118, wherein the nucleic acid construct further comprises a linker sequence encoding a linker peptide between the vIGF2 nucleotide sequence and the nucleic acid sequence encoding a therapeutic protein.
120. The gene therapy vector of claim 119, wherein the linker peptide comprises an amino acid sequence at least 90% identical to SEQ ID NO: 18-21, SEQ ID NO: 33, or SEQ ID NO: 37.
121. The gene therapy vector of claim 119, wherein the linker peptide comprises SEQ ID NO: 18-21, SEQ ID NO: 33, or SEQ ID NO: 37.
122. The gene therapy vector of any one of claims 58 to 121, wherein the therapeutic protein is selected from the group consisting of alpha-galactosidase (A or B), β -galactosidase, β -hexosaminidase (A or B), galactosylceramidase, arylsulfatase (A or B), β -glucocerebrosidase, glucocerebrosidase, lysosomal acid lipase, lysosomal enzyme acid sphingomyelinase, formylglycine-generating enzyme, iduronidase (e.g., alpha-L), acetyl-CoA:alpha-glucosaminide N-acetyltransferase, glycosaminoglycan alpha-L-iduronohydrolase, heparan N-sulfatase, N-acetyl- α -D-glucosaminidase (NAGLU), iduronate-2-sulfatase, galactosamine-6-sulfate sulfatase, N-acetylgalactosamine-6-sulfatase, glycosaminoglycan N-acetylgalactosamine 4-sulfatase, β -glucuronidase, hyaluronidase, alpha-N -acetyl neuraminidase (sialidase), ganglioside sialidase, phosphotransferase, alpha-glucosidase, alpha-D-mannosidase, beta-D-mannosidase, aspartylglucosaminidase, alpha-L-fucosidase, battenin, palmitoyl protein thioesterases, and other Batten-related proteins (e.g., ceroid-lipofuscinosis neuronal protein 6), or an enzymatically active fragment thereof.

123. The gene therapy vector of claim 122, wherein the therapeutic protein is an alpha-glucosidase, or an enzymatically active fragment thereof.
124. The gene therapy vector of claim 122, wherein the enzyme is a palmitoyl protein thioesterase.
125. The gene therapy vector of claim 122, wherein the therapeutic protein is capable of replacing a defective or deficient protein associated with a genetic disorder in a subject having the genetic disorder.
126. The gene therapy vector of claim 125, wherein the genetic disorder is a lysosomal storage disorder.
127. The gene therapy vector of claim 125, wherein the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and neuronal ceroid lipofuscinosis.
128. The gene therapy vector of claim 125, wherein the genetic disorder is Pompe disease.
129. The gene therapy vector of claim 125, wherein the genetic disorder is neuronal ceroid lipofuscinosis.
130. The gene therapy vector of any one of claims 58 to 129, wherein the gene therapy vector is a viral vector.
131. The gene therapy vector of claim 130, wherein the viral vector is an adeno-associated virus vector, a retrovirus vector, a lentivirus vector, a pox virus vector, a vaccinia virus vector, an adenovirus vector, or a herpes virus vector.
132. A pharmaceutical composition comprising (i) a therapeutically effective amount of the gene therapy vector of any one of claims 58 to 131 and (ii) a pharmaceutically acceptable carrier or excipient
133. The pharmaceutical composition of claim 132, wherein the carrier or excipient comprises a non-ionic, low-osmolar compound, a buffer, a polymer, a salt, or a combination thereof.
134. A method for treating a genetic disorder comprising administering to a subject in need thereof the gene therapy vector of any one of claims 58 to 131 or the pharmaceutical composition of claim 132 or claim 133.
135. The method of claim 134, wherein the genetic disorder is a lysosomal storage disorder.

136. The method of claim 134 or claim 135, wherein the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and neuronal ceroid lipofuscinosis.
137. The method of claim 134 or claim 135, wherein the genetic disorder is Pompe disease.
138. The method of claim 134 or claim 135, wherein the genetic disorder is neuronal ceroid lipofuscinosis.
139. The method of any one of claims claim 134 to 138, wherein the administering is performed intrathecally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, intracerebroventricularly, intraparenchymally, ocularly, subcutaneously, or a combination thereof.
140. The method of any one of claims claim 134 to 139, wherein the administering is performed intrathecally.
141. A pharmaceutical composition comprising a gene therapy vector of any one of claims 58 to 131 and a pharmaceutically acceptable carrier or excipient for use in treating a genetic disorder.
142. A pharmaceutical composition comprising a gene therapy vector of any one of claims 58 to 131 and a pharmaceutically acceptable carrier or excipient for use in preparation of a medicament for treatment of a genetic disorder.
143. The pharmaceutical composition of claim 141 or claim 142, wherein the genetic disorder is a lysosomal storage disorder.
144. The pharmaceutical composition of any one of claims 141 to 143, wherein the genetic disorder is selected from the group consisting of aspartylglucosaminuria, Batten disease, cystinosis, Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Pompe disease, Tay Sachs disease, Sandhoff disease, metachromatic leukodystrophy, mucopolipidosis type I, mucopolipidosis type II, mucopolipidosis type III, mucopolipidosis type IV, Hurler disease, Hunter disease, Sanfilippo disease type A, Sanfilippo disease type B, Sanfilippo disease type C, Sanfilippo disease type D, Morquio disease type A, Morquio disease type B, Maroteau-Lamy disease, Sly disease, Niemann-Pick disease type A, Niemann-Pick disease type B, Niemann-Pick disease type C1, Niemann-Pick disease type C2, Schindler disease type I, Schindler disease type II, adenosine deaminase severe combined immunodeficiency (ADA-SCID), and neuronal ceroid lipofuscinosis.

145. The pharmaceutical composition of any one of claims 141 to 144, wherein the genetic disorder is Pompe disease.
146. The pharmaceutical composition of any one of claims 141 to 144, wherein the genetic disorder is neuronal ceroid lipofuscinosis.
147. The pharmaceutical composition of any one of claims 141 to 146, wherein the composition is formulated for administration intrathecally, intravenously, intramuscularly, intraventricularly, intracerebrally, intracerebellarly, ocularly, or subcutaneously.
148. The pharmaceutical composition of any one of claims 141 to 147, wherein the composition is formulated for administration intrathecally.
149. A gene therapy vector comprising a nucleic acid construct comprising:
 - (a) a nucleic acid sequence encoding a therapeutic protein, and
 - (b) a nucleic acid sequence encoding a peptide that increases endocytosis of the therapeutic protein.
150. The gene therapy vector of claim 149, wherein the peptide that increases endocytosis of the therapeutic protein is a peptide that binds to the CI-MPR.

M6P Receptor Chromatography

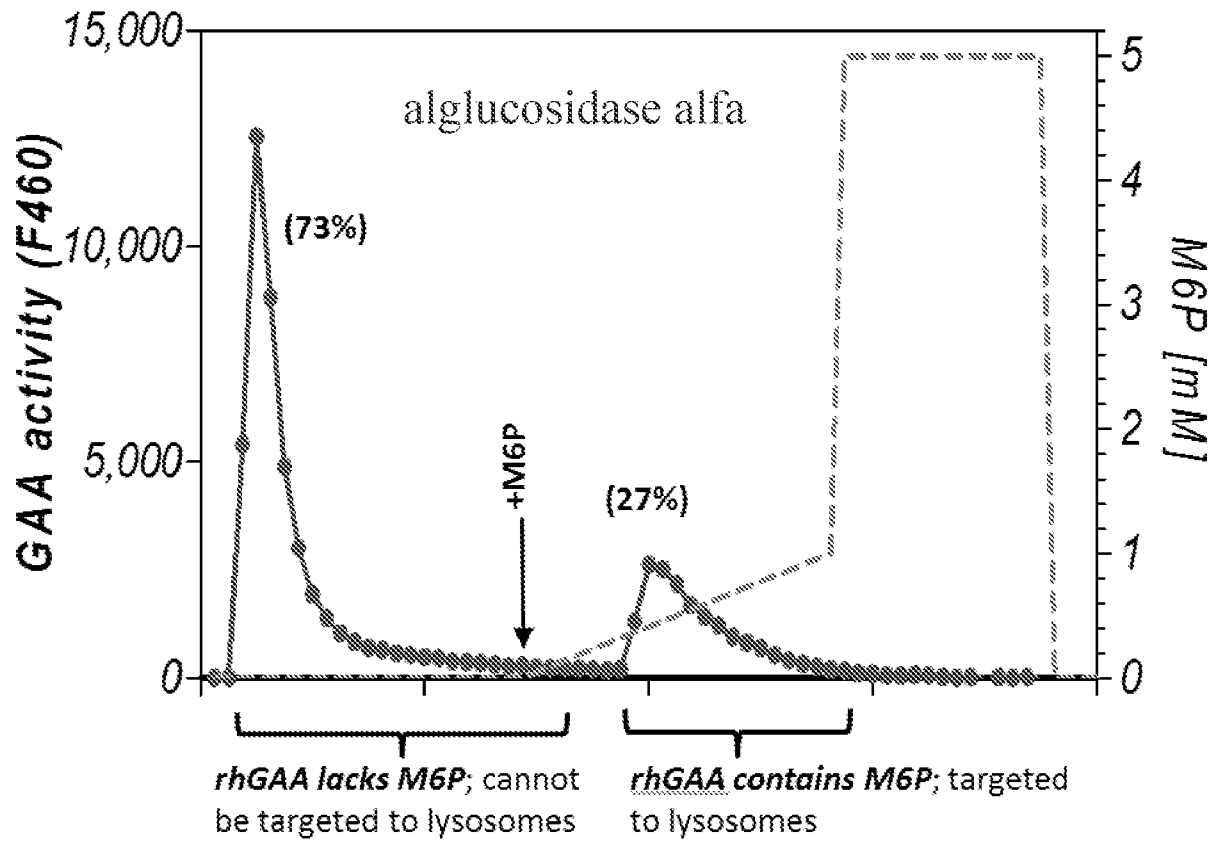


FIG. 1

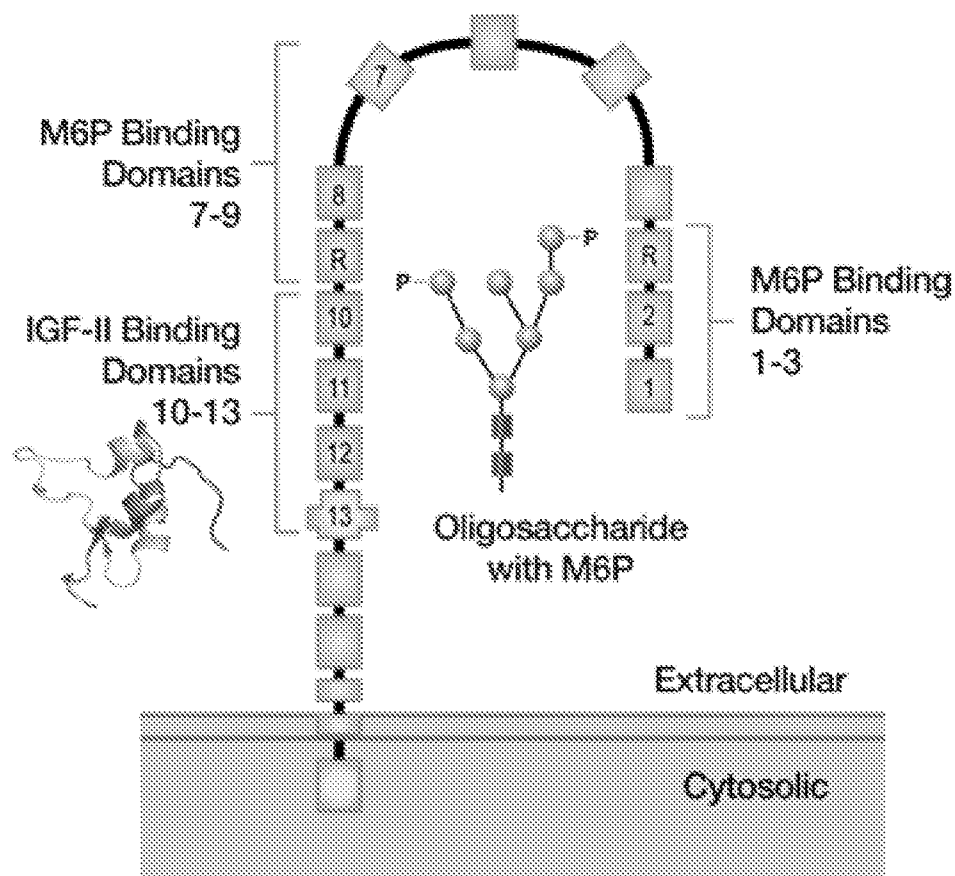


FIG. 2

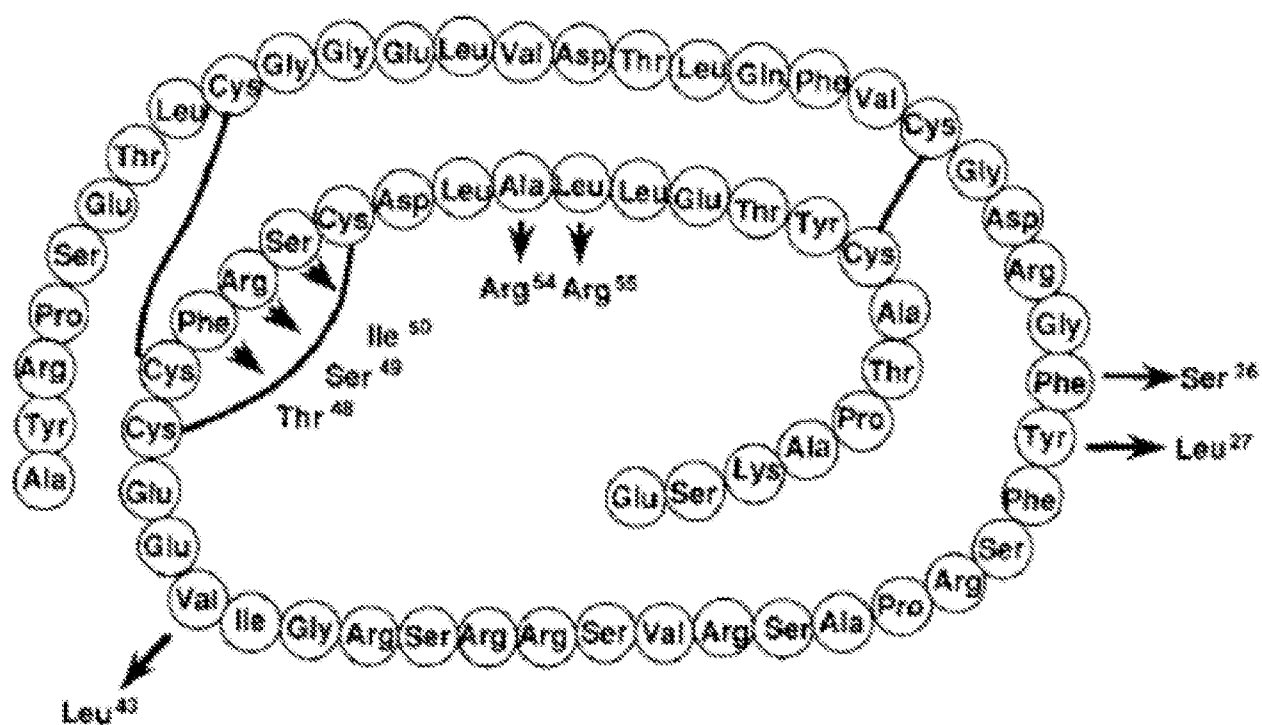


FIG. 3

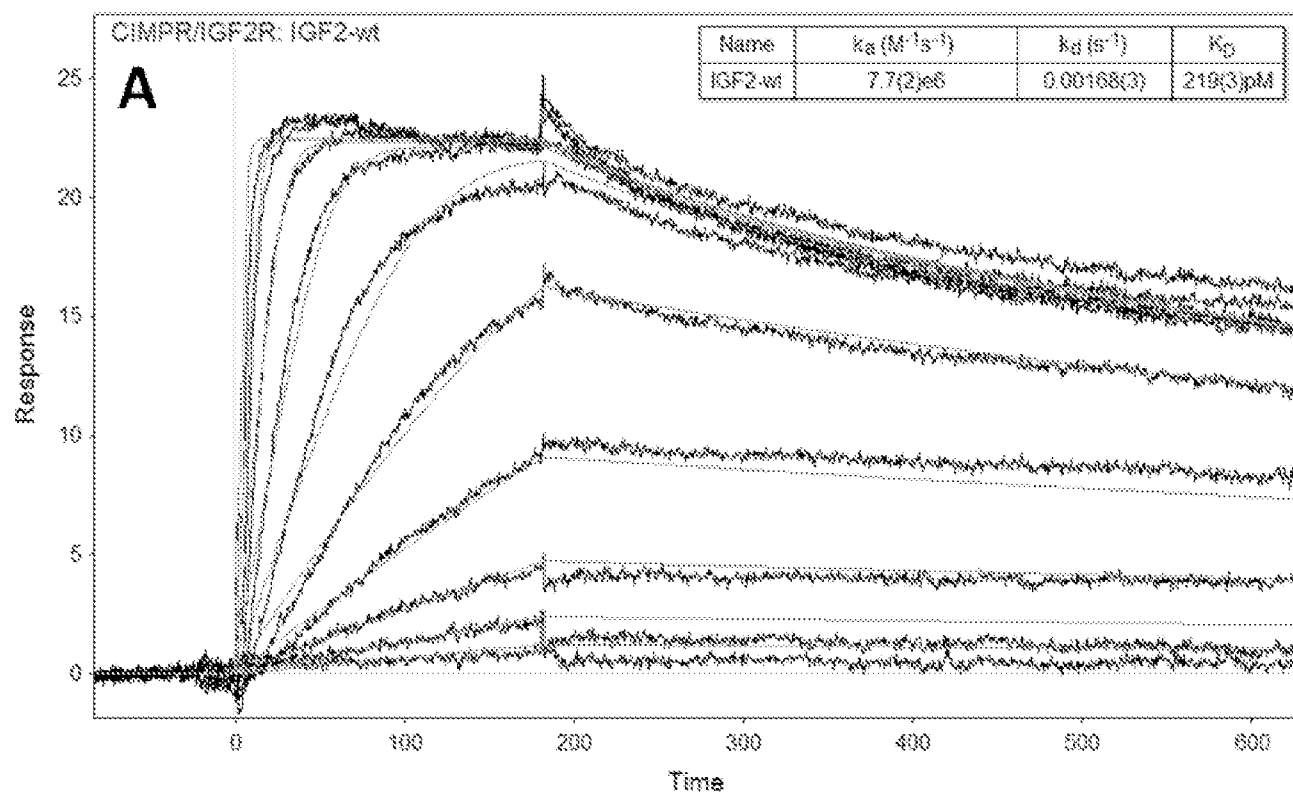


FIG. 4

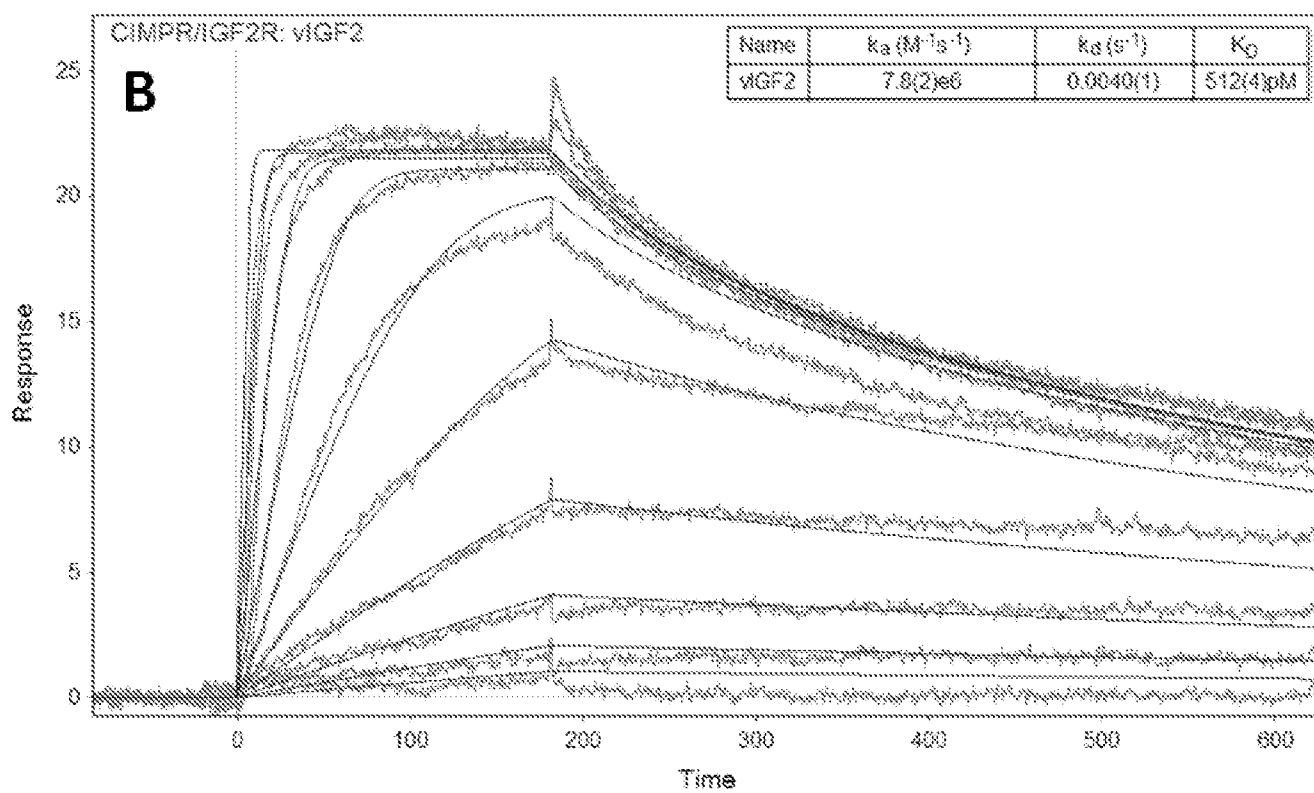


FIG. 5

IGF2/CI-MPR Receptor Binding

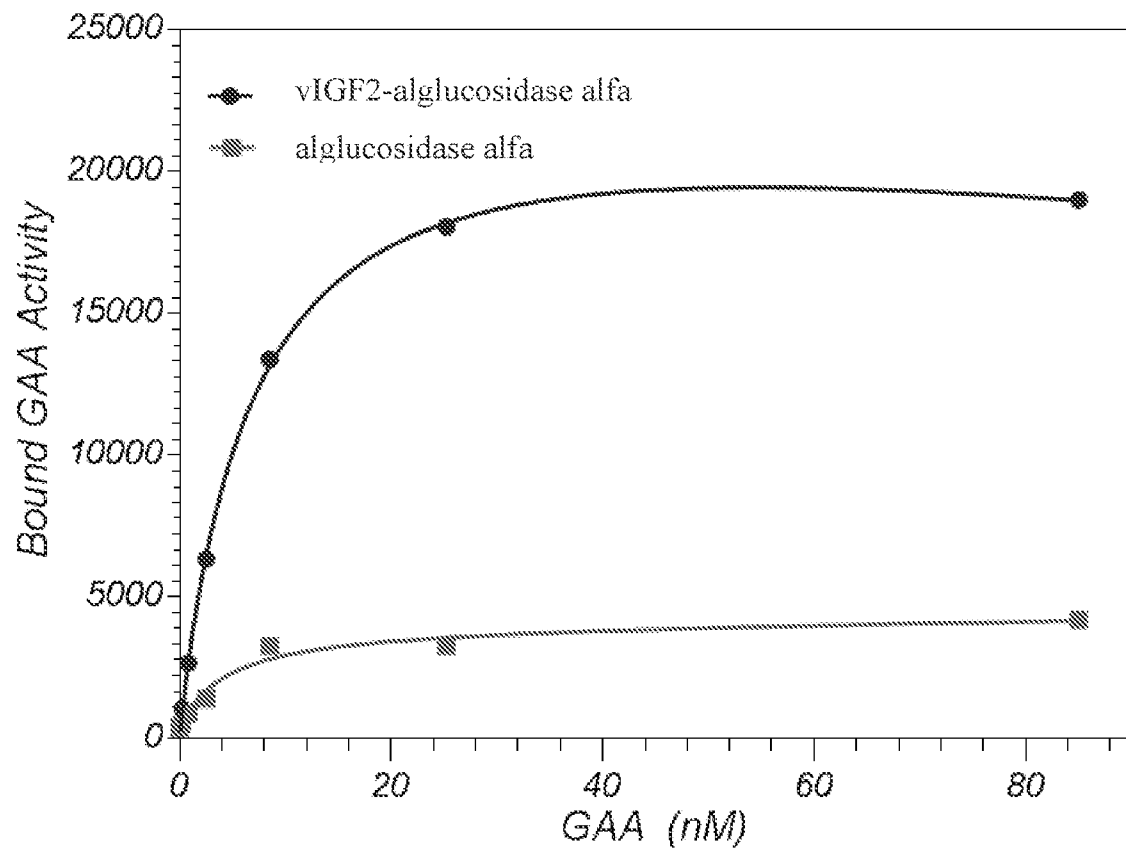


FIG. 6

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IGF2/CI-MPR Receptor Binding

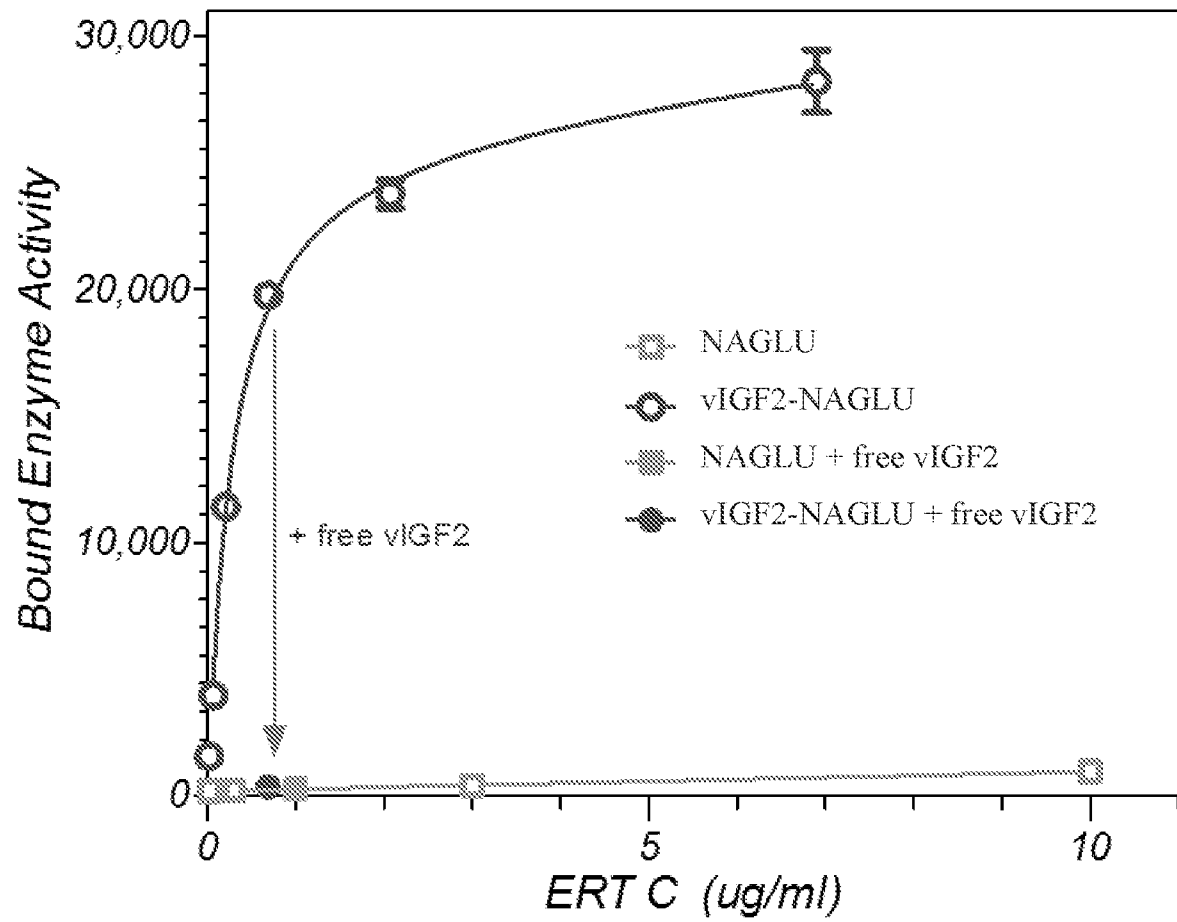


FIG. 7

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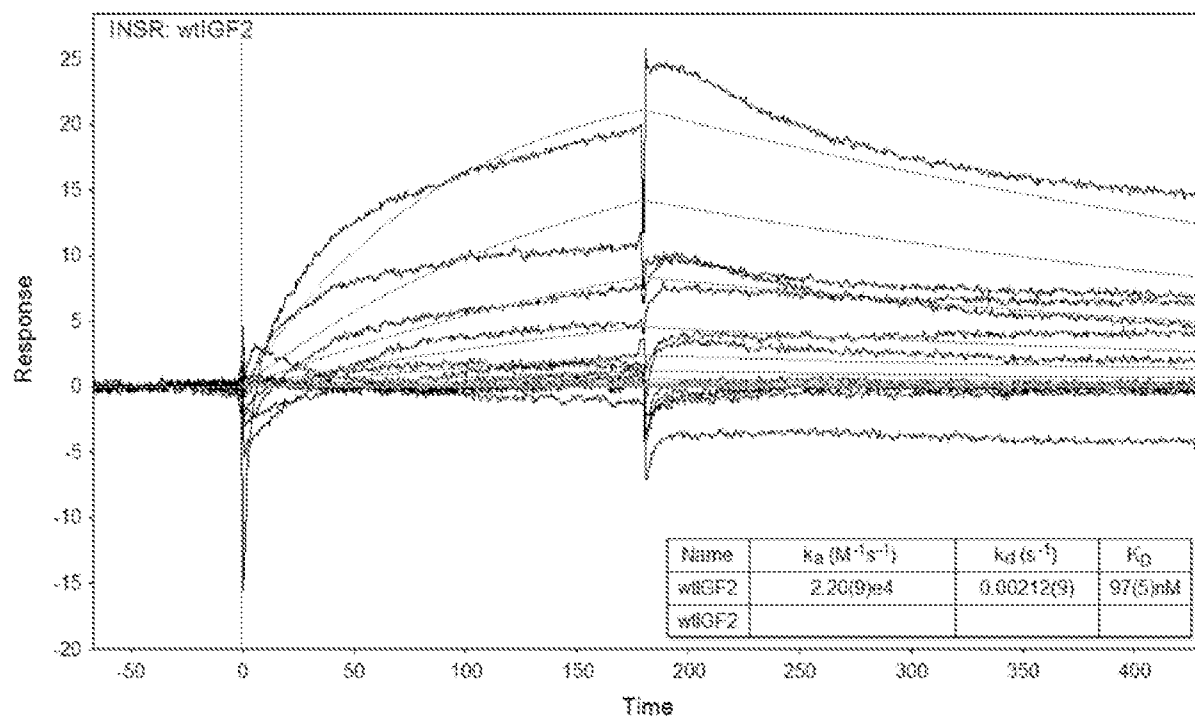


FIG. 8

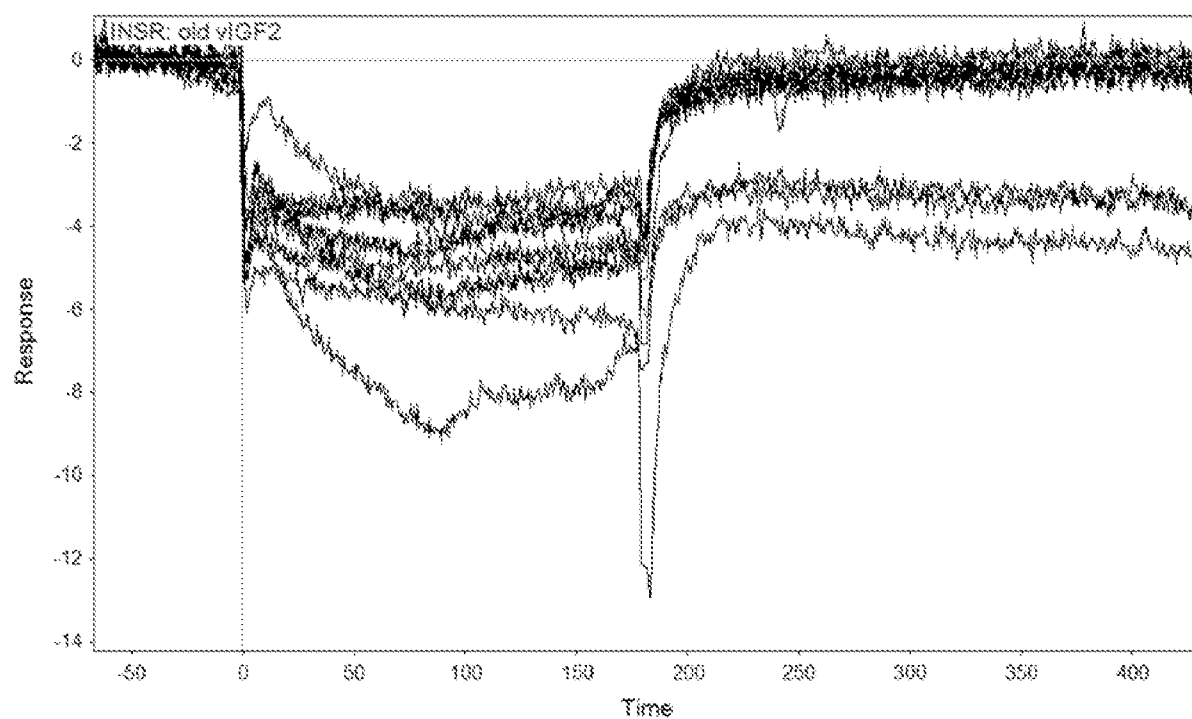


FIG. 9

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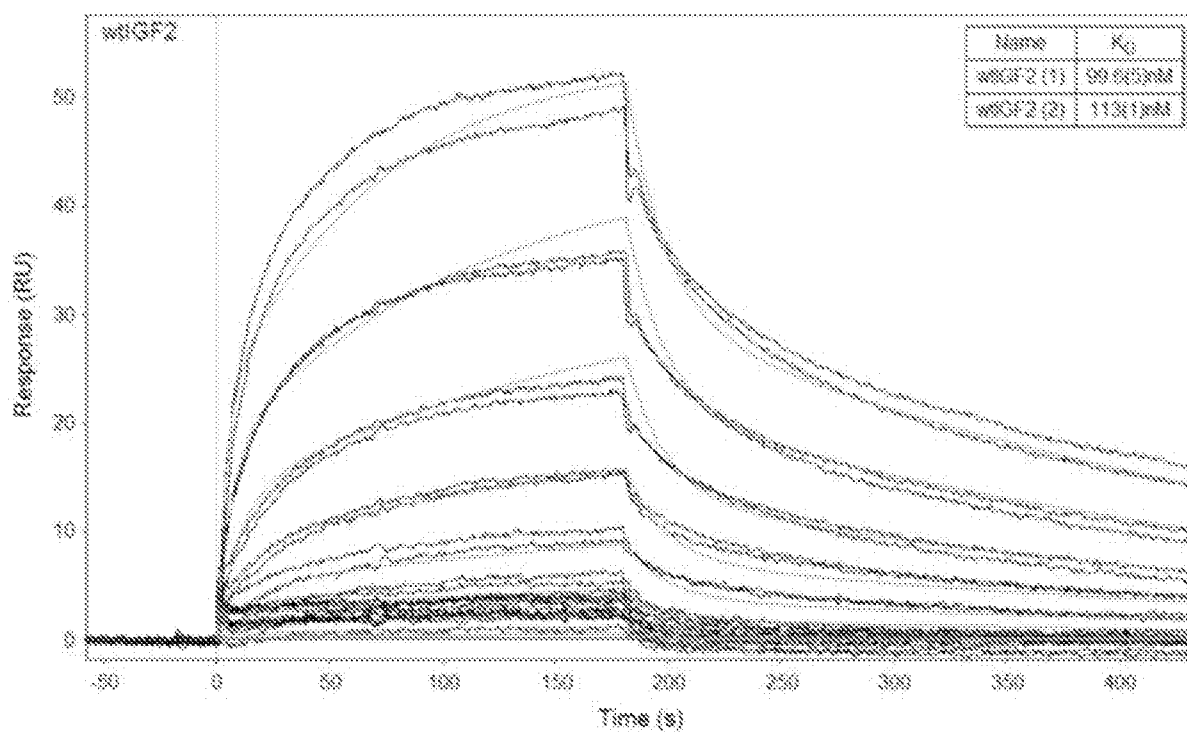


FIG. 10

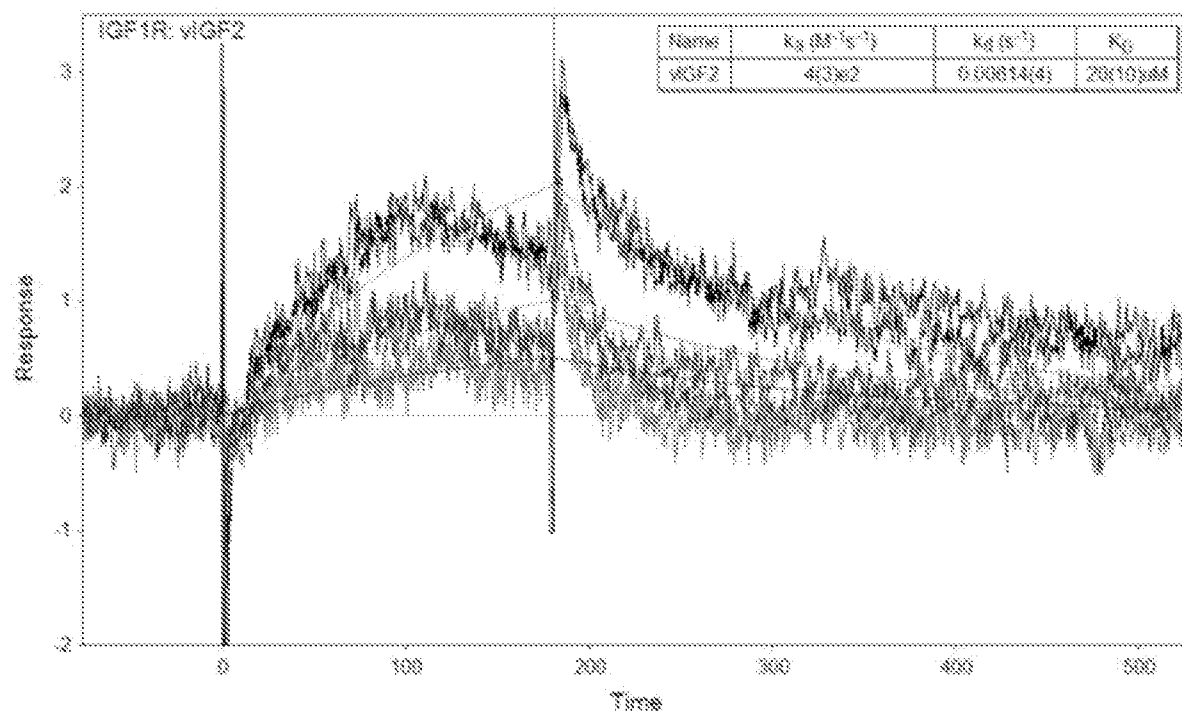


FIG. 11

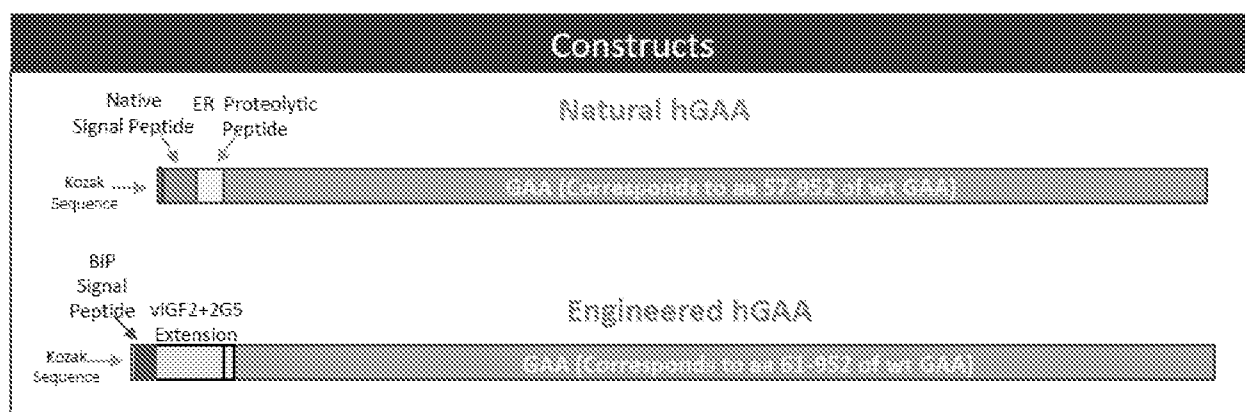


FIG. 12

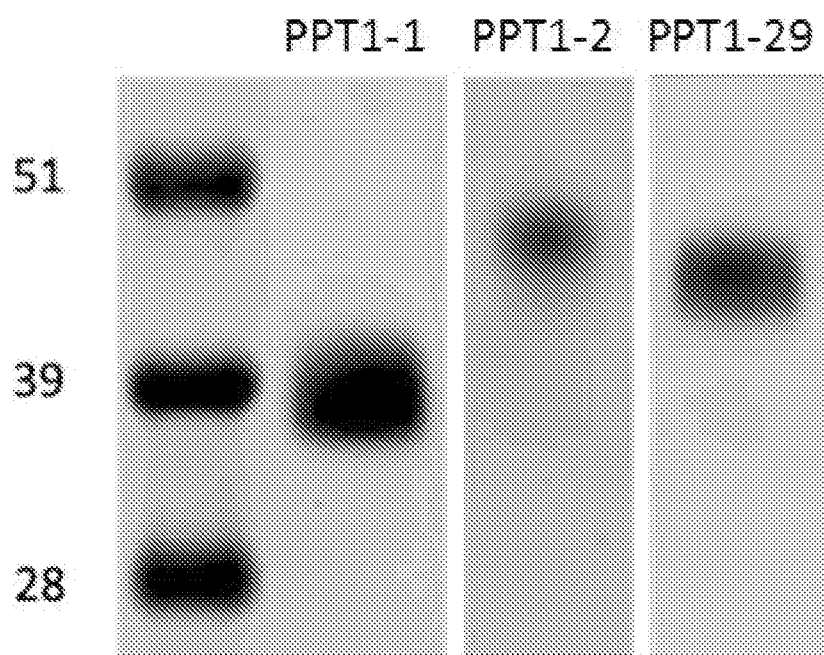


FIG. 13

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Binding of PPT1 constructs to CI-MPR

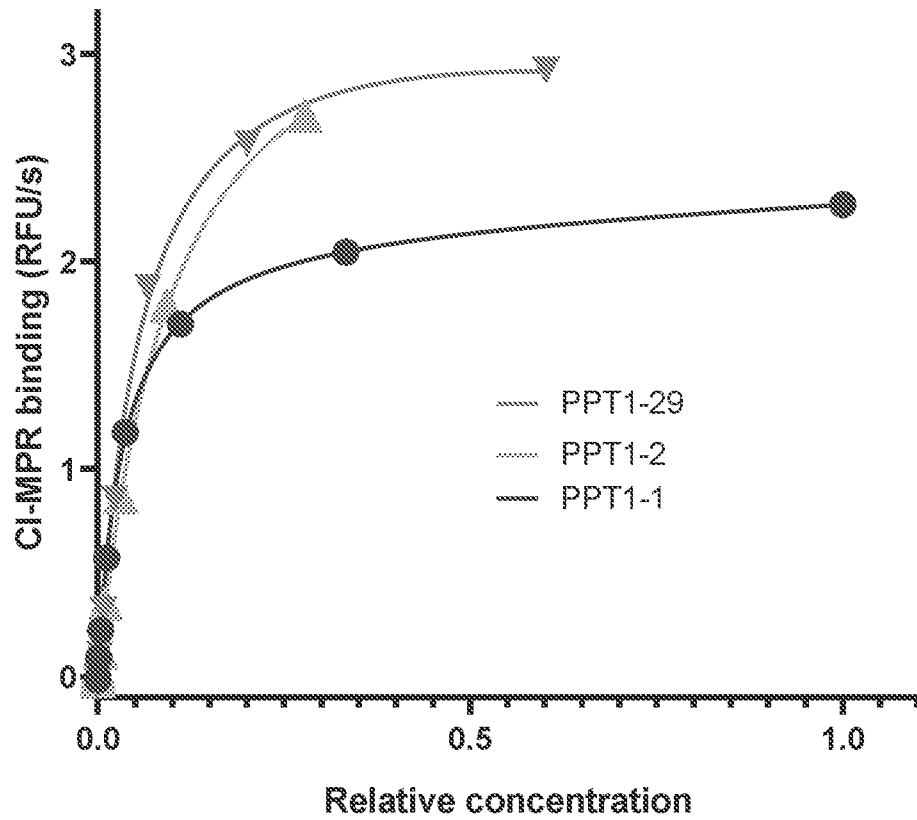


FIG. 14

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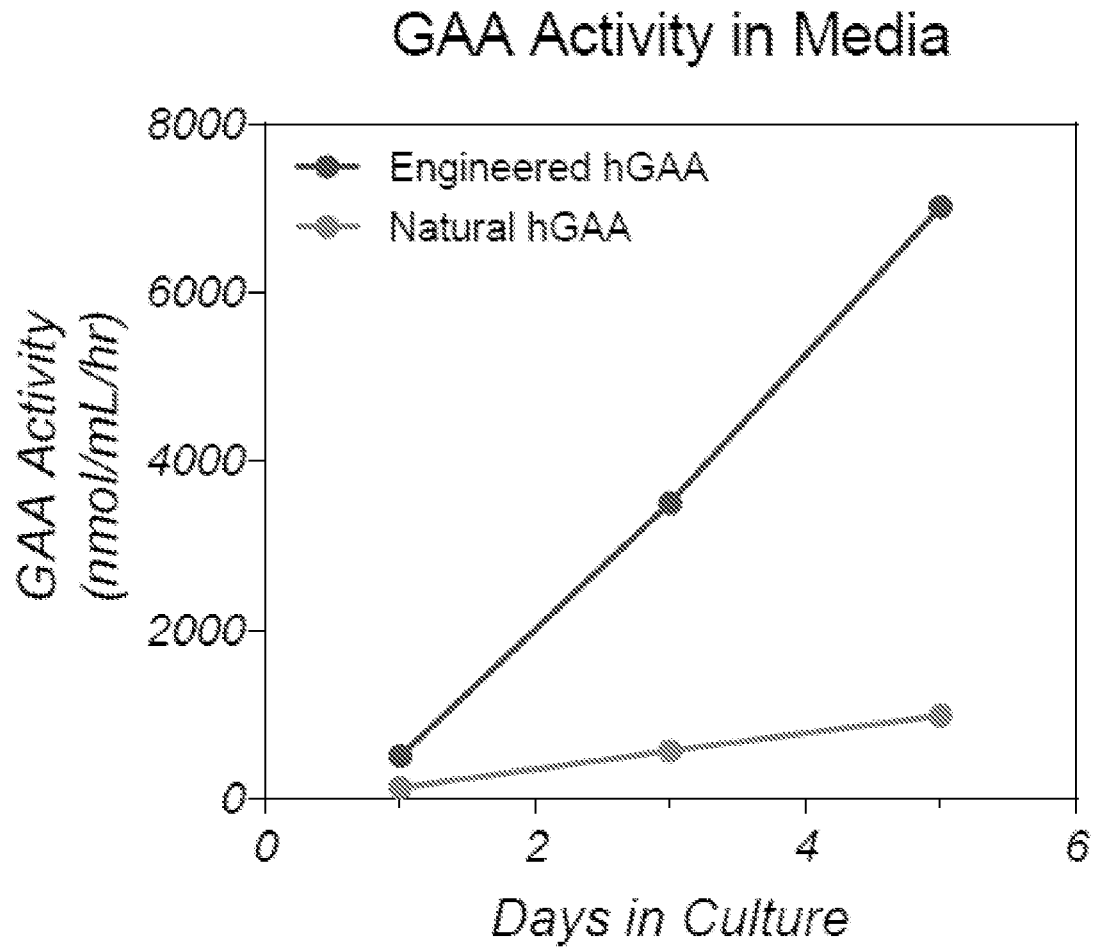


FIG. 15

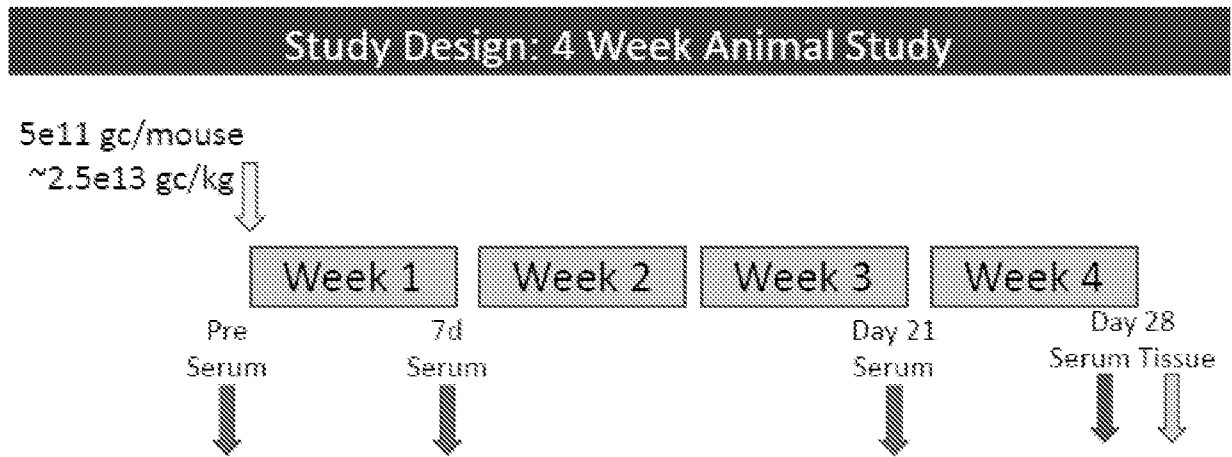


FIG. 16

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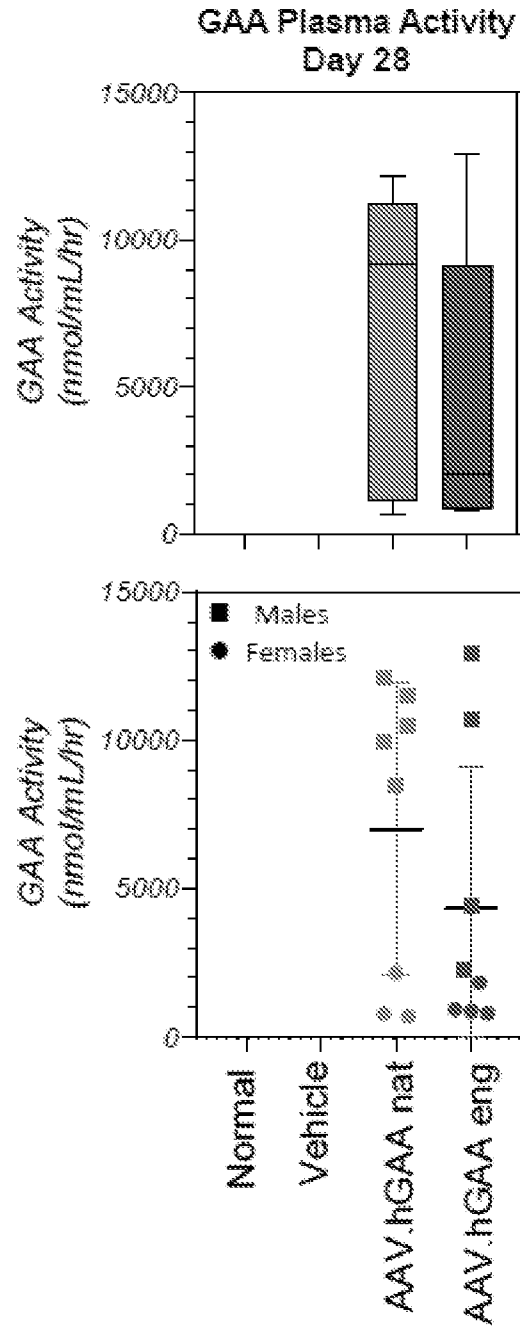


FIG. 17

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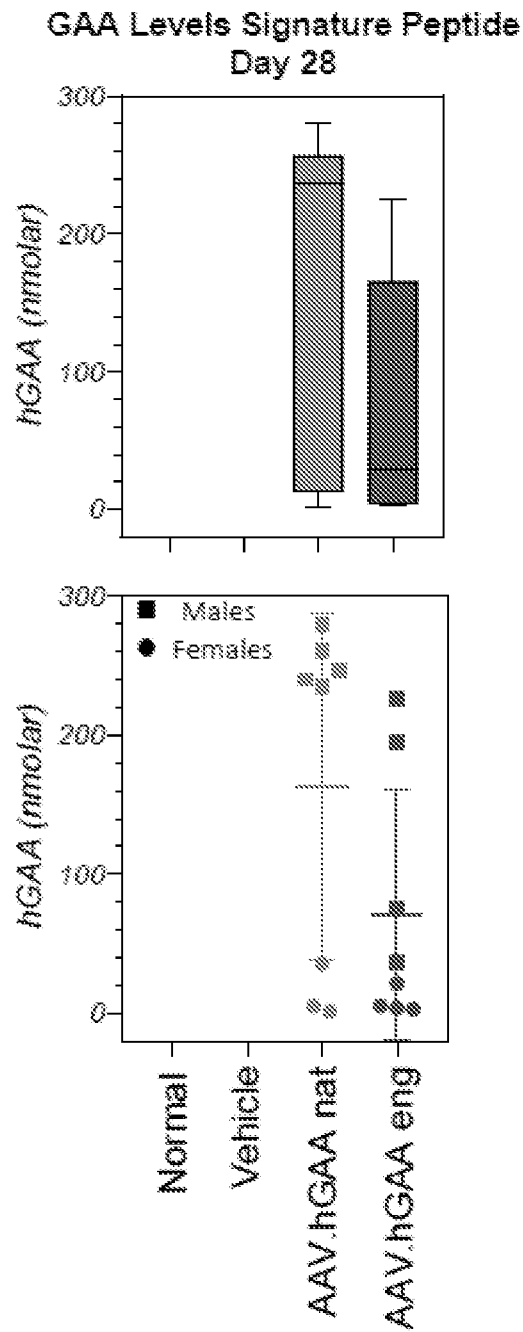
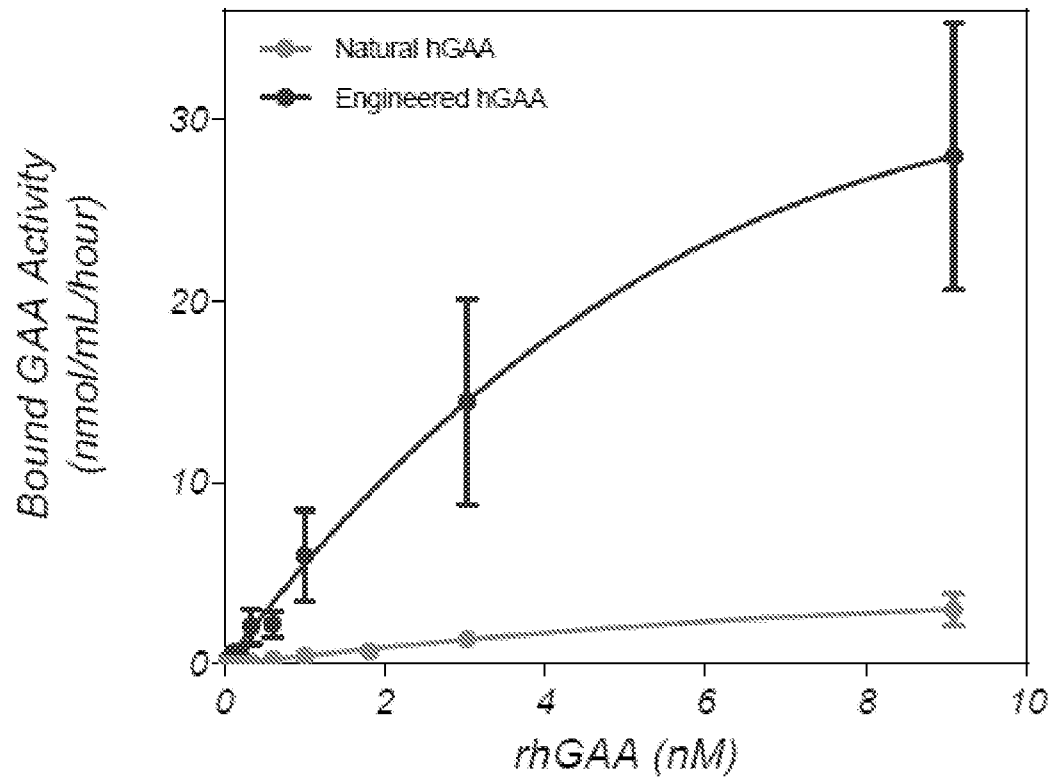


FIG. 18

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Cell-Surface Receptor Binding



*Plasma samples used to evaluate cell
receptor interaction*

FIG. 19

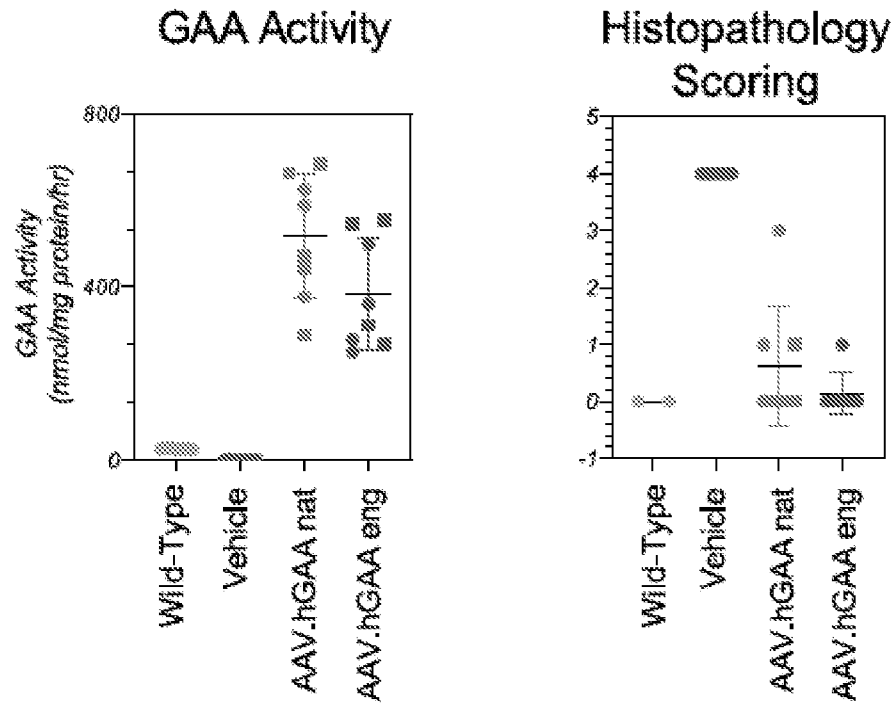


FIG. 20

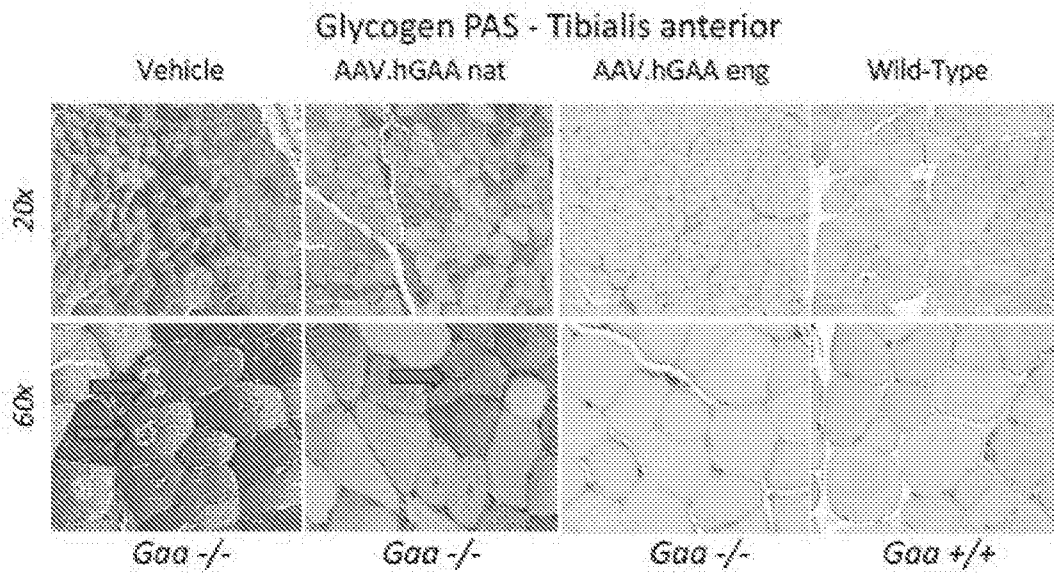


FIG. 21

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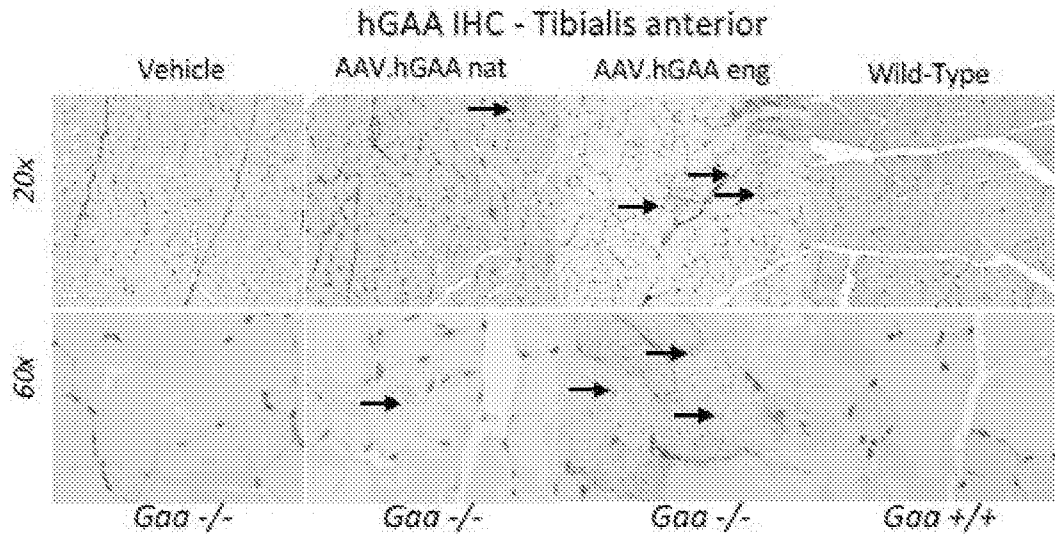


FIG. 22

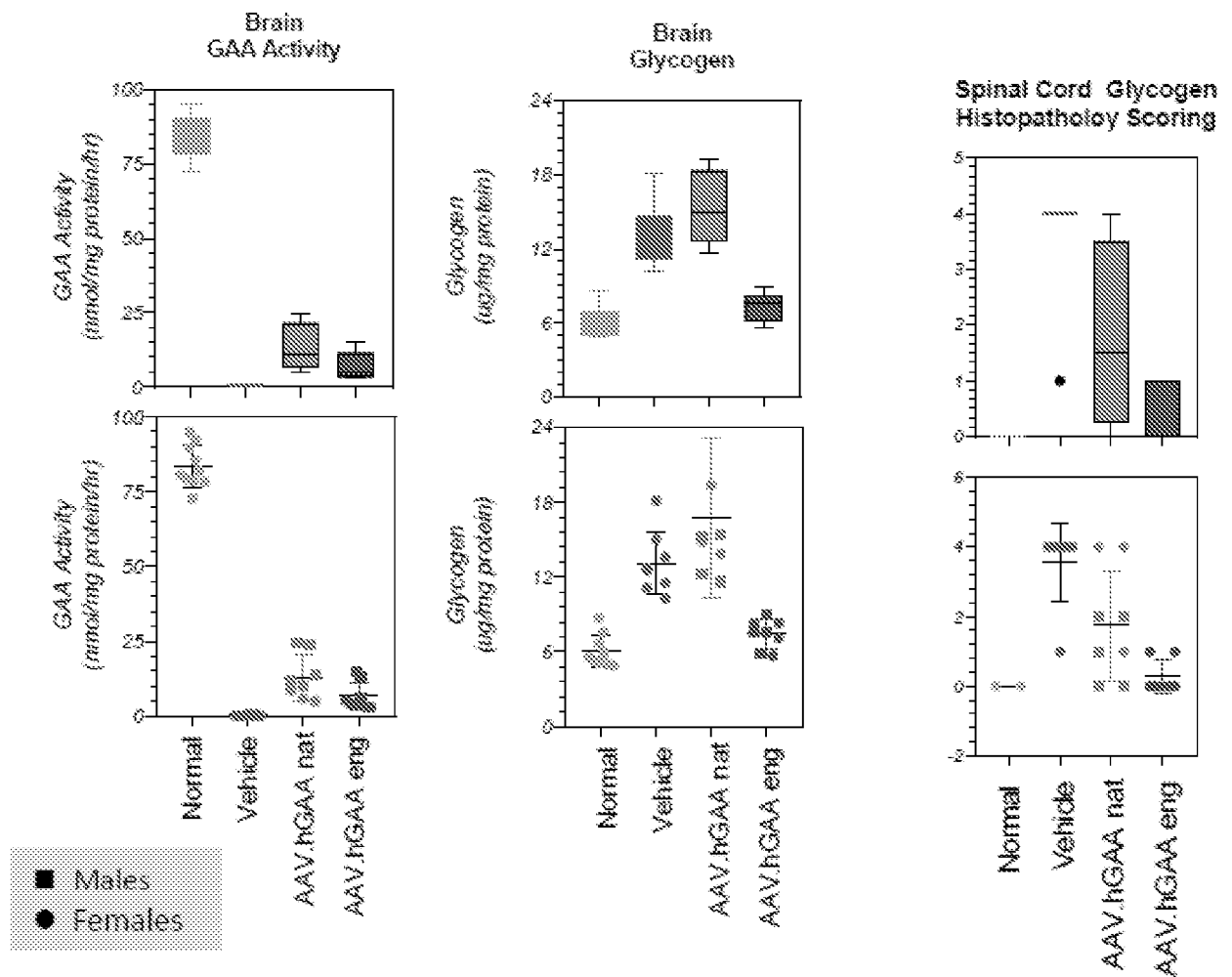


FIG. 23

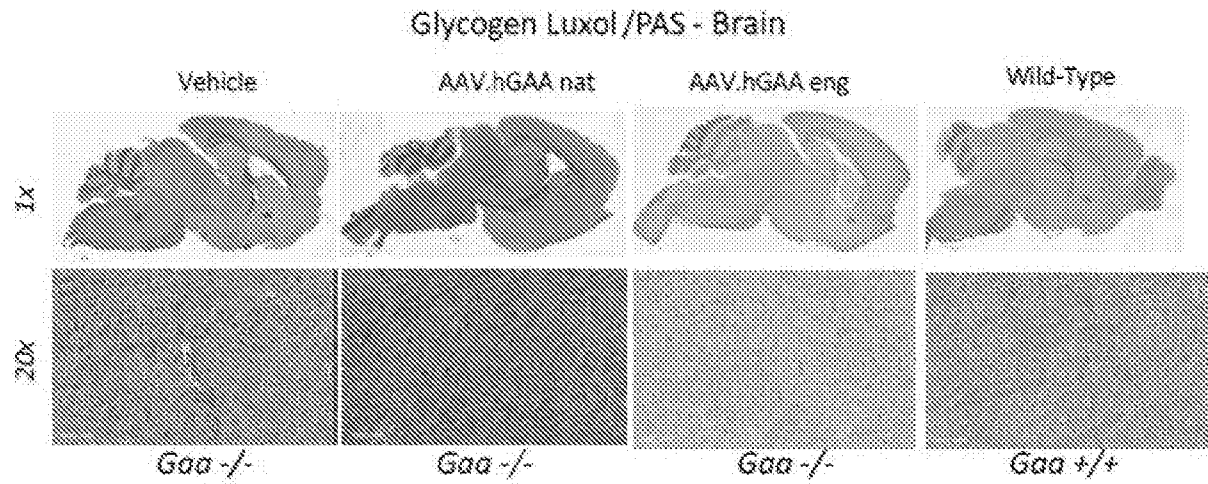


FIG. 24

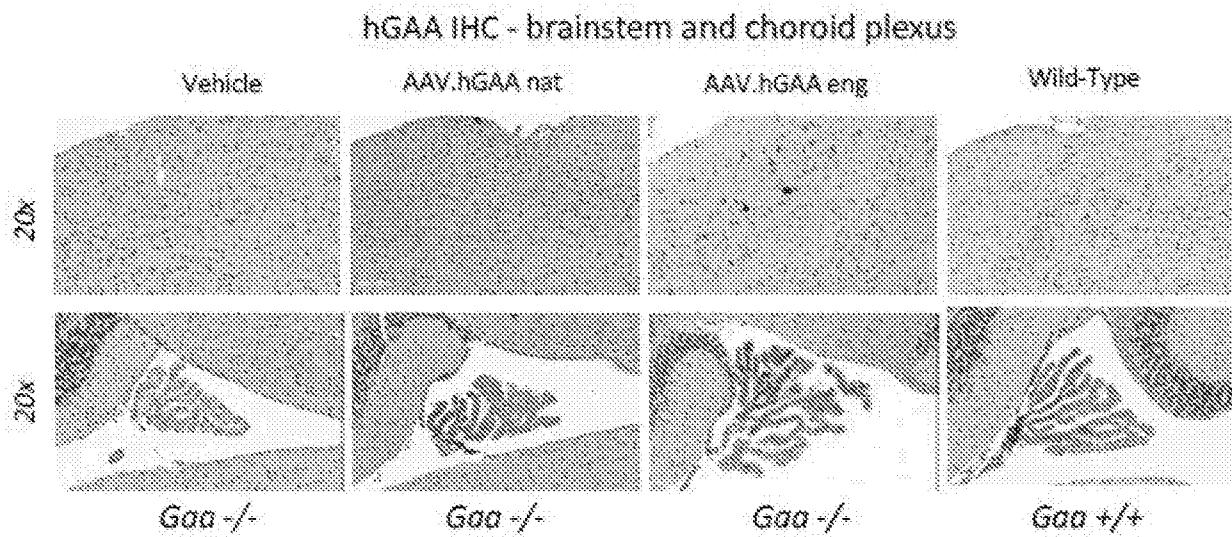


FIG. 25

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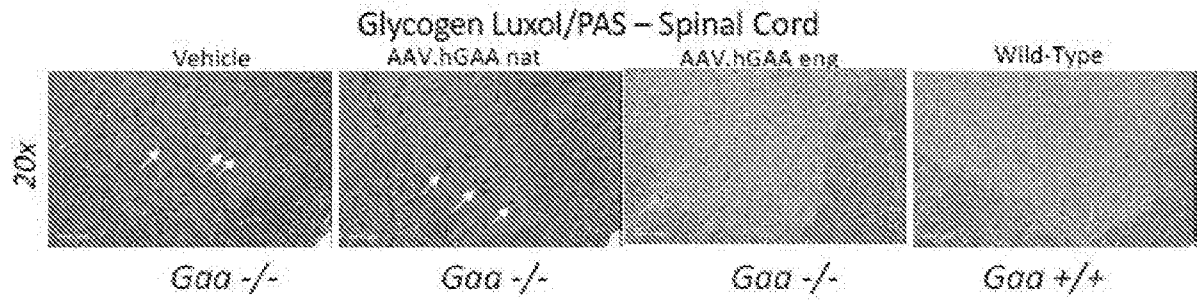


FIG. 26

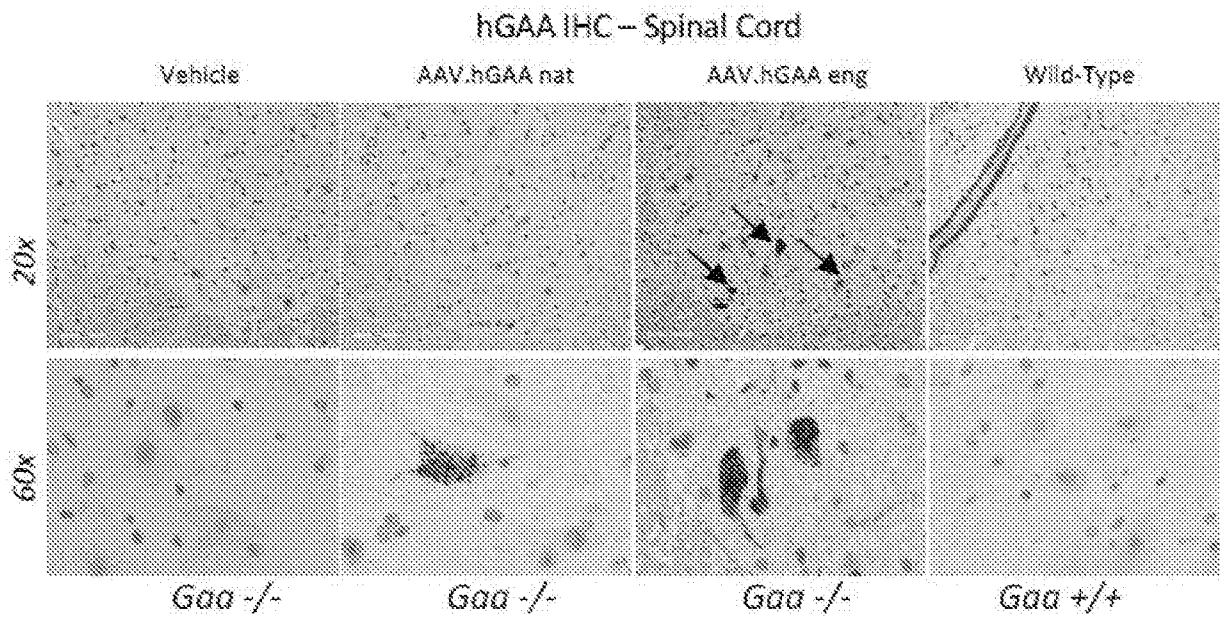


FIG. 27

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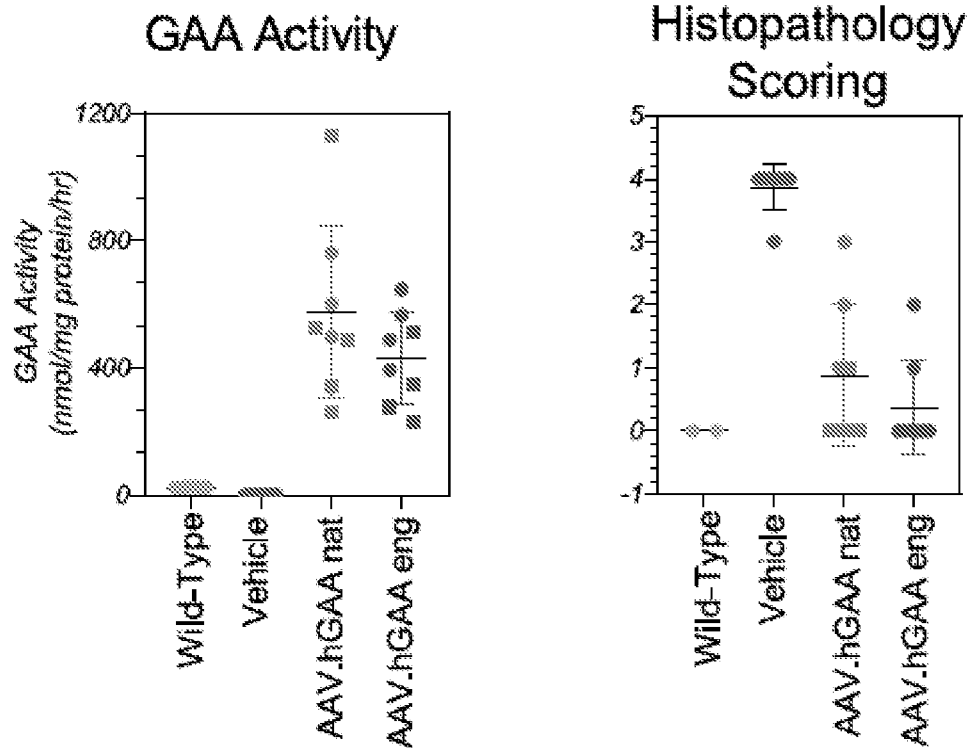


FIG. 28

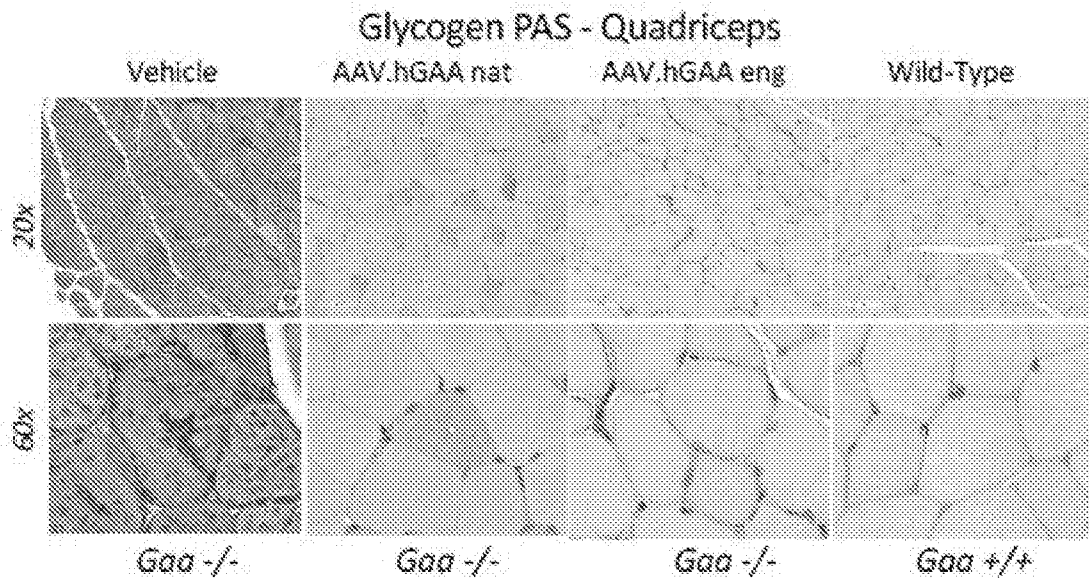


FIG. 29

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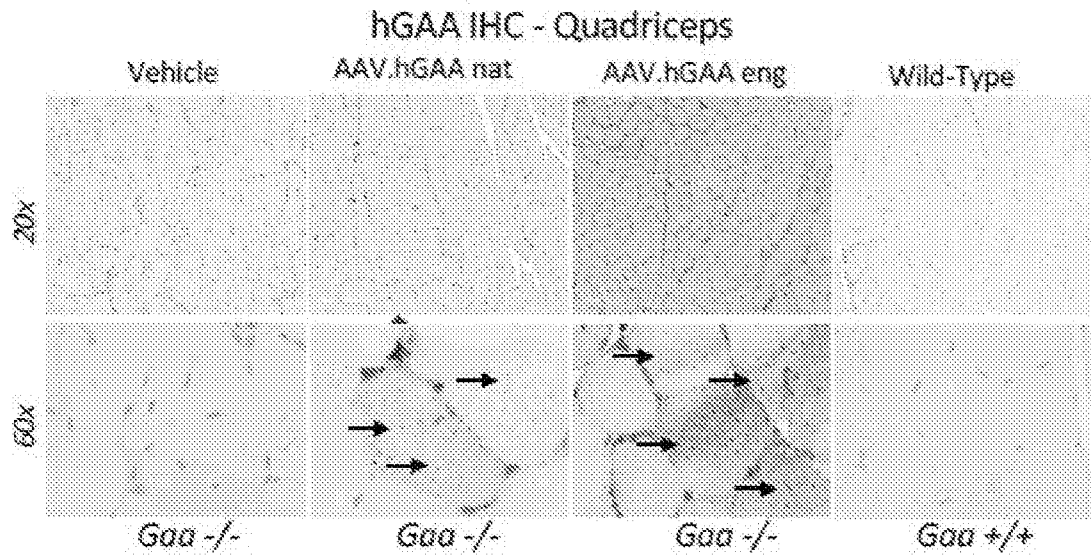


FIG. 30

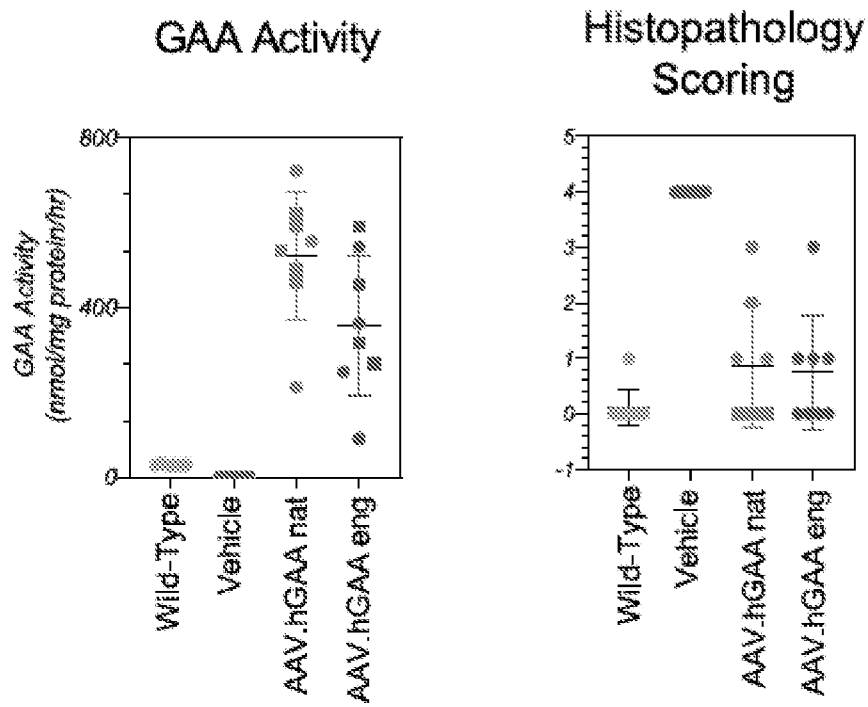


FIG. 31

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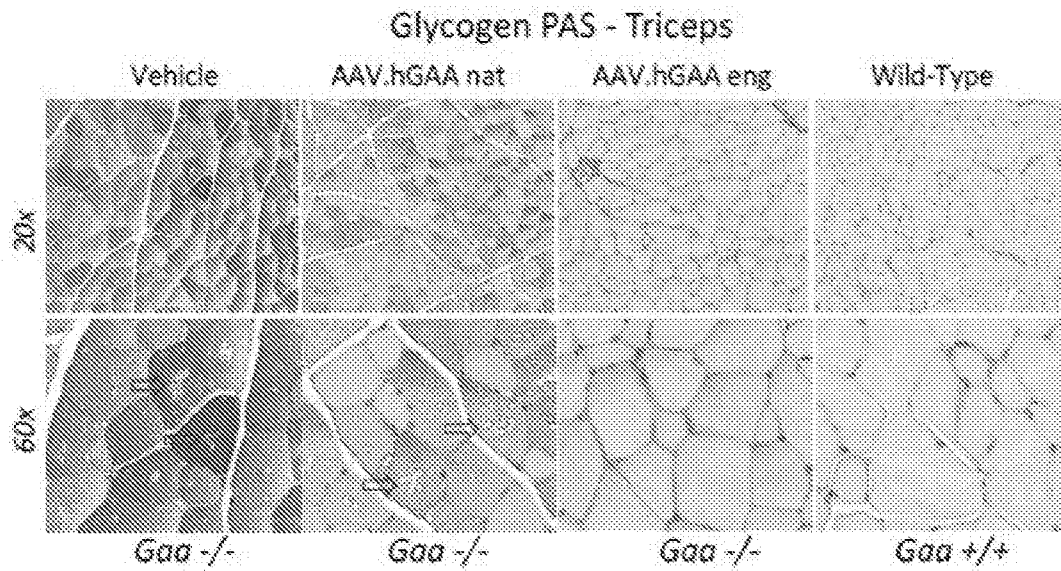


FIG. 32

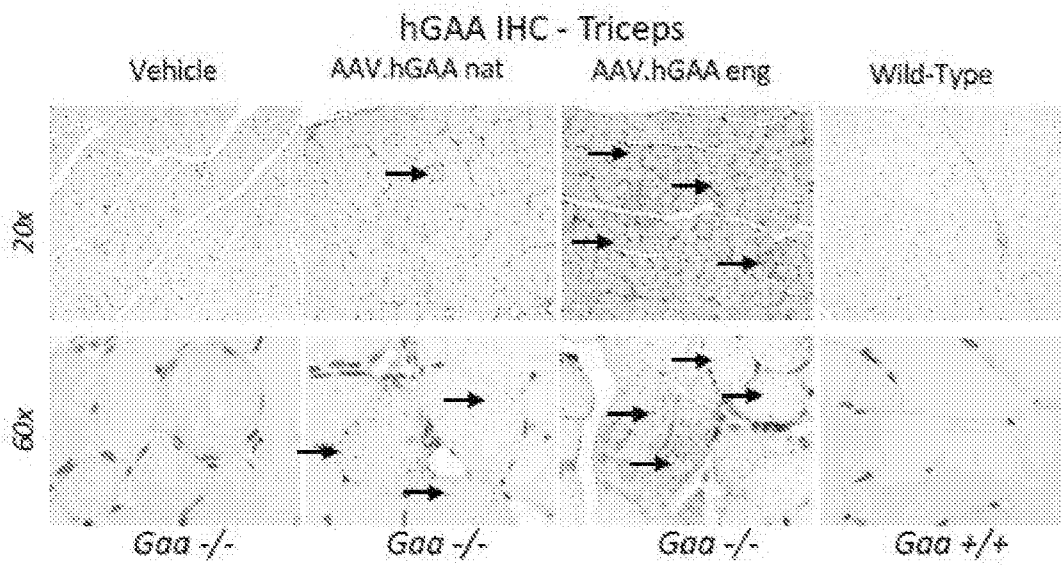


FIG. 33

M6P Receptor Chromatography

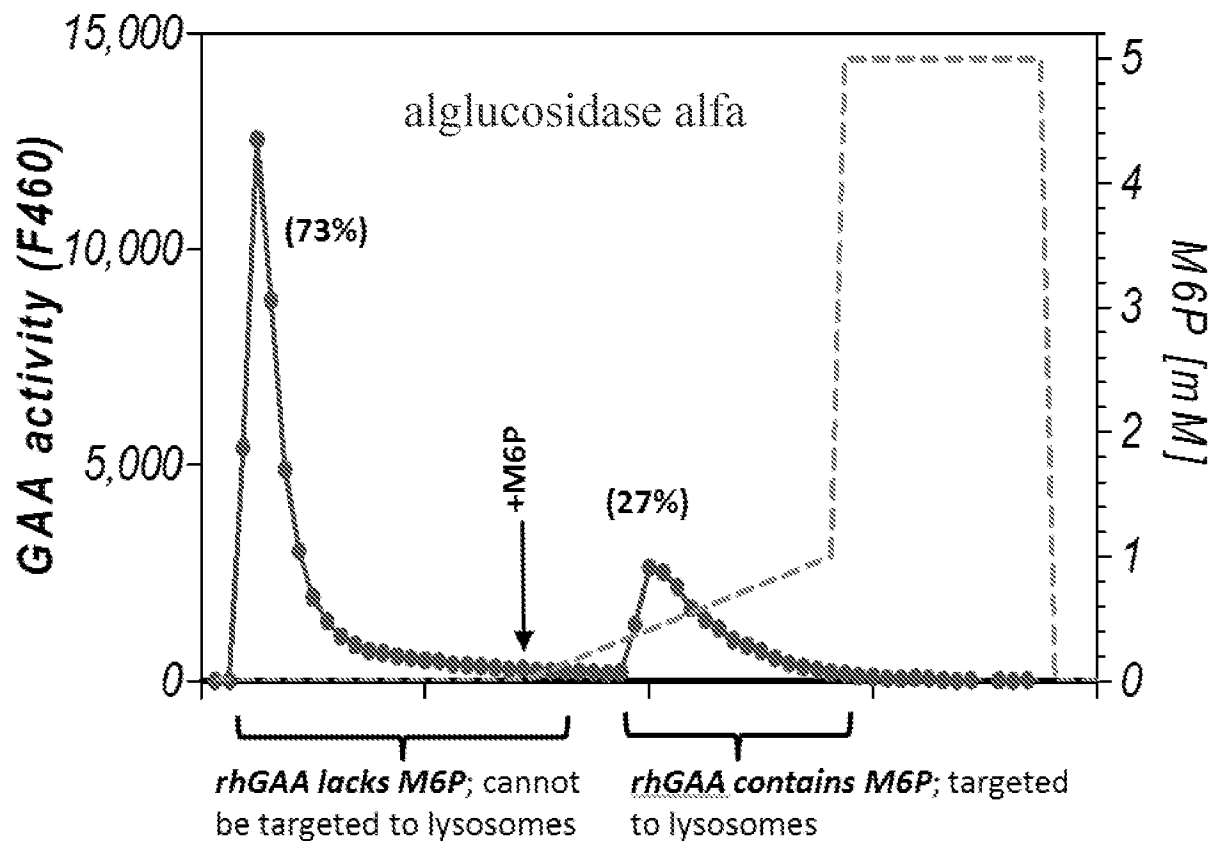


FIG. 1