CLAIMS

1. A polynucleotide encoding a multidomain therapeutic protein comprising a delivery domain and an enzyme domain,

wherein the delivery domain is a single-chain variable fragment (scFv) that binds to CD63,

wherein the enzyme domain comprises an alpha-glucosidase (GAA) or a biologically active portion thereof,

wherein the enzyme domain is attached to the C-terminus of the scFv, and

wherein the polynucleotide further comprises a virus nucleic acid sequence, wherein the virus nucleic acid sequence is an adeno-associated virus (AAV) inverted terminal repeat (ITR) nucleic acid sequence.

2. The polynucleotide of claim 1, wherein:

(i) the delivery domain comprises:

(1) the amino acid sequence set forth in SEQ ID NO: 2;

(2) HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences selected from the group consisting of SEQ ID NOs: 16-18-20-24-26-28, SEQ ID NOs: 32-34-36-40-42-44, SEQ ID NOs: 48-50-52-56-58-60, and SEQ ID NOs: 64-66-68-72-74-76; or

(3) an HCVR/LCVR amino acid pair set forth in SEQ ID NOs: 14/22, SEQ ID NOs: 30/38, SEQ ID NOs: 46/54, or SEQ ID NOs: 62/70,

optionally wherein the HCVR/LCVR amino acid pair comprises from N-terminal to C-terminal: the HCVR, an optional linker, and the LCVR and/or optionally wherein the enzyme domain is attached to the C-terminus of the LCVR, optionally via a linker, and any combination thereof; and/or

(ii) the enzyme domain comprises the amino acid sequence set forth in SEQ ID NO: 1, SEQ ID NO: 13, or SEQ ID NO: 78, or a biologically active fragment thereof, optionally wherein the multidomain therapeutic protein comprises the amino acid sequence set forth in SEQ ID NO: 79.

3. The polynucleotide of claim 1 or 2, wherein:

(i) the polynucleotide further comprises a locus-targeting sequence;

(ii) the inverted terminal repeat sequence comprises the sequence set forth in SEQ ID NO: 6, SEQ ID NO: 7, or both;

(iii) the polynucleotide further comprises a tissue-specific regulatory element, optionally wherein the tissue-specific regulatory element comprises a liver-specific promoter;

(iv) the polynucleotide further comprises a tissue-specific regulatory element comprising the sequence set forth in SEQ ID NO: 8, SEQ ID NO: 9, or both; and/or

(v) the polynucleotide comprises the nucleic acid sequence set forth in SEQ ID NO: 11 and/or encodes the amino acid sequence set forth in SEQ ID NO: 10 or SEQ ID NO: 78.

4. The polynucleotide of any one of claims 1-3, wherein the delivery domain comprises HCDR1-HCDR2-HCDR3-LCDR1-LCDR2-LCDR3 amino acid sequences set forth in SEQ ID NOs: 48-50-52-56-58-60.

5. The polynucleotide of any one of claims 1-4, wherein the delivery domain comprises the HCVR/LCVR pair set forth in SEQ ID NOs: 46/54.

6. The polynucleotide of any one of claims 1-5, wherein the multidomain therapeutic protein comprises the amino acid sequence set forth in SEQ ID NO: 79.

7. A gene therapy vector comprising the polynucleotide of any one of claims 1-6, optionally wherein the gene therapy vector is selected from the group consisting of:

(i) an AAV vector;

(ii) a naked polynucleotide; and

(iii) a polynucleotide complex, optionally wherein the polynucleotide complex is a lipid nanoparticle comprising the polynucleotide and lipids.

8. The gene therapy vector of claim 7, wherein the gene therapy vector is an AAV2/8 chimera or an AAV pseudotyped to a specific tissue, optionally wherein the AAV is pseudotyped to the liver.

9. A recombinant multidomain therapeutic protein comprising a delivery domain and an enzyme domain, wherein the recombinant multidomain therapeutic protein is encoded by the polynucleotide of any one of claims 1-6.

10. A method of expressing in a cell a recombinant multidomain therapeutic protein comprising a delivery domain and an enzyme domain, wherein the cell is *ex vivo*, the method comprising:

(a) contacting the cell with the gene therapy vector of claim 7 or 8 containing the polynucleotide encoding the recombinant multidomain therapeutic protein;

(b) allowing the polynucleotide to integrate into a genomic locus of the cell; and

(c) allowing the cell to produce the recombinant multidomain therapeutic protein.

11. A gene therapy vector for use in a method of expressing in a cell a recombinant multidomain therapeutic protein comprising a delivery domain and an enzyme domain, wherein the cell is *in vivo*, the method comprising:

(a) contacting the cell with the gene therapy vector of claim 7 or 8 containing the polynucleotide encoding the recombinant multidomain therapeutic protein;

(b) allowing the polynucleotide to integrate into a genomic locus of the cell; and

(c) allowing the cell to produce the recombinant multidomain therapeutic protein.

12. The method of claim 10 or the gene therapy vector for use of claim 11, wherein:

(i) the gene therapy vector is an adeno-associated virus (AAV) vector;

(ii) the genomic locus of the cell is a safe harbor locus;

(iii) the genomic locus is at or proximal to a locus selected from the group consisting of an EESYR locus, a SARS locus, position 188,083,272 of human chromosome 1 or

its non-human mammalian orthologue, position 3,046,320 of human chromosome 10 or its non-human mammalian orthologue, position 67,328,980 of human chromosome 17 or its non-human mammalian orthologue, an adeno-associated virus site 1 (AAVS1) on human chromosome 19 or its non-human mammalian orthologue, a chemokine receptor 5 (CCR5) gene, a chemokine receptor gene encoding an HIV-1 coreceptor, a mouse Rosa26 locus or its non-murine mammalian orthologue, and a human albumin (*alb*) locus;

(iv) the cell is a human cell or a mouse cell; and/or

(v) the cell is a liver cell.

13. The gene therapy vector of claim 7 or 8, for use in a method of reducing glycogen accumulation in a tissue in a patient in need thereof, comprising administering to the patient the gene therapy vector.

14. The gene therapy vector for use of claim 13, wherein:

(i) the tissue is selected from the group consisting of heart, liver, and skeletal muscle;

(ii) high serum levels of GAA are maintained for at least 12 weeks, or serum levels of GAA at 12 weeks are 2.5-3-fold higher than serum levels of GAA in a subject administered a gene therapy vector comprising a polynucleotide encoding a GAA without the anti-CD63 scFv delivery domain;

(iii) glycogen levels in heart, skeletal muscle, and liver tissue are reduced to wild-type levels; and/or

(iv) glycogen levels in the tissue are maintained at wild-type levels 3 months after administration of the gene therapy vector.

15. The gene therapy vector of claim 7 or 8 for use in a method of treating enzyme deficiency in a patient in need thereof and/or tolerizing the patient to the enzyme for which it is deficient, comprising administering to the patient the gene therapy vector.

16. The gene therapy vector for use of claim 15, wherein:

(i) the enzyme deficiency is a deficiency in alpha-glucosidase;

(ii) the anti-CD63 scFv-GAA comprises the amino acid sequence set forth in SEQ ID NO: 10 or SEQ ID NO: 79;

(iii) high serum levels of GAA are maintained in the patient for at least 12 weeks after administration of the gene therapy vector;

(iv) serum levels of GAA in the patient at 12 weeks after administration of the gene therapy vector are 2.5-3-fold higher than serum levels of GAA in a subject administered a gene therapy vector comprising a polynucleotide encoding a GAA without the anti-CD63 scFv delivery domain;

(v) glycogen levels in heart, skeletal muscle, and liver tissue in the patient are reduced to wild-type levels;

(vi) glycogen levels in heart, skeletal muscle, and liver tissue in the patient are maintained at wild-type levels 3 months after administration of the gene therapy vector;

(vii) muscle strength of the patient after treatment is restored to wild-type levels;

(viii) the patient has Pompe disease;

(ix) the patient has reduced antibodies to the enzyme compared to a control patient who received the enzyme domain in absence of the delivery domain;

(x) the method further comprises immunosuppressing the patient with one or more immunosuppressant agents;

(xi) the method further comprises as a last step administering to the patient the enzyme in which it is deficient, optionally wherein the patient is administered the enzyme in which it is deficient via a multidomain therapeutic protein, optionally wherein the multidomain therapeutic protein comprises an anti-CD63 antibody or antigen-binding portion thereof; and/or

(xii) the patient is administered a therapeutically effective amount of the polynucleotide that makes unnecessary any further administration of GAA, a biologically active portion thereof, or a multidomain therapeutic polypeptide comprising GAA or a biologically active portion thereof to the patient.

17. A pharmaceutical composition comprising the gene therapy vector of claim 7 or 8 and a pharmaceutically acceptable carrier.