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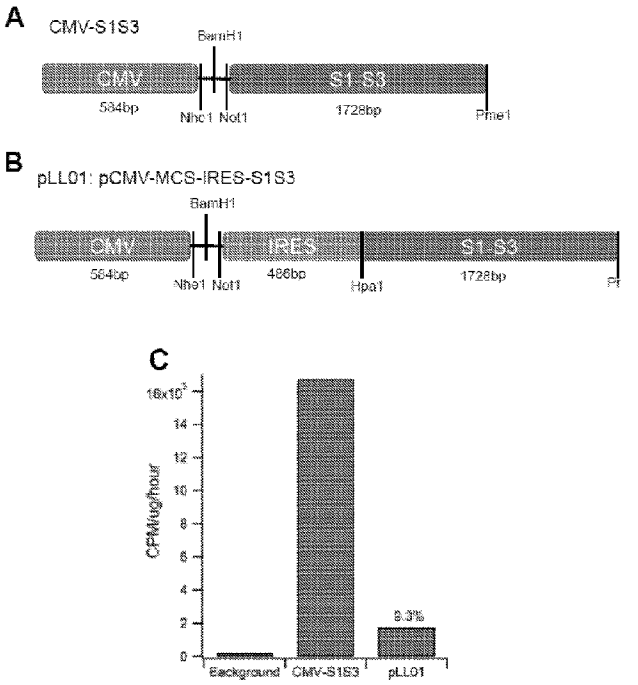
(71) Demandeur/Applicant:
M6P THERAPEUTICS, US

(72) Inventeurs/Inventors:
DO, CUONG, US;
LIU, LIN, US

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : COMPOSITIONS DE VECTEURS ET LEURS PROCEDES D'UTILISATION POUR LE TRAITEMENT DE TROUBLES DU STOCKAGE LYSOSOMAL

(54) Title: VECTOR COMPOSITIONS AND METHODS OF USING SAME FOR TREATMENT OF LYSOSOMAL STORAGE DISORDERS



Figs. 1A-1C

(57) Abrégé/Abstract:
Provided herein are compositions and methods of using a bicistronic vector for treating or preventing a lysosomal storage disorder (LSD) in a subject. The disclosed compositions comprise a bicistronic vector comprising a promoter, an Internal Ribosome Entry Site (IRES), a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase). The present methods comprise administering to the subject a pharmaceutical composition comprising the bicistronic vector as disclosed herein.

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(71) Applicant: **M6P THERAPEUTICS** [US/US]; 88 E. Main Street, No. 514, Mendham, NJ 07945 (US).

(72) Inventors: **DO, Cuong**; 5 India Brook Drive, Mendham, NJ 07945 (US). **LIU, Lin**; 953 Forestlac Court, St. Louis, MO 63141 (US).

(74) Agent: **CHRISTIANSEN, William** et al.; Cooley LLP, 1299 Pennsylvania Aveenue, Nw, Suite 700, Washington, DC 20004 (US).

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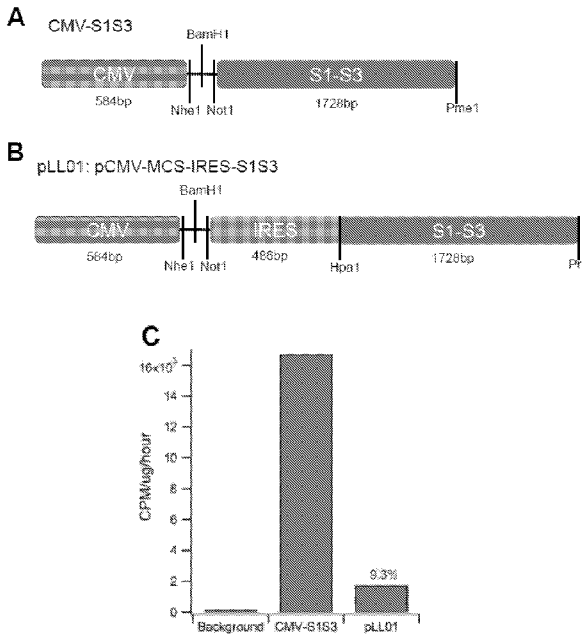
Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) Title: VECTOR COMPOSITIONS AND METHODS OF USING SAME FOR TREATMENT OF LYSOSOMAL STORAGE DISORDERS



Figs. 1A-1C

(57) Abstract: Provided herein are compositions and methods of using a bicistronic vector for treating or preventing a lysosomal storage disorder (LSD) in a subject. The disclosed compositions comprise a bicistronic vector comprising a promoter, an Internal Ribosome Entry Site (IRES), a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase). The present methods comprise administering to the subject a pharmaceutical composition comprising the bicistronic vector as disclosed herein.

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VECTOR COMPOSITIONS AND METHODS OF USING SAME FOR TREATMENT OF LYSOSOMAL STORAGE DISORDERS

RELATED APPLICATIONS

[01] This application claims the benefit of provisional application USSN 62/869,781, filed July 2, 2019 and USSN 62/869,808, filed July 2, 2019, the contents of which are herein incorporated by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

[02] The contents of the text file named "M6PT-002/01WO_SeqList.txt," which was created on July 1, 2020 and is 611 KB in size, are hereby incorporated by reference in their entirety.

TECHNICAL FIELD

[03] The disclosed disclosures relate to compositions and methods for treating lysosomal storage disorders. More particularly, the disclosed disclosures relate to the field of treating lysosomal disorders using improved gene therapy and improved enzyme replacement therapy (ERT).

BACKGROUND

[04] Lysosomal storage disorders (LSDs) relate to inherited metabolic disorders that result from defects in lysosomal function. Currently, about 50 distinct LSDs have been identified but a small number of these (fewer than 10) are reported to have treatments. Therefore, there is an unmet need in the art for safe and effective treatments for LSDs. The disclosure provides two solutions for this unmet need, through either enzyme replacement therapy (ERT) or gene therapy.

SUMMARY

[05] The disclosure provides a composition comprising a vector comprising a sequence encoding a promoter, a first polynucleotide sequence encoding a lysosomal enzyme and a second polynucleotide sequence encoding a modified N-acetylglucosamine-1- phosphotransferase (GlcNAc-1 PTase, PTase), wherein the promoter is capable of driving expression in a mammalian cell and wherein the promoter is operably linked to the first polynucleotide and to the second polynucleotide.

[06] In some embodiments of the compositions of the disclosure, the vector further comprises a sequence encoding an Internal Ribosome Entry Site (IRES). In some embodiments, the sequence encoding the IRES is positioned between the sequence encoding the lysosomal enzyme and the sequence encoding the modified GlcNAc-1 PTase. In some embodiments, the from 5' to 3', the vector comprises the sequence encoding the modified GlcNAc-1 PTase, the sequence encoding the IRES and the sequence encoding the lysosomal enzyme. In some embodiments, the from 5' to 3', the vector comprises the sequence encoding the lysosomal enzyme, the sequence encoding the IRES and the sequence encoding the modified GlcNAc-1 PTase.

[07] In some embodiments of the compositions of the disclosure, the vector further comprises a sequence encoding a cleavage site. In some embodiments, the cleavage site comprise a sequence encoding a 2A self-cleaving peptide.

[08] In some embodiments of the compositions of the disclosure, the vector is an expression vector. In some embodiments, the expression vector comprises a plasmid.

[09] In some embodiments of the compositions of the disclosure, the vector is a delivery vector. In some embodiments, the delivery vector comprises a viral vector. In some embodiments, the viral vector comprises an AAV vector or a lentiviral vector. In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 or AAV9. In some embodiments, the delivery vector comprises a non-viral vector. In some embodiments the non-viral vector comprises a liposome, a lipid nanoparticle (LNP), a micelle, a polymersome, a nanoparticle, a polymer nanoparticle, or an exosome.

[010] In some embodiments of the compositions of the disclosure, the vector is a viral vector. In some embodiments, the viral vector comprises an AAV vector or a lentiviral vector. In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 or AAV9. In some embodiments of the compositions of the disclosure, the vector is a non-viral vector. In some embodiments the non-viral vector comprises a liposome, a lipid nanoparticle (LNP), a micelle, a polymersome, a nanoparticle, a polymer nanoparticle, or an exosome.

[011] In some embodiments of the compositions of the disclosure, the vector is a viral vector. In some embodiments, the vector is a lentiviral vector. In some embodiments, the vector is an adenoviral vector or an adeno-associated viral (AAV) vector. In some embodiments, the AAV vector comprises a serotype selected from the

group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9. In some embodiments, the AAV vector comprises a sequence encoding a capsid isolated or derived from one or more of a serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9. In some embodiments, the AAV vector comprises a sequence encoding at least one inverted terminal repeat (ITR) isolated or derived from one or more of a serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9.

[012] In some embodiments of the compositions of the disclosure, the vector is a bicistronic vector.

[013] In some embodiments of the compositions of the disclosure, the vector is a multicistronic vector.

[014] In some embodiments of the compositions of the disclosure, the promoter comprises a ubiquitous promoter. In some embodiments, the promoter is capable of driving expression in a mammalian cell. In some embodiments, the promoter is capable of driving expression in a human cell.

[015] In some embodiments of the compositions of the disclosure, the promoter comprises a cell type specific promoter. In some embodiments, the promoter is capable of driving expression in a mammalian cell. In some embodiments, the promoter is capable of driving expression in a human cell. In some embodiments, the promoter is capable of driving expression in a neural cell, including but not limited to a neuron or a glial cell. In some embodiments, the promoter is capable of driving expression in a muscle cell, including but not limited to a smooth muscle cell, striated muscle cell or cardiac muscle cell. In some embodiments, the promoter is capable of driving expression in a lung cell. In some embodiments, the promoter is capable of driving expression in a bone cell. In some embodiments, the promoter is capable of driving expression in a blood cell, including but not limited to a red blood cell, white blood cell, progenitor thereof or a hematopoietic stem cell. In some embodiments, the promoter is capable of driving expression in an immune cell, including but not limited to a T-cell, a B-cell or a macrophage. In some embodiments, the promoter is capable of driving expression in a cell of the spleen or pancreas. In some embodiments, the promoter is capable of driving expression in a cell of the kidney.

[016] In some embodiments of the compositions of the disclosure, the promoter is a human T-lymphotropic virus type I (HTLV-I) promoter.

[017] In some embodiments of the compositions of the disclosure, the promoter is a

CBh promoter. In some embodiments, the CBh promoter comprises a CMV early enhancer fused to modified chicken β-actin promoter.

[018] In some embodiments of the compositions of the disclosure, the promoter is a CEF or hCEFI promoter. In some embodiments, the hCEFI promoter comprises a human CMV enhancer operably linked to a human EF1a promoter. In some embodiments, the hCEFI promoter comprises the sequence of SEQ ID NO: 161.

[019] In some embodiments of the compositions of the disclosure, the promoter comprises a constitutive promoter. In some embodiments, the constitutive promoter comprises a Cytomegalovirus (CMV) promoter.

[020] In some embodiments of the compositions of the disclosure, the vector comprises a nucleic acid sequence of SEQ ID NO: 1.

[021] In some embodiments of the compositions of the disclosure, the polynucleotide encoding a modified GlcNAc-1 PTase comprises a nucleic acid sequence of SEQ ID NO: 4.

[022] In some embodiments of the compositions of the disclosure, the lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C.

[023] In some embodiments of the compositions of the disclosure, the lysosomal enzyme comprises at least one lysosomal enzyme listed in Table 1A, Table 1B or Table 1C.

[024] In some embodiments of the compositions of the disclosure, the lysosomal enzyme is selected from the group consisting of β-glucocebrosidase (GCase/GBA, encoded by the GBA gene), Galactosylceremidase (GALC), α-Galactosidase (encoded by the GLA gene), α-N-acetylglucosaminidase (NAGLU), acid α-glucosidase (GAA) and lysosomal acid α-mannosidase (LAMAN).

[025] In some embodiments of the compositions of the disclosure, the lysosomal enzyme comprises β-glucocebrosidase (GCase/GBA). In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 5.

[026] In some embodiments of the compositions of the disclosure, the lysosomal enzyme comprises Galactosylceremidase (GALC). In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 6. In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 23.

[027] In some embodiments of the compositions of the disclosure, the lysosomal

enzyme comprises α-Galactosidase (GLA). In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 7.

[028] In some embodiments of the compositions of the disclosure, the lysosomal enzyme comprises α-N-acetylglucosaminidase (NAGLU). In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 8.

[029] In some embodiments of the compositions of the disclosure, the lysosomal enzyme comprises acid α-glucosidase (GAA). In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 9.

[030] In some embodiments of the compositions of the disclosure, the lysosomal enzyme comprises lysosomal acid α-mannosidase (LAMAN). In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 10.

[031] The disclosure provides a method of treating a lysosomal storage disorder (LSD), the method comprising administering to a subject an effective amount of a composition of the disclosure, wherein the composition increases the phosphorylation of a lysosomal enzyme responsible of the LSD, thereby treating the LSD. The disclosure provides a method of treating a lysosomal storage disorder (LSD), the method comprising administering to a subject an effective amount of a composition of the disclosure, wherein the composition increases the N-linked oligosaccharide phosphorylation of a lysosomal enzyme responsible of the LSD, thereby treating the LSD. In some embodiments, the subject presents a sign or a symptom of the LSD. In some embodiments, the subject has been diagnosed with the LSD.

[032] The disclosure provides a method of preventing an occurrence or an onset of a lysosomal storage disorder (LSD), the method comprising administering to a subject an effective amount of a composition of the disclosure, wherein the composition increases the phosphorylation of a lysosomal enzyme responsible of the LSD, thereby preventing the occurrence of the LSD in the subject. In some embodiments, the subject is at risk of the occurrence or the onset of the LSD. In some embodiments, the subject presents a sign or a symptom of the LSD.

[033] The disclosure provides a method of ameliorating the phosphorylation of a lysosomal enzyme responsible for a lysosomal storage disorder (LSD), the method comprising administering to a subject an effective amount of a composition of the disclosure, wherein the composition increases the phosphorylation of the lysosomal

enzyme. In some embodiments, the subject presents a sign or a symptom of the LSD. In some embodiments, the subject is at risk of the occurrence or the onset of the LSD. In some embodiments, the subject has been diagnosed with the LSD.

[034] The disclosure provides a method of ameliorating the phosphorylation of a lysosomal enzyme responsible for a lysosomal storage disorder (LSD), the method comprising contacting to a cell, an effective amount of a composition of the disclosure, wherein the composition increases the phosphorylation of the lysosomal enzyme. In some embodiments, the cell is in vitro or ex vivo. In some embodiments, the cell is in vivo. In some embodiments, a subject comprises the cell. In some embodiments, the subject presents a sign or a symptom of the LSD. In some embodiments, the subject is at risk of the occurrence or the onset of the LSD.I n some embodiments, the subject has been diagnosed with the LSD.

[035] In some embodiments of the methods of the disclosure, the lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C.

[036] In some embodiments of the methods of the disclosure, the lysosomal enzyme is at least one as listed in Table 1A, Table 1B or Table 1C.

[037] In some embodiments of the methods of the disclosure, the lysosomal enzyme comprises one or more of β-glucocebrosidase (GCase/GBA), Galactosylceremidase (GALC), α-Galactosidase (GLA), α-N-acetylglucosaminidase (NAGLU), acid α-glucosidase (GAA) and lysosomal acid α-mannosidase (LAMAN).

[038] In some embodiments of the methods of the disclosure, the administering comprises a systemic route of administration. In some embodiments, the systemic route of administration is enteral, parenteral, oral, intramuscular (IM), subcutaneous (SC), intravenous (IV), intra-arterial (IA), intraspinal, intraventricular, intrathecal, intracerebroventricular.

[039] In some embodiments of the methods of the disclosure, the administering comprises a local route of administration.

[040] In some embodiments of the methods of the disclosure, the subject is a human. In some embodiments, the subject is a male. In some embodiments, the subject is a female.

BRIEF DESCRIPTION OF THE DRAWINGS

[041] For the purpose of illustrating the disclosure, there are depicted in the drawings certain embodiments of the disclosure. However, the disclosure is not limited to the

precise arrangements and instrumentalities of the embodiments depicted in the drawings.

[042] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[043] Figs. 1A-1C are series of diagrams and a graph depicting the S1-S3 biscistronic vector. Fig. 1A: CMV-S1S3 vector. Fig. 1B: pLL01: pCMV-MCS-IRES-S1S3 vector. Fig. 1C: Graph illustrating the level of expression of CMV-S1S3 and pLL01 (CPM: Counts per minute).

[044] Figs. 2A-2C are series of a diagram and histogram depicting the generation of GBA biscistronic expression plasmid in S1-S3 biscistronic vector. Fig. 2A: pLL11: pCMV-hGBA-IRES-S1S3 vector. Fig. 2B: GBA activity in conditional medium. Fig. 1C: Histogram illustrating the percent of PTase activity.

[045] Figs. 3A-3C are series of graphs and a histogram showing that bicistronic expression increases the phosphorylation of GBA enzyme.

[046] Figs. 4A-4D are series of a diagram, a graph and histograms showing that bicistronic expression increases the phosphorylation of GAA enzyme.

[047] Figs. 5A-5D are series of a diagram, a graph and histograms showing that bicistronic expression increases the phosphorylation of GALC enzyme.

[048] Figs. 6A-6D are series of a diagram, a graph and histograms showing that bicistronic expression increases the phosphorylation of NAGLU enzyme.

[049] Figs. 7A-7D are series of a diagram, a graph and histograms showing that bicistronic expression increases the phosphorylation of GLA enzyme.

[050] Figs. 8A-8D are series of a diagram, a graph and histograms showing that bicistronic expression increases the phosphorylation of LAMAN enzyme.

[051] Figs 9A-9E are series of graphs demonstrating that an S1-S3 PTase bicistronic vector of the disclosure significantly increases the CI-MPR binding of GBA enzyme and its cell uptake in the treatment of Gaucher disease (A-C). Panels D and E demonstrate that a single point mutation in the GBA enzyme increases its stability but does not affect its binding toward CI-MPR.

[052] Figs 10A-10C are series of graphs demonstrating that an S1-S3 PTase bicistronic vector of the disclosure significantly increases the CI-MPR binding of GAA enzyme and its cell uptake in the treatment of Pompe Disease.

[053] Figs 11A-11C are series of graphs demonstrating that an S1-S3 PTase bicistronic vector of the disclosure significantly increases the CI-MPR binding of

GALC enzyme and its cell uptake in the treatment of Krabbe Disease.

[054] Figs 12A-12C are series of graphs demonstrating that an S1-S3 PTase bicistronic vector of the disclosure significantly increases the CI-MPR binding of NAGLU enzyme and its cell uptake in the treatment of MPS IIIB Disease.

[055] Figs 13A-13C are series of graphs demonstrating that an S1-S3 PTase bicistronic vector of the disclosure significantly increases the CI-MPR binding of GLA enzyme and its cell uptake in the treatment of Fabry Disease.

[056] Figs 14A-14C are series of graphs demonstrating that an S1-S3 PTase bicistronic vector of the disclosure significantly increases the CI-MPR binding of LAMAN enzyme and its cell uptake in the treatment of α-Mannosidosis.

[057] Figs 15A-15B are a schematic diagram and a graph demonstrating that an S1-S3 PTase bicistronic vector of the disclosure delivered by AAV9 vector may be used as a gene therapy in the treatment of Mucolipidosis Disease.

[058] Figs 16A-16B are a pair of graphs depicting elevated glucosylceramide levels observed in the liver, lung and spleen of 20 week old Gaucher$^{D409V/null}$ mice. The accumulation of GBA's natural substrate, glucocerebroside was determined in tissue homogenates. The accumulation of GC in the lung is a statistically and therapeutically valuable result, which is a known unmet need of the current standard of care. 20 µL aliquots of tissue homogenates and appropriate controls were glucocylceramides were extracted by adding 200 µL of Methanol/ACN/H2O (v:v:v=85:10:5), a mixing for 5 min at 800 rpm followed by centrifuging for 15 min at 3220 *g* 4 °C; 3). 50 µL of supernatant was recovered, dried with nitrogen and resuspended with Methanol/ACN/H2O (v:v:v=85:10:5) and directly injected for LC-MS/MS analysis.

[059] Figs 17A-17C are a series of graphs demonstrating that GCase^{M6P} has a longer half-life and greater tissue uptake in the GBA$^{D409V/null}$ mouse model compared to imiglucerase. A PK/PD study in the Gaucher D409V/Null mouse model was performed using the standard of care, imiglucerase, and purified GBA produced by transiently co-expressed utilizing the bicistronic vector that encoded for the S1-S3 PTase and a natural variant of GBA in Expi293 cells. This variant of GCase has greater stability at neutral and slightly alkali conditions. Briefly, 3 animals received a tail vein injection of ~ 1.5 mg/kg of recombinant GCase. For the serum pharmacokinetic data, plasma samples were collected at 2, 10, 20, 40 and 60 mins. Activity measured using a synthetic substrate, 4-methylumbelliferyl-beta-D-glucopyranoside (4MU-Glc). The activity was normalized in the individual animals by setting the 2 min time point as 100% activity and subsequent time points are a percent

of the t=2 min time point. The stabilized GCase expressed in the presence of S1-S3 PTase appears to have a longer half-life. This longer half-life is a combination of the enzyme having greater stability and the different clearance pathways. To determine how much GCase was taken up by the tissue, 2 hrs after enzyme injection, tissue was recovered, homogenized and activity measured using the 4MU-Glc substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination. The true advantage of a stabile GCases with appropriate phosphorylation is observed in the tissue uptake data shown. For all tissues evaluated there is more activity found in the stabilized GCase expressed utilizing the bicistronic S1-S3 PTase vector platform S1'S3 PTase. This is most dramatic in the lung, muscle and brain where imiglucerase has little activity. When the tissue and sera data is taken together, the advantage of a more stable GCase with greater N-linked oligosaccharide phosphorylation is apparent for delivering more enzyme to affected tissue. This is the first time that a significant amount of GCase has been delivered to the lung, muscle and heart at these doses.

[060] Figs 18A-18E are a series of photographs and bar graphs demonstrating that $GCase^{M6P}$ ERT reduced tissue macrophages (anti-CD68 staining) better than imiglucerase in the $GBA^{D409V/null}$ mouse model. An efficacy study in the D409V Gaucher mouse model was performed using the standard of care, Cerezyme, and purified GBA (M0111) transiently co-expressed in Expi293 cells utilizing the bicistronic vector that encodes for the S1S3 PTase and a natural variant of GBA with reported greater stability at neutral and slightly alkali conditions. ~20 weeks old Gaucher mice were treated with ~1.5 mg/kg) enzymes weekly for four weeks. Four weeks later, the tissue of Liver and Lung was harvested and fixed in 4% paraformaldehyde-PBS, pH 7.4 for immunohistochemistry with CD68 antibody. M0111 has greater efficacy compared to the current standard of care as evidenced by the reduction of macrophage in affected tissue as visualized by CD68 Ab.

[061] Figs 19A-19C are a series of photographs demonstrating that $GCase^{M6P}$ ERT reduced the number and size of Gaucher storage cells (Hematoxylin and Eosin (H&E) staining) better than imiglucerase in the $GBA^{D409V/null}$ mouse model. An efficacy study in the D409A Gaucher mouse model was performed using the standard of care, Cerezyme, and purified GBA transiently co-expressed in Expi293 cells utilizing the bicistronic vector that encoded for the S1-S3 PTase and a natural variant of GBA with reported greater stability at neutral and slightly alkali conditions. ~20 weeks old Gaucher mice were treated with ~1.5 mg/kg enzymes weekly for four weeks. Four

weeks later, the tissue of Liver and Lung was harvested and fixed in 4% paraformaldehyde-PBS, pH 7.4 for formalin for hematoxylin and eosin (H&E) staining. $GCase^{M6P}$ has greater efficacy compared to the current standard of care as evidenced by the reduction of storage cells in affected tissue as visualized by H&E staining.

[062] Figs 20A-20B are a pair of graphs demonstrating that $GCase^{M6P}$ ERT reduced accumulated substrate better than imiglucerase in the $GBA^{D409V/null}$ mouse model. ~20 weeks old Gaucher mice were treated weekly with ~1.5 mg/kg enzymes for four weeks. Tissue samples were collected and homogenized for glycosylceramide analysis. The accumulation of GCase's natural substrate, glucocerebroside was determined in tissue homogenates. Of significant value is the accumulation of GC in the lung which is a known unmet need for the current standard of care. 20 µL aliquots of tissue homogenates and appropriate controls were glucocylceramides were extracted by adding 200 µL of Methanol/ACN/H2O (v:v:v=85:10:5), mixing for 5 min at 800 rpm followed by centrifuging for 15 min at 3220 *g* 4 °C; 3). 50 µL of supernatant was recovered, dried with nitrogen and resuspended with Methanol/ACN/H2O (v:v:v=85:10:5) and directly injected for LC-MS/MS analysis.. For the two ceramides measured, $GCase^{M6P}$ treated animals had lower levels following ERT therapy over the imiglucerase.

[063] Figs 21A-21D are a series of graphs showing the results of in vivo AAV mediate gene therapy studies for the treatment of Gaucher Disease. To determine the effect of AAV9 gene therapy with the bicistronic expression transgene of stable GBA + S1-S3 PTase with three different promotors. 15 wk old $GBA^{D409V/null}$ mice were dosed with a moderate dose of AAV9-stable GBA+ S1-S3 PTase, 5E11 vg. To determine how much GBA was generated by the tissue, 2 weeks later after AAV9 injection, tissue was recovered, homogenized and activity measured using the 4MU-Glc substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination.

[064] Figs 22A-22C are a series of graphs depicting the results of in vitro studies for the use of lysosomal alpha-mannosidase (LAMAN) as ERT.

[065] Figs 23A-23B is a photograph and corresponding data table depicting LAMAN enzyme expression, purification, and characterization. Two preparations of LAMAN were transiently co-expressed in Expi293 cells with (M0611) or without the bicistronic vector that encoded for the S1-S3 PTases. Both were purified by utilization of the HPC4 affinity tag. The significant increase in phosphorylation was demonstrated by

measuring the amount of LAMAN that kind bind to immobilized cation-independent mannose 6-phosphate receptor in a dose dependent manner. The amount of LAMAN bound was based on its activity using it synthetic substrate 4-Methylumbelliferyl-α-D-Mannopyranoside (4MU-Man). The specificity of binding via phosphorylated oligosaccharides was confirmed by the ability of added mannose 6-phosphate to block binding. Of note is the ability of LAMAN^{M6P} (M0611) to bind the receptor even in the presence of M6P. LAMAN^{M6P} (M0611, P-0030) and LAMAN (P-0031) were chosen for in vivo animal study.

[066] Fig 23C a graph depicting LAMAN^{M6P} (M0611) enzyme expression, purification, and characterization. Two preparations of LAMAN were transiently co-expressed in Expi293 cells with or without the bicistronic vector that encoded for the S1-S3 variant of PTase. Both were purified by utilization of the HPC4 tag. The significant increase in phosphorylation was demonstrated by measuring the amount of LAMAN that kind bind to immobilized cation-independent mannose 6-phosphate receptor in a dose dependent manner. The amount of bound LAMAN was determined by activity using a synthetic substrate 4-Methylumbelliferyl-α-D-Mannopyranoside (4MU-Man). The specificity of binding via phosphorylated oligosaccharides was confirmed by the ability of added mannose 6-phosphate to block binding. Of note is the ability of M0611 to bind the receptor even in the presence of M6P. LAMAN^{M6P} (M0611, P-0030) and LAMAN (P-0031) were chosen for in vivo animal study.

[067] Figs 24A-24B are a pair of graphs demonstrating the biodistribution of LAMAN and LAMAN^{M6P} enzymes in wild type mice for enzyme replacement therapy. To evaluate the difference in tissue uptake between LAMAN and LAMAN^{M6P} (LAMAN co-expressed with S1-S3 PTase), 2 mg/kg of each prep was injected via tail vein into wild type mice (n=4). 2 and 8 hrs after dosing, tissue was recovered, homogenized and activity measured using the 4MU-Man substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination. An advantage of LAMAN^{M6P} (LAMAN co-expressed with S1S3 PTase) is observed in the tissue uptake data. For liver, spleen, heart, lung, and brain there was greater activity in the tissue at 2 hours. This trend was also true at 8 hours with the exception of the lung. This might be a result of the high variation observed in the analysis of this tissue. The only exception to this observation was the kidney. Endogenous LAMAN activity is subtracted from all samples. Higher LAMAN enzyme activity was detected in most tissues of the mice which were injected with our LAMAN^{M6P} enzyme.

[068] Figs 25A-25B are a pair of graphs demonstrating the biodistribution of αLAMAN and LAMAN^{M6P} enzymes in wild type mice for enzyme replacement therapy. To evaluate the difference in tissue uptake between LAMAN and LAMAN^{M6P} (LAMAN co-expressed with S1-S3 PTase), 10 mg/kg of each prep was injected via tail vein into wild type mice (n=4). 2 and 8 hrs after dosing, tissue was recovered, homogenized and activity measured using the 4MU-Man substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination. An advantage of LAMAN^{M6P} (LAMAN co-expressed with S1-S3 PTase) is observed in the tissue uptake data. For liver, spleen, heart, lung, and brain there was greater activity in the tissue at 2 hours. This trend was also true at 8 hours with the exception of the Kidney. This might be a result of the high variation observed in the analysis of this tissue.

[069] Figs 26A-26B is a schematic diagram and a graph depicting the AAV9 design and in vitro testing for a Mucolipidosis gene therapy (GTx). 293T cells was transduced with various M0021 (AAV9-CAGp-S1-S3) virus and cultured for 2 days before PTase activity assay.

[070] Figs 27A-27B are a pair of graphs demonstrating that M0021 treatment decreases the serum lysosomal enzymes level in ML II mouse. To determine the effect of S1-S3 PTase Gene Therapy, a 34 week old female mouse was dose with a moderate dose of M0021 (AAV9-CAGp-S1-S3) , 4e12 vg (2e13 vg/kg). One of the phenotypes of ML II is elevated serum level of lysosomal enzyme due to their inability to be targeted to the lysosome within the cell. An encouraging results was observed when there was a decrease in LAMAN and ManB activity in the serum after just 1 week of receiving the therapy. This result is important since it demonstrates the ability to effect a described phenotype of the MLII mouse model.

[071] Figs 28A-28C are a series of graphs demonstrating that M0021 treatment increases the phosphorylation of lysosomal enzymes in ML II. To further understand the impact on S1-S3 PTase gene therapy in decreasing the serum activity of LAMAN and ManB, CI-MPR binding of the enzyme found in the serum was evaluated using the immobilized receptor binding assay described earlier. Briefly, a known about of activity in added in increasing amounts to immobilized CI-MPR. The unbound enzyme is washed away and the remaining bound enzyme is measured using the appropriate synthetic substrate; Man-b-4MU (ManB, LAMAN 4MU-Man (LAMAN). AAV9-S1S3 Gene therapy in ML II mouse increases the glycan phosphorylation of lysosomal enzymes. The total phosphorylated lysosomal enzymes in serum normalized

to normal levels or slightly higher after 3 weeks.

[072] Figs 29A-29C are a series of graphs depicting enzyme activity and select GCase substrates in the lung and liver 2 weeks post injection of AAV9-hTLV-GBA^{M6P} gene therapy in Gaucher mice. AAV9-hTLV-GBA-S1S3 is otherwise known as AAV9-hTLV-GBA^{M6P} wherein the M6P denotes the S1S3 construct. Two weeks following AAV9 hTLV-GBA or AAV9 hTLV-GBA^{M6P} (transgene with bicistronic vector with GBA and S1-S3 PTase) There was elevated expression in the liver for both constructs (Fig. 29A) When liver glucosyl-β-ceramide levels were measured (Fig, 29B,C), the greatest reduction in accumulated substrate was observed for the AAV9 hTLV-GBA^{M6P} treated animals even though there was lower GCase activity in the liver compared to the AAV9 hTLV-GBA treated animals. This greater substrate reduction with less activity indicates the importance of N-linked oligosaccharide phosphorylation for gene therapy in terms cell uptake and lysosomal targeting. In the lung, the GCase activity for the AAV9 treated animals is low. However, the AAV9-hTLV-GBA^{M6P} treated animals showed significant reduction in the lung for accumulated glucosyl-β-ceramide levels (Fig, 29B, C). Little reduction was observed for the AAV9-hTLV-GBA treated animals. This demonstrates that having a phosphorylated transgene product with high affinity for the CI-MPR can lead to effective therapies even at low activities levels due to efficient cellular uptake and lysosomal targeting.

DETAILED DESCRIPTION

[073] Lysosomal storage disorders (LSDs) relate to inherited metabolic disorders that result from defects in lysosomal function. Currently, about 50 distinct LSDs have been identified but a small number of these (fewer than 10) are reported to have treatments. Patients are currently treated by intravenous infusion of enzyme replacement therapies (ERTs), which supplement the missing enzyme in patients to address their symptoms of disease. The goal of ERT is to introduce sufficient amounts of normal enzyme into the lysosomes of the defective cells to clear the storage material and restore lysosome function. In order to insure efficient uptake of the ERTs into the affected lysosomes, it is imperative that ERTs contain high levels of Mannose 6-phosphate (M6P). Ideally patients with LSDs should be treated by administering the missing enzyme with highly saturated level of M6P to enable effective delivery to lysosomes. However, this process is very challenging as the phosphorylation process that enables the addition of M6P to the lysosomal is inherently inefficient. The recent discovery of S1-S3 variant

of GlcNAc-1PTase significantly improves the phosphorylation process of lysosomal enzymes. Additionally, there is a need for a gene therapy approach that would provide the patient with a long-term cure of LSD.

[074] The disclosure provides expression vectors, compositions and methods for generating lysosomal enzymes operably linked to a S1-S3 variant of GlcNAc-1-Phosphotransferase. The S1-S3 variant of GlcNAc-1-Phosphotransferase significantly increases transport of operably linked lysosomal enzymes into cells and out of the blood serum or the kidneys for increased update, distribution, and lysosomal enzymatic activity.

[075] The disclosure provides gene therapy vectors, compositions and methods for generating lysosomal enzymes operably linked to a S1-S3 variant of GlcNAc-1-Phosphotransferase. The disclosure demonstrates that expression of the S1-S3 variant increases the uptake, distribution and activity of endogenous lysosomal enzymes.

[076] The disclosure provides ERT, vectors, compositions and methods for generating lysosomal enzymes with appropriate phosphorylated N-linked oligosaccharides by co-expression with S1-S3 PTase via a novel bicistronic vector. The bicistronic expression of S1-S3 PTase and lysosomal enzyme significantly increases the M6P content of the lysosomal enzyme being expressed. Having well phosphorylated enzymes allows for the efficient uptake and lysosomal delivery of the enzyme. This enables for better tissue distribution, cellular uptake, lysosomal targeting and substrate reduction. The disclosure provides gene therapy vectors, compositions and methods for generating high levels of expression or high levels of activity of M6P lysosomal enzymes by co-expression the S1-S3 PTase. The bicistronic expression of the S1-S3 variant of PTase significantly increases the M6P content level in lysosomal enzymes. Through the high M6P on the surface of lysosomal enzymes, the enzymes could be delivered to tissue cells with increased uptake, distribution and efficacy in vitro and in vivo.

[077] Vectors, compositions and methods of the disclosure may be used for enzyme replacement therapy (ERT).

[078] Alternatively or in addition, vectors, compositions and methods of the disclosure may be used for gene therapy.

[079] A number of lysosomal enzymes are described and their uses in both ERT and gene therapy are demonstrated. Importantly, the vectors, compositions and methods of the disclosure may be used with any lysosomal enzyme to increase cellular uptake of the lysosomal enzyme and, consequently, increase activity of the lysosomal enzyme in

one or more bodily tissues.

[080] In some embodiments, the compositions and methods of the disclosure comprising the S1-S3 PTase operably linked to a lysosomal protein, increase uptake and activity of the lysosomal protein in one or more of the spleen, the brain, one or more lungs, or one or more muscles of a subject.

[081] In some embodiments, the vectors, compositions and methods of the disclosure comprising the S1-S3 GlcNAc-1-Phosphotransferase, including those embodiments in which a bicistronic vector comprises a sequence encoding the S1-S3GlcNAc-1-Phosphotransferase and a sequence encoding a lysosomal protein, increase uptake and activity of the encoded lysosomal protein in one or more of the spleen, the brain, one or more lungs, or one or more muscles of a subject.

Exemplary Embodiments

[082] The disclosure provides a composition comprising a vector comprising a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase).

[083] The disclosure provides a composition comprising a bicistronic vector comprising a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase).

[084] In some embodiments of the compositions of the disclosure, the bicistronic vector comprises an Internal Ribosome Entry Site (IRES) located before the polynucleotide encoding a modified GlcNAc-1 PTase and after the polynucleotide encoding a lysosomal enzyme. In some embodiments, the bicistronic vector comprises an IRES located after the polynucleotide encoding a modified GlcNAc-1 PTase and before the polynucleotide encoding a lysosomal enzyme.

[085] In some embodiments of the compositions of the disclosure, the bicistronic vector comprises a promoter. In some embodiments, the bicistronic vector comprises a constitutive promoter. In some embodiments, the constitutive promoter comprises a Cytomegalovirus (CMV) promoter. In some embodiments, the promoter is operably linked to the polynucleotide encoding a lysosomal enzyme or the polynucleotide encoding a modified GlcNAc-1 PTase. In some embodiments, the promoter is operably linked to the polynucleotide encoding a lysosomal enzyme and the polynucleotide encoding a modified GlcNAc-1 PTase.

[086] In some embodiments of the compositions of the disclosure, the bicistronic vector comprises a nucleic acid sequence of SEQ ID NO: 1.

[087] In some embodiments of the compositions of the disclosure, the polynucleotide

encoding a modified GlcNAc-1 phosphotransferase comprises a nucleic acid sequence of SEQ ID NO: 4.

[088] In some embodiments of the compositions of the disclosure, the encoded lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1. In some embodiments, the encoded lysosomal enzyme or a variant thereof causes at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C. In some embodiments, an activity or a function of the encoded lysosomal enzyme or a variant thereof is decreased, inhibited or deregulated in at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C.

[089] In some embodiments of the compositions of the disclosure, the lysosomal enzyme comprises a lysosomal enzyme listed in Table 1A, Table 1B or Table 1C. In some embodiments, the lysosomal enzyme comprises at least one lysosomal enzyme listed in Table 1A, Table 1B or Table 1C. In some embodiments, the lysosomal enzyme comprises one or more lysosomal enzyme(s) listed in Table 1A, Table 1B or Table 1C. In some embodiments, the lysosomal enzyme is selected from the group consisting of β-glucocebrosidase (GCase, GBA), Galactosylceremidase (GALC), α-Galactosidase (GLA), α-N-acetylglucosaminidase (NAGLU), acid α-glucosidase (GAA) and lysosomal acid α-mannosidase (LAMAN). In some embodiments, the lysosomal enzyme comprises β-glucocebrosidase (GCase, GBA). In some embodiments, the lysosomal enzyme comprises Galactosylceremidase (GALC). In some embodiments, the lysosomal enzyme comprises α-Galactosidase (GLA). In some embodiments, the lysosomal enzyme comprises α-N-acetylglucosaminidase (NAGLU). In some embodiments, the lysosomal enzyme comprises acid α-glucosidase (GAA). In some embodiments, the lysosomal enzyme comprises lysosomal acid α-mannosidase (LAMAN). In some embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NOs: 5-10.

[090] The disclosure provides a composition comprising a bicistronic vector comprising a constitutive promoter, an Internal Ribosome Entry Site (IRES) and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase).

[091] In some embodiments of the compositions of the disclosure, the composition further comprises a pharmaceutically-acceptable carrier.

[092] In some embodiments of the vectors of the disclosure, the vector is a viral vector. In some embodiments, the viral vector is an adenovirus, an adeno-associated viruses (AAV), a retrovirus or a lentivirus. In some embodiments, the viral vector comprises an adenovirus. In some embodiments, the viral vector comprises an AAV vector. In some

embodiments, the AAV vector comprises a sequence isolated or derived from one or more AAV of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 and AAV9. In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 1 (AAV1). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 2 (AAV2). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 3 (AAV3). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 4 (AAV4). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 5 (AAV5). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 6 (AAV6). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 7 (AAV7). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 8 (AAV8). In some embodiments, the AAV vector comprises a sequence isolated or derived from an AAV of serotype 9 (AAV9).

[093] In some embodiments of the vectors of the disclosure, the vector is an expression vector. In some embodiments, the expression vector comprises the polynucleotide sequence of SEQ ID NO: 1.

[094] The disclosure provides a cell comprising a vector of the disclosure. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a primate cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a cultured cell. In some embodiments, the cell is an immortalized or stabilized cell line. In some embodiments, the cell is a Chinese hamster ovary (CHO) cell. In some embodiments, the cell is a Human embryonic kidney 293 (HEK293) cell.

[095] The disclosure provides a cell comprising a bicistronic vector of the disclosure. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a primate cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a cultured cell. In some embodiments, the cell is an immortalized or stabilized cell line. In some embodiments, the cell is a Chinese hamster ovary (CHO) cell. In some embodiments, the cell is a Human embryonic kidney 293 (HEK293) cell.

[096] The disclosure provides a cell comprising composition of the disclosure. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a primate cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a cultured cell. In some embodiments, the cell is an immortalized or stabilized cell line. In some embodiments, the cell is a Chinese hamster ovary (CHO) cell. In some

embodiments, the cell is a Human embryonic kidney 293 (HEK293) cell.

[097] The disclosure provides a pharmaceutical composition comprising a lysosomal enzyme expressed by a vector of the disclosure and a pharmaceutically acceptable carrier.

[098] The disclosure provides a method of treating a lysosomal storage disorder (LSD), the method comprising administering to a subject a composition of the disclosure, thereby treating the LSD.

[099] The disclosure provides a method of treating a lysosomal storage disorder (LSD), the method comprising administering to a subject a therapeutically-effective amount of a composition of the disclosure, wherein the composition increases phosphorylation of a lysosomal enzyme, thereby treating the LSD.

[0100] The disclosure provides a method of treating a subject suffering from a lysosomal storage disorder (LSD), the method comprising administering to the subject a pharmaceutical composition of the disclosure, thereby increasing the phosphorylation of a lysosomal enzyme and treating the subject.

[0101] The disclosure provides a method of preventing the occurrence of a lysosomal storage disorder (LSD) in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition of the disclosure, thereby increasing the phosphorylation of a lysosomal enzyme and preventing the occurrence of a LSD in the subject.

[0102] The disclosure provides a method of ameliorating the phosphorylation of a lysosomal enzyme responsible for a lysosomal storage disorder (LSD) in a subject in need thereof, the method comprising administering to the subject a composition of the disclosure, wherein the composition increases the phosphorylation of the lysosomal enzyme.

[0103] In some embodiments of the methods of the disclosure, the lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C.

[0104] In some embodiments of the methods of the disclosure, the lysosomal enzyme comprises a lysosomal storage disorder (LSD) listed in Table 1A, Table 1B or Table 1C. In some embodiments, the lysosomal enzyme comprises at least one lysosomal storage disorder (LSD) listed in Table 1A, Table 1B or Table 1C. In some embodiments, the lysosomal enzyme comprises one or more lysosomal storage disorder(s) (LSD(s)) listed in Table 1A, Table 1B or Table 1C.

Enzyme Replacement Therapy (ERT)

[0105] Provided herein are compositions comprising a bicistronic expression vector comprising a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase). In some embodiments, the disclosed bicistronic expression vector comprises an Internal Ribosome Entry Site (IRES) located before the polynucleotide encoding a modified GlcNAc-1 PTase and after the polynucleotide encoding a lysosomal enzyme. In other embodiments, the disclosed bicistronic expression vector comprises an IRES located after the polynucleotide encoding a modified GlcNAc-1 PTase and before the polynucleotide encoding a lysosomal enzyme.

[0106] Provided herein are mammalian cells comprising the disclosed bicistronic expression vector.

[0107] Provided herein are pharmaceutical composition comprising a lysosomal enzyme expressed by the biscistronic vector as disclosed herein and a pharmaceutically acceptable carrier.

[0108] Provided herein are methods for treating a subject suffering from a lysosomal storage disorder (LSD) and methods preventing the occurrence of a lysosomal storage disorder (LSD) in a subject in need thereof.

Gene Therapy

[0109] Provided herein are compositions comprising a bicistronic viral vector comprising a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase). In some embodiments, the disclosed bicistronic viral vector comprises an Internal Ribosome Entry Site (IRES) located before the polynucleotide encoding a modified GlcNAc-1 PTase and after the polynucleotide encoding a lysosomal enzyme. In other embodiments, the disclosed bicistronic viral vector comprises an IRES located after the polynucleotide encoding a modified GlcNAc-1 PTase and before the polynucleotide encoding a lysosomal enzyme. In some embodiments, the viral vector is an adenovirus, an adeno-associated viruses (AAV), a retrovirus or a lentivirus.

[0110] Provided herein are methods for treating a subject suffering from a lysosomal storage disorder (LSD) and methods preventing the occurrence of a lysosomal storage disorder (LSD) in a subject in need thereof by administering to the subject the disclosed bicistronic viral vector.

[0111] Further provided herein are methods for ameliorating the phosphorylation of a lysosomal enzyme responsible for an LSD in a subject in need thereof.

[0112] Provided herein are compositions and methods of using a bicistronic vector for

treating or preventing a lysosomal storage disorder (LSD) in a subject.

[0113] The disclosure provides compositions comprising a bicistronic vector comprising a promoter, an Internal Ribosome Entry Site (IRES), a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase). Methods of the disclosure comprise administering to a subject a pharmaceutical composition comprising the bicistronic vector as disclosed herein.

Definitions

[0114] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

[0115] It is also to be understood that the terminology used herein is for the purpose of describing some embodiments only, and is not intended to be limiting.

[0116] As used herein, the articles "a" and "an" are used to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0117] As used herein when referring to a measurable value such as an amount, a temporal duration, and the like, the term "about" is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0118] The terms "2A" or "2A peptide" or "2A-like peptide" is a self-processing viral peptide. The 2A peptide can separate different protein coding sequences in a single ORF transcription unit (Ryan et al., 1991, J Gen Virol 72:2727-2732). Although termed a "self-cleaving" peptide or protease site, the mechanism by which the 2A sequence generates two proteins from one transcript occurs by ribosome skipping where a normal peptide bond is impaired at 2A, resulting in two discontinuous protein fragments from one translation event. Linking with 2A peptide sequences results in cellular expression of multiple, discrete proteins (in essentially equimolar quantities) derived from a single ORF (de Felipe et al., 2006, Trends Biotechnol 24:68-75).

[0119] The term "biological" or "biological sample" refers to a sample obtained from an organism or from components (e.g., cells) of an organism. The sample may be of

any biological tissue or fluid. Frequently the sample will be a "clinical sample" which is a sample derived from a patient. Such samples include, but are not limited to, bone marrow, cardiac tissue, sputum, blood, lymphatic fluid, blood cells (e.g., white cells), tissue or fine needle biopsy samples, urine, peritoneal fluid, and pleural fluid, or cells therefrom. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes.

[0120] As used herein, the terms "derivative" specifies that a derivative of a virus can have a nucleic acid or amino acid sequence difference in respect to a template viral nucleic acid or amino acid sequence.

[0121] A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

[0122] In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

[0123] "Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide. In some embodiments, the disclosed vector is referred herein as a viral vector. In some embodiments, the disclosed vector is referred herein as an expression vector.

[0124] As used herein, "higher" refers to expression levels which are at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% higher or more, and/or 1.1 fold, 1.2 fold, 1.4 fold, 1.6 fold, 1.8 fold, 2.0 fold higher or more, and any and all whole or partial increments therebetween, than a control reference. A disclosed herein an expression level higher than a reference value refers to an expression level (mRNA or protein) that is higher than a normal or control level from an expression (mRNA or protein) measured in a healthy subject or defined or used in the art.

[0125] As used herein, "lower" refers to expression levels which are at least 10% lower or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% lower or

more, and/or 1.1 fold, 1.2 fold, 1.4 fold, 1.6 fold, 1.8 fold, 2.0 fold lower or more, and any and all whole or partial increments in between, than a control reference. A disclosed herein an expression level lower than a reference value refers to an expression level (mRNA or protein) that is lower than a normal or control level from an expression (mRNA or protein) measured in a healthy subject or defined or used in the art.

[0126] As used herein, the terms "control," or "reference" can be used interchangeably and refer to a value that is used as a standard of comparison.

[0127] As used herein, by "combination therapy" is meant that a first agent is administered in conjunction with another agent. "In combination with" or "In conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in combination with" refers to administration of one treatment modality before, during, or after delivery of the other treatment modality to the individual. Such combinations are considered to be part of a single treatment regimen or regime. For example, a vector or a composition comprising a vector of the disclosure may be provided or administered to a subject in combination with a second therapeutic agent. In some embodiments the vectors and compositions of the disclosure are provided or administered to a subject simultaneously or sequentially with the second therapeutic agent. In some embodiments the vectors and compositions of the disclosure are provided or administered to a subject simultaneously with the second therapeutic agent. In some embodiments the vectors and compositions of the disclosure are provided or administered to a subject sequentially with the second therapeutic agent. In some embodiments the vectors and compositions of the disclosure are provided or administered to a subject prior to administration of the second therapeutic agent. In some embodiments the vectors and compositions of the disclosure are provided or administered to a subject following administration of the second therapeutic agent. In some embodiments, the second therapeutic agent comprises a second vector of composition of the disclosure. In some embodiments, the second therapeutic agent comprises a variant form of a lysosomal enzyme of the disclosure, including a vector or a composition of the disclosure encoding same. In some embodiments, the second therapeutic agent comprises one or more agents to alleviate a sign or symptom of a lysosomal storage disorder. In some embodiments, the second therapeutic agent comprises one or more anti-inflammatory or immunosuppressive agents.

[0128] The term "operably linked," as used herein, means that expression of a nucleic

acid sequence is under the control of a promoter with which it is spatially connected. A promoter may be positioned 5' (upstream) of the nucleic acid sequence under its control.

[0129] As used herein, "primary cells" refer to cells taken directly from living tissue (i.e. biopsy material) and established for growth in vitro, that have undergone very few population doublings and are therefore more representative of the main functional components and characteristics of tissues from which they are derived from, in comparison to continuous tumorigenic or artificially immortalized cell lines.

[0130] As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that may comprise a protein or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[0131] The term "promoter" as used herein, may mean a synthetic or naturally-derived molecule that is capable of conferring, activating or enhancing expression of a nucleic acid. As used herein, the promoter is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

[0132] As used herein, the term "promoter/regulatory sequence" means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

[0133] A "constitutive" promoter is a nucleotide sequence which, when operably

linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

[0134] An "inducible" promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

[0135] The term "RNA" as used herein is defined as ribonucleic acid.

[0136] The term "treatment" as used within the context of the present invention is meant to include therapeutic treatment as well as prophylactic, or suppressive measures for the disease or disorder. As used herein, the term "treatment" and associated terms such as "treat" and "treating" means the reduction of the progression, severity and/or duration of a disease condition or at least one symptom thereof. The term 'treatment' therefore refers to any regimen that can benefit a subject. The treatment may be in respect of an existing condition or may be prophylactic (preventative treatment). Treatment may include curative, alleviative or prophylactic effects. References herein to "therapeutic" and "prophylactic" treatments are to be considered in their broadest context. The term "therapeutic" does not necessarily imply that a subject is treated until total recovery. Similarly, "prophylactic" does not necessarily mean that the subject will not eventually contract a disease condition. Thus, for example, the term treatment includes the administration of an agent prior to or following the onset of a disease or disorder thereby preventing or removing all signs of the disease or disorder. As another example, administration of the agent after clinical manifestation of the disease to combat the symptoms of the disease comprises "treatment" of the disease.

[0137] As used herein, the term "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides. ESTs, chromosomes, cDNAs, mRNAs, and rRNAs are representative examples of molecules that may be referred to as nucleic acids.

[0138] As used herein, the term "pharmaceutical composition" refers to a mixture of at least one compound useful within the invention with other chemical components, such as carriers, stabilizers, diluents, adjuvants, dispersing agents, suspending agents,

thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to: intra-tumoral, intravenous, intrapleural, oral, aerosol, parenteral, ophthalmic, pulmonary and topical administration.

[0139] The language "pharmaceutically acceptable carrier" includes a pharmaceutically acceptable salt, pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound(s) of the present invention within or to the subject such that it may perform its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each salt or carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, and not injurious to the subject. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; diluent; granulating agent; lubricant; binder; disintegrating agent; wetting agent; emulsifier; coloring agent; release agent; coating agent; sweetening agent; flavoring agent; perfuming agent; preservative; antioxidant; plasticizer; gelling agent; thickener; hardener; setting agent; suspending agent; surfactant; humectant; carrier; stabilizer; and other non-toxic compatible substances employed in pharmaceutical formulations, or any combination thereof. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound, and are physiologically acceptable to the subject. Supplementary active compounds may also be incorporated into the compositions.

[0140] As used herein, the term "effective amount" or "therapeutically effective amount" means the amount of the virus particle or infectious units generated from

vector of the invention which is required to prevent the particular disease condition, or which reduces the severity of and/or ameliorates the disease condition or at least one symptom thereof or condition associated therewith.

[0141] A "subject" or "patient," as used therein, may be a human or non-human mammal. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. Preferably, the subject is a human.

[0142] Ranges: throughout this disclosure, some embodiments can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Compositions

[0143] Provided herein are compositions and methods for treating or preventing a lysosomal storage disorder (LSD) in a subject by administering to the subject a pharmaceutical comprising a bicistronic expression vector.

[0144] In some embodiments, the disclosure provides a composition comprising a bicistronic vector comprising a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase). In one embodiment, the polynucleotide encoding a lysosomal enzyme and the polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) are operably linked.

[0145] In some embodiments, the disclosure provides a composition comprising a bicistronic vector comprising a constitutive promoter, an Internal Ribosome Entry Site (IRES) and a polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase).

[0146] In some embodiments, the bicistronic vector comprises an IRES located before the polynucleotide encoding a modified GlcNAc-1 PTase and after the polynucleotide encoding a lysosomal enzyme. In other embodiments, the bicistronic vector comprises an IRES located after the polynucleotide encoding a modified GlcNAc-1 PTase and

before the polynucleotide encoding a lysosomal enzyme.

[0147] The sequence of the IRES can be a sequence known in the art or a variant thereof. The IRES variant be a can be modified or mutated. In one embodiment, the sequence IRES comprises SEQ ID NO: 3. In other embodiment, the sequence of the IRES is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% similar to SEQ ID NO: 3.

[0148] In one embodiment, the polynucleotide of a lysosomal enzyme is operably linked to a 2A DNA encoding a 2A peptide, which is in turn operably the polynucleotide of a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase). Various 2A peptides known in the art can be used in the disclosed bicistronic vector including but not limited to T2A, P2A, E2A and F2A. In some embodiments, the addition of GSG residues can be added to the 5'end of the peptide to improve cleavage efficiency.

[0149] In some embodiments, the bicistronic viral vector comprises a promoter operably linked to the polynucleotide encoding a lysosomal enzyme and the polynucleotide encoding a modified GlcNAc-1 PTase.

[0150] In some embodiments, the bicistronic expression vector comprises a promoter.

[0151] A promoter may be constitutive, inducible/repressible or cell type specific. In certain embodiments, the promoter may be constitutive. Non-limiting examples of constitutive promoters for mammalian cells include CMV, UBC, EF1 a, SV40, PGK, CAG, CBA/CAGGS/ACTB, CBh, MeCP2, U6 and H1. In some embodiments, the presently disclosed bicistronic vector comprises a constitutive promoter. In some embodiments, the constitutive promoter is a Cytomegalovirus (CMV) promoter. In some embodiments, the polynucleotide of CMV promoter comprises a nucleic acid sequence of SEQ ID NO: 2.

[0152] In other embodiments, the promoter may be an inducible promoter. The inducible promoter may be selected from the group consisting of: tetracycline, heat shock, steroid hormone, heavy metal, phorbol ester, adenovirus E1A element, interferon, and serum inducible promoters.

[0153] In different embodiments, the promoter may be cell type specific. For example, cell type specific promoters for neurons (e.g. syapsin), astrocytes (e.g. GFAP), oligodendrocytes (e.g. myelin basic protein), microglia (e.g. CX3CR1), neuroendocrine cells (e.g. chromogranin A), muscle cells (e.g. desmin, Mb), or cardiomyocytes (e.g. alpha myosin heavy-chain promoter) could be used. In an exemplary embodiment, a promoter may be the Nrl (rod photoreceptor-specific)

promoter or the HBB (haemoglobin beta) promoter. A promoter may further comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of a nucleic acid.

[0154] Enhancer sequences found on a vector also regulates expression of the gene contained therein. Typically, enhancers are bound with protein factors to enhance the transcription of a gene. Enhancers may be located upstream or downstream of the gene it regulates. Enhancers may also be tissue-specific to enhance transcription in a specific cell or tissue type. In one embodiment, the present bicistronic vector comprises one or more enhancers to boost transcription of the gene present within the vector. Non- limiting examples of enhancer include the CMV enhancer and the SP1 enhancer.

[0155] In some embodiments more than one promoter can be operably linked to each polynucleotide encoding a polypeptide, the promoters may be the same or different. The distance between the promoter and a nucleic acid sequence to be expressed may be approximately the same as the distance between that promoter and the native nucleic acid sequence it controls. As is known in the art, variation in this distance may be accommodated without loss of promoter function.

[0156] In order to assess the expression of the polypeptides within the bicistronic vector, the vector can also comprise 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 some embodiments, the selectable marker may be carried on a separate piece of DNA and used in a co- transfection procedure. Both selectable markers and reporter genes may be 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.

[0157] Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green

fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter- driven transcription.

[0158] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be 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 can be transferred into a host cell by physical, chemical, or biological means.

[0159] Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

[0160] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[0161] Chemical means 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, 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).

[0162] In some embodiments in which 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 some embodiments, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be 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, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be 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.

[0163] Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, MO; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, NY); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Stock solutions of lipids in chloroform or chloroform/methanol can be 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 can be 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 may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

[0164] Regardless of the method used to introduce exogenous nucleic acids into a host

cell, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, 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 of the disclosure.

Vectors for Gene Therapy

[0165] The vectors to be used for treating or preventing LSDs in a subject as disclosed herein, are suitable for replication and, optionally, integration in eukaryotic cells. Typical vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0166] The vectors of the present disclosure may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the disclosure provides a gene therapy vector.

[0167] The isolated nucleic acid of the disclosure can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[0168] Further, the vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), 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).

[0169] A number of viral 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 can be inserted into a vector and packaged in

retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

[0170] For example, 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 vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. In a preferred embodiment, the composition includes a vector derived from an adeno-associated virus (AAV). Adeno-associated viral (AAV) vectors have become powerful gene delivery tools for the treatment of various disorders. AAV vectors possess a number of features that render them ideally suited for gene therapy, including a lack of pathogenicity, minimal immunogenicity, and the ability to transduce postmitotic cells in a stable and efficient manner. Expression of a particular gene contained within an AAV vector can be specifically targeted to one or more types of cells by choosing the appropriate combination of AAV serotype, promoter, and delivery method

[0171] In some embodiments, the disclosed bicistronic viral vector comprises an adenovirus (e.g. Ad-SYE, AdSur-SYE, Ad5/3-MDA7/IL-24, Ad-SB, Ad-CRISPR, oncolytic Ad); an adeno-associated virus, AAV (e.g. AAV-MeCP2, AAV1, AAV5, Dual AAV9 AAV8, AAV9, AAVrh10, AAVhu37); a herpes simplex virus, HSV (e.g. HSV1, HSV2, HSV-1, HF10 Oncolytic HSV-2); a Rretrovirus (e.g. RRV/ Toca 511, GRV); a lentivirus (e.g. HIV-1, HIV-2); an alphavirus (SFV, M1); a flavivirus (Kunjin virus); a rhabdovirus (VSV); a measles virus (e.g. MV-Edm); a Newcastle disease virus (e.g. NDV90); an anhinga Picornaviruses Coxsackievirus (e.g. CVB3, CAV21, EV1); or a poxvirus (e.g. PANVAC, VV, VV-GLV-1h153, CPXV).

[0172] In one embodiment the disclosed bicistronic viral vector is an adenovirus, an adeno-associated viruses (AAV), an alphavirus, a flavivirus, a herpes simplex virus (HSV), a measles virus, a rhabdovirus, a retrovirus, a lentivirus, a Newcastle disease virus (NDV), a poxvirus, or a picornavirus. In one embodiment the disclosed bicistronic viral vector is an adenovirus, an adeno-associated viruses (AAV), a retrovirus or a lentivirus.

[0173] In one embodiment, the polynucleotide encoding a lysosomal enzyme and a

polynucleotide encoding a modified GlcNAc-1 PTase are contained within an AAV vector. More than 30 naturally occurring serotypes of AAV are available. Many natural variants in the AAV capsid exist, allowing identification and use of an AAV with properties specifically suited for skeletal muscle. AAV viruses may be engineered using conventional molecular biology techniques, making it possible to optimize these particles for cell specific delivery of nucleic acid sequences, for minimizing immunogenicity, for tuning stability and particle lifetime, for efficient degradation, for accurate delivery to the nucleus, to name a few.

[0174] The use of AAVs is a common mode of exogenous delivery of DNA as it is relatively non-toxic, provides efficient gene transfer, and can be easily optimized for specific purposes. Among the serotypes of AAVs isolated from human or non-human primates (NHP) and well characterized, human serotype 2 is the first AAV that was developed as a gene transfer vector; it has been widely used for efficient gene transfer experiments in different target tissues and animal models. Clinical trials of the experimental application of AAV2 based vectors to some human disease models are in progress, and include therapies for diseases such as for example, cystic fibrosis and hemophilia B. Other useful AAV serotypes include AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 and AAV9.

[0175] Desirable AAV fragments for assembly into vectors include the cap proteins, including the vp1, vp2, vp3 and hypervariable regions, the rep proteins, including rep 78, rep 68, rep 52, and rep 40, and the sequences encoding these proteins. These fragments may be readily utilized in a variety of vector systems and host cells. Such fragments may be used alone, in combination with other AAV serotype sequences or fragments, or in combination with elements from other AAV or non-AAV viral sequences. As used herein, artificial AAV serotypes include, without limitation, AAV with a non-naturally occurring capsid protein. Such an artificial capsid may be generated by any suitable technique, using a selected AAV sequence (e.g., a fragment of a vp1 capsid protein) in combination with heterologous sequences which may be obtained from a different selected AAV serotype, non-contiguous portions of the same AAV serotype, from a non-AAV viral source, or from a non-viral source. An artificial AAV serotype may be, without limitation, a chimeric AAV capsid, a recombinant AAV capsid, or a "humanized" AAV capsid. Thus exemplary AAVs, or artificial AAVs, suitable for expression of a lysosomal enzyme of interest and a modified GlcNAc-1 PTase, include AAV2/8 (see U.S. Pat. No. 7,282,199), AAV2/5 (available from the National Institutes of Health), AAV2/9 (International Patent Publication No.

WO2005/033321), AAV2/6 (U.S. Pat. No. 6,156,303), and AAVrh8 (International Patent Publication No. WO2003/042397), among others.

[0176] In one embodiment, the vectors useful in the compositions and methods described herein contain, at a minimum, sequences encoding a selected AAV serotype capsid, e.g., an AAV8 capsid, or a fragment thereof. In another embodiment, useful vectors contain, at a minimum, sequences encoding a selected AAV serotype rep protein, e.g., AAV8 rep protein, or a fragment thereof. Optionally, such vectors may contain both AAV cap and rep proteins. In vectors in which both AAV rep and cap are provided, the AAV rep and AAV cap sequences can both be of one serotype origin, e.g., all AAV8 origin. Alternatively, vectors may be used in which the rep sequences are from an AAV serotype which differs from that which is providing the cap sequences. In one embodiment, the rep and cap sequences are expressed from separate sources (e.g., separate vectors, or a host cell and a vector). In another embodiment, these rep sequences are fused in frame to cap sequences of a different AAV serotype to form a chimeric AAV vector, such as AAV2/8 described in U.S. Pat. No. 7,282,199.

[0177] A suitable recombinant adeno-associated virus (AAV) is generated by culturing a host cell which contains a nucleic acid sequence encoding an adeno-associated virus (AAV) serotype capsid protein, or fragment thereof, as defined herein; a functional rep gene; a minigene composed of, at a minimum, AAV inverted terminal repeats (ITRs) and a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a modified GlcNAc-1 PTase; and sufficient helper functions to permit packaging of the minigene into the AAV capsid protein. The components required to be cultured in the host cell to package an AAV minigene in an AAV capsid may be provided to the host cell in trans. Alternatively, any one or more of the required components (e.g., minigene, rep sequences, cap sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or more of the required components using methods known to those of skill in the art.

[0178] Most suitably, such a stable host cell will contain the required component(s) under the control of a constitutive promoter. However, the required component(s) may be under the control of an inducible promoter. Examples of suitable inducible and constitutive promoters are provided elsewhere herein, and are well known in the art. In still another alternative, a selected stable host cell may contain selected component(s) under the control of a constitutive promoter and other selected component(s) under the control of one or more inducible promoters. For example, a stable host cell may be

generated which is derived from 293 cells (which contain E1 helper functions under the control of a constitutive promoter), but which contains the rep and/or cap proteins under the control of inducible promoters. Still other stable host cells may be generated by one of skill in the art.

[0179] The minigene, rep sequences, cap sequences, and helper functions required for producing the rAAV of the disclosure may be delivered to the packaging host cell in the form of any genetic element which transfers the sequences carried thereon. The selected genetic element may be delivered using any suitable method, including those described herein and any others available in the art. The methods used to construct any embodiment of this disclosure are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques (see, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y). Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present disclosure (see, e.g., K. Fisher et al, 1993 J. Virol., 70:520-532 and U.S. Pat. No. 5,478,745, among others).

[0180] Unless otherwise specified, the AAV ITRs, and other selected AAV components described herein, may be readily selected from among any AAV serotype, including, without limitation, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9 or other known or as yet unknown AAV serotypes. These ITRs or other AAV components may be readily isolated from an AAV serotype using techniques available to those of skill in the art. Such an AAV may be isolated or obtained from academic, commercial, or public sources (e.g., the American Type Culture Collection, Manassas, Va.). Alternatively, the AAV sequences may be obtained through synthetic or other suitable means by reference to published sequences such as are available in the literature or in databases such as, e.g., GenBank, PubMed, or the like.

[0181] In some embodiments, the bicistronic vector comprises a nucleic acid sequence of SEQ ID NO: 1. In other embodiments, the bicistronic vector comprises a nucleic acid sequence having at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% similarity with SEQ ID NO: 1.

[0182] In some embodiments, the encoded lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C below. In other embodiments, the lysosomal enzyme is at least one as listed in Table 1A, Table 1B or Table 1C below.

[0183] Table 1A – ERT Embodiment (enzymes with (Uniprot Accession Nos.))

ENZYMES INVOLVED IN LYSOSOMAL STORAGE DISORDERS	SEQ ID NOs:	DISEASE (LSD)
1. DEFECTS IN GLYCAN DEGRADATION		
1.1. Defects in glycoprotein degradation		
Neuraminidase Q99519	24 and 25	Sialidosis, Type I&II
Cathepsin A P10619	26 and 27	Galactosialidosis
α-Mannosidase O00754	28 and 29	α-Mannosidosis, types I and II
β-Mannosidase O00462	30 and 31	β-Mannosidosis
Glycosylasparaginase P20933	32 and 33	Aspartylglucosaminuria
α-L-Fucosidase P04066	34 and 35	Fucosidosis
α-N-Acetylglucosaminidase P54802	36 and 37	Kanzaki disease, Schindler disease, TypeI&III
1.2. Defects in glycolipid degradation		
1.2a. GM1 Ganglioside		
β-Galactosidase-1 P16278		GM1 gangliosidosis Type I, II & III
Hexosaminidase α-subunit P06865	38 and 39	GM2-gangliosidosis, Tay-Sachs disease
Hexosaminidase β-subunit P07686	40 and 41	GM2-gangliosidosis, Sandhoff disease
GM2 activator protein P17900	42 and 43	GM2 gangliosidosis, AB viriant
Acid beta-glucosidase P04062	44 and 45	Gaucher disease
Saposin C P07602	46 and 47 48, 49 and 50	Gaucher disease, atypical
1.2b. Defects in the degradation of sulfatide		
Arylsulfatase A P15289	52 and 53	Metachromatic leukodystrophy
Saposin B P07602	48, 49 and 51	Metachromatic leukodystrophy
sulfatase-modifying factor-1 Q8NBK3	54 and 55	Multiple sulfatase deficiency
Galactosylceramidase P54803	56 and 57	Krabbe disease
1.2c. Defects in degradation of globotriaosylceramide		
alpha-galactosidase A P06280	58 and 59	Fabry
1.3. Defects in degradation of Glycosaminoglycan (Mucopolysaccharidoses)		
1.3a. Degradation of heparan sulphate		
Iduronate 2-sulfatase P22304	60 and 61	MPS II (Hunter)
alpha-L-iduronidase P35475	62 and 63	MPS I (Hurler, Scheie)
N-sulfoglucosamine sulfohydrolase P51688	64 and 65	MPS IIIa (Sanfilippo A)
heparan acetyl-CoA:alpha-glucosaminide N-acetyltransferase Q68CP4	66 and 67	MPS IIIc (Sanfilippo C)
N-alpha-acetylglucosaminidase P54802		MPS IIIb (Sanfilippo B)

	36 and 37	
β-glucuronidase P08236	68 and 69	MPS VII (Sly)
N-acetyl glucosamine 6-sulfatase P15586	70 and 71	MPS IIId (Sanfilippo D)
1.3bDegradation of other mucopolysaccharides		
N-Acetylgalactosamine 4-sulfatase P15848	72 and 73	MPS VI
galactosamine-6-sulfate sulfatase P34059	129 and 130	MPS IVA (Morquio A)
Hyaluronidase 1 Q12794	74 and 75	MPS IX
1.4. Defects in degradation of glycogen		
acid alpha-1,4-glucosidase P10253	76 and 77	Pompe
2. DEFECTS IN LIPID DEGRADATION		
2.1 Defects in degradation of sphingomyelin		
acid sphingomyelinase P17405	78 and 79	Niemann Pick type A and B
Acid ceramidase Q13510	80 and 81	Farber lipogranulomatosis
2.2 Defects in degradation of triglycerides and cholesteryls ester		
Acid lipase P38571	82 and 83	Wolman and cholesteryl ester storage disease
3. DEFECTS IN PROTEIN DEGRADATION		
Cathepsin K P43235	84 and 85	Pycnodysostosis
Tripeptidyl peptidase O14773	86 and 87	Ceroide lipofuscinosis 2
Palmitoyl-protein thioesterase 1 P50897	88 and 89	Ceroide lipofuscinosis 1
4. DEFECTS IN LYSOSOMAL TRANSPORTERS		
Cystinosin (cystin transport) O60931	90 and 91	Cystinosis
SOLUTE CARRIER FAMILY 17 (ACIDIC SUGAR TRANSPORTER), MEMBER 5 H0UI05	92 and 93	Salla disease
5. DEFECTS IN LYSOSOMAL TRAFFICKING PROTEINS		
UDP-N-acetylglucosamine Q96950	94 and 95	
N-acetylglucosamine-1-phosphotransferase γ-subunit Q9UJJ9	96 and 97	Mucolipidosis III gamma (I-cell)
N-acetylglucosamine-1-phosphotransferase alpha/beta-subunits Q3T906	98 and 99	Mucolipidosis III alpha/beta
Mucolipin-1(cation channel) Q9GZU1	100 and 101	Mucolipidosis IV
Lysosome-associated membrane protein 2 (LAMP-2) P13473	102 and 103	Danon
Niemann-Pick C1 O15118	104 and 105	Niemann Pick type C1 & D
Epididymal secretory protein HE1 P61916	106 and 107	Niemann-pick disease, type C2
ceroid lipofuscinosis-3 Q13286	108 and 109	Ceroid lipofuscinosis, neuronal, 3
ceroid lipofuscinosis-6 Q9NWW5	110 and 111	Ceroid lipofuscinosis 6
ceroid lipofuscinosis-8 Q9UBY8	112 and 113	Ceroid lipofuscinosis 8
Lysosomal trafficking regulator Q99698	114 and 115	Chediak-Higashi

myosin 5A Q9Y4I1	116 and 117	Griscelli Type 1
Ras-associated protein RAB27A P51159	118 and 119	Griscelli Type 2
Melanophilin Q9BV36	120 and 121	Griscelli Type 3
AP3 β-subunit O00203	122 and 123	Hermansky Pudliak 2

[0184] Table 1B – Gene Therapy Embodiment (enzymes with (Uniprot Accession Nos.))

ENZYMES INVOLVED IN LYSOSOMAL STORAGE DISORDERS	SEQ ID NO:	DISEASE (LSD)
1. DEFECTS IN GLYCAN DEGRADATION		
1.1. Defects in glycoprotein degradation		
Neuraminidase Q99519	24 and 25	Sialidosis, Type I&II
Cathepsin A P10619	26 and 27	Galactosialidosis
α-Mannosidase O00754	28 and 29	α-Mannosidosis, types I and II
β-Mannosidase O00462	30 and 31	β-Mannosidosis
Glycosylasparaginase P20933	32 and 33	Aspartylglucosaminuria
α-L-Fucosidase P04066	34 and 35	Fucosidosis
α-N-Acetylglucosaminidase P54802	36 and 37	Kanzaki disease, Schindler disease, TypeI&III
Neuraminidase Q99519	24 and 25	
1.2. Defects in glycolipid degradation		
1.2a. GM1 Ganglioside		
β-Galactosidase-1 P16278		GM1 gangliosidosis Type I, II & III
Hexosaminidase α-subunit P06865	38 and 39	GM2-gangliosidosis, Tay-Sachs disease
Hexosaminidase β-subunit P07686	40 and 41	GM2-gangliosidosis, Sandhoff disease
GM2 activator protein P17900	42 and 43 44 and 45	GM2 gangliosidosis, AB viriant
Acid beta-glucosidase P04062	46 and 47	Gaucher disease
Saposin C P07602	48, 49 and 50	Gaucher disease, atypical
1.2b. Defects in the degradation of sulfatide		
Arylsulfatase A P15289	52 and 53	Metachromatic leukodystrophy
Saposin B P07602	48, 49 and 51	Metachromatic leukodystrophy
sulfatase-modifying factor-1 Q8NBK3	54 and 55	Multiple sulfatase deficiency
Galactosylceramidase P54803	56 and 57	Krabbe disease
1.2c. Defects in degradation of globotriaosylceramide		
alpha-galactosidase A P06280	58 and 59	Fabry
Arylsulfatase A P15289	52 and 53	

1.3. Defects in degradation of Glycosaminoglycan (Mucopolysaccharidoses)		
1.3a. Degradation of heparan sulphate		
Iduronate 2-sulfatase P22304	60 and 61	MPS II (Hunter)
alpha-L-iduronidase P35475	62 and 63	MPS I (Hurler, Scheie)
N-sulfoglucosamine sulfohydrolase P51688	64 and 65	MPS IIIa (Sanfilippo A)
heparan acetyl-CoA:alpha-glucosaminide N-acetyltransferase Q68CP4	66 and 67	MPS IIIc (Sanfilippo C)
N-alpha-acetylglucosaminidase P54802	36 and 37	MPS IIIb (Sanfilippo B)
β-glucuronidase P08236	68 and 69	MPS VII (Sly)
N-acetyl glucosamine 6-sulfatase P15586	70 and 71	MPS IIId (Sanfilippo D)
1.3bDegradation of other mucopolysaccharides		
N-Acetylgalactosamine 4-sulfatase P15848	72 and 73	MPS VI
galactosamine-6-sulfate sulfatase P34059	129 and 130	MPS IVA (Morquio A)
Hyaluronidase 1 Q12794	74 and 75	MPS IX
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2. DEFECTS IN LIPID DEGRADATION		
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acid sphingomyelinase P17405	78 and 79	Niemann Pick type A and B
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2.2 Defects in degradation of triglycerides and cholesteryls ester		
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Tripeptidyl peptidase O14773	86 and 87	Ceroide lipofuscinosis 2
Palmitoyl-protein thioesterase 1 P50897	88 and 89	Ceroide lipofuscinosis 1
4. DEFECTS IN LYSOSOMAL TRANSPORTERS		
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SOLUTE CARRIER FAMILY 17 (ACIDIC SUGAR TRANSPORTER), MEMBER 5 H0UI05	92 and 93	Salla disease
5. DEFECTS IN LYSOSOMAL TRAFFICKING PROTEINS		
UDP-N-acetylglucosamine Q96950	94 and 95	
N-acetylglucosamine-1-phosphotransferase γ-subunit Q9UJJ9	96 and 97	Mucolipidosis III gamma (I-cell)
N-acetylglucosamine-1-phosphotransferase alpha/beta-subunits Q3T906	98 and 99	Mucolipidosis III alpha/beta
Mucolipin-1(cation channel) Q9GZU1	100 and 101	Mucolipidosis IV
Lysosome-associated membrane protein 2		Danon

(LAMP-2) P13473	102 and 103	
Niemann-Pick C1 O15118	104 and 105	Niemann Pick type C1 & D
Epididymal secretory protein HE1 P61916	106 and 107	Niemann-pick disease, type C2
ceroid lipofuscinosis-3 Q13286	108 and 109	Ceroid lipofuscinosis, neuronal, 3
ceroid lipofuscinosis-6 Q9NWW5	110 and 111	Ceroid lipofuscinosis 6
ceroid lipofuscinosis-8 Q9UBY8	112 and 113	Ceroid lipofuscinosis 8
Lysosomal trafficking regulator Q99698	114 and 115	Chediak-Higashi
myosin 5A Q9Y4I1	116 and 117	Griscelli Type 1
Ras-associated protein RAB27A P51159	118 and 119	Griscelli Type 2
Melanophilin Q9BV36	120 and 121	Griscelli Type 3
AP3 β-subunit O00203	122 and 123	Hermansky Pudliak 2

[0185] Table 1C – Lysosomal Disorders (Protein (UniProt Accession No.)

Clinical Name	Subtype	Protein	SEQ ID NO:	Gene	SEQ ID NO:	Exemplary Second Therapeutic Agent
activator deficiency, GM2-gangliosidosis; GM2-gangliosidosis, AB variant	AB variant GM2-gangliosidosis	GM2-activator protein (P17900)	45	GM2A	44	
alpha-mannosidosis	type 1, mild form	α-mannosidase (O00754)	29	MAN2B1	28	
	type 2, moderate form	α-mannosidase (O00754)	29	MAN2B1	28	
	type 3, neonatal, severe	α-mannosidase (O00754)	29	MAN2B1	28	
beta-mannosidosis	beta-mannosidosis	lysosomal ß-mannosidase (O00462)	31	MANBA	30	
aspartylglucosaminuria	aspartylglucosaminuria	Glycosylasparaginase (P20933)	33	AGA	32	
lysosomal acid lipase deficiency	cholesteryl ester storage disease (later-onset)	lysosomal acid lipase (P38571)	83	LIPA	82	sebelipase alfa (Kanuma™)
lysosomal acid lipase deficiency	Wolman disease (infantile)	lysosomal acid lipase (P38571)	83	LIPA	82	sebelipase alfa (Kanuma™)
cystinosis	adult nonnephropathic	Cystinosin (O60931)	91	CTNS	90	cysteamine (Cystagon, Procysbi)
	late-onset juvenile or adolescent nephropathic type	Cystinosin (O60931)	91	CTNS	90	cysteamine (Cystagon, Procysbi)
	infantile nephropathic	Cystinosin (O60931)	91	CTNS	90	
Chanarin-Dorfman syndrome	neutral lipid storage disease with ichthyosis; NLSDI	1-acylglycerol-3-phosphate O-acyltransferase (Q8WTS1)	160	CGI58, ABHD5	159	

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	neutral lipid storage disease with myopathy; NLSDM	adipose triglyceride lipase (Q96AD5)	125	PNPLA2	124	
Danon disease	Danon disease	lysosome-associated membrane protein-2 (P13473)	103	LAMP2	102	
Fabry disease	Fabry disease type I, classic	α-galactosidase A (P06280)	59	GLA	58	agalsidase beta (Fabrazyme®);migalastat (Galafold®)
	Fabry disease type II, late-onset	α-galactosidase A (P06280)	59	GLA	58	agalsidase beta (Fabrazyme®);migalastat (Galafold®)
Farber disease; Farber lipogranulomatosis	acid ceramidase deficiency	acid ceramidase (Q13510)	81	ASAH1	80	
fucosidosis	fucosidosis	α-L-fucosidase (P04066)	35	FUCA1	34	
galactosialidosis (combined neuraminidase & beta-galactosidase deficiency)	cathepsin A deficiency	protective protein/cathepsin A (P10619)	27	CTSA	26	
Gaucher disease	type I Gaucher disease	acid ß-glucosidase (P04062)	47	GBA	46	pharmacologic recombinant human glucocerebrosidase glycoproteins
	type II Gaucher disease	acid ß-glucosidase (P04062)	47	GBA	46	pharmacologic recombinant human glucocerebrosidase glycoproteins
	type III Gaucher disease	acid ß-glucosidase (P04062)	47	GBA	46	pharmacologic recombinant human glucocerebrosidase glycoproteins

	type IIIC Gaucher disease	acid ß-glucosidase (P04062)	47	GBA	46	pharmacologic recombinant human glucocerebrosidase glycoproteins
	Gaucher disease, atypical, due to saposin C deficiency	saposin C (P07602)	50 and 49	PSAP	48	
GM1-gangliosidosis	infantile GM1-gangliosidosis	ß-galactosidase-1 (P16278)	39	GLB1	38	
	late-infantile/juvenile GM1-gangliosidosis	ß-galactosidase-1 (P16278)	39	GLB1	38	
	adult/chronic GM1-gangliosidosis	ß-galactosidase-1 (P16278)	39	GLB1	38	
Globoid cell leukodystrophy, Krabbe disease	Early Infantile Onset	galactosylceramide ß-galactosidase (P54803)	57	GALC	56	hematopoietic stem cell transplantation using umbilical cord blood from healthy donors
	Late infantile onset	galactosylceramide ß-galactosidase (P54803)	57	GALC	56	
	Juvenile Onset	galactosylceramide ß-galactosidase (P54803)	57	GALC	56	
	Adult Onset	galactosylceramide ß-galactosidase (P54803)	57	GALC	56	
	Krabbe disease, atypical, due to saposin A deficiency	Saposin A (P07602)	126	PSAP	48	
Metachromatic Leukodystrophy	late infantile	arylsulfatase A (P15289)	53	ARSA	52	
	juvenile	arylsulfatase A (P15289)	53	ARSA	52	

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	adult	arylsulfatase A (P15289)	53	ARSA	52	
	partial cerebroside sulfate deficiency	arylsulfatase A (P15289)	53	ARSA	52	
	pseudoarylsulfatase A deficiency	arylsulfatase A (P15289)	53	ARSA	52	
	metachromatic leukodystrophy due to saposin B deficiency	saposin B	51	PSAP	48	
Mucopolysaccharidoses disorders:						
MPS I, Hurler syndrome		α-L-iduronidase (P35475)	63	IDUA	62	hematopoietic stem cell transplantation from healthy donors; & laronidase (Aldurazyme®)
MPS I, Hurler-Scheie syndrome		α-L-iduronidase (P35475)	63	IDUA	62	laronidase (Aldurazyme®)
MPS I, Scheie syndrome		α-L-iduronidase (P35475)	63	IDUA	62	laronidase (Aldurazyme®)
MPS II, Hunter syndrome	Classic severe / MPS IIA	iduronate 2-sulfatase (P22304)	61	IDS	60	
MPS II, Hunter syndrome	Attenuated / MPS IIB	iduronate 2-sulfatase (P22304)	61	IDS	60	
Sanfilippo syndrome Type A / MPS IIIA		heparan N-sulfatase (P51688)	128	SGSH	127	rhHNS
Sanfilippo syndrome Type B / MPS IIIB		N-α-acetylglucosaminidase (P54802)	37	NAGLU	36	

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Sanfilippo syndrome Type C / MPS IIIC		heparan acetyl CoA: α-glucosaminide acetyltransferase (Q68CP4)	67	HGSNAT	66	
Sanfilippo syndrome Type D / MPS IIID		N-acetylglucosamine 6-sulfatase (P34059)	130	GNS	129	
Morquio syndrome, type A / MPS IVA		N-acetylglucosamine 6-sulfatase (P34059)	130	GNS	129	elosulfase alfa (VIMIZIM®)
Morquio syndrome, type B / MPS IVB		ß-galactosidase (P16278)	39	GLB1	38	
MPS IX hyaluronidase deficiency		Hyaluronidase (Q12794)	75	HYAL1	74	
MPS VI Maroteaux-Lamy syndrome		arylsulfatase B (P15848)	132	ARSB	131	
MPS VII Sly syndrome		ß-glucuronidase (P08236)	69	GUSB	68	vestronidase alfa (Mepsevii®)
mucolipidosis I, sialidosis	type I	Neuraminidase (Q99519)	25	NEU1	24	
	type II	Neuraminidase (Q99519)	25	NEU1	24	
I-cell disease, Leroy disease, mucolipidosis II		N-acetylglucosamine-1-phosphotransferase subunits alpha/beta (Q3T906)	99	GNPTAB	98	
Pseudo-Hurler polydystrophy / mucolipidosis type III		N-acetylglucosamine-1-phosphotransferase subunits alpha/beta (Q3T906)	99	GNPTAB	98	
mucolipidosis IIIC / ML III GAMMA		gamma subunit of N-acetylglucosamine-1-phosphotransferase (Q9UJJ9)	97	GNPTG	96	

mucolipidosis type IV		mucolipin-1 (Q9GZU1)	101	MCOLN1	100	
multiple sulfatase deficiency	juvenile sulfatidosis	sulfatase-modifying factor-1 (Q8NBK3)	55	SUMF1	54	
Niemann-Pick disease	type A	acid sphingomyelinase (P17405)	79	SMPD1	78	
	type B	acid sphingomyelinase (P17405)	79	SMPD1	78	
	type C1 / chronic neuronopathic form	epididymal secretory protein HE1 (O15118)	134	NPC1	133	2-hydroxy-propyl- beta-cyclodextrin; Vorinostat
	type C2	NPC intracellular cholesterol transporter 2 (P61916)	107	NPC2	106	arimoclomol
	type D / Nova Scotian type	epididymal secretory protein HE1 (also known as NPC intracellular cholesterol transporter 1; (O15118))	134	NPC1	133	
Neuronal Ceroid Lipofuscinoses:						
CLN6 disease - Atypical Late Infantile, Late-Onset variant, Early Juvenile		Ceroid-lipofuscinosis neuronal protein 6 (Q9NWW5)	111	CLN6	110	
Batten-Spielmeyer-Vogt/Juvenile NCL/CLN3 disease		Ceroid-lipofuscinosis neuronal protein 3 (Q13286)	109	CLN3	108	
Finnish Variant Late Infantile CLN5		Ceroid-lipofuscinosis neuronal protein 5 (also known as Ceroid lipofuscinosis-5) (O75503)	136	CLN5	135	
Jansky-Bielschowsky disease/Late infantile CLN2/TPP1 Disease		Tripeptidyl-peptidase 1 (O14773)	139	TPP1	138	
Kufs/Adult-onset NCL/CLN4 disease	type A	Ceroid-lipofuscinosis neuronal protein 6 (Q9NWW5)	111	CLN6	110	
	type B	Ceroid-lipofuscinosis neuronal protein 6 (Q9NWW5)	111	CLN6	110	

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Northern Epilepsy/variant late infantile CLN8		Ceroid-lipofuscinosis neuronal protein 8 (Q9UBY8)	113	CLN8	112	
Santavuori-Haltia/Infantile CLN1/PPT disease		palmitoyl-protein thioesterase-1 (P50897)	89	PPT1	88	
Pompe disease (glycogen storage disease type II)	infantile Pompe disease	acid maltase (acid α-1,4-glucosidase) (P10253)	77	GAA	76	alglucosidase alfa (Lumizyme®)
	late-onset Pompe disease	acid maltase (acid α-1,4-glucosidase) (P10253)	77	GAA	76	alglucosidase alfa (Lumizyme®)
Pycnodysostosis		cathepsin K (P43235)	85	CTSK	84	
Sandhoff disease / GM2 gangliosidosis	infantile	hexosaminidase (P07686)	43	HEXB	42	
Sandhoff disease / GM2 gangliosidosis	juvenile	hexosaminidase (P07686)	43	HEXB	42	
Sandhoff disease / GM2 Gangliosidosis	adult-onset	hexosaminidase B (P07686)	43	HEXB	42	
Schindler disease	type I / infantile	α-N-acetylgalactosaminidase (P17050)	140	NAGA	139	
	type III / intermediate, variable	α-N-acetylgalactosaminidase (P17050)	140	NAGA	139	
Kanzaki disease	Schindler disease type II	α-N-acetylgalactosaminidase (P17050)	140	NAGA	139	
Salla disease	adult form of sialic acid storage disease	Sialin (Q9NRA2)	142	SLC17A5	141	

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infantile free sialic acid storage disease (ISSD)	infantile form of sialic acid storage disease	Sialin (Q9NRA2)	142	SLC17A5	141	
spinal muscular atrophy with progressive myoclonic epilepsy (SMAPME)	myoclonus, hereditary, with progressive distal muscular atrophy	acid ceramidase (Q13510)	81	ASAH1	80	
Tay-Sachs disease / GM2 gangliosidosis	infantile Tay-Sachs disease	hexosaminidase A (P06865)	41	HEXA	40	
	juvenile-onset Tay-Sachs disease	hexosaminidase A (P06865)	41	HEXA	40	
	late-onset Tay-Sachs disease	hexosaminidase A (P06865)	41	HEXA	40	
Christianson syndrome	MRXSCH	monovalent sodium-selective sodium/hydrogen exchanger (NHE) (Q92581)	144	SLC9A6	143	
Lowe oculocerebrorenal syndrome		Inositol polyphosphate 5-phosphatase (also known as PIP(2) 5-phosphatase) (Q01968)	146	OCRL	145	
Charcot-Marie-Tooth type 4J, CMT4J		Polyphosphoinositide phosphatase (Q92562)	148	FIG4	147	
Yunis-Varon syndrome		Polyphosphoinositide phosphatase (Q92562)	148	FIG4	147	
bilateral temporooccipital polymicrogyria (BTOP)		Polyphosphoinositide phosphatase (Q92562)	148	FIG4	147	
X-linked hypercalciuric nephrolithiasis, Dent-1		H(+)/Cl(-) exchange transporter 5 (P51795)	150	CLCN5	149	
Dent disease 2		Inositol polyphosphate 5-phosphatase (also known as PIP(2) 5-phosphatase) (Q01968)	146	OCRL	145	

		Autophagy protein 5 (Q9H1Y0)	152	ATG5	151			
		Ubiquitin-like modifier-activating enzyme ATG7 (O95352)	154	ATG7	153			
		Serine/threonine-protein kinase mTOR (P42345)	156	mTORC1	155			
		Sodium-coupled neutral amino acid transporter 9 (Q8NBW4)	158	SLC38A9	157			

[0186] In some embodiments, the lysosomal enzyme is selected from the group consisting of β-glucocebrosidase (GBA), Galactosylceremidase (GALC), α-Galactosidase (GLA), α-N-acetylglucosaminidase (NAGLU), acid α-glucosidase (GAA) and lysosomal acid α-mannosidase (LAMAN). In yet other embodiments, the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NOs: 5-10. In other embodiments, the lysosomal enzyme is encoded by a polynucleotide having at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% similarity with of SEQ ID NOs: 5-10.

[0187] In some embodiments, the S1-S3 PTase is encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 4. In other embodiments, the GlcNAc-1 PTase is encoded by a polynucleotide having at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% similarity with of SEQ ID NO: 4.

[0188] The present disclosure should also be construed to include any form of a polypeptide or polynucleotide having substantial homology to the ones disclosed herein.

[0189] Preferably, a polypeptide which is "substantially homologous" is about 50% homologous, more preferably about 70% homologous, even more preferably about 80% homologous, more preferably about 90% homologous, even more preferably, about 95% homologous, and even more preferably about 99% homologous to amino acid sequence of the peptides disclosed herein.

[0190] The polypeptide may alternatively be made by recombinant means or by cleavage from a longer polypeptide. The composition of a peptide may be confirmed by amino acid analysis or sequencing. The variants of the polypeptides according to the present disclosure may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, (ii) one in which there are one or more modified amino acid residues, e.g., residues that are modified by the attachment of substituent groups, (iii) one in which the polypeptide is an alternative splice variant of the polypeptide of the present disclosure, (iv) fragments of the polypeptides and/or (v) one in which the polypeptide is fused with another polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification (for example, His-tag) or for detection (for example, Sv5 epitope tag). The fragments include polypeptides generated via proteolytic cleavage (including multi-site proteolysis) of an original sequence. Variants may be post-translationally, or chemically modified. Such

variants are deemed to be within the scope of those skilled in the art from the teaching herein.

[0191] As known in the art the "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to a sequence of a second polypeptide. Variants are defined to include polypeptide sequences different from the original sequence, preferably different from the original sequence in less than 40% of residues per segment of interest, more preferably different from the original sequence in less than 25% of residues per segment of interest, more preferably different by less than 10% of residues per segment of interest, most preferably different from the original protein sequence in just a few residues per segment of interest and at the same time sufficiently homologous to the original sequence to preserve the functionality of the original sequence and/or the ability to bind to ubiquitin or to a ubiquitylated protein. The present disclosure includes amino acid sequences that are at least 60%, 65%, 70%, 72%, 74%, 76%, 78%, 80%, 90%, or 95% similar or identical to the original amino acid sequence. The degree of identity between two polypeptides is determined using computer algorithms and methods that are widely known for the persons skilled in the art. The identity between two amino acid sequences is preferably determined by using the BLASTP algorithm [BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894, Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990)].

[0192] The polypeptides disclosed herein can be post-translationally modified. For example, post-translational modifications that fall within the scope of the present disclosure include signal peptide cleavage, glycosylation, acetylation, isoprenylation, proteolysis, myristoylation, protein folding and proteolytic processing, etc. Some modifications or processing events require introduction of additional biological machinery. For example, processing events, such as signal peptide cleavage and core glycosylation, are examined by adding canine microsomal membranes or Xenopus egg extracts to a standard translation reaction.

[0193] The polypeptides of the disclosure may include unnatural amino acids formed by post-translational modification or by introducing unnatural amino acids during translation. A variety of approaches are available for introducing unnatural amino acids during protein translation.

[0194] The term "functionally equivalent" as used herein refers to a polypeptide that preferably retains at least one biological function or activity of the specific amino acid sequence of a lysosomal enzyme of the disclosure.

[0195] A polypeptide may be conjugated with other molecules, such as proteins, to prepare

fusion proteins. This may be accomplished, for example, by the synthesis of N-terminal or C-terminal fusion proteins provided that the resulting fusion protein retains the functionality of a lysosomal enzyme of the disclosure.

[0196] A polypeptide may be phosphorylated using conventional methods. In one embodiment, the presently disclosed lysosomal enzyme can be phosphorylated thanks to the presently disclosed modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase).

[0197] Cyclic derivatives of the peptides or chimeric proteins are also contemplated herein. Cyclization may allow the peptide or chimeric protein to assume a more favorable conformation for association with other molecules. Cyclization may be achieved using techniques known in the art. For example, disulfide bonds may be formed between two appropriately spaced components having free sulfhydryl groups, or an amide bond may be formed between an amino group of one component and a carboxyl group of another component.

[0198] Cyclization may also be achieved using an azobenzene-containing amino acids. The components that form the bonds may be side chains of amino acids, non-amino acid components or a combination of the two. In one embodiment, cyclic peptides may comprise a beta-turn in the right position. Beta-turns may be introduced into the peptides of the disclosure by adding the amino acids Pro-Gly at the right position. It may be desirable to produce a cyclic peptide which is more flexible than the cyclic peptides containing peptide bond linkages as described above. A more flexible peptide may be prepared by introducing cysteines at the right and left position of the peptide and forming a disulfide bridge between the two cysteines. The two cysteines are arranged so as not to deform the beta-sheet and turn. The peptide is more flexible as a result of the length of the disulfide linkage and the smaller number of hydrogen bonds in the beta-sheet portion. The relative flexibility of a cyclic peptide can be determined by molecular dynamics simulations.

Tags

[0199] In one embodiment, the polypeptides as disclosed herein further comprise the amino acid sequence of a tag. The tag includes but is not limited to: polyhistidine tags (His-tags) (for example H6 and H10, etc.) or other tags for use in IMAC systems, for example, Ni2+ affinity columns, etc., GST fusions, MBP fusions, streptavidine-tags, the BSP biotinylation target sequence of the bacterial enzyme BIRA and tag epitopes that are directed by antibodies (for example c-myc tags, FLAG-tags, HPC4-tag among others). As will be observed by a person skilled in the art, the tag peptide can be used for purification, inspection, selection and/or visualization of the fusion protein of the disclosure. In one embodiment, the tag is a

detection tag and/or a purification tag. It will be appreciated that the tag sequence will not interfere in the function of the protein of the disclosure.

Leader and Secretory Sequence

[0200] Accordingly, the polypeptides of the disclosure can be fused to another polypeptide or tag, such as a leader or secretory sequence or a sequence which is employed for purification or for detection. In some embodiments, the polypeptide of the disclosure comprises the glutathione-S-transferase protein tag which provides the basis for rapid high-affinity purification of the polypeptide of the disclosure. Indeed, this GST-fusion protein can then be purified from cells via its high affinity for glutathione. Agarose beads can be coupled to glutathione, and such glutathione-agarose beads bind GST-proteins. Thus, in a particular embodiment, the polypeptide can be bound to a solid support. In some embodiments, if the polypeptide comprises a GST moiety, the polypeptide is coupled to a glutathione-modified support. In some embodiments, the glutathione modified support is a glutathione-agarose bead. Additionally, a sequence encoding a protease cleavage site can be included between the affinity tag and the polypeptide sequence, thus permitting the removal of the binding tag after incubation with this specific enzyme and thus facilitating the purification of the corresponding protein of interest.

[0201] The polypeptides disclosed herein can also be fused to, or integrated into, a target protein, and/or a targeting domain capable of directing the chimeric protein to a desired cellular component or cell type or tissue. The chimeric proteins may also contain additional amino acid sequences or domains. The chimeric proteins are recombinant in the sense that the various components are from different sources, and as such are not found together in nature (i.e. are heterologous).

[0202] In some embodiments of the compositions of the disclosure, polypeptides comprise peptidomimetics of the lysosomal proteins of the disclosure or a vector encodes a peptidomimetic of the lysosomal proteins of the disclosure. Peptidomimetics are compounds based on, or derived from, peptides and proteins.

[0203] N-terminal or C-terminal fusion proteins comprising a peptide or chimeric protein of the disclosure conjugated with other molecules may be prepared by fusing, through recombinant techniques, the N-terminal or C-terminal of the peptide or chimeric protein, and the sequence of a selected protein or selectable marker with a desired biological function. The resultant fusion proteins contain a lysosomal enzyme comprising peptide or chimeric protein fused to the selected protein or marker protein as described herein. Examples of proteins which may be used to prepare fusion proteins include immunoglobulins, glutathione-S-

transferase (GST), hemagglutinin (HA), and truncated myc.

[0204] The polypeptides and chimeric proteins presently disclosed may be converted into pharmaceutical salts by reacting with inorganic acids such as hydrochloric acid, sulfuric acid, hydrobromic acid, phosphoric acid, etc., or organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid, benezenesulfonic acid, and toluenesulfonic acids.

Modified Cell

[0205] In some embodiments, the disclosure provides a cell comprising a vector of the disclosure. In some embodiments, the vector is a viral vector (e.g., an AAV or a lentiviral vector). In some embodiments, the vector is a non-viral vector (e.g., a liposome, a nanoparticle, a lipid nanoparticle, a micelle, a polymersome, an exosome). .In some embodiments, the vector is an expression vector. In some embodiments, the vector contains at least one element allowing for bicistronic, polycistronic or multicistronic expression of at least two sequences. In some embodiments, the vector comprises a sequence encoding a lysosomal enzyme of the disclosure. Alternatively or in addition, in some embodiments, the vector comprises a sequence encoding a S1S3 construct of the disclosure. In some embodiments, the lysosomal enzyme is one or more of the enzymes listed in Table 1A, Table 1B or Table 1C. In some embodiments, the vector comprises a nucleic acid or amino acid sequence encoding the lysosomal enzyme is one or more of the enzymes listed in Table 1A, Table 1B or Table 1C.

[0206] In some embodiments the cell comprising a vector of the disclosure is a modified cell of the disclosure. In some embodiments, the cell comprising a vector of the disclosure is non-naturally occurring.

[0207] In some embodiments, the cell is a mammalian cell capable of expressing a human sequence and/or producing a human protein. In some embodiments, the mammalian cell is isolated or derived from a mouse, rat, guinea pig, rabbit, cat, dog, or non-human primate.

[0208] In some embodiments, the cell is a human cell capable of expressing a human sequence and/or producing a human protein.

[0209] In some embodiments, the cell is a primary cell, modified to express a vector of the disclosure and cultured ex vivo. In some embodiments, the cultured cell is immortalized or otherwise modified to facilitate propagation of the cell in vitro indefinitely, generating a cultured cell line.

Host Cell

[0210] In some embodiments, the disclosure provides a cell comprising a bicistronic vector of the disclosure. The cell may be a prokaryotic cell or a eukaryotic cell. Appropriate cells include, but are not limited to, bacterial, yeast, fungal, insect, and mammalian cells.

[0211] In some embodiments, the disclosure provides a mammalian cell comprising a bicistronic vector of the disclosure.

[0212] A host cell comprising the disclosed bicistronic vector may be used for protein expression and, optionally, purification. Methods for expressing and, optionally, purifying an expressed protein from a host are standard in the art.

[0213] In some embodiments, the host cell comprising a vector of the disclosure may be used to produce a polypeptide encoded by an enzyme construct of the disclosure. Generally, production of a polypeptide of the disclosure involves transfecting host cells with a vector comprising an enzyme construct and then culturing the cells so that they transcribe and translate the desired polypeptide. The isolated host cells may then be lysed to extract the expressed polypeptide for subsequent purification.

[0214] In some embodiments, the host cell is a prokaryotic cell. Non- limiting examples of suitable prokaryotic cells include E. coli and other Enterobacteriaceae, Escherichia sp., Campylobacter sp., Wolinella sp., Desulfovibrio sp. Vibrio sp., Pseudomonas sp. Bacillus sp., Listeria sp., Staphylococcus sp., Streptococcus sp., Peptostreptococcus sp., Megasphaera sp., Pectinatus sp., Selenomonas sp., Zymophilus sp., Actinomyces sp., Arthrobacter sp., Frankia sp., Micromonospora sp., Nocardia sp., Propionibacterium sp., Streptomyces sp., Lactobacillus sp., Lactococcus sp., Leuconostoc sp., Pediococcus sp., Acetobacterium sp., Eubacterium sp., Heliobacterium sp., Heliospirillum sp., Sporomusa sp., Spiroplasma sp., Ureaplasma sp., Erysipelothrix sp., Corynebacterium sp. Enterococcus sp., Clostridium sp., Mycoplasma sp., Mycobacterium sp., Actinobacteria sp., Salmonella sp., Shigella sp., Moraxella sp., Helicobacter sp, Stenotrophomonas sp., Micrococcus sp., Neisseria sp., Bdellovibrio sp., Hemophilus sp., Klebsiella sp., Proteus mirabilis, Enterobacter cloacae, Serratia sp. , Citrobacter sp. , Proteus sp. , Serratia sp., Yersinia sp., Acinetobacter sp., Actinobacillus sp. Bordetella sp., Brucella sp., Capnocytophaga sp., Cardiobacterium sp., Eikenella sp., Francisella sp., Haemophilus sp., Kingella sp., Pasteurella sp., Flavobacterium sp. Xanthomonas sp., Burkholderia sp., Aeromonas sp., Plesiomonas sp., Legionella sp. and alpha- proteobaeteria such as Wolbachia sp., cyanobacteria, spirochaetes, green sulfur and green non-sulfur bacteria, Gram-negative cocci, Gram negative bacilli which are fastidious, Enterobacteriaceae-glucose-fermenting gram-negative bacilli, Gram negative bacilli-non-glucose fermenters, Gram negative bacilli-glucose fermenting, oxidase positive. Particularly

useful bacterial host cells for protein expression include Gram negative bacteria, such as Escherichia coli, Pseudomonas fiuorescens, Pseudomonas haloplanctis, Pseudomonas putida AC 10, Pseudomonas pseudof lava, Bartonella henselae, Pseudomonas syringae, Caulobacter crescentus, Zymomonas mobilis, Rhizobium meliloti, Myxococcus xanthus and Gram positive bacteria such as Bacillus subtilis, Corynebacterium, Streptococcus cremoris, Streptococcus lividans, and Streptomyces lividans. E. coli is one of the most widely used expression hosts. Accordingly, the techniques for overexpression in E. coli are well developed and readily available to one of skill in the art.

[0215] Further, Pseudomonas fiuorescens, is commonly used for high level production of recombinant proteins (i.e. for the development bio- therapeutics and vaccines).

[0216] In some embodiments, a host cell is a yeast or fungal cell. Particularly useful fungal host cells for protein expression include Aspergillis oryzae, Aspergillis niger, Trichoderma reesei, Aspergillus nidulans, Fusarium graminearum. Particularly useful yeast host cells for protein expression include Candida albicans, Candida maltose, Hansenula polymorpha, Kluyveromyces fragilis, Kluyveromyces lactis, Pichia guillerimondii, Pichia pastoris, Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Yarrowia lipolytica.

[0217] In some embodiments, a host cell is an insect cell. Non-limiting examples include Spodoptera frugiperda cell lines (such as the Sf9 or Sf21), drosophila cell lines, or mosquito cell lines (such as Aedes albopictus derived cell lines).

[0218] In some embodiments, a host cell is a mammalian cell. Useful mammalian host cells for protein expression include Chinese hamster ovary (CHO) cells, HeLa cells, Human embryonic kidney 293 (HEK293) cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (eg. Hep G2), human embryonic kidney cells, Bos primigenius, and Mus musculus. In a specific embodiment, the host cells are CHO cells. Additionally, the mammalian host cell may be an established, commercially- available cell line (e.g., American Type Culture Collection (ATCC), Manassas, VA). The host cell may be an immortalized cell. Alternatively, the host cell may be a primary cell.

[0219] In some embodiments, the host cell has been engineered to produce high levels of a protein of interest.

Methods of the Disclosure

[0220] In some embodiments, the disclosure provides a method of treating a subject suffering from a lysosomal storage disorder (LSD) is disclosed herein. The method comprises administering to the subject a pharmaceutical composition comprising the lysosomal enzyme expressed by the biscistronic vector as disclosed elsewhere herein, thereby increasing the

phosphorylation of a lysosomal enzyme and treating the subject.

[0221] In some embodiments, the disclosure provides a method of preventing the occurrence of a lysosomal storage disorder (LSD) in a subject in need thereof. The method comprises administering to the subject a pharmaceutical composition comprising the lysosomal enzyme expressed by the biscistronic vector as disclosed elsewhere herein, thereby increasing the phosphorylation of a lysosomal enzyme and preventing the occurrence of a LSD in the subject.

[0222] In some embodiments, the lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1. In other embodiments, the lysosomal enzyme is at least one as listed in Table 1.

[0223] In further embodiments, the administering comprises an administration route selected from the group consisting of enteral, parenteral, oral, intramuscular (IM), subcutaneous (SC), intravenous (IV), and intra-arterial (IA). Additional administration routes that can be used for the disclosed methods are described in detail elsewhere herein.

Combination Therapies

[0224] The compositions and methods for treating or preventing LSDs as described herein may be useful when combined with at least one additional compound useful for treating LSDs. The additional compound may comprise a commercially available compound, known to treat, prevent, or reduce the symptoms of LSDs. The compound could be but is not limited to an ERT known in the art.

Pharmaceutical Compositions and Formulations

[0225] Also provided herein is a pharmaceutical composition comprising a lysosomal enzyme expressed by the biscistronic vector of the disclosure.

[0226] Such a pharmaceutical composition is in a form suitable for administration to a subject, or the pharmaceutical composition may further comprise one or more pharmaceutically acceptable carriers, one or more additional ingredients, or some combination of these. The various components of the pharmaceutical composition may be present in the form of a physiologically acceptable salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

[0227] In some embodiments of the disclosure, the pharmaceutical composition useful for practicing the method of the disclosure may be administered to deliver a dose of between 1 ng/kg/day and 100 mg/kg/day. In some embodiments of the disclosure, the pharmaceutical composition useful for practicing the disclosure may be administered to deliver a dose of between 1 ng/kg/day and 500 mg/kg/day. The relative amounts of the active ingredient, the

pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the disclosure will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0228] In some embodiments of the disclosure, the pharmaceutical composition useful for practicing the method of the disclosure may be administered to deliver a dose of between 1 ng/kg and 100 mg/kg. In some embodiments of the disclosure, the pharmaceutical composition useful for practicing the disclosure may be administered to deliver a dose of between 1 ng/kg and 500 mg/kg. In some embodiments of the disclosure, the pharmaceutical composition is provided daily, weekly, bi-weekly, monthly, or annually. The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the disclosure will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0229] Pharmaceutical compositions that are useful in the methods of the disclosure may be suitably developed for inhalational, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic, intrathecal, intravenous or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations. The route(s) of administration is readily apparent to the skilled artisan and depends upon any number of factors including the type and severity of the disease being treated, the type and age of the veterinary or human patient being treated, and the like.

[0230] The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit. In some embodiments, the presently disclosed compositions can be formulated in a natural capsid, a modified capsid, as a naked RNA, or encapsulated in a protective coat.

[0231] The amount of the active ingredient is generally equal to the dosage of the active ingredient that would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. The unit dosage form may be for

a single daily dose or one of multiple daily doses (e.g., about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

[0232] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions suitable for ethical administration to humans, it is understood by the skilled artisan that such compositions are generally suitable for administration to animals of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions of the disclosure is contemplated include, but are not limited to, humans and other primates, mammals including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, and dogs. In one embodiment, the subject is a human or a non-human mammal such as but not limited to an equine, an ovine, a bovine, a porcine, a canine, a feline and a murine. In one embodiment, the subject is a human.

[0233] In one embodiment, the compositions are formulated using one or more pharmaceutically acceptable excipients or carriers. In some embodiments, the disclosure provides a pharmaceutical composition for treating a subject suffering from LSDs. In some embodiments, the disclosure provides a pharmaceutical composition comprising a lysosomal enzyme expressed by a biscistronic vector of the disclosure and a pharmaceutically acceptable carrier.

[0234] Pharmaceutically acceptable carriers, which are useful, include, but are not limited to, glycerol, water, saline, ethanol and other pharmaceutically acceptable salt solutions such as phosphates and salts of organic acids. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some embodiments, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about

by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

[0235] Formulations may be employed in admixtures with conventional excipients, i.e., pharmaceutically acceptable organic or inorganic carrier substances suitable for oral, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, e.g., other analgesic agents.

[0236] The disclosed composition may comprise a preservative from about 0.005% to 2.0% by total weight of the composition. The preservative is used to prevent spoilage in the case of exposure to contaminants in the environment. Examples of preservatives useful in accordance with the disclosure included but are not limited to those selected from the group consisting of benzyl alcohol, sorbic acid, parabens, imidurea and combinations thereof. In some embodiments, the preservative is a combination of about 0.5% to 2.0% benzyl alcohol and 0.05% to 0.5% sorbic acid.

[0237] The composition may include an antioxidant and a chelating agent which inhibit the degradation of the compound. Preferred antioxidants for some compounds are BHT, BHA, alpha-tocopherol and ascorbic acid in the preferred range of about 0.01% to 0.3% and more preferably BHT in the range of 0.03% to 0.1% by weight by total weight of the composition. Preferably, the chelating agent is present in an amount of from 0.01% to 0.5% by weight by total weight of the composition. Particularly preferred chelating agents include edetate salts (e.g. disodium edetate) and citric acid in the weight range of about 0.01% to 0.20% and more preferably in the range of 0.02% to 0.10% by weight by total weight of the composition. The chelating agent is useful for chelating metal ions in the composition which may be detrimental to the shelf life of the formulation. In some embodiments, BHT and disodium edetate are the antioxidant and the chelating agent respectively for some compounds, however, other suitable and equivalent antioxidants and chelating agents may be substituted therefore as would be known to those skilled in the art.

Administration/Dosing

[0238] The regimen of administration may affect what constitutes an effective amount. For example, the therapeutic formulations may be administered to the patient subject either prior to or after a surgical intervention related to a lysosomal storage disorder (LSD), or shortly

after the patient was diagnosed with a lysosomal storage disorder (LSD). Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

[0239] Administration of the compositions of the present disclosure to a patient subject, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a lysosomal storage disorder (LSD) in the subject. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the disclosure is from about 0.01 and 50 mg/kg of body weight/per day.

[0240] The compound can be administered to a subject as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on. The frequency of the dose is readily apparent to the skilled artisan and depends upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, and the type and age of the animal. Actual dosage levels of the active ingredients in the pharmaceutical compositions of this disclosure may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. A medical doctor, e.g., physician or veterinarian, having ordinary skill in

the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the disclosure employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0241] In some embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the disclosure are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of LSDs.

Routes of Administration

[0242] One skilled in the art will recognize that although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route.

[0243] Routes of administration of the disclosed compositions include inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (e.g., sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (e.g., trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, intra-cisterna magna (ICM), intraspinal, intraventricular, intracerebroventricular, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration. Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present disclosure are not limited to the particular formulations and compositions that are described herein. In one embodiment, the treatment of LSD comprises an administration route selected from the group consisting of inhalation, oral, rectal, vaginal, parenteral, topical, transdermal, pulmonary, intranasal, buccal, ophthalmic,

intra-hepatic arterial, intrapleural, intrathecal, intra-tumoral, intravenal and any combination thereof.

Gene Therapy Administration

[0244] One skilled in the art recognizes that different methods of delivery may be utilized to administer a vector into a cell. Examples include: (1) methods utilizing physical means, such as electroporation (electricity), a gene gun (physical force) or applying large volumes of a liquid (pressure); and (2) methods wherein the vector is complexed to another entity, such as a liposome, aggregated protein or transporter molecule.

[0245] Furthermore, the actual dose and schedule can vary depending on whether the compositions are administered in combination with other pharmaceutical compositions, or depending on interindividual differences in pharmacokinetics, drug disposition, and metabolism. Similarly, amounts can vary in in vitro applications depending on the particular cell line utilized (e.g., based on the number of vector receptors present on the cell surface, or the ability of the particular vector employed for gene transfer to replicate in that cell line). Furthermore, the amount of vector to be added per cell will likely vary with the length and stability of the therapeutic gene inserted in the vector, as well as also the nature of the sequence, and is particularly a parameter which needs to be determined empirically, and can be altered due to factors not inherent to the methods of the present disclosure (for instance, the cost associated with synthesis). One skilled in the art can easily make any necessary adjustments in accordance with the exigencies of the particular situation.

[0246] Cells containing the therapeutic agent may also contain a suicide gene i.e., a gene which encodes a product that can be used to destroy the cell. In many gene therapy situations, it is desirable to be able to express a gene for therapeutic purposes in a host, cell but also to have the capacity to destroy the host cell at will. The therapeutic agent can be linked to a suicide gene, whose expression is not activated in the absence of an activator compound. When death of the cell in which both the agent and the suicide gene have been introduced is desired, the activator compound is administered to the cell thereby activating expression of the suicide gene and killing the cell. Examples of suicide gene/prodrug combinations which may be used are herpes simplex virus-thymidine kinase (HSV-tk) and ganciclovir, acyclovir; oxidoreductase and cycloheximide; cytosine deaminase and 5-fluorocytosine; thymidine kinase thymidilate kinase (Tdk::Tmk) and AZT; and deoxycytidine kinase and cytosine arabinoside.

Therapeutic

[0247] The present disclosure encompasses a method to treat a deficient lysosomal enzyme in

a subject diagnosed with LSD or in a subject at risk for developing an LDS. The method improves phosphorylation of lysosomal enzymes thereby treating the subject or preventing the occurrence of the LSD in the subject. Further, the method improves quality of life in a patient. In one embodiment, the method of the present disclosure comprises administering to a subject, a composition comprising a polynucleotide encoding a lysosomal enzyme and a polynucleotide encoding a GlcNAc-1 PTase.

[0248] *Nucleic Acid Sequences:*

[0249] pLL01 bicistronic vector sequence (SEQ ID NO:1) (CMV promoter: *italic and underline*. IRES: ***bold and italic***. S1-S3: **bold and underline**.)

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   1 GACGGATCGG GAGATCTCCC GATCCCCTAT GGTGCACTCT CAGTACAATC TGCTCTGATG
  61 CCGCATAGTT AAGCCAGTAT CTGCTCCCTG CTTGTGTGTT GGAGGTCGCT GAGTAGTGCG
 121 CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC
 181 TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT
 241 GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
 301 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC
 361 CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC
 421 ATTGACGTCA ATGGGTGGAG TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT
 481 ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT
 541 ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
 601 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG
 661 ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC
 721 AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG
 781 GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA
 841 CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC
 901 GTTTAAACTT AAGCTTGGTA CCGAGCTCGG ATCCACTAGT CCAGTGTGGT GGAATTCTGC
 961 AGATATCCAG CACAGTGGCG GCCGCtgatt aacctcagga ctagtGGTTA TTTTCCACCA
1021 TATTGCCGTC TTTTGGCAAT GTGAGGGCCC GGAAACCTGG CCCTGTCTTC TTGACGAGCA
1081 TTCCTAGGGG TCTTTCCCCT CTCGCCAAAG GAATGCAAGG TCTGTTGAAT GTCGTGAAGG
1141 AAGCAGTTCC TCTGGAAGCT TCTTGAAGAC AAACAACGTC TGTAGCGACC CTTTGCAGGC
1201 AGCGGAACCC CCCACCTGGC GACAGGTGCC TCTGCGGCCA AAAGCCACGT GTATAAGATA
1261 CACCTGCAAA GGCGGCACAA CCCCAGTGCC ACGTTGTGAG TTGGATAGTT GTGGAAAGAG
1321 TCAAATGGCT CACCTCAAGC GTATTCAACA AGGGGCTGAA GGATGCCCAG AAGGTACCCC
1381 ATTGTATGGG ATCTGATCTG GGGCCTCGGT GCACATGCTT TACATGTGTT TAGTCGAGGT
1441 TAAAAAACGT CTAGGCCCCC CGAACCACGG GGACGTGGTT TTCCTTTGAA AGTTTGTTAA
1501 Catgctgttc aagctcctgc agagacagac ctatacctgc ctgtcccaca ggtatgggct
1561 ctacgtgtgc ttcttgggcg tcgttgtcac catcgtctcc gccttccagt tcggagaggt
1621 ggttctggaa tggagccgag atcaatacca tgttttgttt gattcctata gagacaatat
1681 tgctggaaag tcctttcaga atcggctttg tctgcccatg ccgattgacg ttgtttacac
1741 ctgggtgaat ggcacagatc ttgaactact gaaggaacta ACAGAATTAA AAAGATCAAA
1801 ACGTGATCCA TTAATACCAG AATGTCAAGG TAAACAAACA CCAGAAAAAG ATAAATGTTA
1861 TAGAGATgac atctctgcca gtcgttttga agataacgaa gaactgaggt actcattgcg
1921 atctatcgag aggcatgcac catgggttcg gaatattttc attgtcacca acgggcagat
1981 tccatcctgg ctgaaccttg acaatcctcg agtgacaata gtaacacacc aggatgtttt
2041 tcgaaatttg agccacttgc ctacctttag ttcacctgct attgaaagtc acattcatcg
2101 catcgaaggg ctgtcccaga agtttattta cctaaatgat gatgtcatgt ttgggaagga
2161 tgtctggcca gatgattttt acagtcactc caaaggccag aaggtttatt tgacatggcc
2221 tgtgccaaac GGAGGTAGCG GAGGTgatac atttgcagat tccctcagat atgtaaataa
2281 aattctaaat agcaagtttg gattcacatc gcggaaagtc cctgctcaca tgcctcacat
2341 gattgaccgg attgttatgc aagaactgca agatatgttc cctgaagaat ttgacaagac
2401 gtcatttcac aaagtgcgcc attctgagga tatgcagttt gccttctctt atttttatta
2461 tctcatgagt gcagtgcagc cactgaatat atctcaagtc tttgatgaag ttgatacaga
2521 tcaatctggt gtcttgtctg acagagaaat ccgaacactg gctaccagaa ttcacgaact
2581 gccgttaagt ttgcaggatt tgacaggtct ggaacacatg ctaataaatt gctcaaaaat
2641 gcttcctgct gatatcacgc agctaaataa tattccacca actcaggaat cctactatga
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2701 **tcccaacctg ccaccggtca ctaaaagtct agtaacaaac tgtaaaccag taactgacaa**
2761 **aatccacaaa gcatataagg acaaaaacaa atataggttt gaaatcatgg gagaagaaga**
2821 **aatcgctttt aaaatgattc gtaccaacgt ttctcatgtg gttggccagt tggatgacat**
2881 **aagaaaaaac cctaggaagt ttgtttgcct gaatgacaac attgaccaca atcataaaga**
2941 **tgctcagaca gtgaaggctg ttctcaggga cttctatgaa tccatgttcc ccataccttc**
3001 **ccaatttgaa ctgccaagag agtatcgaaa ccgtttcctt catatgcatg agctgcagga**
3061 **atggagggct tatcgagaca aattgaagtt ttggacccat tgtgtactag caacattgat**
3121 **tatgtttact atattctcat tttttgctga gcagttaatt gcacttaagc ggaagatatt**
3181 **tcccagaagg aggatacaca aagaagctag tcccaatcga atcagagta**T CTAGAGGAgg
3241 taagcctatc cctaaccctc tcctcggtct cgattctacg tgaGTTTAAA CCCGCTGATC
3301 AGCCTCGACT GTGCCTTCTA GTTGCCAGCC ATCTGTTGTT TGCCCCTCCC CCGTGCCTTC
3361 CTTGACCCTG GAAGGTGCCA CTCCCACTGT CCTTTCCTAA TAAAATGAGG AAATTGCATC
3421 GCATTGTCTG AGTAGGTGTC ATTCTATTCT GGGGGGTGGG GTGGGGCAGG ACAGCAAGGG
3481 GGAGGATTGG GAAGACAATA GCAGGCATGC TGGGGATGCG GTGGGCTCTA TGGCTTCTGA
3541 GGCGGAAAGA ACCAGCTGGG GCTCTAGGGG GTATCCCCAC GCGCCCTGTA GCGGCGCATT
3601 AAGCGCGGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT ACACTTGCCA GCGCCCTAGC
3661 GCCCGCTCCT TTCGCTTTCT TCCCTTCCTT TCTCGCCACG TTCGCCGGCT TTCCCCGTCA
3721 AGCTCTAAAT CGGGGGCTCC CTTTAGGGTT CCGATTTAGT GCTTTACGGC ACCTCGACCC
3781 CAAAAAACTT GATTAGGGTG ATGGTTCACG TAGTGGGCCA TCGCCCTGAT AGACGGTTTT
3841 TCGCCCTTTG ACGTTGGAGT CCACGTTCTT TAATAGTGGA CTCTTGTTCC AAACTGGAAC
3901 AACACTCAAC CCTATCTCGG TCTATTCTTT TGATTTATAA GGGATTTTGC CGATTTCGGC
3961 CTATTGGTTA AAAAATGAGC TGATTTAACA AAAATTTAAC GCGAATTAAT TCTGTGGAAT
4021 GTGTGTCAGT TAGGGTGTGG AAAGTCCCCA GGCTCCCCAG CAGGCAGAAG TATGCAAAGC
4081 ATGCATCTCA ATTAGTCAGC AACCAGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
4141 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCATAG TCCCGCCCCT AACTCCGCCC
4201 ATCCCGCCCC TAACTCCGCC CAGTTCCGCC CATTCTCCGC CCCATGGCTG ACTAATTTTT
4261 TTTATTTATG CAGAGGCCGA GGCCGCCTCT GCCTCTGAGC TATTCCAGAA GTAGTGAGGA
4321 GGCTTTTTTG GAGGCCTAGG CTTTTGCAAA AAGCTCCCGG GAGCTTGTAT ATCCATTTTC
4381 GGATCTGATC AAGAGACAGG ATGAGGATCG TTTCGCATGA TTGAACAAGA TGGATTGCAC
4441 GCAGGTTCTC CGGCCGCTTG GGTGGAGAGG CTATTCGGCT ATGACTGGGC ACAACAGACA
4501 ATCGGCTGCT CTGATGCCGC CGTGTTCCGG CTGTCAGCGC AGGGGCGCCC GGTTCTTTTT
4561 GTCAAGACCG ACCTGTCCGG TGCCCTGAAT GAACTGCAGG ACGAGGCAGC GCGGCTATCG
4621 TGGCTGGCCA CGACGGGCGT TCCTTGCGCA GCTGTGCTCG ACGTTGTCAC TGAAGCGGGA
4681 AGGGACTGGC TGCTATTGGG CGAAGTGCCG GGGCAGGATC TCCTGTCATC TCACCTTGCT
4741 CCTGCCGAGA AAGTATCCAT CATGGCTGAT GCAATGCGGC GGCTGCATAC GCTTGATCCG
4801 GCTACCTGCC CATTCGACCA CCAAGCGAAA CATCGCATCG AGCGAGCACG TACTCGGATG
4861 GAAGCCGGTC TTGTCGATCA GGATGATCTG GACGAAGAGC ATCAGGGGCT CGCGCCAGCC
4921 GAACTGTTCG CCAGGCTCAA GGCGCGCATG CCCGACGGCG AGGATCTCGT CGTGACCCAT
4981 GGCGATGCCT GCTTGCCGAA TATCATGGTG GAAAATGGCC GCTTTTCTGG ATTCATCGAC
5041 TGTGGCCGGC TGGGTGTGGC GGACCGCTAT CAGGACATAG CGTTGGCTAC CCGTGATATT
5101 GCTGAAGAGC TTGGCGGCGA ATGGGCTGAC CGCTTCCTCG TGCTTTACGG TATCGCCGCT
5161 CCCGATTCGC AGCGCATCGC CTTCTATCGC CTTCTTGACG AGTTCTTCTG AGCGGGACTC
5221 TGGGGTTCGA AATGACCGAC CAAGCGACGC CCAACCTGCC ATCACGAGAT TTCGATTCCA
5281 CCGCCGCCTT CTATGAAAGG TTGGGCTTCG GAATCGTTTT CCGGGACGCC GGCTGGATGA
5341 TCCTCCAGCG CGGGGATCTC ATGCTGGAGT TCTTCGCCCA CCCCAACTTG TTTATTGCAG
5401 CTTATAATGG TTACAAATAA AGCAATAGCA TCACAAATTT CACAAATAAA GCATTTTTTT
5461 CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATGT ATCTTATCAT GTCTGTATAC
5521 CGTCGACCTC TAGCTAGAGC TTGGCGTAAT CATGGTCATA GCTGTTTCCT GTGTGAAATT
5581 GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT AAAGCCTGGG
5641 GTGCCTAATG AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC GCTTTCCAGT
5701 CGGGAAACCT GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG AGAGGCGGTT
5761 TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG GTCGTTCGGC
5821 TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA GAATCAGGGG
5881 ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG
5941 CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC AAAAATCGAC
6001 GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCCCCCTG
6061 GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC CTGTCCGCCT
6121 TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT CTCAGTTCGG
6181 TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG CCCGACCGCT
6241 GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC
6301 TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT GCTACAGAGT

6361 TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGAAC AGTATTTGGT ATCTGCGCTC
6421 TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC AAACAAACCA
6481 CCGCTGGTAG CGGTTTTTTT GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC
6541 AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT
6601 AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA
6661 AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT
6721 GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT
6781 GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG
6841 CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAGCCAG
6901 CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA
6961 ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG
7021 CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG
7081 GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT
7141 CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA
7201 TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG
7261 GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC
7321 CGGCGTCAAT ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG
7381 GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA
7441 TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG
7501 GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT
7561 GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC
7621 TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA
7681 CATTTCCCCG AAAAGTGCCA CCTGACGTC.

[0250] CMV sequence (SEQ ID NO: 2)

1 CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC CCCGCCCATT
61 GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC ATTGACGTCA
121 ATGGGTGGAG TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC
181 AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
241 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC
301 CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG
361 ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC AAAATCAACG
421 GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT
481 ACGGTGGGAG GTCTATATAA GCAGAGCT.

[0251] IRES sequence (SEQ ID NO: 3)

1 GGTTATTTTC CACCATATTG CCGTCTTTTG GCAATGTGAG GGCCCGGAAA CCTGGCCCTG
61 TCTTCTTGAC GAGCATTCCT AGGGGTCTTT CCCCTCTCGC CAAAGGAATG CAAGGTCTGT
121 TGAATGTCGT GAAGGAAGCA GTTCCTCTGG AAGCTTCTTG AAGACAAACA ACGTCTGTAG
181 CGACCCTTTG CAGGCAGCGG AACCCCCCAC CTGGCGACAG GTGCCTCTGC GGCCAAAAGC
241 CACGTGTATA AGATACACCT GCAAAGGCGG CACAACCCCA GTGCCACGTT GTGAGTTGGA
301 TAGTTGTGGA AAGAGTCAAA TGGCTCACCT CAAGCGTATT CAACAAGGGG CTGAAGGATG
361 CCCAGAAGGT ACCCCATTGT ATGGGATCTG ATCTGGGGCC TCGGTGCACA TGCTTTACAT
421 GTGTTTAGTC GAGGTTAAAA AACGTCTAGG CCCCCCGAAC CACGGGGACG TGGTTTTCCT
481 TTGAAA.

[0252] Modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase), S1-S3 sequence (SEQ ID No: 4)

1 atgctgttca agctcctgca gagacagacc tatacctgcc tgtcccacag gtatgggctc
61 tacgtgtgct tcttgggcgt cgttgtcacc atcgtctccg ccttccagtt cggagaggtg
121 gttctggaat ggagccgaga tcaataccat gttttgtttg attcctatag agacaatatt
181 gctggaaagt cctttcagaa tcggctttgt ctgcccatgc cgattgacgt tgtttacacc
241 tgggtgaatg gcacagatct tgaactactg aaggaactaA CAGAATTAAA AAGATCAAAA
301 CGTGATCCAT TAATACCAGA ATGTCAAGGT AAACAAACAC CAGAAAAAGA TAAATGTTAT
361 AGAGATgaca tctctgccag tcgttttgaa gataacgaag aactgaggta ctcattgcga
421 tctatcgaga ggcatgcacc atgggttcgg aatattttca ttgtcaccaa cgggcagatt
481 ccatcctggc tgaaccttga caatcctcga gtgacaatag taacacacca ggatgttttt
541 cgaaatttga gccacttgcc tacctttagt tcacctgcta ttgaaagtca cattcatcgc

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 601 atcgaagggc tgtcccagaa gtttatttac ctaaatgatg atgtcatgtt tgggaaggat
 661 gtctggccag atgattttta cagtcactcc aaaggccaga aggtttattt gacatggcct
 721 gtgccaaacG GAGGTAGCGG AGGTgataca tttgcagatt ccctcagata tgtaaataaa
 781 attctaaata gcaagtttgg attcacatcg cggaaagtcc ctgctcacat gcctcacatg
 841 attgaccgga ttgttatgca agaactgcaa gatatgttcc ctgaagaatt tgacaagacg
 901 tcatttcaca aagtgcgcca ttctgaggat atgcagtttg ccttctctta tttttattat
 961 ctcatgagtg cagtgcagcc actgaatata tctcaagtct ttgatgaagt tgatacagat
1021 caatctggtg tcttgtctga cagagaaatc cgaacactgg ctaccagaat tcacgaactg
1081 ccgttaagtt tgcaggattt gacaggtctg gaacacatgc taataaattg ctcaaaaatg
1141 cttcctgctg atatcacgca gctaaataat attccaccaa ctcaggaatc ctactatgat
1201 cccaacctgc caccggtcac taaaagtcta gtaacaaact gtaaaccagt aactgacaaa
1261 atccacaaag catataagga caaaaacaaa tataggtttg aaatcatggg agaagaagaa
1321 atcgctttta aaatgattcg taccaacgtt tctcatgtgg ttggccagtt ggatgacata
1381 agaaaaaacc ctaggaagtt tgtttgcctg aatgacaaca ttgaccacaa tcataaagat
1441 gctcagacag tgaaggctgt tctcagggac ttctatgaat ccatgttccc cataccttcc
1501 caatttgaac tgccaagaga gtatcgaaac cgtttccttc atatgcatga gctgcaggaa
1561 tggagggctt atcgagacaa attgaagttt tggacccatt gtgtactagc aacattgatt
1621 atgtttacta tattctcatt ttttgctgag cagttaattg cacttaagcg gaagatattt
1681 cccagaagga ggatacacaa agaagctagt cccaatcgaa tcagagta.
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[0253] hGBA wild type sequence (SEQ ID NO: 5):

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   1 ATGGAGTTTT CAAGTCCTTC CAGAGAGGAA TGTCCCAAGC CTTTGAGTAG GGTAAGCATC
  61 ATGGCTGGCA GCCTCACAGG ATTGCTTCTA CTTCAGGCAG TGTCGTGGGC ATCAGGTGCC
 121 CGCCCCTGCA TCCCTAAAAG CTTCGGCTAC AGCTCGGTGG TGTGTGTCTG CAATGCCACA
 181 TACTGTGACT CCTTTGACCC CCCGACCTTT CCTGCCCTTG GTACCTTCAG CCGCTATGAG
 241 AGTACACGCA GTGGGCGACG GATGGAGCTG AGTATGGGGC CCATCCAGGC TAATCACACG
 301 GGCACAGGCC TGCTACTGAC CCTGCAGCCA GAACAGAAGT TCCAGAAAGT GAAGGGATTT
 361 GGAGGGGCCA TGACAGATGC TGCTGCTCTC AACATCCTTG CCCTGTCACC CCCTGCCCAA
 421 AATTTGCTAC TTAAATCGTA CTTCTCTGAA GAAGGAATCG GATATAACAT CATCCGGGTA
 481 CCCATGGCCA GCTGTGACTT CTCCATCCGC ACCTACACCT ATGCAGACAC CCCTGATGAT
 541 TTCCAGTTGC ACAACTTCAG CCTCCCAGAG GAAGATACCA AGCTCAAGAT ACCCCTGATT
 601 CACCGAGCCC TGCAGTTGGC CCAGCGTCCC GTTTCACTCC TTGCCAGCCC CTGGACATCA
 661 CCCACTTGGC TCAAGACCAA TGGAGCGGTG AATGGGAAGG GGTCACTCAA GGGACAGCCC
 721 GGAGACATCT ACCACCAGAC CTGGGCCAGA TACTTTGTGA AGTTCCTGGA TGCCTATGCT
 781 GAGCACAAGT TACAGTTCTG GGCAGTGACA GCTGAAAATG AGCCTTCTGC TGGGCTGTTG
 841 AGTGGATACC CCTTCCAGTG CCTGGGCTTC ACCCCTGAAC ATCAGCGAGA CTTCATTGCC
 901 CGTGACCTAG GTCCTACCCT CGCCAACAGT ACTCACCACA ATGTCCGCCT ACTCATGCTG
 961 GATGACCAAC GCTTGCTGCT GCCCCACTGG GCAAAGGTGG TACTGACAGA CCCAGAAGCA
1021 GCTAAATATG TTCATGGCAT TGCTGTACAT TGGTACCTGG ACTTTCTGGC TCCAGCCAAA
1081 GCCACCCTAG GGGAGACACA CCGCCTGTTC CCCAACACCA TGCTCTTTGC CTCAGAGGCC
1141 TGTGTGGGCT CCAAGTTCTG GGAGCAGAGT GTGCGGCTAG GCTCCTGGGA TCGAGGGATG
1201 CAGTACAGCC ACAGCATCAT CACGAACCTC CTGTACCATG TGGTCGGCTG GACCGACTGG
1261 AACCTTGCCC TGAACCCCGA AGGAGGACCC AATTGGGTGC GTAACTTTGT CGACAGTCCC
1321 ATCATTGTAG ACATCACCAA GGACACGTTT TACAAACAGC CCATGTTCTA CCACCTTGGC
1381 CACTTCAGCA AGTTCATTCC TGAGGGCTCC CAGAGAGTGG GGCTGGTTGC CAGTCAGAAG
1441 AACGACCTGG ACGCAGTGGC ACTGATGCAT CCCGATGGCT CTGCTGTTGT GGTCGTGCTA
1501 AACCGCTCCT CTAAGGATGT GCCTCTTACC ATCAAGGATC CTGCTGTGGG CTTCCTGGAG
1561 ACAATCTCAC CTGGCTACTC CATTCACACC TACCTGTGGC GTCGCCAGTG A.
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[0254] hGBA natural variant sequence (SEQ ID NO: 162): hGBA (K360N) sequence. Bolded and underlined nucleotide at the mutation site.

```
   1 ATGGAGTTTT CAAGTCCTTC CAGAGAGGAA TGTCCCAAGC CTTTGAGTAG GGTAAGCATC
  61 ATGGCTGGCA GCCTCACAGG TTTGCTTCTA CTTCAGGCAG TGTCGTGGGC ATCAGGTGCC
 121 CGCCCCTGCA TCCCTAAAAG CTTCGGCTAC AGCTCGGTGG TGTGTGTCTG CAATGCCACA
 181 TACTGTGACT CCTTTGACCC CCCGACCTTT CCTGCCCTTG GTACCTTCAG CCGCTATGAG
 241 AGTACACGCA GTGGGCGACG GATGGAGCTG AGTATGGGGC CCATCCAGGC TAATCACACG
 301 GGCACAGGCC TGCTACTGAC CCTGCAGCCA GAACAGAAGT TCCAGAAAGT GAAGGGATTT
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 361 GGAGGGGCCA TGACAGATGC TGCTGCTCTC AACATCCTTG CCCTGTCACC CCCTGCCCAA
 421 AATTTGCTAC TTAAATCGTA CTTCTCTGAA GAAGGAATCG GATATAACAT CATCCGGGTA
 481 CCCATGGCCA GCTGTGACTT CTCCATCCGC ACCTACACCT ATGCAGACAC CCCTGATGAT
 541 TTCCAGTTGC ACAACTTCAG CCTCCCAGAG GAAGATACCA AGCTCAAGAT ACCCCTGATT
 601 CACCGAGCCC TGCAGTTGGC CCAGCGTCCC GTTTCACTCC TTGCCAGCCC CTGGACATCA
 661 CCCACTTGGC TCAAGACCAA TGGAGCGGTG AATGGGAAGG GGTCACTCAA GGGACAGCCC
 721 GGAGACATCT ACCACCAGAC CTGGGCCAGA TACTTTGTGA AGTTCCTGGA TGCCTATGCT
 781 GAGCACAAGT TACAGTTCTG GGCAGTGACA GCTGAAAATG AGCCTTCTGC TGGGCTGTTG
 841 AGTGGATACC CCTTCCAGTG CCTGGGCTTC ACCCCTGAAC ATCAGCGAGA CTTCATTGCC
 901 CGTGACCTAG GTCCTACCCT CGCCAACAGT ACTCACCACA ATGTCCGCCT ACTCATGCTG
 961 GATGACCAAC GCTTGCTGCT GCCCCACTGG GCAAAGGTGG TACTGACAGA CCCAGAAGCA
1021 GCTAAATATG TTCATGGCAT TGCTGTACAT TGGTACCTGG ACTTTCTGGC TCCAGCCAAC
1081 GCCACCCTAG GGGAGACACA CCGCCTGTTC CCCAACACCA TGCTCTTTGC CTCAGAGGCC
1141 TGTGTGGGCT CCAAGTTCTG GGAGCAGAGT GTGCGGCTAG GCTCCTGGGA TCGAGGGATG
1201 CAGTACAGCC ACAGCATCAT CACGAACCTC CTGTACCATG TGGTCGGCTG GACCGACTGG
1261 AACCTTGCCC TGAACCCCGA AGGAGGACCC AATTGGGTGC GTAACTTTGT CGACAGTCCC
1321 ATCATTGTAG ACATCACCAA GGACACGTTT TACAAACAGC CCATGTTCTA CCACCTTGGC
1381 CACTTCAGCA AGTTCATTCC TGAGGGCTCC CAGAGAGTGG GGCTGGTTGC CAGTCAGAAG
1441 AACGACCTGG ACGCAGTGGC ACTGATGCAT CCCGATGGCT CTGCTGTTGT GGTCGTGCTA
1501 AACCGCTCCT CTAAGGATGT GCCTCTTACC ATCAAGGATC CTGCTGTGGG CTTCCTGGAG
1561 ACAATCTCAC CTGGCTACTC CATTCACACC TACCTGTGGC GTCGCCAGTG A.
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[0255] hGBA engineered variant sequence (SEQ ID NO: 163): hGBA (C165S) sequence. Bolded and underlined nucleotide at the mutation site.

```
   1 ATGGAGTTTT CAAGTCCTTC CAGAGAGGAA TGTCCCAAGC CTTTGAGTAG GGTAAGCATC
  61 ATGGCTGGCA GCCTCACAGG TTTGCTTCTA CTTCAGGCAG TGTCGTGGGC ATCAGGTGCC
 121 CGCCCCTGCA TCCCTAAAAG CTTCGGCTAC AGCTCGGTGG TGTGTGTCTG CAATGCCACA
 181 TACTGTGACT CCTTTGACCC CCCGACCTTT CCTGCCCTTG GTACCTTCAG CCGCTATGAG
 241 AGTACACGCA GTGGGCGACG GATGGAGCTG AGTATGGGGC CCATCCAGGC TAATCACACG
 301 GGCACAGGCC TGCTACTGAC CCTGCAGCCA GAACAGAAGT TCCAGAAAGT GAAGGGATTT
 361 GGAGGGGCCA TGACAGATGC TGCTGCTCTC AACATCCTTG CCCTGTCACC CCCTGCCCAA
 421 AATTTGCTAC TTAAATCGTA CTTCTCTGAA GAAGGAATCG GATATAACAT CATCCGGGTA
 481 CCCATGGCCA GCTCCGACTT CTCCATCCGC ACCTACACCT ATGCAGACAC CCCTGATGAT
 541 TTCCAGTTGC ACAACTTCAG CCTCCCAGAG GAAGATACCA AGCTCAAGAT ACCCCTGATT
 601 CACCGAGCCC TGCAGTTGGC CCAGCGTCCC GTTTCACTCC TTGCCAGCCC CTGGACATCA
 661 CCCACTTGGC TCAAGACCAA TGGAGCGGTG AATGGGAAGG GGTCACTCAA GGGACAGCCC
 721 GGAGACATCT ACCACCAGAC CTGGGCCAGA TACTTTGTGA AGTTCCTGGA TGCCTATGCT
 781 GAGCACAAGT TACAGTTCTG GGCAGTGACA GCTGAAAATG AGCCTTCTGC TGGGCTGTTG
 841 AGTGGATACC CCTTCCAGTG CCTGGGCTTC ACCCCTGAAC ATCAGCGAGA CTTCATTGCC
 901 CGTGACCTAG GTCCTACCCT CGCCAACAGT ACTCACCACA ATGTCCGCCT ACTCATGCTG
 961 GATGACCAAC GCTTGCTGCT GCCCCACTGG GCAAAGGTGG TACTGACAGA CCCAGAAGCA
1021 GCTAAATATG TTCATGGCAT TGCTGTACAT TGGTACCTGG ACTTTCTGGC TCCAGCCAAA
1081 GCCACCCTAG GGGAGACACA CCGCCTGTTC CCCAACACCA TGCTCTTTGC CTCAGAGGCC
1141 TGTGTGGGCT CCAAGTTCTG GGAGCAGAGT GTGCGGCTAG GCTCCTGGGA TCGAGGGATG
1201 CAGTACAGCC ACAGCATCAT CACGAACCTC CTGTACCATG TGGTCGGCTG GACCGACTGG
1261 AACCTTGCCC TGAACCCCGA AGGAGGACCC AATTGGGTGC GTAACTTTGT CGACAGTCCC
1321 ATCATTGTAG ACATCACCAA GGACACGTTT TACAAACAGC CCATGTTCTA CCACCTTGGC
1381 CACTTCAGCA AGTTCATTCC TGAGGGCTCC CAGAGAGTGG GGCTGGTTGC CAGTCAGAAG
1441 AACGACCTGG ACGCAGTGGC ACTGATGCAT CCCGATGGCT CTGCTGTTGT GGTCGTGCTA
1501 AACCGCTCCT CTAAGGATGT GCCTCTTACC ATCAAGGATC CTGCTGTGGG CTTCCTGGAG
1561 ACAATCTCAC CTGGCTACTC CATTCACACC TACCTGTGGC GTCGCCAGTG A.
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[0256] mGALC sequence (SEQ ID NO: 6):

```
   1  ATGGCTAACA GCCAACCTAA GGCTTCCCAG CAACGCCAAG CAAAAGTCAT GACCGCCGCC
  61  GCGGGCTCGG CGAGCCGTGT TGCGGTGCCC TTATTGTTGT GTGCGCTGCT AGTGCCCGGT
 121  GGCGCCTACG TGCTGGACGA CTCTGACGGC CTGGGCAGAG AGTTCGACGG CATCGGCGCT
 181  GTGTCTGGCG GCGGAGCCAC AAGCAGACTG CTGGTCAACT ACCCCGAGCC CTACAGAAGC
 241  GAGATCCTGG ACTACCTGTT CAAGCCCAAC TTCGGCGCCA GCCTGCACAT CCTGAAGGTG
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 301  GAAATCGGCG GCGACGGCCA GACCACCGAC GGCACAGAGC CCAGCCACAT GCACTACGAG
 361  CTGGATGAGA ACTACTTCAG AGGCTACGAG TGGTGGCTGA TGAAGGAAGC CAAGAAGAGA
 421  AACCCCGACA TCATCCTGAT GGGCCTGCCT TGGAGCTTCC CCGGCTGGCT GGGCAAGGGC
 481  TTCAGCTGGC CCTACGTGAA CCTGCAGCTG ACCGCCTACT ACGTCGTGCG GTGGATTCTG
 541  GGCGCCAAGC ACTACCACGA CCTGGACATC GACTACATCG GCATCTGGAA CGAGAGGCCC
 601  TTCGACGCCA ACTACATCAA AGAACTGAGG AAGATGCTGG ATTACCAGGG CCTGCAGAGA
 661  GTGCGGATCA TTGCCAGCGA CAACCTGTGG GAGCCCATCA GCAGCTCCCT GCTGCTGGAC
 721  CAGGACCTGT GGAAGGTCGT CGACGTGATC GGCGCCCACT ACCCTGGCAC CTACACCGTG
 781  TGGAACGCCA AGATGAGCGG CAAGAAGCTG TGGTCCAGCG AGGACTTCAG CACCATCAAC
 841  AGCAACGTGG GAGCCGGCTG CTGGTCCAGA ATCCTGAACC AGAATTACAT CAACGGCAAC
 901  ATGACCAGCA CAATCGCCTG GNAACCTGGN GGCCAGCTAC TACGAGGACT GCCCTACGGC
 961  AGATCCGGCC TGATGACCGC CCAGGAACCT TGGAGCGGCC ACTACGTGGT GGCTTCCCCA
1021  ATCTGGGTGT CCGCCCACAC CACCCAGTTC ACCCAGCCTG GCTGGTACTA CCTGAAAACC
1081  GTGGGCCACC TGGAAAAGGG CGGCAGCTAC GTGGCCCTGA CCGATGGCCT GGGCAACCTG
1141  ACCATCATCA TCGAGACAAT GAGCCACCAG CACAGCATGT GCATCAGACC CTACCTGCCC
1201  TACTACAACG TGTCCCACCA GCTGGCCACA TTCACCCTGA AGGGCAGCCT GAGAGAGATC
1261  CAGGAACTGC AGGTCTGGTA CACCAAGCTG GGCACCCCCC AGCAGAGACT GCACTTCAAG
1321  CAGCTGGACA CCCTGTGGCT GCTGGACGGC AGCGGCAGCT TCACCCTGGA ACTGGAAGAG
1381  GACGAAATCT TCACCCTGAC CACACTGACC ACCGGCAGAA AGGGCAGCTA CCCCCCACCT
1441  CCTAGCAGCA AGCCATTCCC CACCAACTAC AAGGACGACT TCAACGTGGA ATACCCCCTG
1501  TTCAGCGAGG CCCCCAACTT CGCCGACCAG ACCGGCGTGT TCGAGTACTA CATGAACAAC
1561  GAGGACAGAG AGCACAGGTT CACCCTGAGA CAGGTGCTGA ACCAGAGGCC CATCACCTGG
1621  GCTGCCGACG CCAGCAGCAC CATCTCCGTG ATCGGGGACC ACCACTGGAC CAACATGACC
1681  GTGCAGTGCG ACGTGTACAT CGAGACACCT AGAAGCGGCG GAGTGTTTAT CGCCGGCAGA
1741  GTGAACAAGG GCGGCATCCT GATCAGATCC GCTACAGGCG TGTTCTTCTG GATCTTCGCC
1801  AACGGCAGCT ACAGAGTGAC CGCCGACCTG GGCGGCTGGA TCACATACGC CTCTGGCCAC
1861  GCCGACGTGA CCGCCAAGAG ATGGTACACC CTGACCCTGG GCATCAAGGG CTACTTCGCC
1921  TTCGGCATGC TGAACGGCAC CATCCTGTGG AAGAACGTGC GCGTGAAGTA CCCCGGCCAC
1981  GGCTGGGCTG CCATCGGCAC CCACACATTC GAGTTCGCCC AGTTCGACAA CTTTCGCGTG
2041  GAAGCTGCTC GC.
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[0257] hGLA sequence (SEQ ID NO: 7):

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   1 ATGCAGCTGA GGAACCCAGA ACTACATCTG GGCTGCGCGC TTGCGCTTCG CTTCCTGGCC
  61 CTCGTTTCCT GGGACATCCC TGGGGCTAGA GCACTGGACA ATGGATTGGC AAGGACGCCT
 121 ACCATGGGCT GGCTGCACTG GGAGCGCTTC ATGTGCAACC TTGACTGCCA GGAAGAGCCA
 181 GATTCCTGCA TCAGTGAGAA GCTCTTCATG GAGATGGCAG AGCTCATGGT CTCAGAAGGC
 241 TGGAAGGATG CAGGTTATGA GTACCTCTGC ATTGATGACT GTTGGATGGC TCCCCAAAGA
 301 GATTCAGAAG GCAGACTTCA GGCAGACCCT CAGCGCTTTC CTCATGGGAT TCGCCAGCTA
 361 GCTAATTATG TTCACAGCAA AGGACTGAAG CTAGGGATTT ATGCAGATGT TGGAAATAAA
 421 ACCTGCGCAG GCTTCCCTGG GAGTTTTGGA TACTACGACA TTGATGCCCA GACCTTTGCT
 481 GACTGGGGAG TAGATCTGCT AAAATTTGAT GGTTGTTACT GTGACAGTTT GGAAAATTTG
 541 GCAGATGGTT ATAAGCACAT GTCCTTGGCC CTGAATAGGA CTGGCAGAAG CATTGTGTAC
 601 TCCTGTGAGT GGCCTCTTTA TATGTGGCCC TTTCAAAAGC CCAATTATAC AGAAATCCGA
 661 CAGTACTGCA ATCACTGGCG AAATTTTGCT GACATTGATG ATTCCTGGAA AAGTATAAAG
 721 AGTATCTTGG ACTGGACATC TTTTAACCAG GAGAGAATTG TTGATGTTGC TGGACCAGGG
 781 GGTTGGAATG ACCCAGATAT GTTAGTGATT GGCAACTTTG GCCTCAGCTG GAATCAGCAA
 841 GTAACTCAGA TGGCCCTCTG GGCTATCATG GCTGCTCCTT TATTCATGTC TAATGACCTC
 901 CGACACATCA GCCCTCAAGC CAAAGCTCTC CTTCAGGATA AGGACGTAAT TGCCATCAAT
 961 CAGGACCCCT TGGGCAAGCA AGGGTACCAG CTTAGACAGG GAGACAACTT TGAAGTGTGG
1021 GAACGACCTC TCTCAGGCTT AGCCTGGGCT GTAGCTATGA TAAACCGGCA GGAGATTGGT
1081 GGACCTCGCT CTTATACCAT CGCAGTTGCT TCCCTGGGTA AAGGAGTGGC CTGTAATCCT
1141 GCCTGCTTCA TCACACAGCT CCTCCCTGTG AAAAGGAAGC TAGGGTTCTA TGAATGGACT
1201 TCAAGGTTAA GAAGTCACAT AAATCCCACA GGCACTGTTT TGCTTCAGCT AGAAAATACA
1261 ATGCAGATGT CATTAAAAGA CTTACTTTAA.
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[0258] hNAGLU sequence (SEQ ID NO: 8):

```
   1 ATGGAGGCGG TGGCGGTGGC CGCGGCGGTG GGGGTCCTTC TCCTGGCCGG GGCCGGGGGC
  61 GCGGCAGGCG ACGAGGCCCG GGAGGCGGCG GCCGTGCGGG CGCTCGTGGC CCGGCTGCTG
 121 GGGCCAGGCC CCGCGGCCGA CTTCTCCGTG TCGGTGGAGC GCGCTCTGGC TGCCAAGCCG
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 181 GGCTTGGACA CCTACAGCCT GGGCGGCGGC GGCGCGGCGC GCGTGCGGGT GCGCGGCTCC
 241 ACGGGCGTGG CGGCCGCCGC GGGGCTGCAC CGCTACCTGC GCGACTTCTG TGGCTGCCAC
 301 GTGGCCTGGT CCGGCTCTCA GCTGCGCCTG CCGCGGCCAC TGCCAGCCGT GCCGGGGGAG
 361 CTGACCGAGG CCACGCCCAA CAGGTACCGC TATTACCAGA ATGTGTGCAC GCAAAGCTAC
 421 TCTTTCGTGT GGTGGGACTG GGCCCGCTGG GAGCGAGAGA TAGACTGGAT GGCGCTGAAT
 481 GGCATCAACC TGGCACTGGC CTGGAGCGGC CAGGAGGCCA TCTGGCAGCG GGTGTACCTG
 541 GCCTTGGGCC TGACCCAGGC AGAGATCAAT GAGTTCTTTA CTGGTCCTGC CTTCCTGGCC
 601 TGGGGGCGAA TGGGCAACCT GCACACCTGG GATGGCCCCC TGCCCCCCTC CTGGCACATC
 661 AAGCAGCTTT ACCTGCAGCA CCGGGTCCTG GACCAGATGC GCTCCTTCGG CATGACCCCA
 721 GTGCTGCCTG CATTCGCGGG GCATGTTCCC GAGGCTGTCA CCAGGGTGTT CCCTCAGGTC
 781 AATGTCACGA AGATGGGCAG TTGGGGCCAC TTTAACTGTT CCTACTCCTG CTCCTTCCTT
 841 CTGGCTCCGG AAGACCCCAT ATTCCCCATC ATCGGGAGCC TCTTCCTGCG AGAGCTGATC
 901 AAAGAGTTTG GCACAGACCA CATCTATGGG GCCGACACTT TCAATGAGAT GCAGCCACCT
 961 TCCTCAGAGC CCTCCTACCT TGCCGCAGCC ACCACTGCCG TCTATGAGGC CATGACTGCA
1021 GTGGATACTG AGGCTGTGTG GCTGCTCCAA GGCTGGCTCT TCCAGCACCA GCCGCAGTTC
1081 TGGGGGCCCG CCCAGATCAG GGCTGTGCTG GGAGCTGTGC CCCGTGGCCG CCTCCTGGTT
1141 CTGGACCTGT TTGCTGAGAG CCAGCCTGTG TATACCCGCA CTGCCTCCTT CCAGGGCCAG
1201 CCCTTCATCT GGTGCATGCT GCACAACTTT GGGGGAAACC ATGGTCTTTT TGGAGCCCTA
1261 GAGGCTGTGA ACGGAGGCCC AGAAGCTGCC CGCCTCTTCC CCAACTCCAC CATGGTAGGC
1321 ACGGGCATGG CCCCCGAGGG CATCAGCCAG AACGAAGTGG TCTATTCCCT CATGGCTGAG
1381 CTGGGCTGGC GAAAGGACCC AGTGCCAGAT TTGGCAGCCT GGGTGACCAG CTTTGCCGCC
1441 CGGCGGTATG GGGTCTCCCA CCCGGACGCA GGGGCAGCGT GGAGGCTACT GCTCCGGAGT
1501 GTGTACAACT GCTCCGGGGA GGCCTGCAGG GGCCACAATC GTAGCCCGCT GGTCAGGCGG
1561 CCGTCCCTAC AGATGAATAC CAGCATCTGG TACAACCGAT CTGATGTGTT TGAGGCCTGG
1621 CGGCTGCTGC TCACATCTGC TCCCTCCCTG GCCACCAGCC CCGCCTTCCG CTACGACCTG
1681 CTGGACCTCA CTCGGCAGGC AGTGCAGGAG CTGGTCAGCT TGTACTATGA GGAGGCAAGA
1741 AGCGCCTACC TGAGCAAGGA GCTGGCCTCC CTGTTGAGGG CTGGAGGCGT CCTGGCCTAT
1801 GAGCTGCTGC CGGCACTGGA CGAGGTGCTG GCTAGTGACA GCCGCTTCTT GCTGGGCAGC
1861 TGGCTAGAGC AGGCCCGAGC AGCGGCAGTC AGTGAGGCCG AGGCCGATTT CTACGAGCAG
1921 AACAGCCGCT ACCAGCTGAC CTTGTGGGGG CCAGAAGGCA ACATCCTGGA CTATGCCAAC
1981 AAGCAGCTGG CGGGGTTGGT GGCCAACTAC TACACCCCTC GCTGGCGGCT TTTCCTGGAG
2041 GCGCTGGTTG ACAGTGTGGC CCAGGGCATC CCTTTCCAAC AGCACCAGTT TGACAAAAAT
2101 GTCTTCCAAC TGGAGCAGGC CTTCGTTCTC AGCAAGCAGA GGTACCCCAG CCAGCCGCGA
2161 GGAGACACTG TGGACCTGGC CAAGAAGATC TTCCTCAAAT ATTACCCCCG CTGGGTGGCC
2221 GGCTCTTGGT GA.
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[0259] hGAA sequence (SEQ ID NO: 9):

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   1 ATGGGAGTGA GGCACCCGCC CTGCTCCCAC CGGCTCCTGG CCGTCTGCGC CCTCGTGTCC
  61 TTGGCAACCG CTGCACTCCT GGGGCACATC CTACTCCATG ATTTCCTGCT GGTTCCCCGA
 121 GAGCTGAGTG GCTCCTCCCC AGTCCTGGAG GAGACTCACC CAGCTCACCA GCAGGGAGCC
 181 AGCAGACCAG GGCCCCGGGA TGCCCAGGCA CACCCCGGCC GTCCCAGAGC AGTGCCCACA
 241 CAGTGCGACG TCCCCCCCAA CAGCCGCTTC GATTGCGCCC CTGACAAGGC CATCACCCAG
 301 GAACAGTGCG AGGCCCGCGG CTGTTGCTAC ATCCCTGCAA AGCAGGGGCT GCAGGGAGCC
 361 CAGATGGGGC AGCCCTGGTG CTTCTTCCCA CCCAGCTACC CCAGCTACAA GCTGGAGAAC
 421 CTGAGCTCCT CTGAAATGGG CTACACGGCC ACCCTGACCC GTACCACCCC CACCTTCTTC
 481 CCCAAGGACA TCCTGACCCT GCGGCTGGAC GTGATGATGG AGACTGAGAA CCGCCTCCAC
 541 TTCACGATCA AAGATCCAGC TAACAGGCGC TACGAGGTGC CCTTGGAGAC CCCGCATGTC
 601 CACAGCCGGG CACCGTCCCC ACTCTACAGC GTGGAGTTCT CCGAGGAGCC CTTCGGGGTG
 661 ATCGTGCGCC GGCAGCTGGA CGGCCGCGTG CTGCTGAACA CGACGGTGGC GCCCCTGTTC
 721 TTTGCGGACC AGTTCCTTCA GCTGTCCACC TCGCTGCCCT CGCAGTATAT CACAGGCCTC
 781 GCCGAGCACC TCAGTCCCCT GATGCTCAGC ACCAGCTGGA CCAGGATCAC CCTGTGGAAC
 841 CGGGACCTTG CGCCCACGCC CGGTGCGAAC CTCTACGGGT CTCACCCTTT CTACCTGGCG
 901 CTGGAGGACG GCGGGTCGGC ACACGGGGTG TTCCTGCTAA ACAGCAATGC CATGGATGTG
 961 GTCCTGCAGC CGAGCCCTGC CCTTAGCTGG AGGTCGACAG GTGGGATCCT GGATGTCTAC
1021 ATCTTCCTGG GCCCAGAGCC CAAGAGCGTG GTGCAGCAGT ACCTGGACGT TGTGGGATAC
1081 CCGTTCATGC CGCCATACTG GGGCCTGGGC TTCCACCTGT GCCGCTGGGG CTACTCCTCC
1141 ACCGCTATCA CCCGCCAGGT GGTGGAGAAC ATGACCAGGG CCCACTTCCC CCTGGACGTC
1201 CAGTGGAACG ACCTGGACTA CATGGACTCC CGGAGGGACT TCACGTTCAA CAAGGATGGC
1261 TTCCGGGACT TCCCGGCCAT GGTGCAGGAG CTGCACCAGG GCGGCCGGCG CTACATGATG
1321 ATCGTGGATC CTGCCATCAG CAGCTCGGGC CCTGCCGGGA GCTACAGGCC CTACGACGAG
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1381 GGTCTGCGGA GGGGGGTTTT CATCACCAAC GAGACCGGCC AGCCGCTGAT TGGGAAGGTA
1441 TGGCCCGGGT CCACTGCCTT CCCCGACTTC ACCAACCCCA CAGCCCTGGC CTGGTGGGAG
1501 GACATGGTGG CTGAGTTCCA TGACCAGGTG CCCTTCGACG GCATGTGGAT TGACATGAAC
1561 GAGCCTTCCA ACTTCATCAG GGGCTCTGAG GACGGCTGCC CCAACAATGA GCTGGAGAAC
1621 CCACCCTACG TGCCTGGGGT GGTTGGGGGG ACCCTCCAGG CGGCCACCAT CTGTGCCTCC
1681 AGCCACCAGT TTCTCTCCAC ACACTACAAC CTGCACAACC TCTACGGCCT GACCGAAGCC
1741 ATCGCCTCCC ACAGGGCGCT GGTGAAGGCT CGGGGGACAC GCCCATTTGT GATCTCCCGC
1801 TCGACCTTTG CTGGCCACGG CCGATACGCC GGCCACTGGA CGGGGGACGT GTGGAGCTCC
1861 TGGGAGCAGC TCGCCTCCTC CGTGCCAGAA ATCCTGCAGT TTAACCTGCT GGGGGTGCCT
1921 CTGGTCGGGG CCGACGTCTG CGGCTTCCTG GGCAACACCT CAGAGGAGCT GTGTGTGCGC
1981 TGGACCCAGC TGGGGGCCTT CTACCCCTTC ATGCGGAACC ACAACAGCCT GCTCAGTCTG
2041 CCCCAGGAGC CGTACAGCTT CAGCGAGCCG GCCCAGCAGG CCATGAGGAA GGCCCTCACC
2101 CTGCGCTACG CACTCCTCCC CCACCTCTAC ACACTGTTCC ACCAGGCCCA CGTCGCGGGG
2161 GAGACCGTGG CCCGGCCCCT CTTCCTGGAG TTCCCCAAGG ACTCTAGCAC CTGGACTGTG
2221 GACCACCAGC TCCTGTGGGG GGAGGCCCTG CTCATCACCC CAGTGCTCCA GGCCGGGAAG
2281 GCCGAAGTGA CTGGCTACTT CCCCTTGGGC ACATGGTACG ACCTGCAGAC GGTGCCAGTA
2341 GAGGCCCTTG GCAGCCTCCC ACCCCCACCT GCAGCTCCCC GTGAGCCAGC CATCCACAGC
2401 GAGGGGCAGT GGGTGACGCT GCCGGCCCCC CTGGACACCA TCAACGTCCA CCTCCGGGCT
2461 GGGTACATCA TCCCCCTGCA GGGCCCTGGC CTCACAACCA CAGAGTCCCG CCAGCAGCCC
2521 ATGGCCCTGG CTGTGGCCCT GACCAAGGGT GGGGAGGCCC GAGGGGAGCT GTTCTGGGAC
2581 GATGGAGAGA GCCTGGAAGT GCTGGAGCGA GGGGCCTACA CACAGGTCAT CTTCCTGGCC
2641 AGGAATAACA CGATCGTGAA TGAGCTGGTA CGTGTGACCA GTGAGGGAGC TGGCCTGCAG
2701 CTGCAGAAGG TGACTGTCCT GGGCGTGGCC ACGGCGCCCC AGCAGGTCCT CTCCAACGGT
2761 GTCCCTGTCT CCAACTTCAC CTACAGCCCC GACACCAAGG TCCTGGACAT CTGTGTCTCG
2821 CTGTTGATGG GAGAGCAGTT TCTCGTCAGC TGGTGTTAG.
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[0260] hGAA (SEQ ID NO: 164; UniProt Accession No. P10253-1)

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  1 MGVRHPPCSH RLLAVCALVS LATAALLGHI LLHDFLLVPR ELSGSSPVLE ETHPAHQQGA
 61 SRPGPRDAQA HPGRPRAVPT QCDVPPNSRF DCAPDKAITQ EQCEARGCCY IPAKQGLQGA
121 QMGQPWCFFP PSYPSYKLEN LSSSEMGYTA TLTRTTPTFF PKDILTLRLD VMMETENRLH
181 FTIKDPANRR YEVPLETPHV HSRAPSPLYS VEFSEEPFGV IVRRQLDGRV LLNTTVAPLF
241 FADQFLQLST SLPSQYITGL AEHLSPLMLS TSWTRITLWN RDLAPTPGAN LYGSHPFYLA
301 LEDGGSAHGV FLLNSNAMDV VLQPSPALSW RSTGGILDVY IFLGPEPKSV VQQYLDVVGY
361 PFMPPYWGLG FHLCRWGYSS TAITRQVVEN MTRAHFPLDV QWNDLDYMDS RRDFTFNKDG
421 FRDFPAMVQE LHQGGRRYMM IVDPAISSSG PAGSYRPYDE GLRRGVFITN ETGQPLIGKV
481 WPGSTAFPDF TNPTALAWWE DMVAEFHDQV PFDGMWIDMN EPSNFIRGSE DGCPNNELEN
541 PPYVPGVVGG TLQAATICAS SHQFLSTHYN LHNLYGLTEA IASHRALVKA RGTRPFVISR
601 STFAGHGRYA GHWTGDVWSS WEQLASSVPE ILQFNLLGVP LVGADVCGFL GNTSEELCVR
661 WTQLGAFYPF MRNHNSLLSL PQEPYSFSEP AQQAMRKALT LRYALLPHLY TLFHQAHVAG
721 ETVARPLFLE FPKDSSTWTV DHQLLWGEAL LITPVLQAGK AEVTGYFPLG TWYDLQTVPV
781 EALGSLPPPP AAPREPAIHS EGQWVTLPAP LDTINVHLRA GYIIPLQGPG LTTTESRQQP
841 MALAVALTKG GEARGELFWD DGESLEVLER GAYTQVIFLA RNNTIVNELV RVTSEGAGLQ
901 LQKVTVLGVA TAPQQVLSNG VPVSNFTYSP DTKVLDICVS LLMGEQFLVS WC.
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[0261] hLAMAN sequence (SEQ ID NO: 10):

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   1 ATGGGCGCCT ACGCGCGGGC TTCGGGGGTC TGCGCTCGCG GCTGCCTGGA CTCAGCAGGC
  61 CCCTGGACCA TGTCCCGCGC CCTGCGGCCA CCGCTCCCGC CTCTCTGCTT TTTCCTTTTG
 121 TTGCTGGCGG CTGCCGGTGC TCGGGCCGGG GGATACGAGA CATGCCCCAC AGTGCAGCCG
 181 AACATGCTGA ACGTGCACCT GCTGCCTCAC ACACATGATG ACGTGGGCTG GCTCAAAACC
 241 GTGGACCAGT ACTTTTATGG AATCAAGAAT GACATCCAGC ACGCCGGTGT GCAGTACATC
 301 CTGGACTCGG TCATCTCTGC CTTGCTGGCA GATCCCACCC GTCGCTTCAT TTACGTGGAG
 361 ATTGCCTTCT TCTCCCGTTG GTGGCACCAG CAGACAAATG CCACACAGGA AGTCGTGCGA
 421 GACCTTGTGC GCCAGGGGCG CCTGGAGTTC GCCAATGGTG GCTGGGTGAT GAACGATGAG
 481 GCAGCCACCC ACTACGGTGC CATCGTGGAC CAGATGACAC TTGGGCTGCG CTTTCTGGAG
 541 GACACATTTG GCAATGATGG GCGACCCCGT GTGGCCTGGC ACATTGACCC CTTCGGCCAC
 601 TCTCGGGAGC AGGCCTCGCT GTTTGCGCAG ATGGGCTTCG ACGGCTTCTT CTTTGGGCGC
 661 CTTGATTATC AAGATAAGTG GGTACGGATG CAGAAGCTGG AGATGGAGCA GGTGTGGCGG
 721 GCCAGCACCA GCCTGAAGCC CCCGACCGCG GACCTCTTCA CTGGTGTGCT TCCCAATGGT
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781 TACAACCCGC CAAGGAATCT GTGCTGGGAT GTGCTGTGTG TCGATCAGCC GCTGGTGGAG
841 GACCCTCGCA GCCCCGAGTA CAACGCCAAG GAGCTGGTCG ATTACTTCCT AAATGTGGCC
901 ACTGCCCAGG GCCGGTATTA CCGCACCAAC CACACTGTGA TGACCATGGG CTCGGACTTC
961 CAATATGAGA ATGCCAACAT GTGGTTCAAG AACCTTGACA AGCTCATCCG GCTGGTAAAT
1021 GCGCAGCAGG CAAAAGGAAG CAGTGTCCAT GTTCTCTACT CCACCCCCGC TTGTTACCTC
1081 TGGGAGCTGA ACAAGGCCAA CCTCACCTGG TCAGTGAAAC ATGACGACTT CTTCCCTTAC
1141 GCGGATGGCC CCCACCAGTT CTGGACCGGT TACTTTTCCA GTCGGCCGGC CCTCAAACGC
1201 TACGAGCGCC TCAGCTACAA CTTCCTGCAG GTGTGCAACC AGCTGGAGGC GCTGGTGGGC
1261 CTGGCGGCCA ACGTGGGACC CTATGGCTCC GGAGACAGTG CACCCCTCAA TGAGGCGATG
1321 GCTGTGCTCC AGCATCACGA CGCCGTCAGC GGCACCTCCC GCCAGCACGT GGCCAACGAC
1381 TACGCGCGCC AGCTTGCGGC AGGCTGGGGG CCTTGCGAGG TTCTTCTGAG CAACGCGCTG
1441 GCGCGGCTCA GAGGCTTCAA AGATCACTTC ACCTTTTGCC AACAGCTAAA CATCAGCATC
1501 TGCCCGCTCA GCCAGACGGC GGCGCGCTTC CAGGTCATCG TTTATAATCC CCTGGGGCGG
1561 AAGGTGAATT GGATGGTACG GCTGCCGGTC AGCGAAGGCG TTTTCGTTGT GAAGGACCCC
1621 AATGGCAGGA CAGTGCCCAG CGATGTGGTA ATATTTCCCA GCTCAGACAG CCAGGCGCAC
1681 CCTCCGGAGC TGCTGTTCTC AGCCTCACTG CCCGCCCTGG GCTTCAGCAC CTATTCAGTA
1741 GCCCAGGTGC CTCGCTGGAA GCCCCAGGCC CGCGCACCAC AGCCCATCCC CAGAAGATCC
1801 TGGTCCCCTG CTTTAACCAT CGAAAATGAG CACATCCGGG CAACGTTTGA TCCTGACACA
1861 GGGCTGTTGA TGGAGATTAT GAACATGAAT CAGCAACTCC TGCTGCCTGT TCGCCAGACC
1921 TTCTTCTGGT ACAACGCCAG TATAGGTGAC AACGAAAGTG ACCAGGCCTC AGGTGCCTAC
1981 ATCTTCAGAC CCAACCAACA GAAACCGCTG CCTGTGAGCC GCTGGGCTCA GATCCACCTG
2041 GTGAAGACAC CCTTGGTGCA GGAGGTGCAC CAGAACTTCT CAGCTTGGTG TTCCCAGGTG
2101 GTTCGCCTGT ACCCAGGACA GCGGCACCTG GAGCTAGAGT GGTCGGTGGG GCCGATACCT
2161 GTGGGCGACA CCTGGGGGAA GGAGGTCATC AGCCGTTTTG ACACACCGCT GGAGACAAAG
2221 GGACGCTTCT ACACAGACAG CAATGGCCGG GAGATCCTGG AGAGGAGGCG GGATTATCGA
2281 CCCACCTGGA AACTGAACCA GACGGAGCCC GTGGCAGGAA ACTACTATCC AGTCAACACC
2341 CGGATTTACA TCACGGATGG AAACATGCAG CTGACTGTGC TGACTGACCG CTCCCAGGGG
2401 GGCAGCAGCC TGAGAGATGG CTCGCTGGAG CTCATGGTGC ACCGAAGGCT GCTGAAGGAC
2461 GATGGACGCG GAGTATCGGA GCCACTAATG GAGAACGGGT CGGGGGCGTG GGTGCGAGGG
2521 CGCCACCTGG TGCTGCTGGA CACAGCCCAG GCTGCAGCCG CCGGACACCG GCTCCTGGCG
2581 GAGCAGGAGG TCCTGGCCCC TCAGGTGGTG CTGGCCCCGG GTGGCGGCGC CGCCTACAAT
2641 CTCGGGGCTC CTCCGCGCAC GCAGTTCTCA GGGCTGCGCA GGGACCTGCC GCCCTCGGTG
2701 CACCTGCTCA CGCTGGCCAG CTGGGGCCCC GAAATGGTGC TGCTGCGCTT GGAGCACCAG
2761 TTTGCCGTAG GAGAGGATTC CGGACGTAAC CTGAGCGCCC CCGTTACCTT GAACTTGAGG
2821 GACCTGTTCT CCACCTTCAC CATCACCCGC CTGCAGGAGA CCACGCTGGT GGCCAACCAG
2881 CTCCGCGAGG CAGCCTCCAG GCTCAAGTGG ACAACAAACA CAGGCCCCAC ACCCCACCAA
2941 ACTCCGTACC AGCTGGACCC GGCCAACATC ACGCTGGAAC CCATGGAAAT CCGCACTTTC
3001 CTGGCCTCAG TTCAATGGAA GGAGGTGGAT GGT.

[0262] hGALC sequence (SEQ ID NO: 23; GenBank Accession No: BC036518.2):

1 aaaagctatg actgcggccg cgggttcggc gggccgcgcc gcggtgccct tgctgctgtg
61 tgcgctgctg gcgcccggcg gcgcgtacgt gctcgacgac tccgacgggc tgggccggga
121 gttcgacggc atcggcgcgg tcagcggcgg cggggcaacc tcccgacttc tagtaaatta
181 cccagagccc tatcgttctc agatattgga ttatctcttt aagccgaatt ttggtgcctc
241 tttgcatatt ttaaaagtgg aaataggtgg tgatgggcag acaacagatg gcactgagcc
301 ctcccacatg cattatgcac tagatgagaa ttatttccga ggatacgagt ggtggttgat
361 gaaagaagct aagaagagga atcccaatat tacactcatt gggttgccat ggtcattccc
421 tggatggctg ggaaaaggtt tcgactggcc ttatgtcaat cttcagctga ctgcctatta
481 tgtcgtgacc tggattgtgg gcgccaagcg ttaccatgat ttggacattg attatattgg
541 aatttggaat gagaggtcat ataatgccaa ttatattaag atattaagaa aaatgctgaa
601 ttatcaaggt ctccagcgag tgaaaatcat agcaagtgat aatctctggg agtccatctc
661 tgcatccatg ctccttgatg ccgaactctt caaggtggtt gatgttatag gggctcatta
721 tcctggaacc cattcagcaa aagatgcaaa gttgactggg aagaagcttt ggtcttctga
781 agactttagc actttaaata gtgacatggg tgcaggctgc tggggtcgca ttttaaatca
841 gaattatatc aatggctata tgacttccac aatcgcatgg aatttagtgg ctagttacta
901 tgaacagttg ccttatggga gatgcgggtt gatgacggcc caggagccat ggagtgggca
961 ctacgtggta gaatctcctg tctgggtatc agctcatacc actcagttta ctcaacctgg
1021 ctggtattac ctgaagacag ttggccattt agagaaagga ggaagctacg tagctctgac
1081 tgatggctta gggaacctca ccatcatcat tgaaaccatg agtcataaac attctaagtg
1141 catacggcca tttcttcctt atttcaatgt gtcacaacaa tttgccacct ttgttcttaa
1201 gggatctttt agtgaaatac cagagctaca ggtatggtat accaaacttg gaaaaacatc

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1261 cgaaagattt ctttttaagc agctggattc tctatggctc cttgacagcg atggcagttt
1321 cacactgagc ctgcatgaag atgagctgtt cacactcacc actctcacca ctggtcgcaa
1381 aggcagctac ccgcttcctc caaaatccca gcccttccca agtacctata aggatgattt
1441 caatgttgat tacccatttt ttagtgaagc tccaaacttt gctgatcaaa ctggtgtatt
1501 tgaatatttt acaaatattg aagaccctgg cgagcatcac ttcacgctac gccaagttct
1561 caaccagaga cccattacgt gggctgccga tgcatccaac acaatcagta ttataggaga
1621 ctacaactgg accaatctga ctacaaagtg tgatgtttac atagagaccc ctgacacagg
1681 aggtgtgttc attgcaggaa gagtaaataa aggtggtatt ttgattagaa gtgccagagg
1741 aattttcttc tggatttttg caaatggatc ttacagggtt acaggtgatt tagctggatg
1801 gattatatat gctttaggac gtgttgaagt tacagcaaaa aaatggtata cactcacgtt
1861 aactattaag ggtcatttcg cctctggcat gctgaatgac aagtctctgt ggacagacat
1921 ccctgtgaat tttccaaaga atggctgggc tgcaattgga actcactcct ttgaatttgc
1981 acagtttgac aactttcttg tggaagccac acgctaatac ttaacagggc atcatagaat
2041 actctggatt ttcttccctt ctttttggtt ttggttcaga gccaattctt gtttcattgg
2101 aacagtatat gaggcttttg agactaaaaa taatgaagag taaaagggga gagaaattta
2161 tttttaattt accctgtgga agattttatt agaattaatt ccaaggggaa aactggtgaa
2221 tctttaacat tacctggtgt gttccctaac attcaaactg tgcattggcc atacccttag
2281 gagtggtttg agtagtacag acctcgaagc cttgctgcta acactgaggt agctctcttc
2341 atcttatttg caagcggtcc tgtagatggc agtaacttga tcatcactga gatgtattta
2401 tgcatgctga ccgtgtgtcc aagtgagcca gtgtcttcat cacaagatga tgctgccata
2461 atagaaagct gaagaacact agaagtagct ttttgaaaac cacttcaacc tgttatgctt
2521 tatgctctaa aaagtatttt ttttatttcc ctttttaaga tgatactttt gaaatgcagg
2581 atatgatgag tgggatgatt ttaaaaatgc ctctttaata aactacctct aacactattt
2641 ctgtggtaat agatattagc agattaattg ggttatttgc attatttaat ttttttgatt
2701 ccaagttttg gtcttgtaac cactataact ctctgtgaac atttttccag gtggctggaa
2761 gaaggaagaa aacctgatat agccaatgct gttgtagtcg tttcctcagc ctcatctcac
2821 tgtgctgtgg tctgtcctca catgtgcact ggtaacagac tcacacagct gatgaatgct
2881 tttctctcct tatgtgtgga aggaggggag cacttagaca tttgctaact cccagaattg
2941 gatcatctcc taagatgtac ttacttttta aagtccaaat atgtttatat ttaaatatac
3001 gtgagcatgt tcatcatgtt gtatgattta tactaagcat taatgtggct ctatgtagca
3061 aatcagttat tcatgtaggt aaagtaaatc tagaattatt tataagaatt actcattgaa
3121 ctaattctac tatttaggaa tttgtaagag tctaacatag gcttagctac agtgaagttt
3181 tgcattgctt ttgaagacaa gaagataagt gctagaataa ataagattac agagaaaatt
3241 ttttgttaaa accaagtgat ttccagctga tgtatctaat atttttaaa acgaacatta
3301 tagaggtgta atttatttac aataaaatgt tcctacttta aatatacaat tcagtgagtt
3361 ttgataaatt gatataccca tgtaaccaac actccagtca agcttcagaa tatttccatc
3421 accccagaag gttctcttgt atacctgctc agtcagttcc tttcactccc gattgttggc
3481 agccattgat aggaattcta tcactatagg ttagttttct ttgttccaga acatcatgaa
3541 agcggcgtca tgtactgtgt attcttatga atggtttctt tccatcagca taatgatttg
3601 agatttgtcc atgttgtgtg attcagtggt ttgttccttc ttatttctga agagttttcc
3661 attgtatgaa tataccacaa tttgtttcct ccccaccagt ttctgatact acaattaaaa
3721 ctgtctacat ttacaaaaaa aaaaaaaaa.
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[0263] CEF promoter sequence (SEQ ID NO: 161):

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  1 gttacataac ttatggtaaa tggcctgcct ggctgactgc ccaatgaccc ctgcccaatg
 61 atgtcaataa tgatgtatgt tcccatgtaa tgccaatagg gactttccat tgatgtcaat
121 gggtggagta tttatggtaa ctgcccactt ggcagtacat caagtgtatc atatgccaag
181 tatgcccccc attgatgtca atgatggtaa atggcctgcc tggcattatg cccagtacat
241 gaccttatgg gactttccta cttggcagta catctatgta ttagtcattg ctattaccat
301 gggaattcac tagtggagaa gagcatgctt gagggctgag tgcccctcag tgggcagaga
361 gcacatggcc cacagtccct gagaagttgg ggggaggggt gggcaattga actggtgcct
421 agagaaggtg gggcttgggt aaactgggaa agtgatgtgg tgtactggct ccacctttt
481 ccccagggtg ggggagaacc atatataagt gcagtagtct ctgtgaacat tc.
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[0264] Table 2: Primers used in the study.

Primer	Sequence	SEQ ID NO:	Restriction Enzyme

GBA-F	ctgctagccaccATGGAGTTTTCAAGTCCTTC	11	NheI
GBA-R	atagcggccgcTCACTGGCGACGCCACAGGT	12	NotI
GAA-F	ctgctagccaccATGGGAGTGAGGCACCCGCCCTG	13	NheI
GAA-R	atagcggccgctcaACACCAGCTGACGAGAAACTGCTC	14	NotI
GALC-F	ctgctagccaccATGGCTAACAGCCAACCTAAGGC	15	NheI
GALC-R	atagcggccgctcaGCGAGCAGCTTCCACGCGAAAGTTG	16	NotI
NAGLU-F	ctgctagccaccATGGAAGCCGTGGCTGTCGCAG	17	NheI
NAGLU-R	atagcggccgctcaCCAACTACCAGCCACCCATCTAG	18	NotI
GLA-F	ctggatccaccATGCAGCTGAGGAACCCAGAAC	19	BamHI
GLA-R	atagcggccgctcaAAGTAAGTCTTTTAATGACATCTG	20	NotI
LAMAN-F	ctgctagccaccATGGGCGCCTACGCGCGGGCTTC	21	NheI
LAMAN-R	atagcggccgctcaACCATCCACCTCCTTCCATTGAAC	22	NotI

EXAMPLES

[0265] The disclosure is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only and the disclosure should in no way be construed as being limited to these Examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

[0266] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present disclosure and practice the claimed methods. The following working examples therefore, specifically point out the preferred embodiments of the present disclosure, and are not to be construed as limiting in any way the remainder of the disclosure.

[0267] The materials and methods employed in these experiments are now described.

[0268] *Cell lines*: The *HEK293T* cells were maintained in DMEM (Corning) containing 0.11 g/L sodium pyruvate and 4.5 g/L glucose, supplemented with 10% (vol/vol) FBS (Gibco), 100,000 U/L penicillin, 100 mg/L streptomycin (Invitrogen) and 2 mM L-glutamine (Invitrogen). Expi293 cells (Invitrogen) were grown in suspension in Expi293 expression medium (Invitrogen).

[0269] *DNA constructs*: The CMV-S1S3 plasmid was provided by Prof. Stuart Kornfeld at Washington University School of Medicine in St. Louis. Bicistronic vector pLL01 was created in two steps as follows: in the first step, a 486 bp IRES sequence was amplified from the Ptase α/β and γ bicistronic construct (provided by Prof. Stuart Kornfeld) and the S1-S3 gene fragment was obtained from plasmid CMV-S1S3 by PCR. These two fragments were linked together subsequently in the second step by overlap extension PCR to form IRES-S1S3

fragment. The IRES-S1S3 fragment was digested with HpaI and PmeI restriction enzymes (NEB) and ligated into pcDNA3.1(+) vector. To generate pLL11, pLL21, pLL31, pLL41, pLL51 and pLL61 bicistronic plasmids, hGBA, hGAA, mGALC, hNAGLU, hGLA and hLAMAN gene were amplified by their specific primers (Table 1) and inserted into the bicistronic vector (pLL01).

[0270] *Phosphotransferase Assay*: HEK293T or Expi293 cells were harvested and lysed in lysis buffer (25 mM Tris-Cl, pH 7.2, 150 mM NaCl, 1% Triton X-100, and protease inhibitor cocktail). 5 µl of cell extract was incubated in phosphotransferase assay buffer (50 mM Tris-Cl, pH 7.4, 10 mM MgCl2, 10 mM MnCl2, 2 mg/mL BSA, 2 mM ATP) in the presence of 75 mM UDP-GlcNAc, 1 mCi UDP-[3H]GlcNAc, and 100 mM aMM in a final volume of 50 µL for 0.5 hour at 37°C. The reactions were stopped by the addition of 1 mL of 2 mM EDTA, pH 8.0, and the samples were subjected to QAE-Sephadex chromatography.

[0271] *Enzyme Production*: Expi293 cells were transfected with empty vector, bicistronic plasmids or its single expression plasmid. The media was harvested after 2–3 days. For the production of GBA, the conditional medium containing 30 uM of isofagomine during cell culture to stabilize the secreted enzyme was dialyzed in PBS buffer at 4 °C overnight to remove isofagomine for enzyme activity assay.

[0272] *Enzyme activity assay*: The following substrates are used for enzymes activity assay: 4-methylumbelliferyl [3-D-glucopyranoside (GCase/GBA enzyme substrate, M3633, Sigma,), 4-methylumbelliferyl α-D-glucopyranoside (GAA enzyme substrate, M9766, Sigma), 6-Hexadecanoylamino-4-methylumbelliferyl [3-D-galactopyranoside (GALC enzyme substrate, EH05989, Carbosynth), 4-methylumbelliferyl-N-acetyl-α-D-glucosaminide (NAGLU enzyme substrate, 474500, Millipore), 4-methylumbelliferyl α-D-galactopyranoside (GLA enzyme substrate, M7633, Sigma), and 4-methylumbelliferyl α-D-mannopyranoside (LAMAN enzyme substrate, M3657,Sigma). GBA enzyme activity was assayed in citrate-phosphate buffer, pH5.0, 0.25% TX-100, 0.25% Na Taurocholate with 1 mM GBA substrate. GAA enzyme activity was carried in citrate buffer, pH4.0, 0.25% TX-100 with 1 mM GAA substrate. GALC enzyme activity was performed in citrate-phosphate buffer, pH4.0, 0.25% TX-100, 0.6% Na Taurocholate, 0.2% Oleic acid with 0.1 mM GALC substrate. NAGLU enzyme activity was assayed in citrate buffer, pH4.0, 0.25% TX-100 with 1 mM NAGLU substrate. GLA enzyme activity was assayed in citrate buffer, pH4.5, 0.25% TX-100 with 1 mM GLA substrate. LAMAN enzyme activity was assayed in citrate buffer, pH4.0, 0.25% TX-100 with 1 mM LAMAN substrate.

[0273] *CI-MPR binding assay*: CI-MPR binding was performed in high binding 96 well

plate (Costar 3601). The plate was immobilized with 50 µl purified bovine CI-MPR at 10 µg/ml at room temperature (RT) for 1 hour and blocked by 2% BSA at RT for another 1 hour. Aliquots of conditional media from transfected Expi293 cells were diluted with Hepes buffer (40 mM Hepes, pH6.8, 150 mM NaCl, 0.05% Tween-20) and incubated with the immobilized CI-MPR at RT for 1 hour to bind the phosphorylated lysosomal enzymes. After three times wash, the lysosomal enzyme activity was assayed by 4-Methylumbelliferone method.

Example 1: Generation of an empty bicistronic vector containing phosphotransferase (S1-S3) for lysosomal enzyme expression

[0274] The GlcNAc-1-phosphotransferase (GlcNAc-1-PTase, also referred to as Ptase), which is an $\alpha 2\beta 2\gamma 2$ hexamer encoded by two genes (GNPTAB and GNPTG), is involved in the generation of phosphorylated oligosaccharide that is required for lysosomal targeting via the cation-independent mannose 6-phosphate receptor (CI-MPR). The phosphorylation of expressed lysosomal enzymes significantly increases by co-transfection with an engineered truncated Ptase (S1-S3). This study utilizes a S1-S3 construct for the production of phosphorylated lysosomal enzymes for the treatment of lysosomal storage diseases (LSD, such as but not limited to Gaucher disease, Pompe disease, and α-Mannosidosis).

[0275] To produce highly phosphorylated therapeutic lysosomal enzymes for enzyme replacement therapy (ERT), a therapeutic lysosomal enzyme and S1-S3 is co-expressed simultaneously in the same cells. Since the S1-S3 and lysosomal enzyme are expressed in different vectors, in order to produce highly phosphorylated therapeutic lysosomal enzyme, a stable cell line with expression of lysosomal enzyme and S1-S3 are generated by two steps: (a) create a stable cell line expressing Ptase S1-S3; (b) based on the S1-S3 stable cell line, generate a second cell line which add the expression of therapeutic lysosomal enzyme into it. To avoid this two-step and time-consuming procedure, disclosed herein is a bicistronic vector by introducing an Internal Ribosome Entry Site (IRES), which is able to express two separate genes under a single promoter.

[0276] Bicistronic expression may also be applied gene therapy for lysosomal storage diseases (LSD). An empty bicistronic vector - pLL01 containing a 486bp IRES sequence and S1-S3 gene under cytomegalovirus (CMV) promoter in pcDNA3.1(+) plasmid vector (Figure 1B). The bicistronic vector pLL01 has three unique restriction enzyme cleavage sites in the multi-cloning sites which are located in front of IRES sequence and allowed to insert therapeutic lysosomal enzyme gene. To examine the expression of S1-S3 using the bicistronic vector pLL01, HEK293 cells were transfected with equivalent amount plasmid

of pcDNA3.1(+), CMV-S1S3 (Figure 1A) or pLL01. 48 hour later, cells were harvested and lysed in lysis buffer (25 mM Tris buffer, pH7.4, 150 mM NaCl, 1% TX-100 with protease inhibitor cocktail). Phosphotransferase activity analysis of whole cell extracts expressing pcDNA3.1(+), CMV-S1S3 or pLL01 was performed to determine the expression of S1-S3. As shown in Figure 1C, comparing to sample CMV-S1S3, the phosphotransferase activity in pcDNA3.1(+) sample is negligible, but the bicistronic vector pLL01 maintains 9.3% activity.

Example 2: Bicistronic expression enhances phosphorylation of therapeutic lysosomal enzymes

[0277] Since the expression of S1-S3 in the bicistronic vector was low (9.3%) (see Example 1), this study was designed to determine whether the low S1-S3 activity would be enough to phosphorylate lysosomal enzymes. Six different lysosomal enzymes were tested in the present bicistronic vector. The enzymes were as follow: acid β-Glucosidase (GBA), acid α-Glucosidase (GAA), Galactosylceramidase (GALC), α-N-acetylglucosaminidase (NAGLU), α-Galactosidase (GLA) and acid α-mannosidase (LAMAN).

[0278] *Acid β-Glucosidase (GBA)*: GBA is a lysosomal enzyme which degrades its substrate glycocerebroside in lysosome. The deficiency of GBA in lysosome causes Gaucher disease which is the most common lysosomal storage disease (LSD). To test the phosphorylation of GBA in the presently disclosed bicistronic vector, GBA bicistronic plasmid - pLL11 was generated by inserting a 1611 bp human GBA cDNA sequence with a stop codon into the bicistronic empty vector - pLL01 through NheI and NotI restriction sites (Figure 2A). The same amount of pLL11 and GBA plasmid with or without CMV-S1S3 plasmid were transfected into Expi293 cells. 48 hours later, the cells and conditional medium were harvested separately. Surprisingly, the GBA activity in the pLL11 conditional medium is 240 nmol/hour/ml which is more than 2 times higher than the medium prepared by GBA alone (96 nmol/hour/ml) or GBA and S1-S3 co-transfection (90 nmol/hour/ml, Figure 2B). In addition to the GBA expression, the S1-S3 expression was quantified by phosphotransferase assay using cell extract. Similar to the bicistronic vector pLL01 lacking GBA, pLL11 sample has 7.5% phosphotransferase expression, comparing to the co-transfection sample of GBA&S1-S3 (Figure 2C).

[0279] Since the S1-S3 expression was decreased in the bicistronic vector, the consequence of the low phosphotransferase expression on the phosphorylation of GBA was determined. For this purpose, the conditional medium of pLL11, GBA alone and GBA co-transfected with S1-S3 were harvested and the degree of phosphorylation was quantitated by performing

cation-independent mannose 6-phospohate receptor (CI-MPR) binding experiment. The GBA produced in the presently disclosed bicistronic vector has even higher binding to CI-MPR in the plateau phase (Figure 3A). Nevertheless, when the percentage of receptor binding was calculated by using the linear range points, 44% of GBA generated in the disclosed bicistronic vector were bound to the CI-MPR which is the same as the GBA produced by co-transfection with S1-S3 (43%) and is ten times higher than the GBA produced by endogenous phosphotransferase (4.5%, Figure 3B).

[0280] Titration have been widely used in the art to determine the concentration of an identified analyte. The concentration of CI-MPR in the binding experiment was titrated. Serial diluted CI-MPR was immobilized in 96 well plate, and similar amount of GBA enzyme which was produced by the presently disclosed bicistronic vector or endogenous phosphatase (Ptase) was added into the plate for receptor binding assay. As shown in figure 3C, the binding of GBA from the pLL11 sample was dependent on the concentration of CI-MPR, and it saturated when the receptor concentration reached 15 μg/ml, while the binding of GBA produced by endogenous Ptase stays in the low level. The present data indicated that the disclosed bicistronic vector greatly elevates the phosphorylation level of GBA enzyme.

[0281] *Acid α-Glucosidase (GAA):* Lysosomal enzyme GAA is essential for the degradation of glycogen to glucose in lysosome. Mutation in GAA gene is associated with a lysosomal storage disorder - Pompe disease. In order to create GAA bicistronic plasmid - pLL21, a 2859 base pair (bp) human GAA gene fragment containing stop codon was amplified and inserted into bicistronic vector pLL01 after digestion by restriction enzymes NheI and NotI (Figure 4A). Sequence verified pLL21 and GAA plasmids were transfected in Expi293 cells. 48 hours later, conditional medium was collected for GAA activity and CI-MPR binding experiments. Similar to GBA, the GAA activity in pLL21 conditional medium was higher than GAA single expression (Figure 4B). The binding of pLL21 conditional medium was faster and higher than GAA single conditional medium (Figure 4C). During 1 hour incubation time, 72.5% of GAA from pLL21 conditional medium binds to CI-MPR, but the CI-MPR binding of GAA from GAA single expression is only 21.5% (Figure 4D). These data suggested that the presently disclosed bicistronic expression platform can greatly increase the phosphorylation of GAA enzyme.

[0282] *Galactosylceramidase (GALC)*: In lysosome, GALC enzyme is responsible for the catabolism of galactosylceramide by removing galactose from ceramide derivatives. Genetic deficiency of GALC enzyme is responsible for Krabbe disease. To test GALC enzyme in the presently disclosed bicistronic expression, bicistronic plasmid pLL31 was generated by

inserting a mouse GALC gene into vector pLL01 (Figure 5A). The GALC enzyme activity in pLL31 conditional medium which was harvested in pLL31 transfected Expi293 cells is similar to GALC alone medium (0.86 nmol/μl/h vs 0.62 nmol/μl/h, Figure 5B). CI-MPR receptor binding results showed that the bicistronic expression of GALC with S1-S3 increases its CI-MPR binding from 28.4% to 56.8% (Figure 5C&D).

[0283] *α-N-acetylglucosaminidase (NAGLU)*: NAGLU gene encodes an enzyme that degrades heparin sulfate in lysosome. Defect in the NAGLU enzyme results in Sanfilippo syndrome type B, also known as Mucopolysaccharidosis (MPS) IIIB. When the NAGLU enzyme produced in cell line for ERT does not have any phosphate in the mannose residues. And the clinical trials for its ERT failed early this year. To express NAGLU in the presently disclosed bicistronic vector, the same procedure as described above was used. A 2229 bp human NAGLU gene was inserted into pLL01 bicistronic vector (Figure 6A), and the NAGLU bicistronic plasmid -pLL41 and NAGLU single expression plasmid were transfected into Expi293 cells. By using the conditional medium, the NAGLU activity in sample pLL41 was shown to be higher than NAGLU single expression sample (Figure 6B). In term of CI-MPR binding, hardly any NAGLU binding was detected from NAGLU single expression sample, even though we put a high amount enzyme (up to 9 nmol/hour, Figures 6C-6D). However, the NAGLU produced by the bicistronic vector binds to CI-MPR up to 25% (Figures 6C-6D).

[0284] *α-Galactosidase (GLA)*: Lysosomal enzyme GLA hydrolyzes melibiose into galactose and glucose and is able to metabolize globotriaosylceramide (GL-3). A deficiency of GLA enzyme activity causes an X-linker disorder – Fabry disease. To make GLA bicistronic plasmid – pLL51, human GLA gene fragment and bicistronic vector pLL01 were digested with BamHI and NotI, and ligated by T4 ligase (Figure 7A). Correct pLL51 clone and GLA single plasmid are transfected and expressed in Expi293 cells. GLA activity assay and CI-MPR binding experiments are carried by using their conditional mediums. As shown in Figure 7B, the GLA activity in either GLA alone or pLL51 conditional medium are similar. The titration curves using these two mediums suggest pLL51 sample binds to CI-MPR more and faster than GLA sample (Figure 7C). The overall binding percentage for pLL51 sample is 62.1%, which is almost double of GLA sample (33.1%, Figure 7D).

[0285] *acid α-mannosidase (LAMAN):* The genetic disease α-Mannosidosis is caused by defect in the Lysosomal enzyme LAMAN which is encoded by the MAN2B1 gene. Since the human LAMAN enzyme is barely phosphorylated, hLAMAN is a good candidate for the disclosed bicistronic expression. 3033 bp human LAMAN gene was inserted into pLL01

bicistronic vector (Figure 8A) and expressed in Expi293 cells for later study. The LAMAN activity in LAMAN bicistronic plasmid pLL61 conditional medium is slightly lower than LAMAN single expression (Figure 8B). When their binding to CI-MPR was titrated, LAMAN enzyme binding to CI-MPR was hardly detected by using LAMAN single expression sample, but a large amount of LAMAN enzyme from pLL61 sample was found to interact with CI-MPR (Figure 8C). The binding of LAMAN to CI-MPR increase from 1.6% to 75.2% with S1-S3 bicistronic expression (Figure 8D).

[0286] The above six enzymes can be categorized into two groups based on their basal phosphorylation levels. Group one is low phosphorylation lysosomal enzymes (GBA, NAGLU and LAMAN) which are poor substrates for wild-type Ptase during enzyme production. The second group is high phosphorylation enzymes (GAA, GALC and GLA). The enzymes are considered as good substrates for wild-type Ptase and received a fair amount of phosphate. The presently disclosed bicistronic expression of S1-S3 was shown to significantly increase the phosphorylation of six lysosomal enzymes, independent of their basal phosphorylation level. In view of these findings, the bicistronic vector pLL01 disclosed herein can be used to product highly phosphorylated lysosomal enzymes to treat all lysosomal storage diseases. Clearly, the presently disclosed bicistronic vector greatly benefits ERT and gene therapy for the treatment of lysosomal storage disorders.

Example 3: Treatment of Gaucher Disease

[0287] *Enzyme Replacement Therapy (ERT)*

[0288] An expression vector comprising a sequence encoding GBA and a sequence encoding a S1-S3 Ptase may be used to treat or prevent a sign or symptom of Gaucher Disease. The following studies demonstrate that expression of (GCase/GBA)-S1-S3 in the art-recognized standard mouse model of Gaucher Disease leads to expression of the (GCase/GBA)-S1-S3, transportation of the v-S1-S3 into cells from the circulating blood stream and an increased activity of v in cells taking up the v-S1-S3 complex. A small increase in (GCase/GBA) activity resulting from the expression and uptake of the GBA-S1-S3 complex leads to a significant functional recovery of function in the mouse model.

[0289] The expression of GBA utilizing the bicistronic expression vector with S1-S3 PTase, generates a recombinant protein with higher levels of phosphorylated oligosaccharides that can be used to treat or prevent a sign or symptom of Gaucher Disease. The following studies demonstrate that ERT using recombinant protein expressed using the bicistronic vector with S1-S3 PTase in the art-recognized standard mouse model of Gaucher Disease leads to a

longer half-life, greater uptake by tissue, greater substrate reduction and better correction of tissue pathology compared to the current standard of care.

[0290] Figs 16A-16B are a pair of graphs depicting elevated glucosylceramide levels observed in the liver, lung and spleen of 20 week old Gaucher$^{D409V/null}$ mice. The accumulation of GBA's natural substrate, glucocerebroside was determined in tissue homogenates. The accumulation of GC in the lung is a statistically and therapeutically valuable result, which is a known unmet need of the current standard of care. 20 µL aliquots of tissue homogenates and appropriate controls were glucocylceramides were extracted by adding 200 µL of Methanol/ACN/H2O (v:v:v=85:10:5), a mixing for 5 min at 800 rpm followed by centrifuging for 15 min at 3220 *g* 4 °C; 3). 50 µL of supernatant was recovered, dried with nitrogen and resuspended with Methanol/ACN/H2O (v:v:v=85:10:5) and directly injected for LC-MS/MS analysis.

[0291] Figs 17A-17C are a series of graphs demonstrating that GCase^{M6P} has a longer half-life and greater tissue uptake in the GBA$^{D409V/null}$ mouse model compared to imiglucerase. A PK/PD study in the Gaucher D409V/Null mouse model was performed using the standard of care, imiglucerase, and purified GBA produced by transiently co-expressed utilizing the bicistronic vector that encoded for the S1-S3 PTase and a natural variant of GBA in Expi293 cells. This variant of GCase has greater stability at neutral and slightly alkali conditions. Briefly, 3 animals received a tail vein injection of ~ 1.5 mg/kg of recombinant GCase. For the serum pharmacokinetic data, plasma samples were collected at 2, 10, 20, 40 and 60 mins. Activity measured using a synthetic substrate, 4-methylumbelliferyl-beta-D-glucopyranoside (4MU-Glc). The activity was normalized in the individual animals by setting the 2 min time point as 100% activity and subsequent time points are a percent of the t=2 min time point. The stabilized GCase expressed in the presence of S1-S3 PTase appears to have a longer half-life. This longer half-life is a combination of the enzyme having greater stability and the different clearance pathways. To determine how much GCase was taken up by the tissue, 2 hrs after enzyme injection, tissue was recovered, homogenized and activity measured using the 4MU-Glc substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination. The true advantage of a stabile GCases with appropriate phosphorylation is observed in the tissue uptake data shown. For all tissues evaluated there is more activity found in the stabilized GCase expressed utilizing the bicistronic S1-S3 PTase vector platform S1'S3 PTase. This is most dramatic in the lung, muscle and brain where imiglucerase has little activity. When the tissue and sera data is taken together, the advantage of a more stable GCase with greater N-linked oligosaccharide

phosphorylation is apparent for delivering more enzyme to affected tissue. This is the first time that a significant amount of GCase has been delivered to the lung, muscle and heart at these doses.

[0292] Figs 18A-18E are a series of photographs and bar graphs demonstrating that $GCase^{M6P}$ ERT reduced tissue macrophages (anti-CD68 staining) better than imiglucerase in the $GBA^{D409V/null}$ mouse model. An efficacy study in the D409V Gaucher mouse model was performed using the standard of care, Cerezyme, and purified GBA (M0111) transiently co-expressed in Expi293 cells utilizing the bicistronic vector that encodes for the S1S3 PTase and a natural variant of GBA with reported greater stability at neutral and slightly alkali conditions. ~20 weeks old Gaucher mice were treated with ~1.5 mg/kg) enzymes weekly for four weeks. Four weeks later, the tissue of Liver and Lung was harvested and fixed in 4% paraformaldehyde-PBS, pH 7.4 for immunohistochemistry with CD68 antibody. M0111 has greater efficacy compared to the current standard of care as evidenced by the reduction of macrophage in affected tissue as visualized by CD68 Ab.

[0293] Figs 19A-19C are a series of photographs demonstrating that $GCase^{M6P}$ ERT reduced the number and size of Gaucher storage cells (Hematoxylin and Eosin (H&E) staining) better than imiglucerase in the $GBA^{D409V/null}$ mouse model. An efficacy study in the D409A Gaucher mouse model was performed using the standard of care, Cerezyme, and purified GBA transiently co-expressed in Expi293 cells utilizing the bicistronic vector that encoded for the S1-S3 PTase and a natural variant of GBA with reported greater stability at neutral and slightly alkali conditions. ~20 weeks old Gaucher mice were treated with ~1.5 mg/kg enzymes weekly for four weeks. Four weeks later, the tissue of Liver and Lung was harvested and fixed in 4% paraformaldehyde-PBS, pH 7.4 for formalin for hematoxylin and eosin (H&E) staining. $GCase^{M6P}$ has greater efficacy compared to the current standard of care as evidenced by the reduction of storage cells in affected tissue as visualized by H&E staining.

[0294] Figs 20A-20B are a pair of graphs demonstrating that $GCase^{M6P}$ ERT reduced accumulated substrate better than imiglucerase in the $GBA^{D409V/null}$ mouse model. ~20 weeks old Gaucher mice were treated weekly with ~1.5 mg/kg enzymes for four weeks. Tissue samples were collected and homogenized for glycosylceramide analysis. The accumulation of GCase's natural substrate, glucocerebroside was determined in tissue homogenates. Of significant value is the accumulation of GC in the lung which is a known unmet need for the current standard of care. 20 μL aliquots of tissue homogenates and appropriate controls were glucocylceramides were extracted by adding 200 μL of Methanol/ACN/H2O (v:v:v=85:10:5), mixing for 5 min at 800 rpm followed by centrifuging for 15 min at 3220 *g* 4

°C; 3). 50 µL of supernatant was recovered, dried with nitrogen and resuspended with Methanol/ACN/H2O (v:v:v=85:10:5) and directly injected for LC-MS/MS analysis. For the two ceramides measured, GCase^{M6P} treated animals had lower levels following ERT therapy over the imiglucerase.

[0295] *Gene Therapy*

[0296] An delivery vector with a bicistronic vector comprising a sequence encoding GBA and a sequence encoding the S1-S3 PTase may be used to treat or prevent a sign or symptom of Gaucher Disease. In some embodiments, the delivery vector is a viral vector. In some embodiments, the viral vector is an AAV vector. In some embodiments, the AAV vector is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 or AAV9 vector. In some embodiments the viral vector is a lentiviral vector. In some embodiments, the vector is a non-viral vector. In some embodiments, the non-viral vector is a liposome, an LNP, a polymer nanoparticle, a nanoparticle, a micelle, an polymersome or an exosome. The following studies demonstrate that expression of GBA and S1-S3 PTase utilizing the bicistronic vector in the art-recognized standard mouse model of Gaucher Disease leads to expression of GBA^{M6P}, increased activity in tissue and serum, and reduced substrate. This demonstrates that having a phosphorylated transgene product with high affinity for the CI-MPR can lead to effective therapies even at low activities levels due to efficient cellular uptake and lysosomal targeting.

[0297] Figs 21A-21D are a series of graphs showing the results of in vivo AAV mediate gene therapy studies for the treatment of Gaucher Disease. To determine the effect of AAV9 gene therapy with the bicistronic expression transgene of stable GBA + S1-S3 PTase with three different promotors. 15 wk old GBA$^{D409V/null}$ mice were dosed with a moderate dose of AAV9-stable GBA+ S1-S3 PTase, 5E11 vg. To determine how much GBA was generated by the tissue, 2 weeks later after AAV9 injection, tissue was recovered, homogenized and activity measured using the 4MU-Glc substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination.

[0298] Figs 29A-29C are a series of graphs depicting enzyme activity and select GCase substrates in the lung and liver 2 weeks post injection of AAV9-hTLV-GBA^{M6P} gene therapy in Gaucher mice. AAV9-hTLV-GBA-S1S3 is otherwise known as AAV9-hTLV-GBA^{M6P} wherein the M6P denotes the S1S3 construct. Two weeks following AAV9 hTLV-GBA or AAV9 hTLV-GBA^{M6P} (transgene with bicistronic vector with GBA and S1-S3 PTase) There was elevated expression in the liver for both constructs (Fig. 29A) When liver glucosyl-β-ceramide levels were measured (Fig, 29B and C), the greatest reduction in accumulated

substrate was observed for the AAV9 hTLV-GBA^{M6P} treated animals even though there was lower GCase activity in the liver compared to the AAV9 hTLV-GBA treated animals. This greater substrate reduction with less activity indicates the importance of N-linked oligosaccharide phosphorylation for gene therapy in terms cell uptake and lysosomal targeting. In the lung, the GCase activity for the AAV9 treated animals is low. However, the AAV9-hTLV-GBA^{M6P} treated animals showed significant reduction in the lung for accumulated glucosyl-β-ceramide levels (Fig, 29B, C). Little reduction was observed for the AAV9-hTLV-GBA treated animals. This demonstrates that having a phosphorylated transgene product with high affinity for the CI-MPR can lead to effective therapies even at low activities levels due to efficient cellular uptake and lysosomal targeting.

Example 4: Treatment of α-Mannosidosis

[0299] *Enzyme Replacement Therapy (ERT)*

[0300] An expression vector comprising a sequence encoding LAMAN and a sequence encoding a S1-S3 Ptase may be used to treat or prevent a sign or symptom of α-Mannosidosis. The following studies demonstrate that expression of LAMAN-S1-S3 in a mouse model leads to expression of the LAMAN-S1-S3, transportation of the LAMAN-S1-S3 into cells from the circulating blood stream and an increased activity of LAMAN in cells taking up the LAMAN-S1-S3 complex. A small increase in LAMAN resulting from the expression and uptake of the LAMAN-S1-S3 complex leads to a significant functional recovery of function in the mouse model.

[0301] The expression of LAMAN utilizing the bicistronic expression vector with S1-S3 PTase, generates a recombinant protein with higher levels of phosphorylated oligosaccharides that can be used to treat or prevent a sign or symptom of α-Mannosidosis. The following studies demonstrate that ERT using recombinant LAMAN protein expressed using the bicistronic vector with S1-S3 PTase in the wild type mice leads to a greater uptake and boarder distribution in tissues.

[0302] Figs 22A-22C are a series of graphs depicting the results of in vitro studies for the use of lysosomal alpha-mannosidase (LAMAN) as ERT.

[0303] Figs 23A-23B is a photograph and corresponding data table depicting LAMAN enzyme expression, purification, and characterization. Two preparations of LAMAN were transiently co-expressed in Expi293 cells with (M0611) or without the bicistronic vector that encoded for the S1-S3 PTases. Both were purified by utilization of the HPC4 affinity tag. The significant increase in phosphorylation was demonstrated by measuring the amount of LAMAN that kind bind to immobilized cation-independent mannose 6-phosphate receptor in

a dose dependent manner. The amount of LAMAN bound was based on its activity using it synthetic substrate 4-Methylumbelliferyl-α-D-Mannopyranoside (4MU-Man). The specificity of binding via phosphorylated oligosaccharides was confirmed by the ability of added mannose 6-phosphate to block binding. Of note is the ability of LAMAN^{M6P} (M0611) to bind the receptor even in the presence of M6P. LAMAN^{M6P} (M0611, P-0030) and LAMAN (P-0031) were chosen for in vivo animal study.

[0304] Fig 23C a graph depicting LAMAN^{M6P} (M0611) enzyme expression, purification, and characterization. Two preparations of LAMAN were transiently co-expressed in Expi293 cells with or without the bicistronic vector that encoded for the S1-S3 variant of PTase. Both were purified by utilization of the HPC4 tag. The significant increase in phosphorylation was demonstrated by measuring the amount of LAMAN that kind bind to immobilized cation-independent mannose 6-phosphate receptor in a dose dependent manner. The amount of bound LAMAN was determined by activity using a synthetic substrate 4-Methylumbelliferyl-α-D-Mannopyranoside (4MU-Man). The specificity of binding via phosphorylated oligosaccharides was confirmed by the ability of added mannose 6-phosphate to block binding. Of note is the ability of M0611 to bind the receptor even in the presence of M6P. LAMAN^{M6P} (M0611, P-0030) and LAMAN (P-0031) were chosen for in vivo animal study.

[0305] Figs 24A-24B are a pair of graphs demonstrating the biodistribution of LAMAN and LAMAN^{M6P} enzymes in wild type mice for enzyme replacement therapy. To evaluate the difference in tissue uptake between LAMAN and LAMAN^{M6P} (LAMAN co-expressed with S1-S3 PTase), 2 mg/kg of each prep was injected via tail vein into wild type mice (n=4). 2 and 8 hrs after dosing, tissue was recovered, homogenized and activity measured using the 4MU-Man substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination. An advantage of LAMAN^{M6P} (LAMAN co-expressed with S1S3 PTase) is observed in the tissue uptake data. For liver, spleen, heart, lung, and brain there was greater activity in the tissue at 2 hours. This trend was also true at 8 hours with the exception of the lung. This might be a result of the high variation observed in the analysis of this tissue. The only exception to this observation was the kidney. Endogenous LAMAN activity is subtracted from all samples. Higher LAMAN enzyme activity was detected in most tissues of the mice which were injected with our LAMAN^{M6P} enzyme.

[0306] Figs 25A-25B are a pair of graphs demonstrating the biodistribution of αLAMAN and LAMAN^{M6P} enzymes in wild type mice for enzyme replacement therapy. To evaluate the difference in tissue uptake between LAMAN and LAMAN^{M6P} (LAMAN co-expressed with

S1-S3 PTase), 10 mg/kg of each prep was injected via tail vein into wild type mice (n=4). 2 and 8 hrs after dosing, tissue was recovered, homogenized and activity measured using the 4MU-Man substrate. The activity was normalized to total protein in the homogenate as determined by the BCA method for protein determination. An advantage of LAMAN^{M6P} (LAMAN co-expressed with S1-S3 PTase) is observed in the tissue uptake data. For liver, spleen, heart, lung, and brain there was greater activity in the tissue at 2 hours. This trend was also true at 8 hours with the exception of the Kidney. This might be a result of the high variation observed in the analysis of this tissue.

[0307] *Gene Therapy*

[0308] A delivery vector comprising a sequence encoding LAMAN and a sequence encoding the S1-S3 modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) may be used to treat or prevent a sign or symptom of α-Mannosidosis. In some embodiments, the delivery vector is a viral vector. In some embodiments, the viral vector is an AAV vector. In some embodiments, the AAV vector is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 or AAV9 vector. In some embodiments the viral vector is a lentiviral vector. In some embodiments, the vector is a non-viral vector. In some embodiments, the non-viral vector is a liposome, an LNP, a polymer nanoparticle, a nanoparticle, a micelle, an polymersome or an exosome. The following studies demonstrate that expression of LAMAN-S1-S3 in a mouse model of α-Mannosidosis leads to expression of the LAMAN-S1-S3, transportation of the LAMAN-S1-S3 into cells from the circulating blood stream and an increased activity of LAMAN in cells taking up the LAMAN-S1-S3 complex. A small increase in v resulting from the expression and uptake of the LAMAN-S1-S3 complex leads to a significant functional recovery of function in the mouse model.

[0309] Alternatively or in addition, a delivery vector comprising a sequence encoding the S1-S3 modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) may be used to treat or prevent a sign or symptom of *α-Mannosidosis*. The expression of S1-S3 may increase the uptake of endogenous LAMAN by body tissues, thereby inducing a significant functional recovery of function in the mouse model.

Example 5: Treatment of Mucolipidosis

[0310] *Enzyme Replacement Therapy (ERT)*

[0311] An expression vector comprising a sequence encoding the S1-S3 modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) may be used to treat or prevent a sign or symptom of Mucolipidosis. The following studies demonstrate that expression of S1-S3 leads to

expression of the S1-S3, transportation of the S1-S3 as well as one or more lysosomal enzymes into cells from the circulating blood stream and an increased activity of one or more lysosomal enzymes in cells taking up the S1-S3 complex. A small increase in the S1-S3 complex resulting from the expression and uptake of the S1-S3 complex and one or more lysosomal enzymes leads to a significant functional recovery of function.

[0312] *Gene Therapy*

[0313] A delivery vector comprising a sequence encoding a S1-S3 modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) may be used to treat or prevent a sign or symptom of Mucolipidosis. In some embodiments, the delivery vector is a viral vector. In some embodiments, the viral vector is an AAV vector. In some embodiments, the AAV vector is an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 or AAV9 vector. In some embodiments the viral vector is a lentiviral vector. In some embodiments, the vector is a non-viral vector. In some embodiments, the non-viral vector is a liposome, an LNP, a polymer nanoparticle, a nanoparticle, a micelle, an polymersome or an exosome. A delivery vector comprising a sequence encoding a soluble S1-S3 modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) may be used to treat or prevent a sign or symptom of Mucolipidosis. A delivery vector comprising a sequence encoding a S1-S3 modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) may be used to treat or prevent a sign or symptom of Mucolipidosis. The following studies demonstrate that expression of S1-S3 PTase leads to expression of the S1-S3 PTase, S1-S3 cellular activity results in the correction of serum level of mis-trafficked lysosomal enzymes by increasing their N-linked oligosccharide phosphorylation allowing for efficient targeting to the lysosome.

[0314] Alternatively or in addition, a delivery vector comprising a sequence encoding the S1-S3 modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase) may be used to treat or prevent a sign or symptom of Mucolipidosis. The expression of S1-S3 PTase may increase the uptake of one or more endogenous lysosomal enzymes by body tissues, thereby inducing a significant functional recovery of function in the mouse model.

[0315] Figs 26A-26B is a schematic diagram and a graph depicting the AAV9 design and in vitro testing for a Mucolipidosis gene therapy (GTx). 293T cells was transduced with various M0021 (AAV9-CAGp-S1-S3) virus and cultured for 2 days before PTase activity assay.

[0316] Figs 27A-27B are a pair of graphs demonstrating that M0021 treatment decreases the serum lysosomal enzymes level in ML II mouse. To determine the effect of S1-S3 PTase Gene Therapy, a 34 week old female mouse was dose with a moderate dose of M0021 (AAV9-CAGp-S1-S3) , 4e12 vg (2e13 vg/kg). One of the phenotypes of ML II is elevated

serum level of lysosomal enzyme due to their inability to be targeted to the lysosome within the cell. An encouraging results was observed when there was a decrease in LAMAN and ManB activity in the serum after just 1 week of receiving the therapy. This result is important since it demonstrates the ability to effect a described phenotype of the MLII mouse model.

[0317] Figs 28A-28C are a series of graphs demonstrating that M0021 treatment increases the phosphorylation of lysosomal enzymes in ML II. To further understand the impact on S1-S3 PTase gene therapy in decreasing the serum activity of LAMAN and ManB, CI-MPR binding of the enzyme found in the serum was evaluated using the immobilized receptor binding assay described earlier. Briefly, a known about of activity in added in increasing amounts to immobilized CI-MPR. The unbound enzyme is washed away and the remaining bound enzyme is measured using the appropriate synthetic substrate; Man-b-4MU (ManB, LAMAN 4MU-Man (LAMAN). AAV9-S1S3 Gene therapy in ML II mouse increases the glycan phosphorylation of lysosomal enzymes. The total phosphorylated lysosomal enzymes in serum normalized to normal levels or slightly higher after 3 weeks.

[0318] The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this disclosure has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this disclosure may be devised by others skilled in the art without departing from the true spirit and scope of the disclosure. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

What is claimed:

1. A composition comprising a vector comprising a sequence encoding a promoter, a first polynucleotide encoding a lysosomal enzyme and a second polynucleotide encoding a modified GlcNAc-1 phosphotransferase (GlcNAc-1 PTase), wherein the promoter is capable of driving expression in a mammalian cell and wherein the promoter is operably linked to the first polynucleotide and to the second polynucleotide.

2. The composition of claim 1, wherein the vector further comprises a sequence encoding an Internal Ribosome Entry Site (IRES).

3. The composition of claim 2, wherein the sequence encoding the IRES is positioned between the sequence encoding the lysosomal enzyme and the sequence encoding the modified GlcNAc-1 PTase.

4. The composition of claim 2 or 3, wherein from 5' to 3', the vector comprises the sequence encoding the modified GlcNAc-1 PTase, the sequence encoding the IRES and the sequence encoding the lysosomal enzyme.

5. The composition of claim 2 or 3, wherein from 5' to 3', the vector comprises the sequence encoding the lysosomal enzyme, the sequence encoding the IRES and the sequence encoding the modified GlcNAc-1 PTase.

6. The composition of claim 1, wherein the vector further comprises a sequence encoding a cleavage site.

7. The composition of claim 6, wherein the cleavage site comprises a sequence encoding a 2A self-cleaving peptide.

8. The composition of any one of claims 1-7, wherein the vector is an expression vector.

9. The composition of any one of claims 1-7, wherein the vector is a delivery vector.

10. The composition of any one of claims 1-9, wherein the vector is a non-viral vector.

11. The composition of any one of claims 1-10, wherein the vector is a viral vector.

12. The composition of claim 11, wherein the vector is a lentiviral vector.

13. The composition of claim 11, wherein the vector is an adenoviral vector or an adeno-associated viral (AAV) vector.

14. The composition of claim 13, wherein the AAV vector comprises a serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9.

15. The composition of claim 13 or 14, wherein the AAV vector comprises a sequence encoding a capsid isolated or derived from one or more of a serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9.

16. The composition of any one of claims 13-15, wherein the AAV vector comprises a sequence encoding at least one inverted terminal repeat (ITR) isolated or derived from one or more of a serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, and AAV9.

17. The composition of any one of claims 1-16, wherein the vector is a bicistronic vector

18. The composition of any one of claims 1-16, wherein the vector is a multicistronic vector

19. The composition any one of claims 1-18, wherein the promoter comprises a constitutive promoter.

20. The composition of claim 19, wherein the constitutive promoter comprises a Cytomegalovirus (CMV) promoter.

21. The composition of any one of claims 1-20, wherein the vector comprises a nucleic acid sequence of SEQ ID NO: 1.

22. The composition of any one claims 1-21, wherein the polynucleotide encoding a modified GlcNAc-1 phosphotransferase comprises a nucleic acid sequence of SEQ ID NO: 4.

23. The composition of any one claims 1-22, wherein the lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C.

24. The composition of claim 23, wherein the lysosomal enzyme comprises at least one lysosomal enzyme listed in Table 1A, Table 1B or Table 1C.

25. The composition of any one claims 1-21 or 24, wherein the lysosomal enzyme is selected from the group consisting of β-glucocebrosidase (GBA), Galactosylceremidase (GALC), α-Galactosidase (GLA), α-N-acetylglucosaminidase (NAGLU), acid α-glucosidase (GAA) and lysosomal acid α-mannosidase (LAMAN).

26. The composition of any one claims 1-21 or 24, wherein the lysosomal enzyme comprises β-glucocebrosidase (GBA).

27. The composition of claim 26, wherein the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 5.

28. The composition of any one claims 1-21 or 24, wherein the lysosomal enzyme comprises Galactosylceremidase (GALC).

29. The composition of claim 28, wherein the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 6.

30. The composition of claim 29, wherein the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 23.

31. The composition of any one claims 1-21 or 24, wherein the lysosomal enzyme comprises α-Galactosidase (GLA).

32. The composition of claim 31, wherein the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 7.

33. The composition of any one claims 1-21 or 24, wherein the lysosomal enzyme comprises α-N-acetylglucosaminidase (NAGLU).

34. The composition of claim 33, wherein the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 8.

35. The composition of any one claims 1-21 or 24, wherein the lysosomal enzyme comprises acid α-glucosidase (GAA)

36. The composition of claim 35, wherein the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 9.

37. The composition of any one claims 1-21 or 24, wherein the lysosomal enzyme comprises lysosomal acid α-mannosidase (LAMAN).

38. The composition of claim 37, wherein the polynucleotide encoding the lysosomal enzyme comprises a nucleic acid sequence of SEQ ID NO: 10.

39. A method of treating a lysosomal storage disorder (LSD), the method comprising administering to a subject an effective amount of a composition of any one of claims 1-38, wherein the composition increases the phosphorylation of a lysosomal enzyme responsible of the LSD, thereby treating the LSD.

40. The method of claim 39, wherein the subject presents a sign or a symptom of the LSD.

41. The method of claim 39 or 40, wherein the subject has been diagnosed with the LSD.

42. A method of preventing an occurrence or an onset of a lysosomal storage disorder (LSD), the method comprising administering to a subject an effective amount of a composition of any one of claims 1-38, wherein the composition increases the phosphorylation of a lysosomal enzyme responsible of the LSD, thereby preventing the occurrence of the LSD in the subject.

43. The method of claim 42, wherein the subject is at risk of the occurrence or the onset of the LSD.

44. The method of claim 42 or 43, wherein the subject presents a sign or a symptom of the LSD.

45. A method of ameliorating the phosphorylation of a lysosomal enzyme responsible for a lysosomal storage disorder (LSD) , the method comprising administering to a subject an effective amount of a composition of any one of claims 1-38, wherein the composition increases the phosphorylation of the lysosomal enzyme .

46. The method of claim 45, wherein the subject presents a sign or a symptom of the LSD.

47. The method of claim 45 or 46, wherein the subject is at risk of the occurrence or the onset of the LSD.

48. The method of claim 45 or 46, wherein the subject has been diagnosed with the LSD.

49. A method of ameliorating the phosphorylation of a lysosomal enzyme responsible for a lysosomal storage disorder (LSD) , the method comprising contacting to a cell, an effective amount of a composition of any one of claims 1-38, wherein the composition increases the phosphorylation of the lysosomal enzyme.

50. The method of claim 49, wherein the cell is in vitro or ex vivo.

51. The method of claim 49, wherein the cell is in vivo.

52. The method of any one of claims 49-51, wherein a subject comprises the cell.

53. The method of claim 52, wherein the subject presents a sign or a symptom of the LSD.

54. The method of claim 52 or 53, wherein the subject is at risk of the occurrence or the onset of the LSD.

55. The method of claim 52 or 53, wherein the subject has been diagnosed with the LSD.

56. The method of any one of claims 39-55, wherein the lysosomal enzyme is involved in at least one lysosomal storage disorder (LSD) as listed in Table 1A, Table 1B or Table 1C.

57. The method of any one of claims 39-56, wherein the lysosomal enzyme is at least one as listed in Table 1A, Table 1B or Table 1C.

58. The method of any one of claims 39-56, wherein the lysosomal enzyme comprises one or more of β-glucocebrosidase (GBA), Galactosylceremidase (GALC), α-Galactosidase (GLA), α-N-acetylglucosaminidase (NAGLU), acid α-glucosidase (GAA) and lysosomal acid α-mannosidase (LAMAN).

59. The method of any one of claims 39-58, wherein the administering comprises a systemic route of administration.

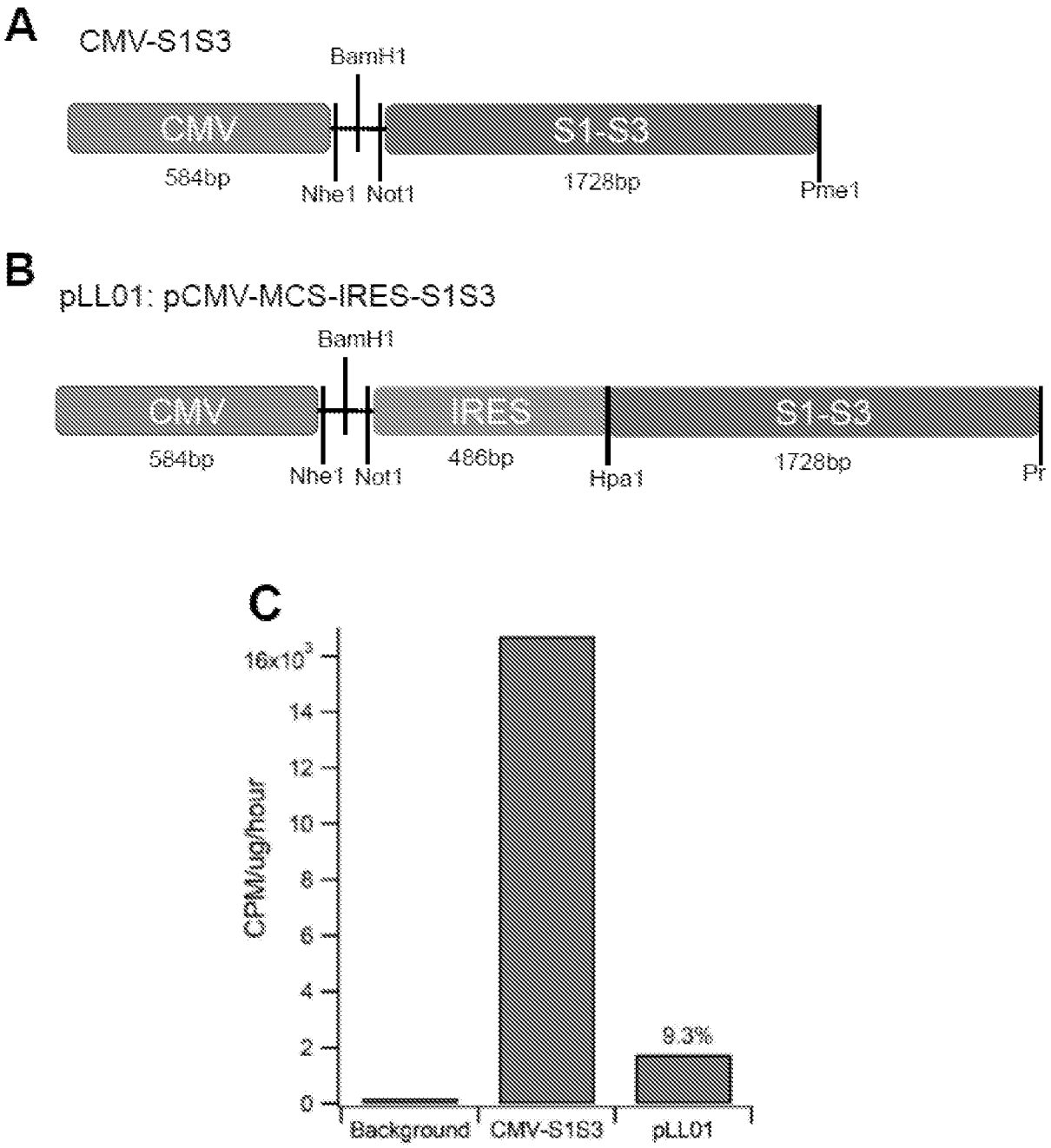
60. The method of claim 59, wherein the systemic route of administration is enteral, parenteral, oral, intramuscular (IM), subcutaneous (SC), intravenous (IV), intra-arterial (IA), intrathecal, intraspinal, or intraventricular.

61. The method of any one of claims 39-58, wherein the administering comprises a local route of administration.

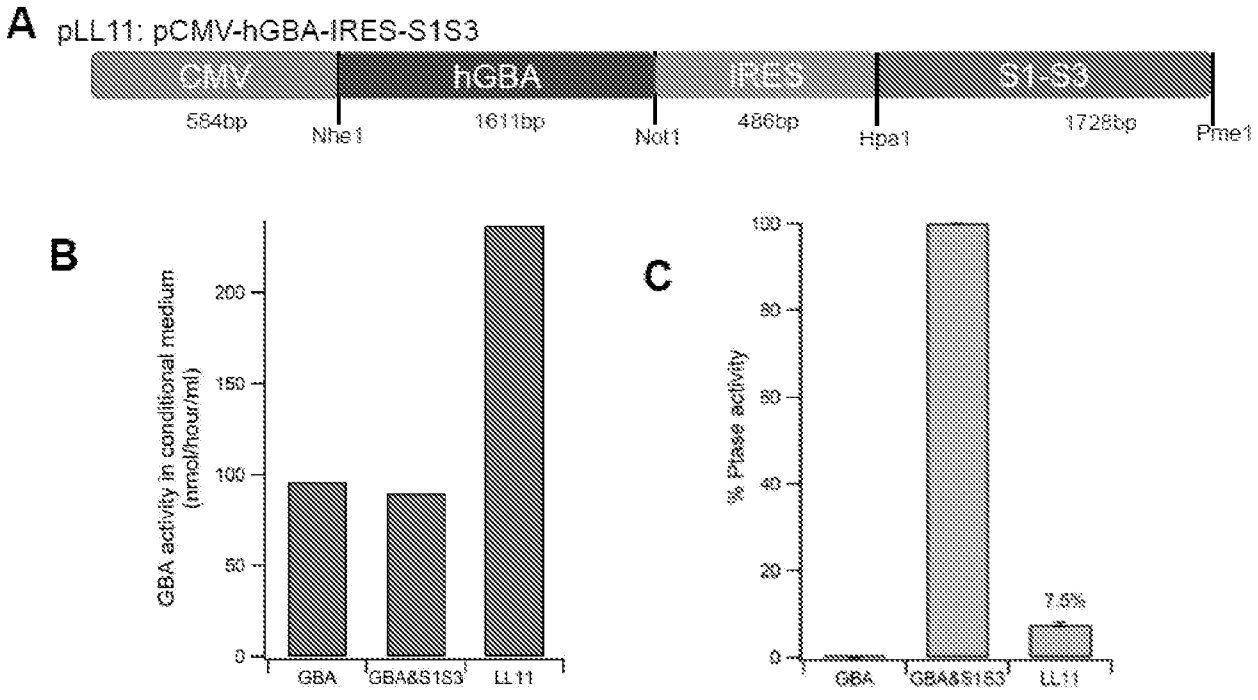
62. The method of any one of claims 39-61, wherein the subject is a human.

63. The method of any one of claims 39-62, wherein the subject is a male.

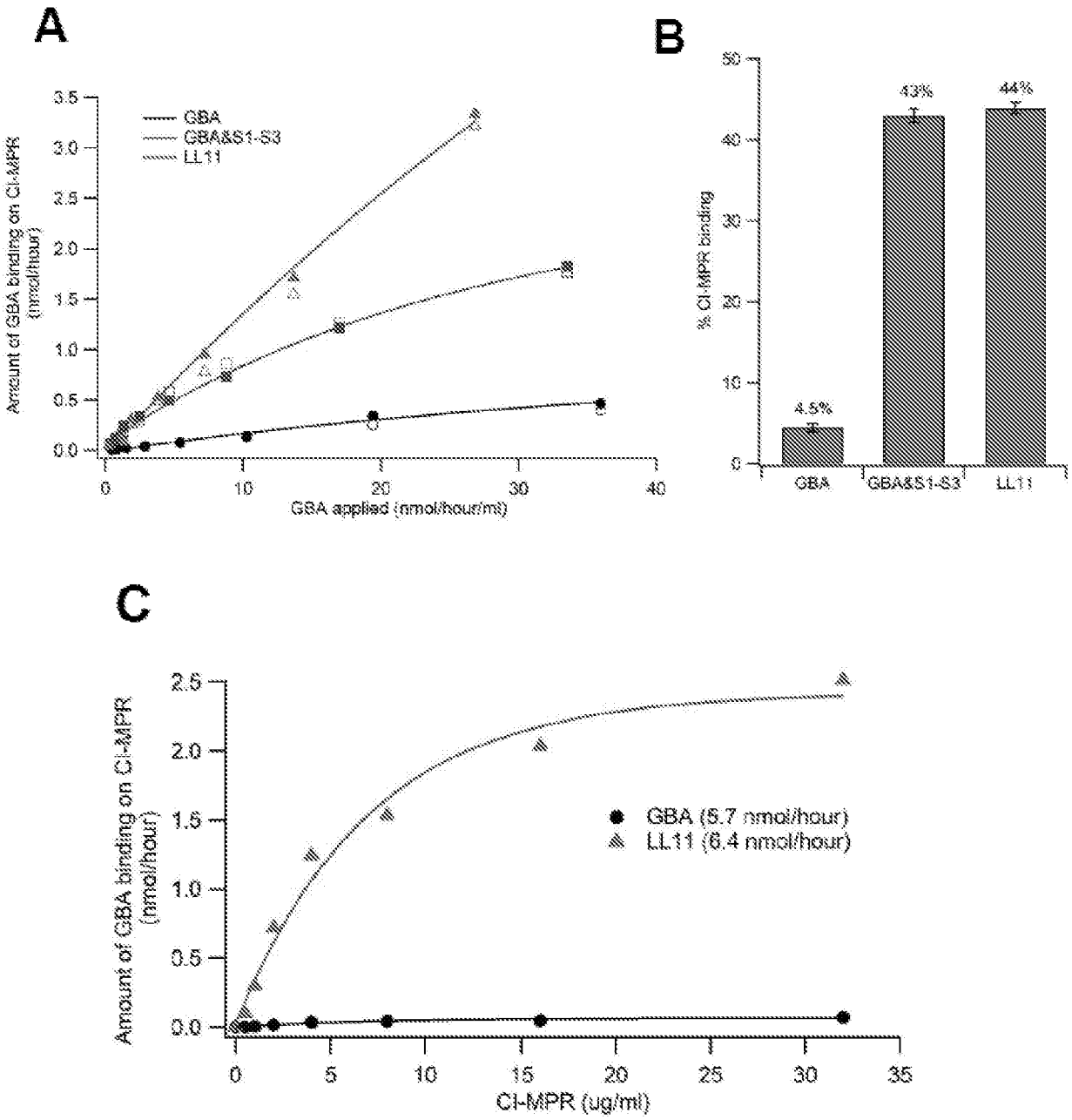
64. The method of any one of claims 39-62, wherein the subject is a female.


A
CMV-S1S3
BamH1
CMV
S1-S3
584bp
Nhe1 Not1
1728bp
Pme1
B
pLL01: pCMV-MCS-IRES-S1S3
BamH1
CMV
IRES
S1-S3
584bp
Nhe1 Not1
486bp
Hpa1
1728bp
Pr
C
16x10³
14
12
10
8
6
4
2
0
CPM/ug/hour
9.3%
Background
CMV-S1S3
pLL01

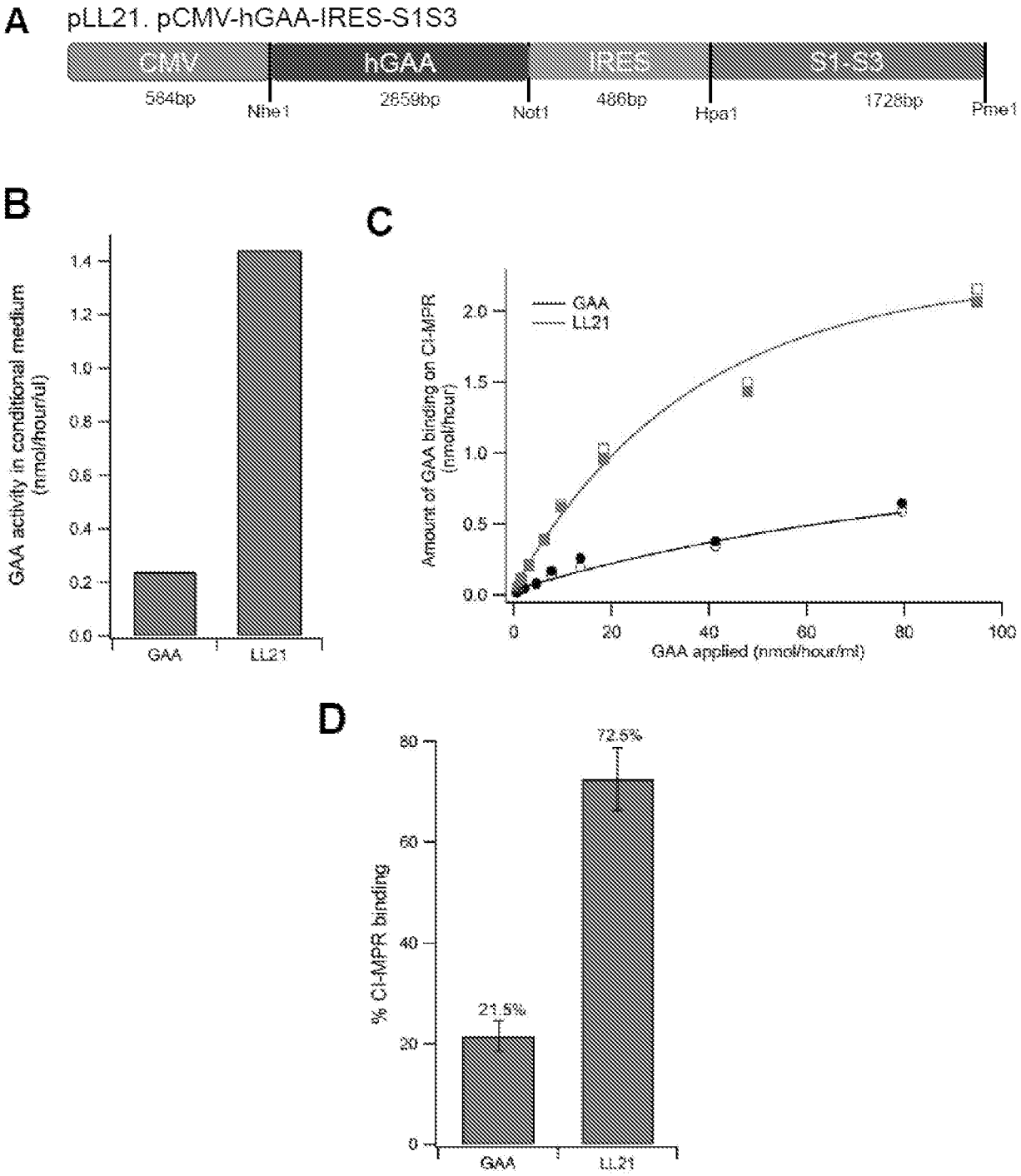
Figs. 1A-1C


A
pLL11: pCMV-hGBA-IRES-S1S3
CMV
hGBA
IRES
S1-S3
584bp
Nhe1
1611bp
Not1
486bp
Hpa1
1728bp
Pme1
B
GBA activity in conditional medium (nmol/hour/ml)
200
150
100
50
0
GBA
GBA&S1S3
LL11
C
% Ptase activity
100
80
60
40
20
0
7.6%
GBA
GBA&S1S3
LL11

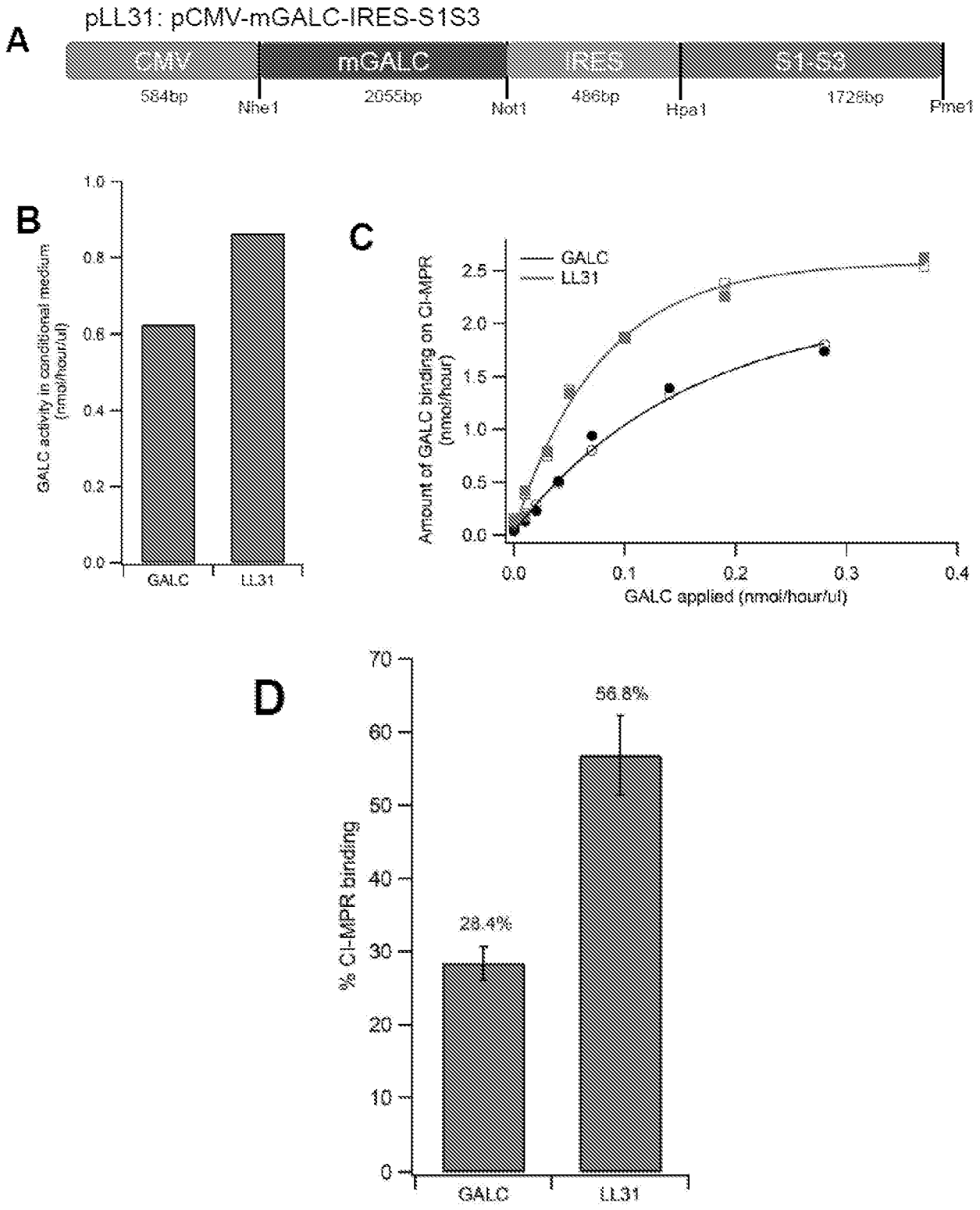
Figs. 2A-2C


A
GBA
GBA&S1-S3
LL11
Amount of GBA binding on CI-MPR (nmol/hour)
GBA applied (nmol/hour/ml)
B
% CI-MPR binding
4.5%
43%
44%
GBA
GBA&S1-S3
LL11
C
Amount of GBA binding on CI-MPR (nmol/hour)
GBA (5.7 nmol/hour)
LL11 (6.4 nmol/hour)
CI-MPR (ug/ml)

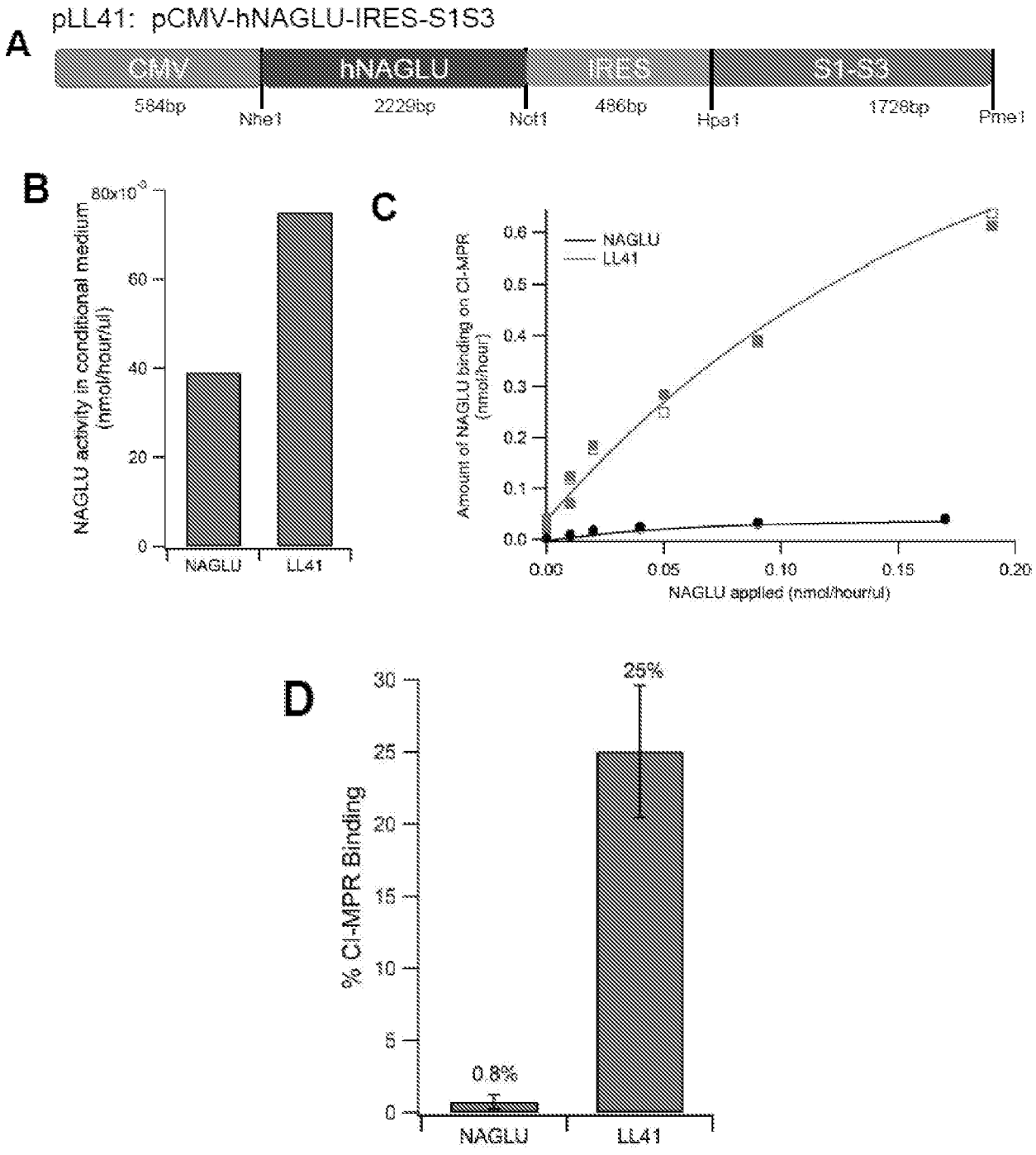
Figs. 3A-3C


A
pLL21. pCMV-hGAA-IRES-S1S3
CMV
hGAA
IRES
S1-S3
584bp
Nhe1
2859bp
Not1
486bp
Hpa1
1728bp
Pme1
B
GAA activity in conditional medium (nmol/hour/ul)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
GAA
LL21
C
Amount of GAA binding on CI-MPR (nmol/hour)
2.0
1.5
1.0
0.5
0.0
GAA
LL21
0
20
40
60
80
100
GAA applied (nmol/hour/ml)
D
% CI-MPR binding
80
60
40
20
0
72.6%
21.5%
GAA
LL21

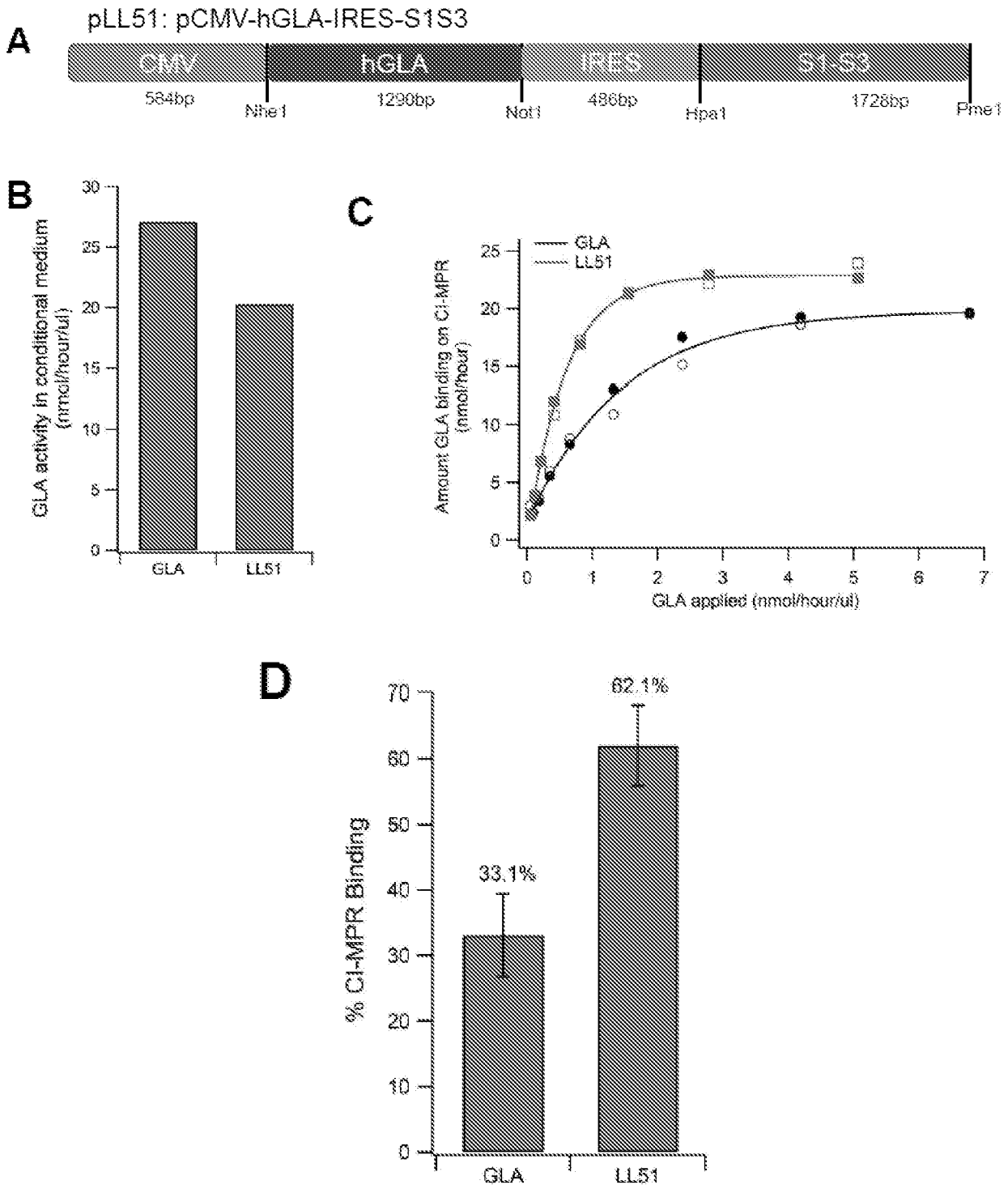
Figs. 4A-4D


A
pLL31: pCMV-mGALC-IRES-S1S3
CMV
mGALC
IRES
S1-S3
584bp
Nhe1
2055bp
Not1
486bp
Hpa1
1728bp
Pme1
B
GALC activity in conditional medium (nmol/hour/ul)
1.0
0.8
0.6
0.4
0.2
0.0
GALC
LL31
C
Amount of GALC binding on CI-MPR (nmol/hour)
GALC
LL31
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
GALC applied (nmol/hour/ul)
D
% CI-MPR binding
70
60
50
40
30
20
10
0
56.8%
28.4%
GALC
LL31

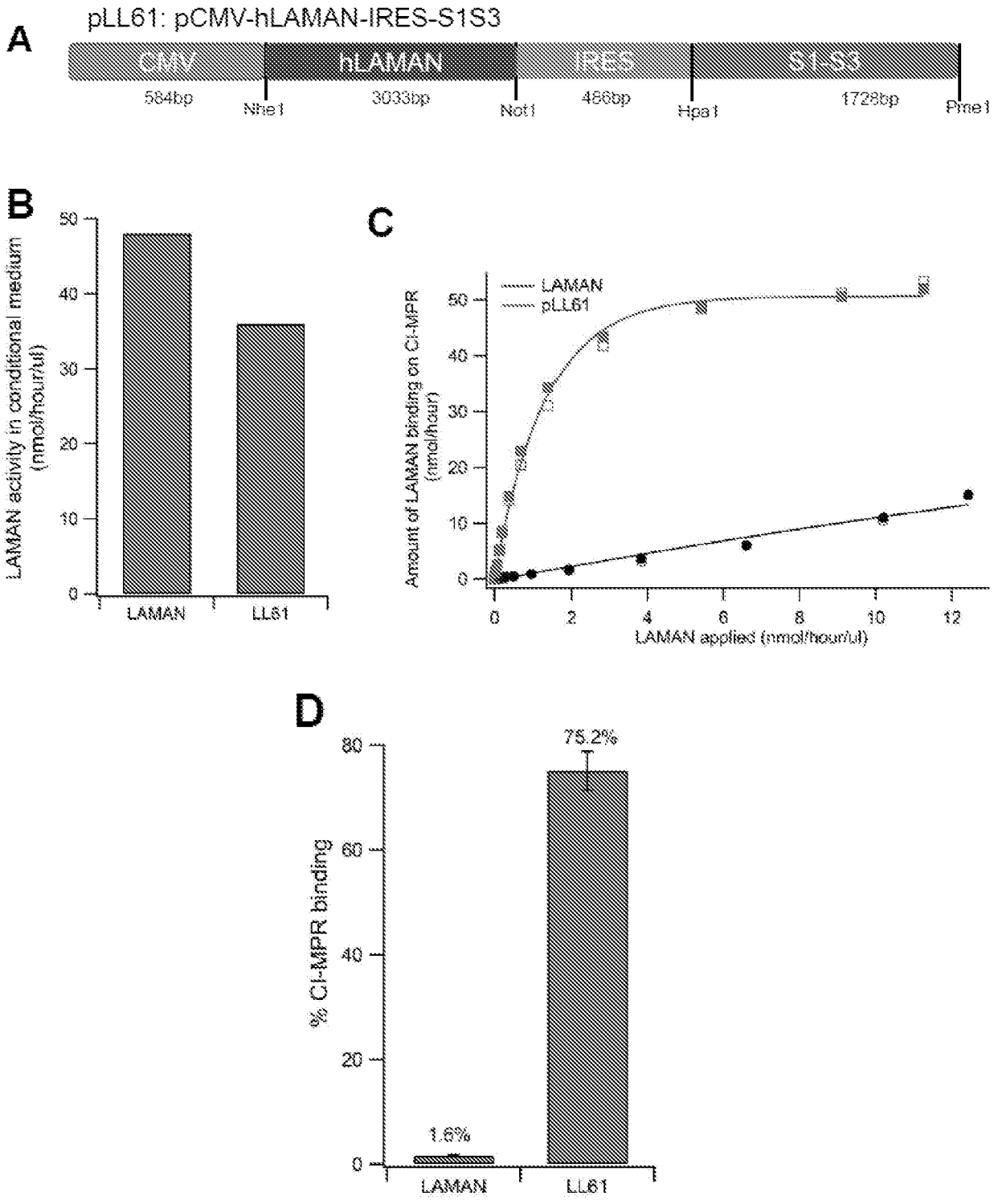
Figs. 5A-5D


A
pLL41: pCMV-hNAGLU-IRES-S1S3
CMV
hNAGLU
IRES
S1-S3
584bp
Nhe1
2229bp
Not1
486bp
Hpa1
1728bp
Pme1
B
NAGLU activity in conditional medium (nmol/hour/ul)
80x10^-3
60
40
20
0
NAGLU
LL41
C
Amount of NAGLU binding on CI-MPR (nmol/hour)
NAGLU
LL41
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.00
0.05
0.10
0.15
0.20
NAGLU applied (nmol/hour/ul)
D
% CI-MPR Binding
30
25
20
15
10
5
0
25%
0.8%
NAGLU
LL41

Figs. 6A-6D


A
pLL51: pCMV-hGLA-IRES-S1S3
CMV
hGLA
IRES
S1-S3
584bp
Nhe1
1290bp
Not1
486bp
Hpa1
1728bp
Pme1
B
GLA activity in conditional medium (nmol/hour/ul)
GLA
LL51
C
GLA
LL51
Amount GLA binding on CI-MPR (nmol/hour)
GLA applied (nmol/hour/ul)
D
% CI-MPR Binding
33.1%
62.1%
GLA
LL51

Figs. 7A-7D


A
pLL61: pCMV-hLAMAN-IRES-S1S3
CMV
hLAMAN
IRES
S1-S3
584bp
Nhe1
3033bp
Not1
486bp
Hpa1
1728bp
Pme1
B
LAMAN activity in conditional medium (nmol/hour/ul)
0
10
20
30
40
50
LAMAN
LL61
C
Amount of LAMAN binding on CI-MPR (nmol/hour)
LAMAN
pLL61
0
10
20
30
40
50
0
2
4
6
8
10
12
LAMAN applied (nmol/hour/ul)
D
% CI-MPR binding
0
20
40
60
80
1.6%
75.2%
LAMAN
LL61

Figs. 8A-8D

Figs. 9A-9E

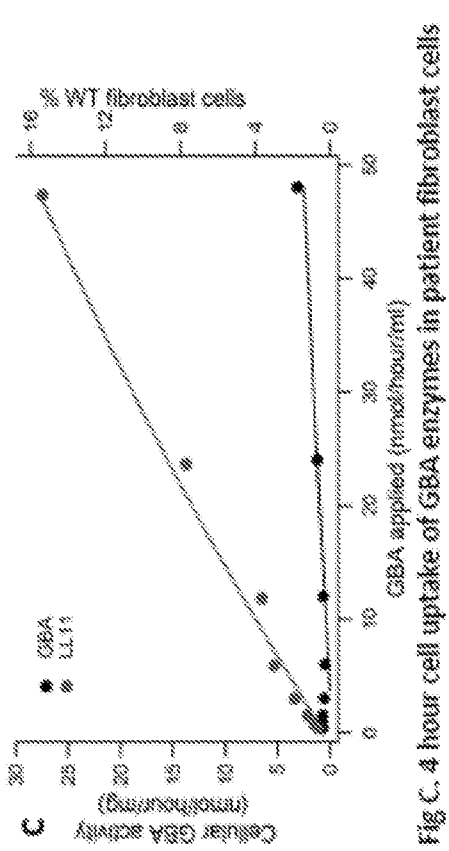


Fig A&B. Binding of expressed GBA enzymes to MGP receptor

Fig C. 4 hour cell uptake of GBA enzymes in patient fibroblast cells

Fig D. Enzyme thermostability study at 37C, neutral pH

Fig E. Binding of expressed GBA enzymes to MGP receptor

Figs. 10A-10C

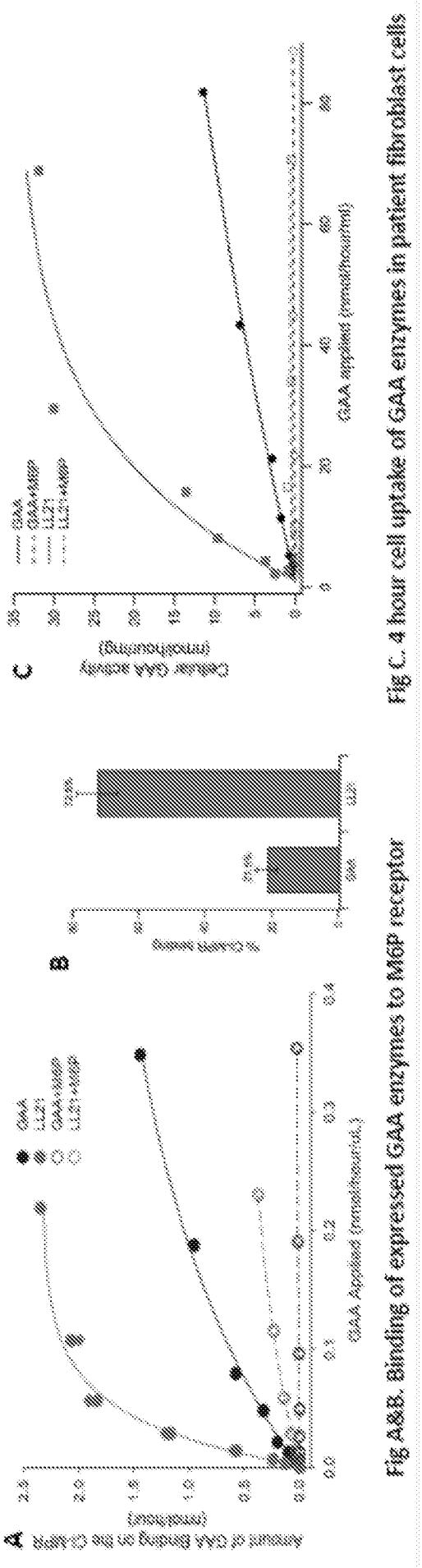


Fig A&B. Binding of expressed GAA enzymes to M6P receptor

Fig C. 4 hour cell uptake of GAA enzymes in patient fibroblast cells

Figs. 11A-11C

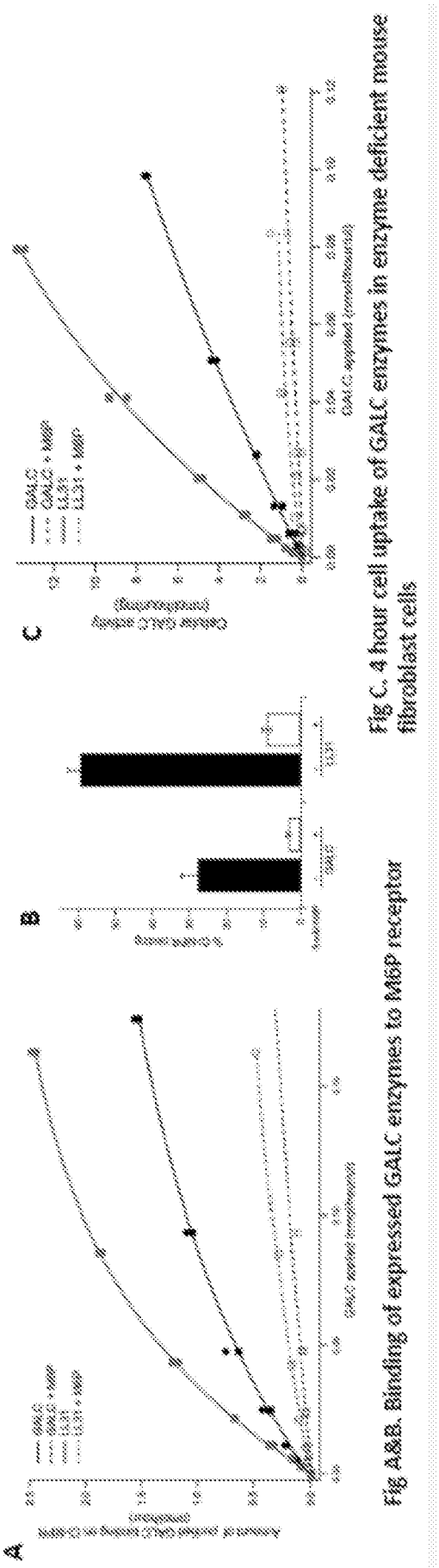


Fig A&B. Binding of expressed GALC enzymes to M6P receptor

Fig C. 4 hour cell uptake of GALC enzymes in enzyme deficient mouse fibroblast cells

Figs 12A-12C

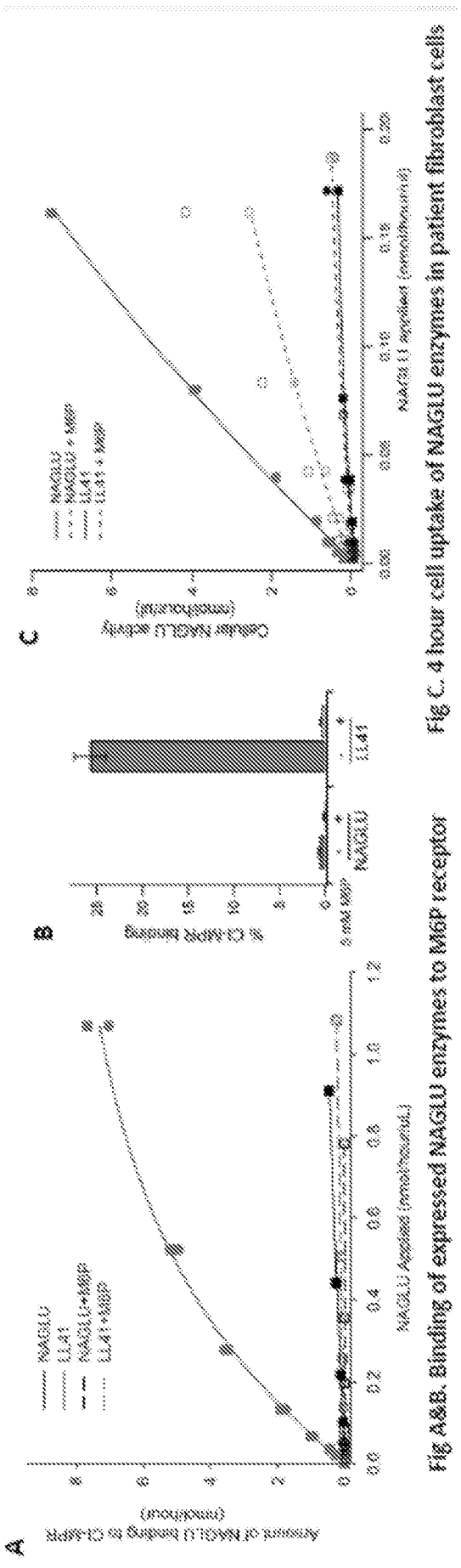


Fig A&B. Binding of expressed NAGLU enzymes to MPR receptor Fig C. 4 hour cell uptake of NAGLU enzymes in patient fibroblast cells

Figs 13A-13C

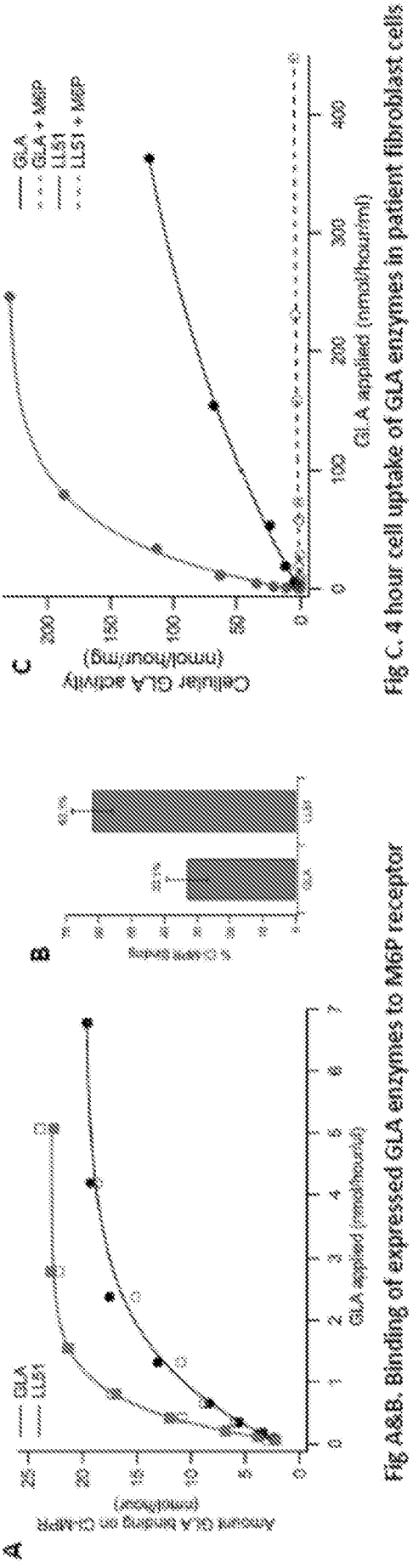


Fig A&B. Binding of expressed GLA enzymes to M6P receptor

Fig C. 4 hour cell uptake of GLA enzymes in patient fibroblast cells

Figs14A-14C

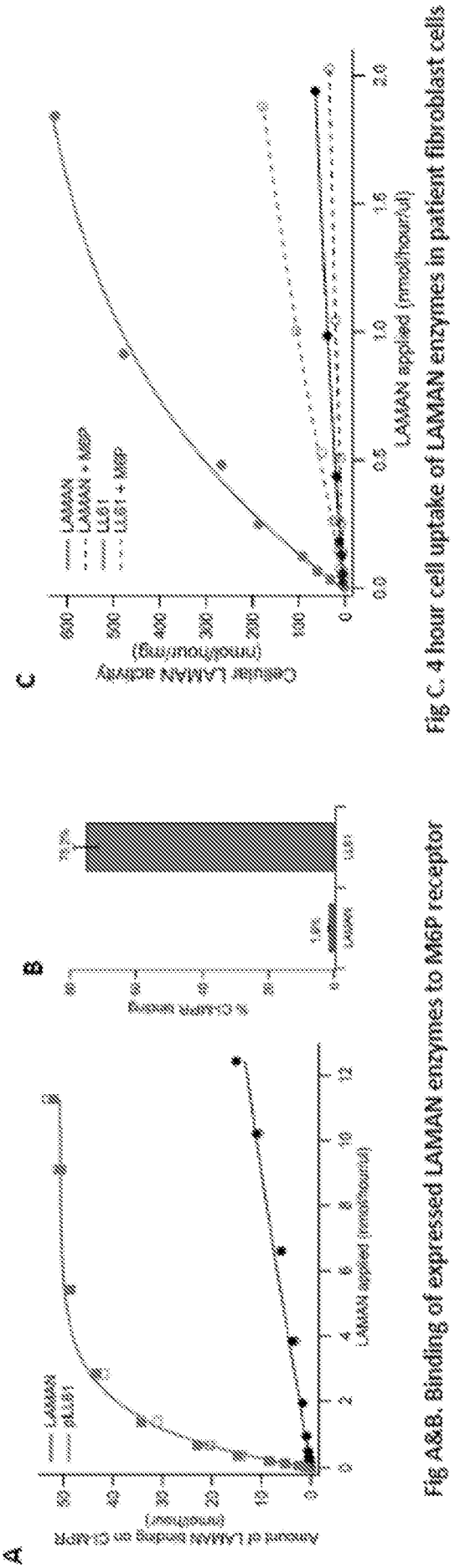


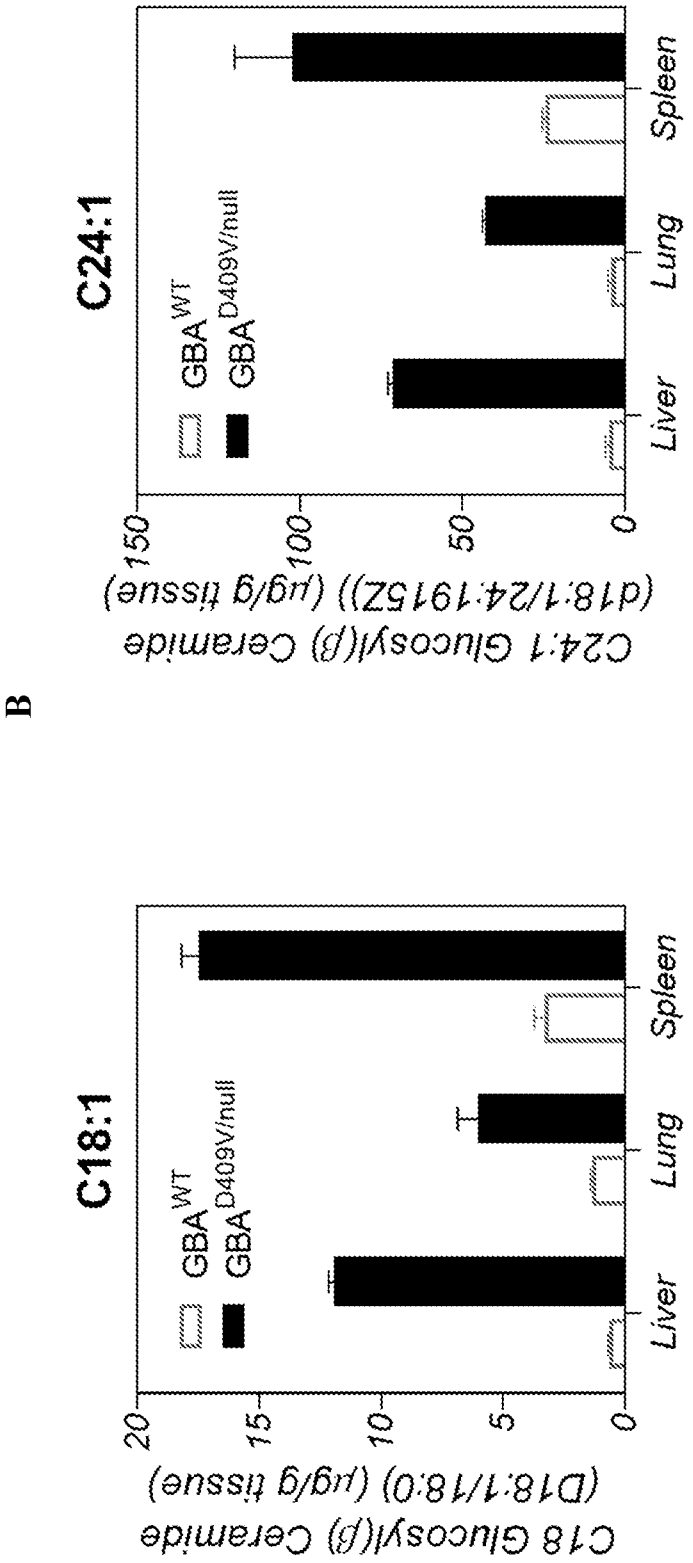
Fig A&B. Binding of expressed LAMAN enzymes to M6P receptor

Fig C. 4 hour cell uptake of LAMAN enzymes in patient fibroblast cells

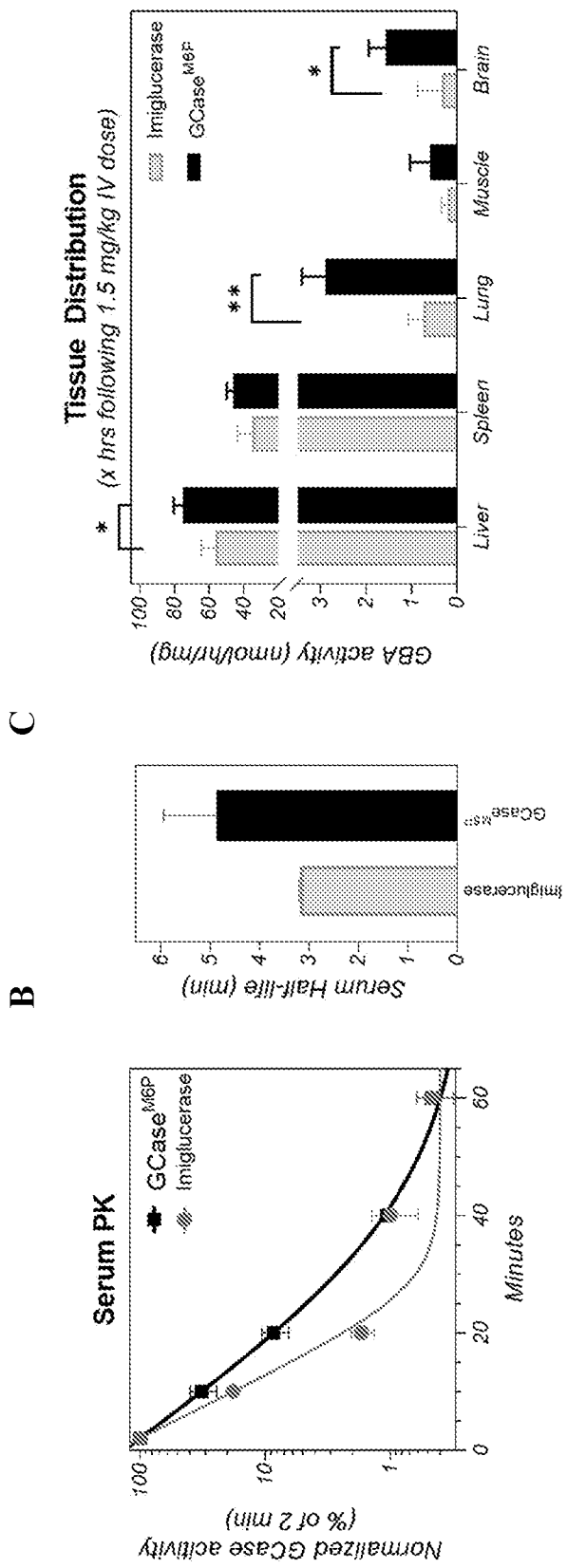
Figs 15A-15B

S1-S3 is suitable for the gene therapy of Mucolipidosis.
1. Full activity compare to full length GNPTAB gene.
2. Small gene size (~1.7 Kb) for AAV package.

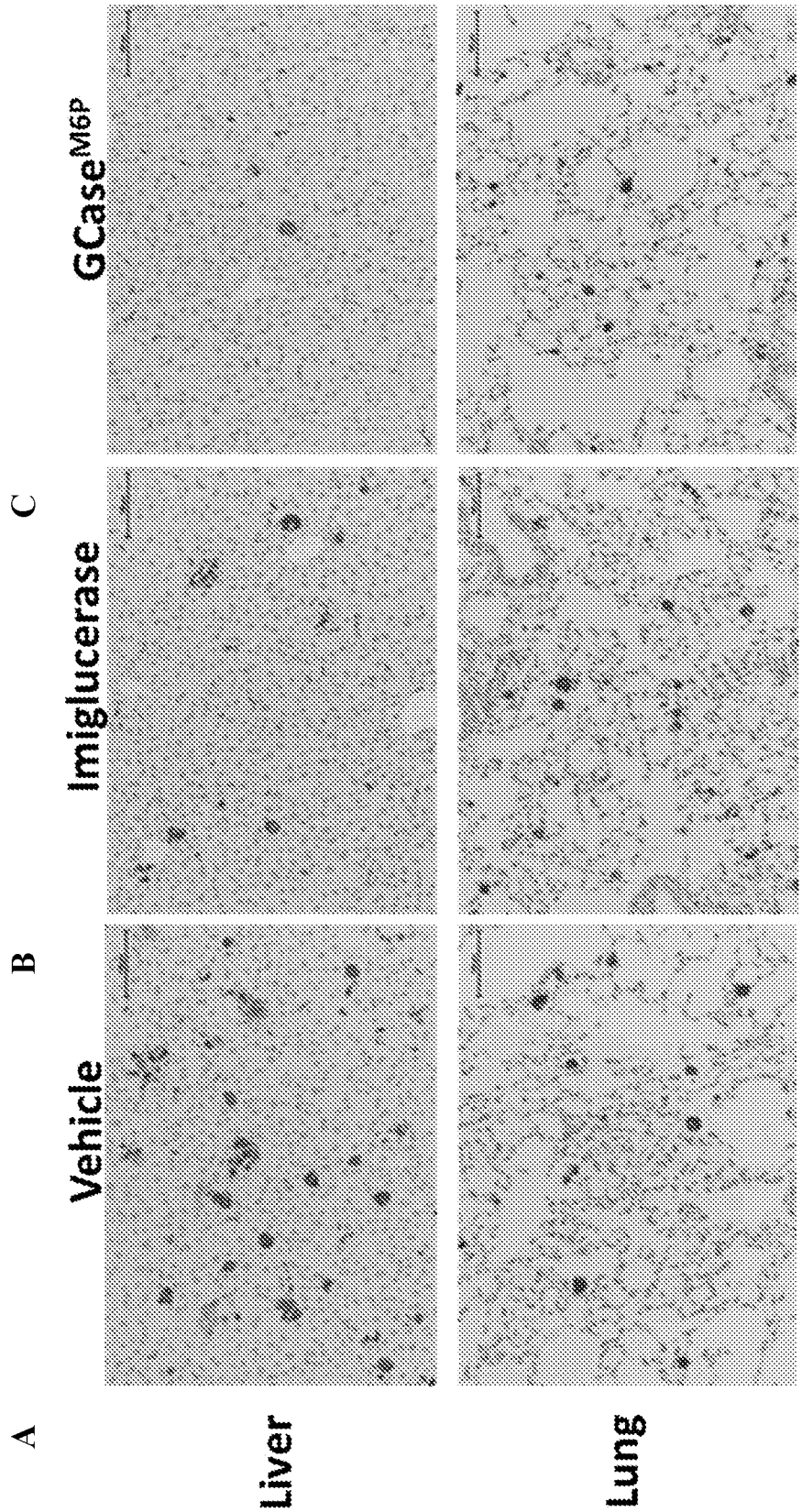
Figs 16A-16B


A
C18 Glucosyl(β) Ceramide
(D18:1/18:0) (μg/g tissue)
0
5
10
15
20
C18:1
GBA^WT
GBA^D409V/null
Liver
Lung
Spleen
B
C24:1 Glucosyl(β) Ceramide
(d18:1/24:1915Z)) (μg/g tissue)
0
50
100
150
C24:1
GBA^WT
GBA^D409V/null
Liver
Lung
Spleen

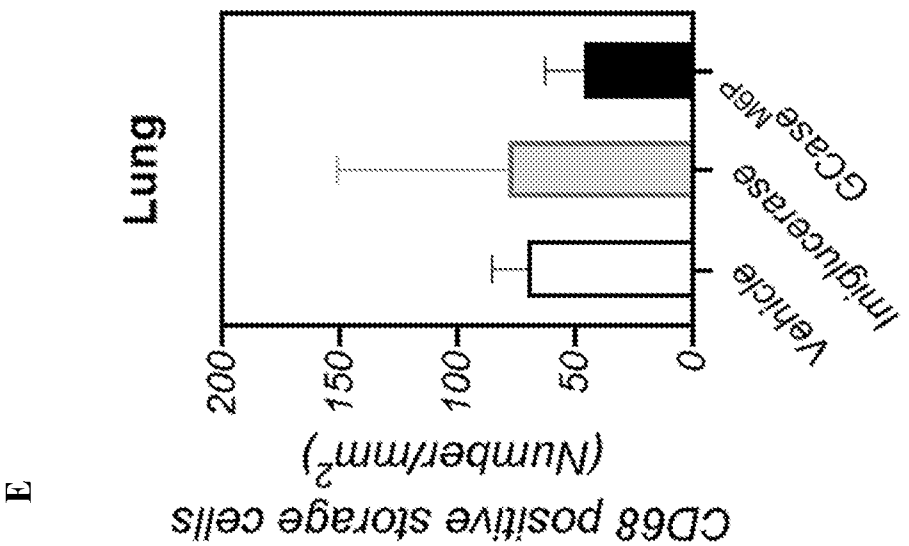
Figs 17A-17C

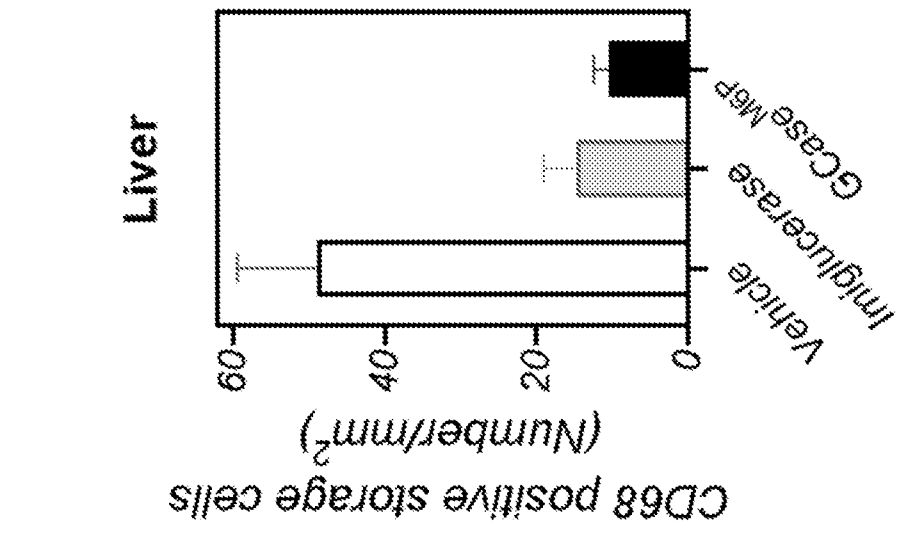

A
Serum PK
GCase^M6P
Imiglucerase
Normalized GCase activity
(% of 2 min)
Minutes
B
Serum Half-life (min)
Imiglucerase
GCase^M6P
C
Tissue Distribution
(x hrs following 1.5 mg/kg IV dose)
Imiglucerase
GCase^M6P
GBA activity (nmol/hr/mg)
Liver
Spleen
Lung
Muscle
Brain

Figs 18A-18C

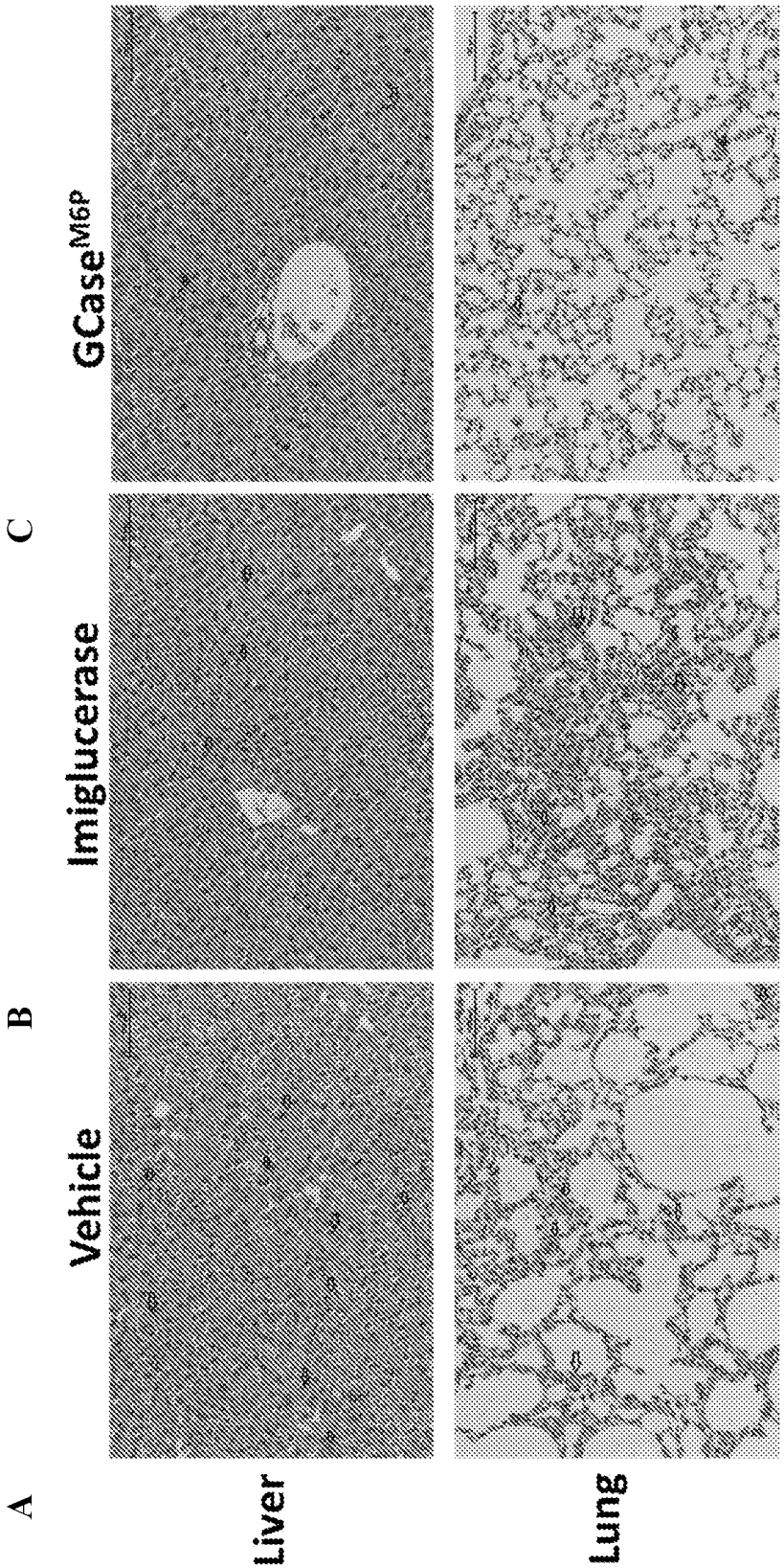


Figs 18D-18E

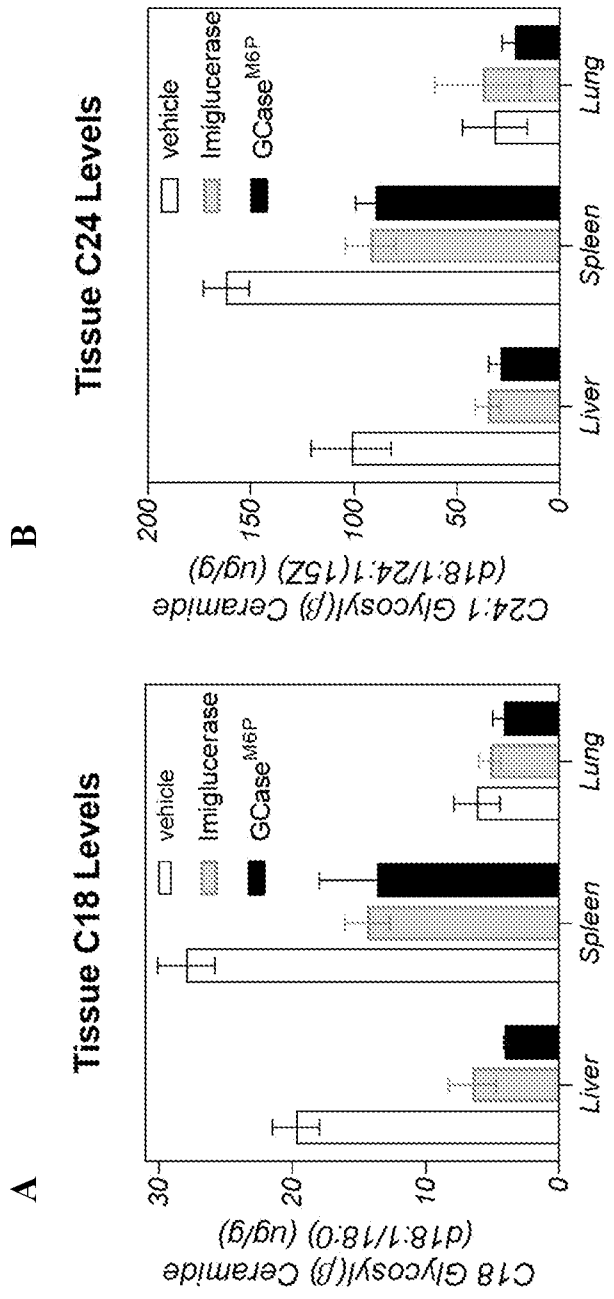

E
Lung
CD68 positive storage cells
(Number/mm²)
0
50
100
150
200
Vehicle
Imiglucerase
GCase Mbp


D
Liver
CD68 positive storage cells
(Number/mm²)
0
20
40
60
Vehicle
Imiglucerase
GCase Mbp

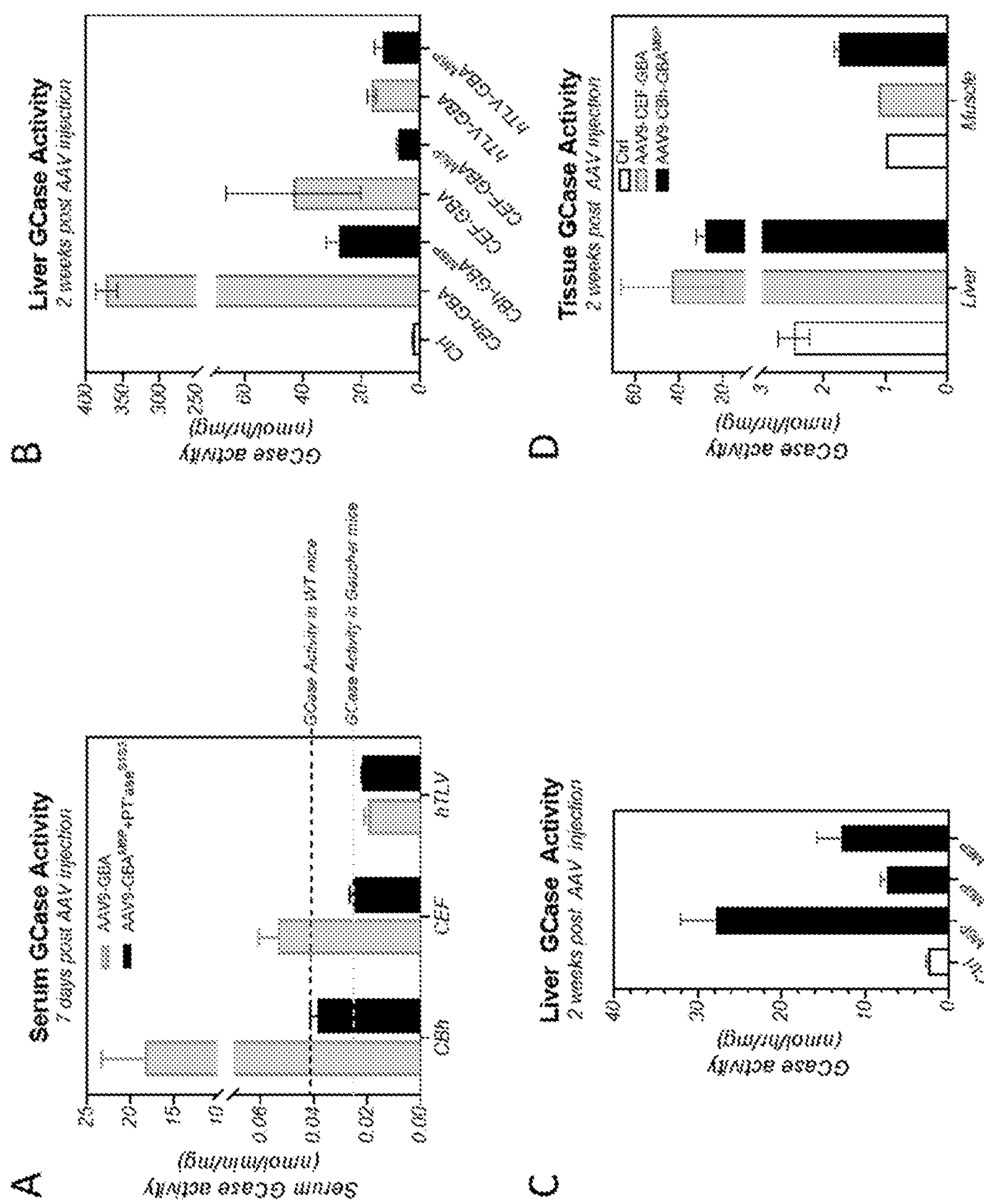
Figs 19A-19C



Figs 20A-20B


A
Tissue C18 Levels
C18 Glycosyl(β) Ceramide (d18:1/18:0) (ug/g)
30
20
10
0
vehicle
Imiglucerase
GCase M6P
Liver
Spleen
Lung
B
Tissue C24 Levels
C24:1 Glycosyl(β) Ceramide (d18:1/24:1(15Z) (ug/g)
200
150
100
50
0
vehicle
Imiglucerase
GCase M6P
Liver
Spleen
Lung

Figs 21A-21D


A
Serum GCase Activity
7 days post AAV injection
AAV9-GBA
GCase Activity in WT mice
GCase Activity in Gaucher mice
Serum GCase activity
(nmol/min/mg)
CBh
CEF
hTLV
B
Liver GCase Activity
2 weeks post AAV injection
GCase activity
(nmol/hr/mg)
Ctrl
CBh-GBA
CEF-GBA
hTLV-GBA
C
Liver GCase Activity
2 weeks post AAV injection
GCase activity
(nmol/hr/mg)
Ctrl
D
Tissue GCase Activity
2 weeks post AAV injection
Ctrl
AAV9-CEF-GBA
GCase activity
(nmol/hr/mg)
Liver
Muscle

Figs 22A-22C

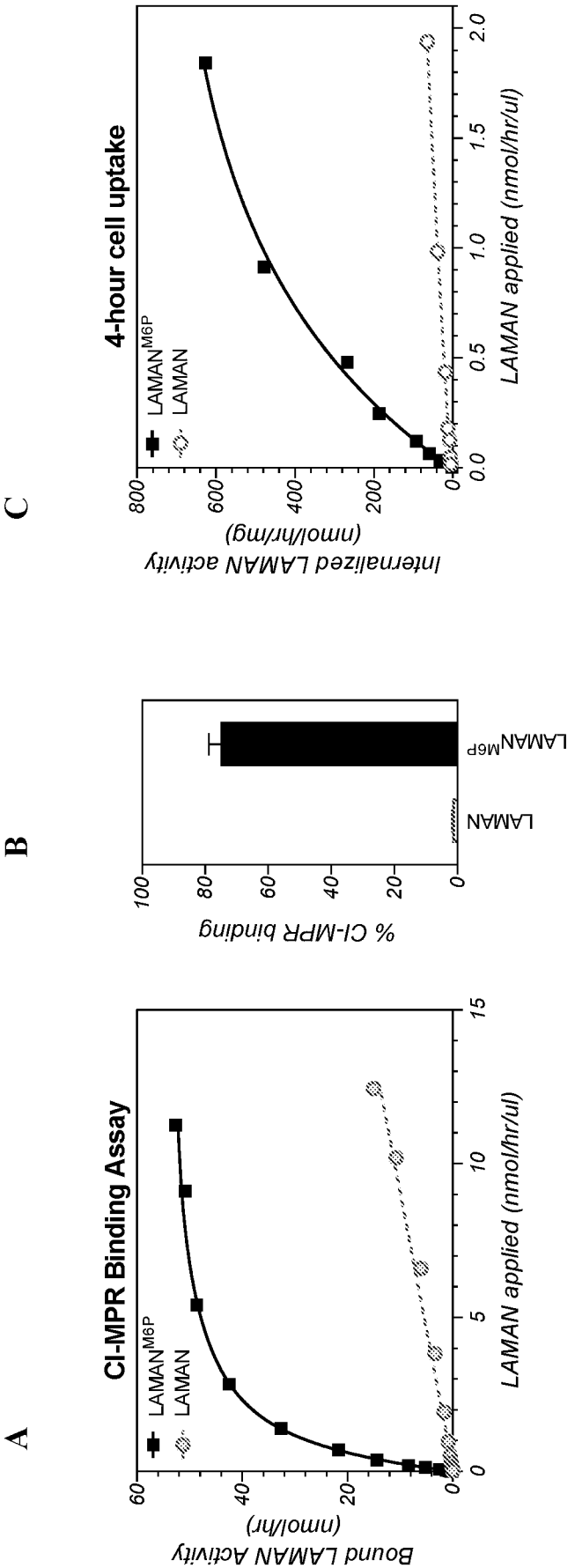

A
CI-MPR Binding Assay
LAMAN^M6P
LAMAN
Bound LAMAN Activity (nmol/hr)
LAMAN applied (nmol/hr/ul)
B
% CI-MPR binding
LAMAN
LAMAN^M6P
C
4-hour cell uptake
LAMAN^M6P
LAMAN
Internalized LAMAN activity (nmol/hr/mg)
LAMAN applied (nmol/hr/ul)

Fig 23A-23B

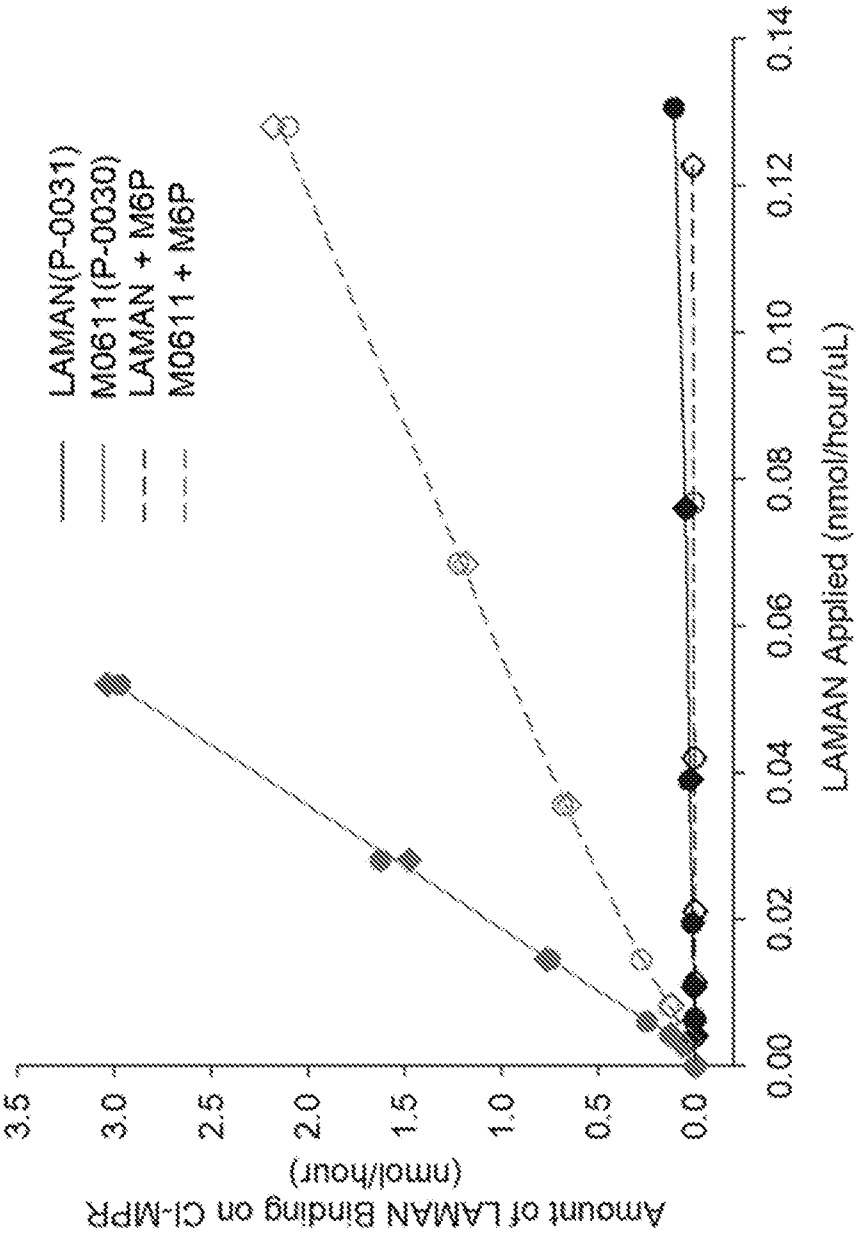
A

B

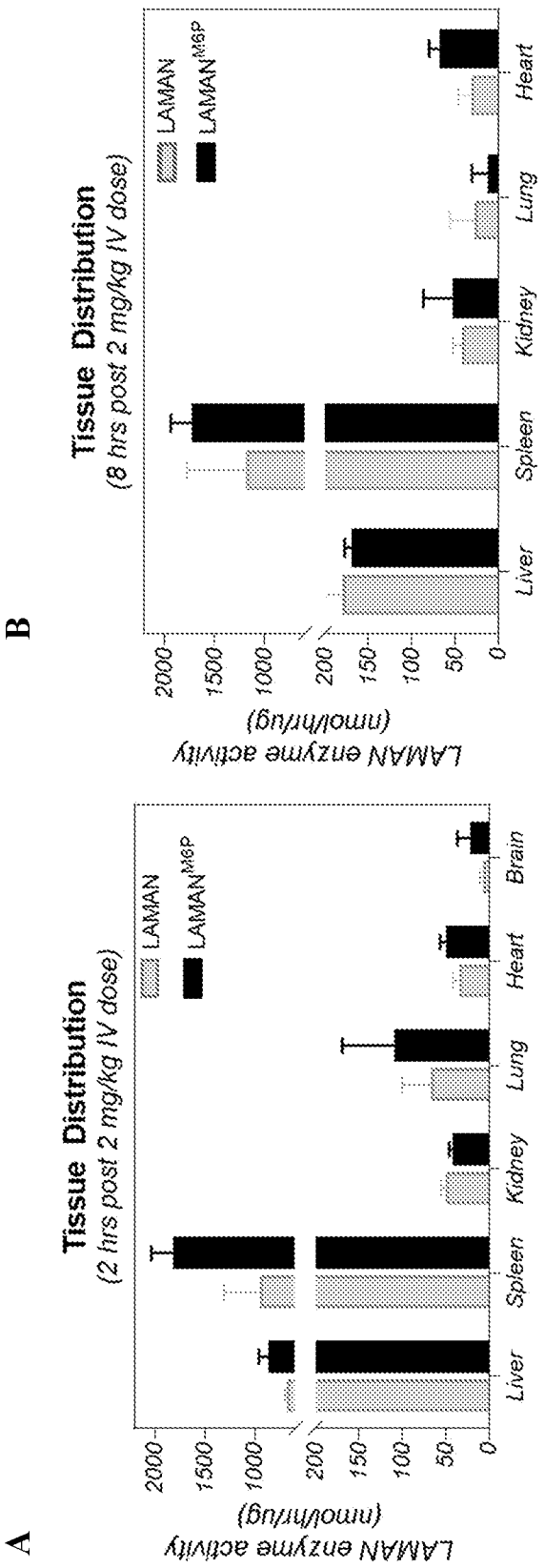
	Protein Conc. (mg/mL)	Absolute Activity (nmol/hr/mg)	Yield (mg)	SDS-PAGE Gel (Lane)	Batch No
M0611	16.0	343,000	15.2	2	P-0030
LAMAN	18.4	404,000	17.4	3	P-0031
LAMAN	20.4	568,000	19	4	P-0032

Fig 23C

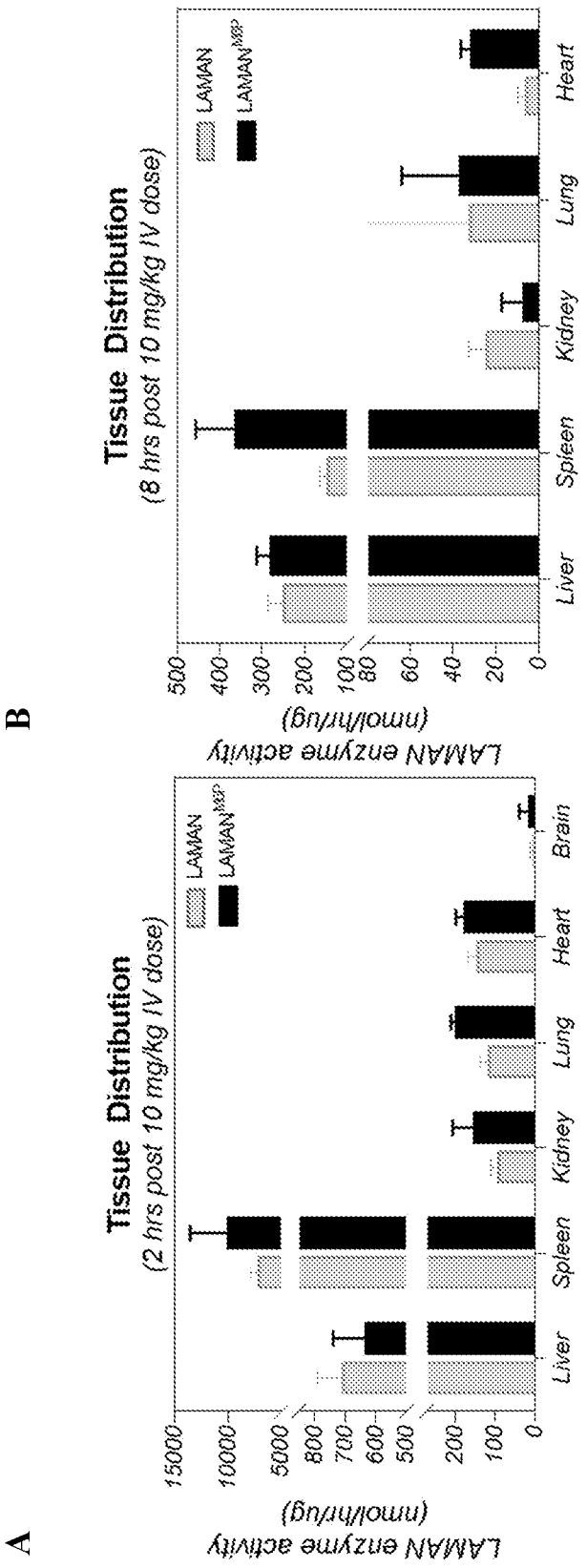
C


LAMAN(P-0031)
M0611(P-0030)
LAMAN + M6P
M0611 + M6P
Amount of LAMAN Binding on CI-MPR (nmol/hour)
LAMAN Applied (nmol/hour/uL)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14

Figs 24A-24B

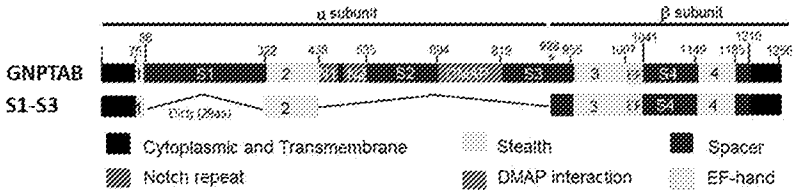

A
Tissue Distribution
(2 hrs post 2 mg/kg IV dose)
LAMAN
LAMAN^{M6P}
LAMAN enzyme activity
(nmol/hr/ug)
2000
1500
1000
200
150
100
50
0
Liver
Spleen
Kidney
Lung
Heart
Brain
B
Tissue Distribution
(8 hrs post 2 mg/kg IV dose)
LAMAN
LAMAN^{M6P}
LAMAN enzyme activity
(nmol/hr/ug)
2000
1500
1000
200
150
100
50
0
Liver
Spleen
Kidney
Lung
Heart

Figs 25A-25B


A
Tissue Distribution
(2 hrs post 10 mg/kg IV dose)
LAMAN
LAMAN^MBP
LAMAN enzyme activity
(nmol/hr/ug)
15000
10000
5000
800
700
600
500
200
100
0
Liver
Spleen
Kidney
Lung
Heart
Brain
B
Tissue Distribution
(8 hrs post 10 mg/kg IV dose)
LAMAN
LAMAN^MBP
LAMAN enzyme activity
(nmol/hr/ug)
500
400
300
200
100
80
60
40
20
0
Liver
Spleen
Kidney
Lung
Heart

Figs 26A-26B

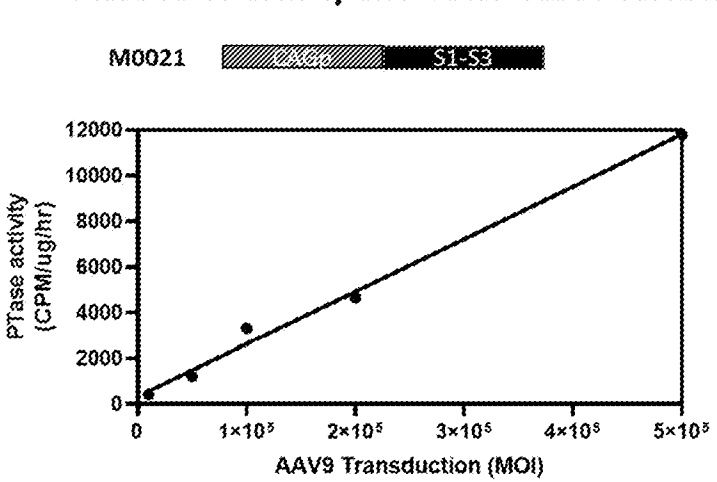
A



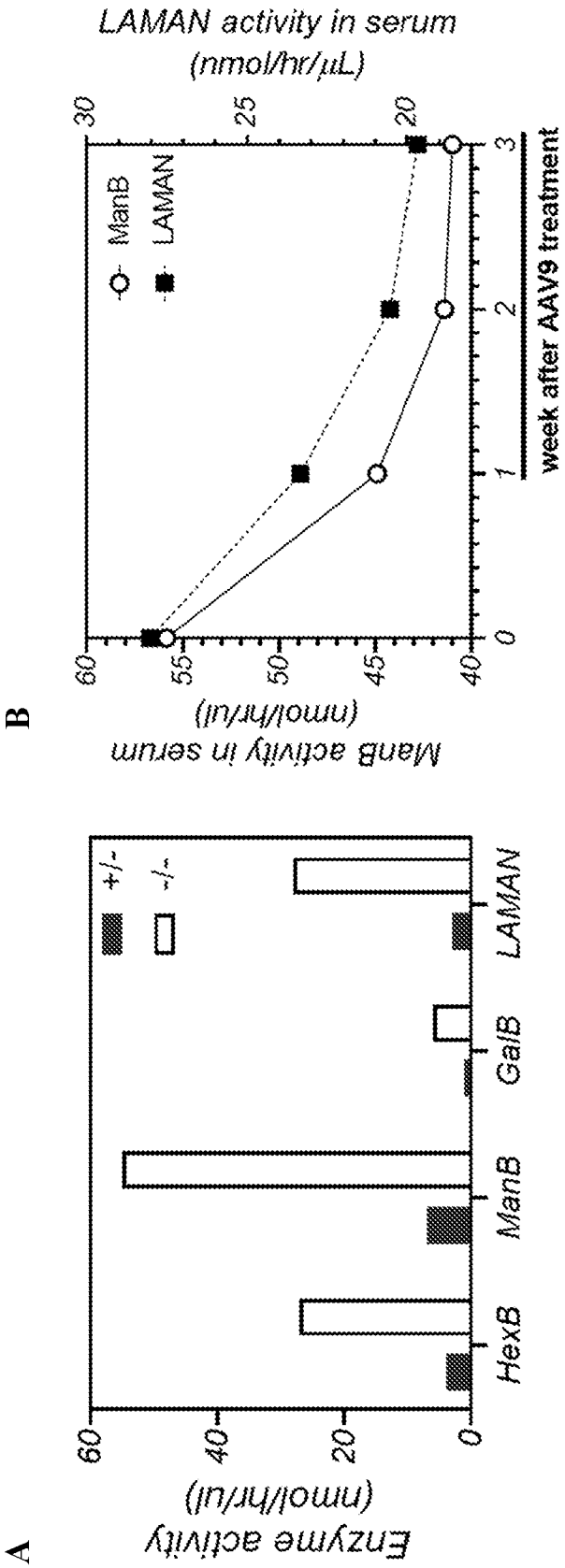
S1-S3 is suitable for the gene therapy of ML II disease

1. Full activity comparing to full length GNPTAB gene.
2. Small gene size (~1.7Kb) for AAV package.

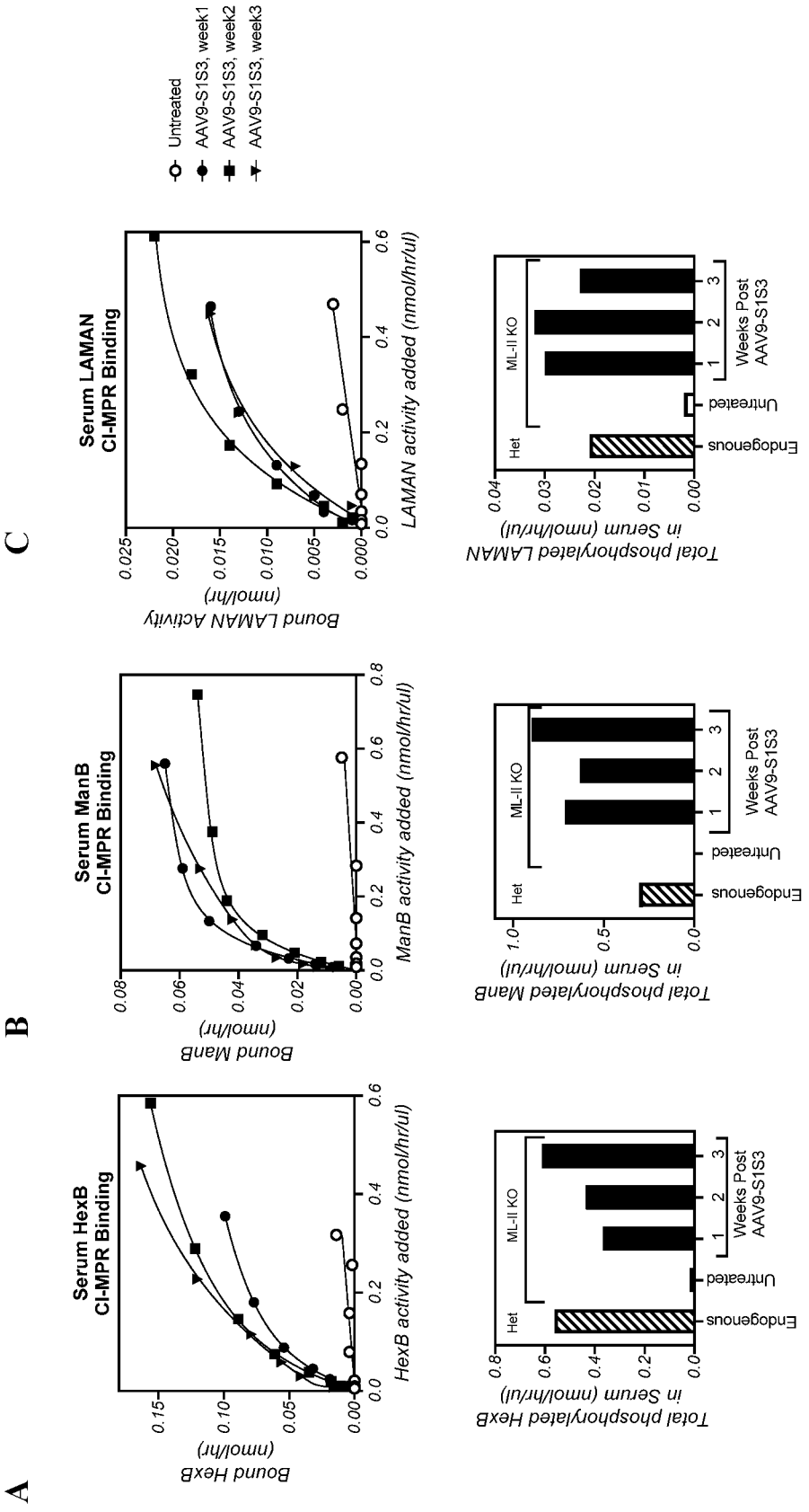
B



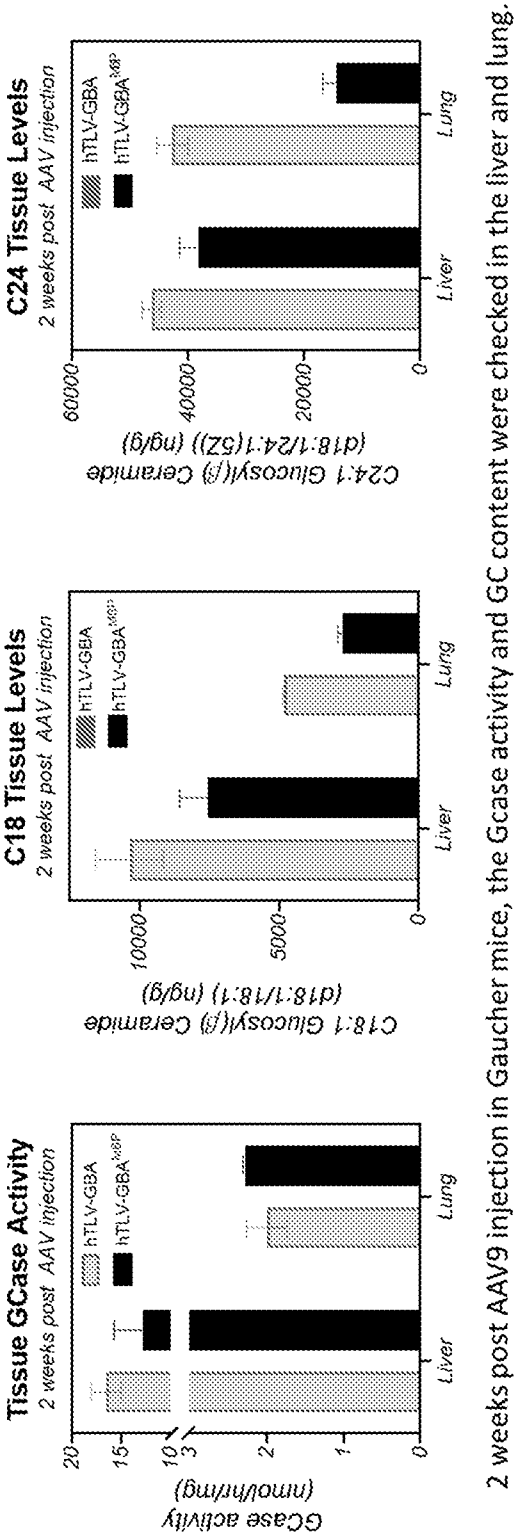
Figs 27A-27B


A
Enzyme activity
(nmol/hr/ul)
60
40
20
0
+/-
-/-
HexB
ManB
GalB
LAMAN
B
ManB activity in serum
(nmol/hr/ul)
60
55
50
45
40
ManB
LAMAN
LAMAN activity in serum
(nmol/hr/μL)
30
25
20
0
1
2
3
week after AAV9 treatment

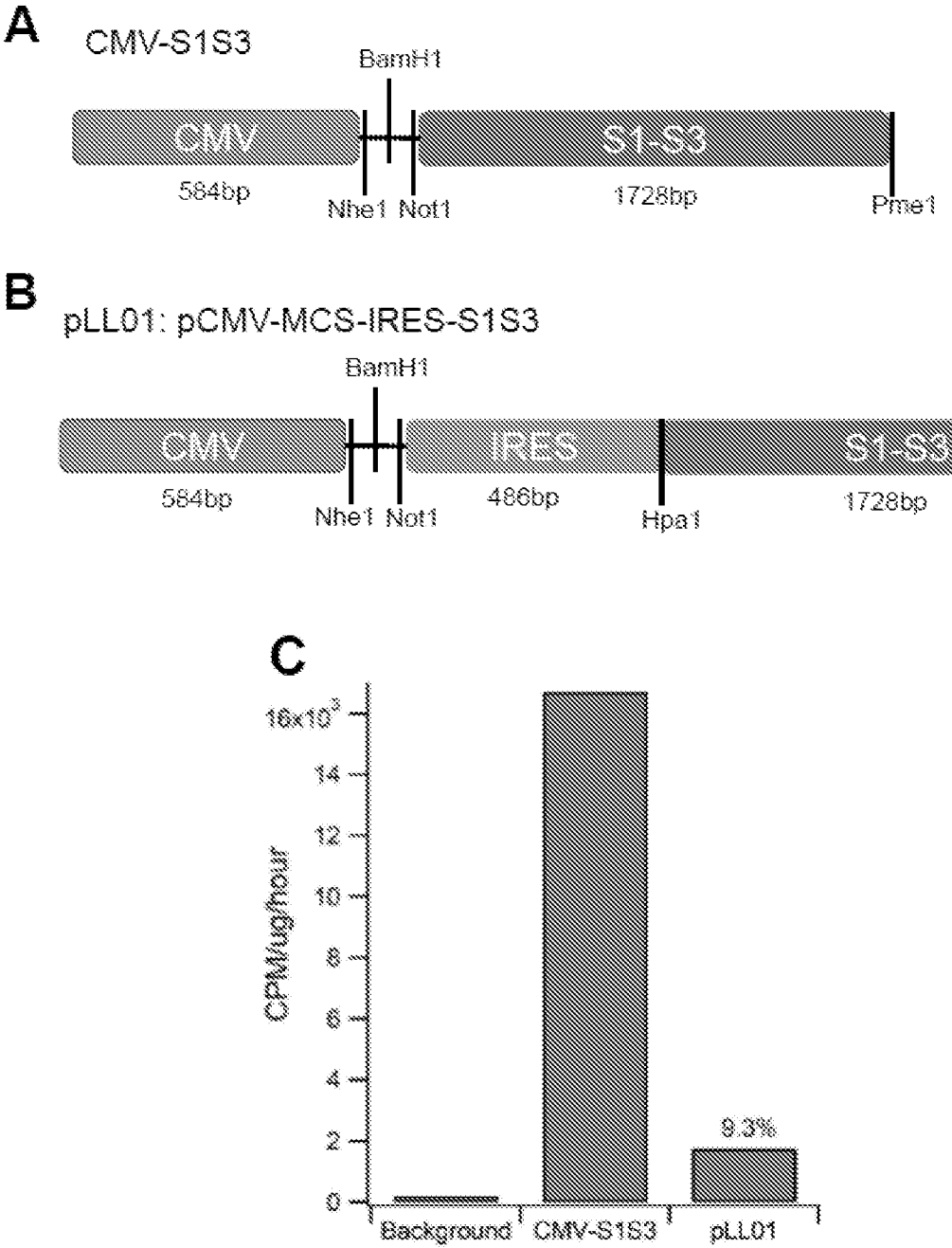
Figs 28A-28C


A
B
C
Serum HexB
CI-MPR Binding
Bound HexB (nmol/hr)
HexB activity added (nmol/hr/ul)
Serum ManB
CI-MPR Binding
Bound ManB (nmol/hr)
ManB activity added (nmol/hr/ul)
Serum LAMAN
CI-MPR Binding
Bound LAMAN Activity (nmol/hr)
LAMAN activity added (nmol/hr/ul)
Untreated
AAV9-S1S3, week1
AAV9-S1S3, week2
AAV9-S1S3, week3
Total phosphorylated HexB in Serum (nmol/hr/ul)
Total phosphorylated ManB in Serum (nmol/hr/ul)
Total phosphorylated LAMAN in Serum (nmol/hr/ul)
Het
ML-II KO
Endogenous
Untreated
Weeks Post AAV9-S1S3

Figs 29A-29C



2 weeks post AAV9 injection in Gaucher mice, the Gcase activity and GC content were checked in the liver and lung.


A
CMV-S1S3
BamH1
CMV
S1-S3
584bp
Nhe1 Not1
1728bp
Pme1
B
pLL01: pCMV-MCS-IRES-S1S3
BamH1
CMV
IRES
S1-S3
584bp
Nhe1 Not1
486bp
Hpa1
1728bp
C
16x10³
14
12
10
8
6
4
2
0
CPM/ug/hour
9.3%
Background
CMV-S1S3
pLL01

Figs. 1A-1C