SUMMARY

[0004] Provided herein are fusion proteins comprising enzyme replacement therapy (ERT) enzymes and methods of use thereof for treating lysosomal storage disorders (LSDs).

[0004a] In a first aspect, the present disclosure provides a protein comprising:

(a) a first Fc polypeptide that is linked to an enzyme replacement therapy (ERT) enzyme, wherein the ERT enzyme is an enzyme that is deficient in a lysosomal storage disorder; and

(b) a second Fc polypeptide that forms an Fc dimer with the first Fc polypeptide,

wherein the first Fc polypeptide and/or the second Fc polypeptide is a modified Fc polypeptide capable of specifically binding to a transferrin receptor (TfR), and wherein the first Fc polypeptide and/or the second Fc polypeptide comprises 11 residues that confer the specific binding to TfR as follows: Trp, Leu, or Glu at position 380; Tyr or Phe at position 384; Thr at position 386; Glu at position 387; Trp at position 388; Ser, Ala, Val, or Asn at position 389; Ser or Asn at position 390; Thr or Ser at position 413; Glu or Ser at position 415; Glu at position 416; and Phe at position 421, according to EU numbering; and

wherein the protein does not include an immunoglobulin heavy and/or light chain variable region sequence or an antigen-binding portion thereof.

[0004b] In a second aspect, the present disclosure provides a polynucleotide comprising a nucleic acid sequence encoding the polypeptides of the disclosure.

[0004c] In a third aspect, the present disclosure provides a pair of polynucleotides comprising nucleic acid sequences encoding the polypeptides as described in the disclosure.

[0004d] In a fourth aspect, the present disclosure provides one or more vector(s) comprising the polynucleotide or pair of polynucleotides of the second or third aspects.

[0004e] In a fifth aspect, the present disclosure provides a host cell comprising the polynucleotide of the second aspect, the pair of polynucleotides of the third aspect, or the one or more vector(s) of the fourth aspect.

[0004f] In a sixth aspect, the present disclosure provides a method for producing polypeptides, comprising culturing a host cell under conditions in which the polypeptides encoded by the polynucleotide of the second aspect are expressed or the polypeptides encoded by the pair of polynucleotides of the third aspect are expressed.

[0004g] In a seventh aspect, the present disclosure provides a protein as described in the disclosure for use in treating a lysosomal storage disorder (LSD) in a patient in need thereof or decreasing the accumulation of a toxic metabolic product in a patient having an LSD.

[0004h] In an eighth aspect, the present disclosure provides a use of the protein of the disclosure in the manufacture of a medicament for treating a lysosomal storage disorder (LSD) and/or decreasing the accumulation of a toxic metabolic product in a patient having an LSD.

[0004i] In a ninth aspect, the present disclosure provides a pharmaceutical composition comprising the protein of the disclosure and a pharmaceutically acceptable excipient.

[0005] In some examples, provided herein is a protein comprising:

(a) a first Fc polypeptide that is linked to an ERT enzyme, an ERT enzyme variant, or a catalytically active fragment thereof; and

(b) a second Fc polypeptide that forms an Fc dimer with the first Fc polypeptide.

[0006] In some examples, the first Fc polypeptide and/or the second Fc polypeptide does not include an immunoglobulin heavy and/or light chain variable region sequence or an antigen-binding portion thereof.

[0007] In some examples, the ERT enzyme is iduronate 2-sulfatase (IDS), an IDS variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:91, 92, 114, 230, and 234. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:91, 92, 114, 230, and 234.

[0008] In some examples, the ERT enzyme is N-sulfoglucosamine sulfohydrolase (SGSH), an SGSH variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:119 and 120. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:119 and 120.

[0009] In some examples, the ERT enzyme is acid sphingomyelinase (ASM), an ASM variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:121, 122, and 123. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:121, 122, and 123.

[0010] In some examples, the ERT enzyme is β-glucocerebrosidase (GBA), a GBA variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme

comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:93 and 94. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:93 and 94.

[0011] In some examples, the first Fc polypeptide is a fusion polypeptide that is linked to the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof by a peptide bond or by a polypeptide linker. In some examples, the polypeptide linker is a flexible polypeptide linker. In some examples, the flexible polypeptide linker is a glycine-rich linker. In some examples, the glycine-rich linker is G_4S (SEQ ID NO:239) or $(G_4S)_2$ (SEQ ID NO:240). In some examples, the first Fc polypeptide is not linked to the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof by a chemical cross-linking agent, *e.g.*, the fusion polypeptide does not include a non-peptide bond or a non-polypeptide linker.

[0012] In certain examples, the fusion polypeptide comprises from N- to C-terminus: the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof; the polypeptide linker; and the first Fc polypeptide.

[0013] In some examples, the second Fc polypeptide is linked to an ERT enzyme, an ERT enzyme variant, or a catalytically active fragment thereof. In some examples, the second Fc polypeptide is a fusion polypeptide that is linked to the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof by a peptide bond or by a polypeptide linker. In some examples, the polypeptide linker is a flexible polypeptide linker. In some examples, the flexible polypeptide linker is a glycine-rich linker. In some examples, the glycine-rich linker is G_4S (SEQ ID NO:239) or $(G_4S)_2$ (SEQ ID NO:240). In some examples, the second Fc polypeptide is not linked to the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof by a chemical cross-linking agent, *e.g.*, the fusion polypeptide does not include a non-peptide bond or a non-polypeptide linker.

[0014] In some examples, the N-terminus of the first Fc polypeptide and/or the N-terminus of the second Fc polypeptide is linked to the ERT enzyme. In some examples, the N-terminus of the first Fc polypeptide is linked to one ERT enzyme and the N-terminus of the second Fc polypeptide is linked to the other ERT enzyme.

[0015] In some examples, the C-terminus of the first Fc polypeptide and/or the C-terminus of the second Fc polypeptide is linked to the ERT enzyme. In some examples, the C-terminus of the first Fc polypeptide is linked to one ERT enzyme and the C-terminus of the second Fc polypeptide is linked to the other ERT enzyme.

[0016] In some examples, the N-terminus of the first Fc polypeptide is linked to one ERT enzyme and the C-terminus of the second Fc polypeptide is linked to the other ERT enzyme. In some examples, the C-terminus of the first Fc polypeptide is linked to one ERT enzyme and the N-terminus of the second Fc polypeptide is linked to the other ERT enzyme.

[0017] In some examples, the protein comprises a single ERT enzyme, and the N-terminus or the C-terminus of the first Fc polypeptide is linked to the ERT enzyme. In some examples, the protein comprises two ERT enzymes (*e.g.,* exactly two ERT enzymes). In some examples, the protein comprises exactly one or exactly two ERT enzymes, enzyme variants, or catalytically active fragments thereof.

[0018] In some examples, the first Fc polypeptide is a modified Fc polypeptide and/or the second Fc polypeptide is a modified Fc polypeptide.

[0019] In some examples, the first Fc polypeptide and the second Fc polypeptide each contain modifications that promote heterodimerization. In some examples, the Fc dimer is an Fc heterodimer. In some examples, one of the Fc polypeptides has a T366W substitution and the other Fc polypeptide has T366S, L368A, and Y407V substitutions, according to EU numbering. In some examples, the first Fc polypeptide contains the T366S, L368A, and Y407V substitutions and the second Fc polypeptide contains the T366W substitution. In some examples, the first Fc polypeptide is linked to the ERT enzyme IDS and comprises the amino acid sequence of any one of SEQ ID NOS:117, 232, and 236. In some examples, the first Fc polypeptide contains the T366W substitution and the second Fc polypeptide contains the T366S, L368A, and Y407V substitutions. In some examples, the first Fc polypeptide is linked to the ERT enzyme IDS and comprises the amino acid sequence of any one of SEQ ID NOS:118, 233, and 237.

[0020] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises a native FcRn binding site. In some examples, the first Fc polypeptide and the

second Fc polypeptide do not have effector function. In some examples, the first Fc polypeptide and/or the second Fc polypeptide includes a modification that reduces effector function. In some examples, the modification that reduces effector function is the substitutions of Ala at position 234 and Ala at position 235, according to EU numbering. In some examples, the modification that reduces effector function further comprises a substitution of Gly at position 329, according to EU numbering. In some examples, the first Fc polypeptide is linked to the ERT enzyme IDS and comprises the amino acid sequence of any one of SEQ ID NOS:115, 231, and 235. In some examples, the first Fc polypeptide is linked to the ERT enzyme SGSH and comprises the amino acid sequence of any one of SEQ ID NOS:149, 150, 152, and 153.

[0021] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises amino acid changes relative to the native Fc sequence that extend serum half-life. In some examples, the amino acid changes comprise substitutions of Tyr at position 252, Thr at position 254, and Glu at position 256, according to EU numbering. Alternatively, in other examples, the amino acid changes comprise substitutions of Leu at position 428 and Ser at position 434, according to EU numbering. Alternatively, in further examples, the amino acid changes comprise a substitution of Ser or Ala at position 434, according to EU numbering.

[0022] In some examples, the first Fc polypeptide and/or the second Fc polypeptide specifically binds to a transferrin receptor (TfR).

[0023] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises at least two substitutions at positions selected from the group consisting of 384, 386, 387, 388, 389, 390, 413, 416, and 421, according to EU numbering. In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises at least three, four, five, six, seven, eight, or nine substitutions at the positions.

[0024] In some examples, the first Fc polypeptide and/or the second Fc polypeptide further comprises one, two, three, or four substitutions at positions comprising 380, 391, 392, and 415, according to EU numbering. In some examples, the first Fc polypeptide and/or the second Fc polypeptide further comprises one, two, or three substitutions at positions comprising 414, 424, and 426, according to EU numbering.

[0025] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises Trp at position 388. In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises an aromatic amino acid at position 421. In some examples, the aromatic amino acid at position 421 is Trp or Phe.

[0026] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises at least one position selected from the following: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0027] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 positions selected from the following: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0028] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises 11 positions as follows: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0029] In some examples, the first Fc polypeptide and/or the second Fc polypeptide has a CH3 domain with at least 85% identity, at least 90% identity, or at least 95% identity to amino acids 111-217 of any one of SEQ ID NOS:34-38, 58, and 60-90, 151, and 156-229. In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:156-229. In some examples, the residues at at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 of the positions corresponding to EU index positions 380, 384, 386, 387, 388, 389, 390, 391, 392, 413, 414, 415, 416, 421, 424 and 426 of any one of SEQ ID NOS:34-38, 58, and 60-90, 151, and 156-229 are not deleted or substituted.

[0030] In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:157. In some examples, the first Fc

polypeptide and/or the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:169. In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:181. In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:193. In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:205. In some examples, the first Fc polypeptide and/or the second Fc polypeptide comprises the amino acid sequence of SEQ ID NO:217.

[0031] In some examples, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:115, and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:205 and 228 (*e.g.*, SEQ ID NO:228). In other examples, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:115, and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:169 and 229 (*e.g.*, SEQ ID NO:229).

[0032] In some examples, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:231, and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:205 and 228 (*e.g.*, SEQ ID NO:228). In other examples, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:231, and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:169 and 229 (*e.g.*, SEQ ID NO:229).

[0033] In some examples, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:235, and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:205 and 228 (*e.g.*, SEQ ID NO:228). In other examples, the first Fc polypeptide comprises the amino acid sequence of SEQ ID NO:235, and the second Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:169 and 229 (*e.g.*, SEQ ID NO:229).

[0034] In some examples, the first Fc polypeptide and/or the second Fc polypeptide binds to the apical domain of TfR. In some examples, the binding of the protein to TfR does not substantially inhibit binding of transferrin to TfR.

[0035] In some examples, the first Fc polypeptide and/or the second Fc polypeptide has an amino acid sequence identity of at least 75%, or at least 80%, 85%, 90%, 92%, or 95%, as compared to the corresponding wild-type Fc polypeptide. In some examples, the corresponding wild-type Fc polypeptide is a human IgG1, IgG2, IgG3, or IgG4 Fc polypeptide.

[0036] In some examples, uptake of the ERT enzyme into the brain (*e.g.*, using an appropriate animal model such as those described herein) is greater than the uptake of the ERT enzyme in the absence of the first Fc polypeptide and/or the second Fc polypeptide or the uptake of the ERT enzyme without the modifications to the first Fc polypeptide and/or the second Fc polypeptide that result in TfR binding. In some examples, uptake of the ERT enzyme into the brain is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100-fold greater as compared to the uptake of the ERT enzyme in the absence of the first Fc polypeptide and/or the second Fc polypeptide or as compared to the uptake of the ERT enzyme without the modifications to the first Fc polypeptide and/or the second Fc polypeptide that result in TfR binding.

[0037] In some examples, the first Fc polypeptide is not modified to bind to a blood-brain barrier (BBB) receptor and the second Fc polypeptide is modified to specifically bind to TfR. In some examples, the first Fc polypeptide is modified to specifically bind to TfR and the second Fc polypeptide is not modified to bind to a BBB receptor.

[0038] In some examples, the protein does not include an immunoglobulin heavy and/or light chain variable region sequence or an antigen-binding portion thereof.

[0039] In some examples, provided herein is a polypeptide comprising an Fc polypeptide that is linked to an ERT enzyme, an ERT enzyme variant, or a catalytically active fragment thereof, wherein the Fc polypeptide contains one or more modifications that promote its heterodimerization to another Fc polypeptide.

[0040] In some examples, the ERT enzyme is IDS, an IDS variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:91, 92, 114, 230, and 234. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:91, 92, 114, 230, and 234.

[0041] In some examples, the ERT enzyme is SGSH, an SGSH variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:119 and 120. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:119 and 120.

[0042] In some examples, the ERT enzyme is ASM, an ASM variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:121, 122, and 123. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:121, 122, and 123.

[0043] In some examples, the ERT enzyme is GBA, a GBA variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:93 and 94. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:93 and 94.

[0044] In some examples, the Fc polypeptide is a fusion polypeptide that is linked to the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof by a peptide bond or by a polypeptide linker. In some examples, the polypeptide linker is a flexible polypeptide linker. In some examples, the flexible polypeptide linker is a glycine-rich linker. In some examples, the glycine-rich linker is G_4S (SEQ ID NO:239) or $(G_4S)_2$ (SEQ ID NO:240). In some examples, the Fc polypeptide is not linked to the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof by a chemical cross-linking agent, *e.g.*, the fusion polypeptide does not include a non-peptide bond or a non-polypeptide linker.

[0045] In certain examples, the fusion polypeptide comprises from N- to C-terminus: the ERT enzyme, the ERT enzyme variant, or the catalytically active fragment thereof; the polypeptide linker; and the first Fc polypeptide.

[0046] In some examples, the Fc polypeptide contains T366S, L368A, and Y407V substitutions, according to EU numbering. In some examples, the polypeptide comprises

the amino acid sequence of any one of SEQ ID NOS:115, 117, 231, 232, 235, and 236. In some examples, the polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:149 and 150. In some examples, the Fc polypeptide contains a T366W substitution. In some examples, the polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:118, 233, and 237. In some examples, the polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:152-155. In some examples, the polypeptide further comprises the other Fc polypeptide. In some examples, the other Fc polypeptide contains a T366W substitution or contains T366S, L368A, and Y407V substitutions and forms an Fc dimer with the ERT enzyme-Fc fusion polypeptide.

[0047] In some examples, the Fc polypeptide comprises a native FcRn binding site. In some examples, the Fc polypeptide does not have effector function. In some examples, the Fc polypeptide includes a modification that reduces effector function. In some examples, the modification that reduces effector function is the substitutions of Ala at position 234 and Ala at position 235, according to EU numbering. In some examples, the modification that reduces effector function further comprises a substitution of Gly at position 329, according to EU numbering.

[0048] In some examples, the Fc polypeptide comprises amino acid changes relative to the native Fc sequence that extend serum half-life. In some examples, the amino acid changes comprise substitutions of Tyr at position 252, Thr at position 254, and Glu at position 256, according to EU numbering.

[0049] In some examples, the Fc polypeptide specifically binds to TfR.

[0050] In some examples, the Fc polypeptide comprises at least two substitutions at positions selected from the group consisting of 384, 386, 387, 388, 389, 390, 413, 416, and 421, according to EU numbering. In some examples, the Fc polypeptide comprises at least three, four, five, six, seven, eight, or nine substitutions at the positions.

[0051] In some examples, the Fc polypeptide further comprises one, two, three, or four substitutions at positions comprising 380, 391, 392, and 415, according to EU numbering. In some examples, the Fc polypeptide further comprises one, two, or three substitutions at positions comprising 414, 424, and 426, according to EU numbering.

[0052] In some examples, the Fc polypeptide comprises Trp at position 388. In some examples, the Fc polypeptide comprises an aromatic amino acid at position 421. In some examples, the aromatic amino acid at position 421 is Trp or Phe.

[0053] In some examples, the Fc polypeptide comprises at least one position selected from the following: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0054] In some examples, the Fc polypeptide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 positions selected from the following: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0055] In some examples, the Fc polypeptide comprises 11 positions as follows: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0056] In some examples, the Fc polypeptide has a CH3 domain with at least 85% identity, at least 90% identity, or at least 95% identity to amino acids 111-217 of any one of SEQ ID NOS:34-38, 58, and 60-90. In some examples, the residues at at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 of the positions corresponding to EU index positions 380, 384, 386, 387, 388, 389, 390, 391, 392, 413, 414, 415, 416, 421, 424 and 426 of any one of SEQ ID NOS:34-38, 58, and 60-90 are not deleted or substituted.

[0057] In some examples, the Fc polypeptide binds to the apical domain of TfR. In some examples, the binding of the protein to TfR does not substantially inhibit binding of transferrin to TfR.

[0058] In some examples, the Fc polypeptide has an amino acid sequence identity of at least 75%, or at least 80%, 85%, 90%, 92%, or 95%, as compared to the corresponding wild-

type Fc polypeptide. In some examples, the corresponding wild-type Fc polypeptide is a human IgG1, IgG2, IgG3, or IgG4 Fc polypeptide.

[0059] In some examples, the Fc polypeptide does not include an immunoglobulin heavy and/or light chain variable region sequence or an antigen-binding portion thereof.

[0060] In some examples, provided herein is a polynucleotide comprising a nucleic acid sequence encoding a polypeptide comprising an Fc polypeptide that is linked to an ERT enzyme, an ERT enzyme variant, or a catalytically active fragment thereof, wherein the Fc polypeptide contains one or more modifications that promote its heterodimerization to another Fc polypeptide. In some examples, provided herein is a vector comprising the polynucleotide. In some examples, provided herein is a host cell comprising the polynucleotide or the vector. In some examples, the host cell further comprises a polynucleotide comprising a nucleic acid sequence encoding the other Fc polypeptide. In some examples, provided herein is a method for producing the polypeptide described herein, comprising culturing a host cell under conditions in which the polypeptide encoded by the polynucleotide is expressed.

[0061] In some examples, provided herein is a protein comprising:

(a) a first polypeptide chain that comprises a modified Fc polypeptide that specifically binds to TfR;

(b) a second polypeptide chain that comprises an Fc polypeptide, wherein the first and second polypeptide chains form an Fc dimer; and

(c) an ERT enzyme, an ERT enzyme variant, or a catalytically active fragment thereof, that is linked to the modified Fc polypeptide of (a) or to the Fc polypeptide of (b).

[0062] In some examples, the ERT enzyme is IDS, an IDS variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:91, 92, 114, 230, and 234. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:91, 92, 114, 230, and 234.

[0063] In some examples, the ERT enzyme is SGSH, an SGSH variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid

sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:119 and 120. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:119 and 120.

[0064] In some examples, the ERT enzyme is ASM, an ASM variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:121, 122, and 123. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:121, 122, and 123.

[0065] In some examples, the ERT enzyme is GBA, a GBA variant, or a catalytically active fragment thereof. In some examples, the ERT enzyme comprises an amino acid sequence having at least 80%, 85%, 90%, or 95% identity to the amino acid sequence of any one of SEQ ID NOS:93 and 94. In some examples, the ERT enzyme comprises the amino acid sequence of any one of SEQ ID NOS:93 and 94.

[0066] In some examples, the ERT enzyme is linked to the modified Fc polypeptide of (a). In some examples, the ERT enzyme is linked to the Fc polypeptide of (b). In some examples, the Fc polypeptide of (b) is not modified to bind to a BBB receptor. In some examples, the Fc polypeptide of (b) is a modified Fc polypeptide that specifically binds to TfR.

[0067] In some examples, the ERT enzyme is linked (*e.g.*, fused) to the modified Fc polypeptide of (a) or to the Fc polypeptide of (b) by a peptide bond or by a polypeptide linker to form a fusion polypeptide. In some examples, the polypeptide linker is a flexible polypeptide linker. In some examples, the flexible polypeptide linker is a glycine-rich linker. In some examples, the glycine-rich linker is G_4S (SEQ ID NO:239) or $(G_4S)_2$ (SEQ ID NO:240). In some examples, the ERT enzyme is not linked to the modified Fc polypeptide of (a) or to the Fc polypeptide of (b) by a chemical cross-linking agent, *e.g.*, the fusion polypeptide does not include a non-peptide bond or a non-polypeptide linker.

[0068] In some examples, the ERT enzyme is linked to the N-terminus of the modified Fc polypeptide of (a) or to the N-terminus of the Fc polypeptide of (b). In some examples,

the ERT enzyme is linked to the C-terminus of the modified Fc polypeptide of (a) or to the C-terminus of the Fc polypeptide of (b).

[0069] In some examples, the protein comprises two ERT enzymes. In some examples, one ERT enzyme is linked to the modified Fc polypeptide of (a) and the other ERT enzyme is linked to the Fc polypeptide of (b). In some examples, the ERT enzymes are both linked to the N-terminus or are both linked to the C-terminus of the respective Fc polypeptides. In some examples, one ERT enzyme is linked to the N-terminus of the modified Fc polypeptide of (a) and the other ERT enzyme is linked to the C-terminus of the Fc polypeptide of (b). In some examples, one ERT enzyme is linked to the C-terminus of the modified Fc polypeptide of (a) and the other ERT enzyme is linked to the N-terminus of the Fc polypeptide of (b).

[0070] In some examples, the Fc polypeptides of (a) and (b) each contain modifications that promote heterodimerization. In some examples, one of the Fc polypeptides has a T366W substitution and the other Fc polypeptide has T366S, L368A, and Y407V substitutions, according to EU numbering. In some examples, the modified Fc polypeptide of (a) contains the T366W substitution and the Fc polypeptide of (b) contains the T366S, L368A, and Y407V substitutions. In some examples, the Fc polypeptide of (b) is linked to the ERT enzyme IDS and comprises the amino acid sequence of any one of SEQ ID NOS:117, 232, and 236. In some examples, the modified Fc polypeptide of (a) contains the T366S, L368A, and Y407V substitutions and the Fc polypeptide of (b) contains the T366W substitution. In some examples, the Fc polypeptide of (b) is linked to the ERT enzyme IDS and comprises the amino acid sequence of any one of SEQ ID NOS:118, 233, and 237.

[0071] In some examples, the modified Fc polypeptide of (a) and/or the Fc polypeptide of (b) comprises a native FcRn binding site. In some examples, the modified Fc polypeptide of (a) and the Fc polypeptide of (b) do not have effector function. In some examples, the modified Fc polypeptide of (a) and/or the Fc polypeptide of (b) includes a modification that reduces effector function. In some examples, the modification that reduces effector function is the substitutions of Ala at position 234 and Ala at position 235, according to EU numbering. In some examples, the modification that reduces effector function further comprises a substitution of Gly at position 329, according to EU numbering.

In some examples, the Fc polypeptide of (b) is linked to the ERT enzyme IDS and comprises the amino acid sequence of any one of SEQ ID NOS:115, 231, and 235. In some examples, the Fc polypeptide of (b) is linked to the ERT enzyme SGSH and comprises the amino acid sequence of any one of SEQ ID NOS:149, 150, 152, and 153.

[0072] In some examples, the modified Fc polypeptide of (a) and/or the Fc polypeptide of (b) comprises amino acid changes relative to the native Fc sequence that extend serum half-life. In some examples, the amino acid changes comprise substitutions of Tyr at position 252, Thr at position 254, and Glu at position 256, according to EU numbering.

[0073] In some examples, the modified Fc polypeptide comprises at least two substitutions at positions selected from the group consisting of 384, 386, 387, 388, 389, 390, 413, 416, and 421, according to EU numbering. In some examples, the modified Fc polypeptide comprises at least three, four, five, six, seven, eight, or nine substitutions at the positions.

[0074] In some examples, the modified Fc polypeptide further comprises one, two, three, or four substitutions at positions comprising 380, 391, 392, and 415, according to EU numbering. In some examples, the modified Fc polypeptide further comprises one, two, or three substitutions at positions comprising 414, 424, and 426, according to EU numbering.

[0075] In some examples, the modified Fc polypeptide comprises Trp at position 388. In some examples, the modified Fc polypeptide comprises an aromatic amino acid at position 421. In some examples, the aromatic amino acid at position 421 is Trp or Phe.

[0076] In some examples, the modified Fc polypeptide comprises at least one position selected from the following: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0077] In some examples, the modified Fc polypeptide comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 positions selected from the following: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser,

Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0078] In some examples, the modified Fc polypeptide comprises 11 positions as follows: position 380 is Trp, Leu, or Glu; position 384 is Tyr or Phe; position 386 is Thr; position 387 is Glu; position 388 is Trp; position 389 is Ser, Ala, Val, or Asn; position 390 is Ser or Asn; position 413 is Thr or Ser; position 415 is Glu or Ser; position 416 is Glu; and position 421 is Phe.

[0079] In some examples, the modified Fc polypeptide has a CH3 domain with at least 85% identity, at least 90% identity, or at least 95% identity to amino acids 111-217 of any one of SEQ ID NOS:34-38, 58, and 60-90, 151, and 156-229. In some examples, the modified Fc polypeptide comprises the amino acid sequence of any one of SEQ ID NOS:156-229. In some examples, the residues at at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 of the positions corresponding to EU index positions 380, 384, 386, 387, 388, 389, 390, 391, 392, 413, 414, 415, 416, 421, 424 and 426 of any one of SEQ ID NOS:34-38, 58, and 60-90, 151, and 156-229 are not deleted or substituted.

[0080] In some examples, the modified Fc polypeptide comprises the amino acid sequence of SEQ ID NO:157. In some examples, the modified Fc polypeptide comprises the amino acid sequence of SEQ ID NO:169. In some examples, the modified Fc polypeptide comprises the amino acid sequence of SEQ ID NO:181. In some examples, the modified Fc polypeptide comprises the amino acid sequence of SEQ ID NO:193. In some examples, the modified Fc polypeptide comprises the amino acid sequence of SEQ ID NO:205. In some examples, the modified Fc polypeptide comprises the amino acid sequence of SEQ ID NO:217.

[0081] In some examples, the first polypeptide chain comprises the amino acid sequence of any one of SEQ ID NOS:205 and 228 (*e.g.*, SEQ ID NO:228) and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:115. In other examples, the first polypeptide chain comprises the amino acid sequence of any one of SEQ ID NOS:169 and 229 (*e.g.*, SEQ ID NO:229) and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:115.

[0082] In some examples, the first polypeptide chain comprises the amino acid sequence of any one of SEQ ID NOS:205 and 228 (*e.g.*, SEQ ID NO:228) and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:231. In other examples, the first polypeptide chain comprises the amino acid sequence of any one of SEQ ID NOS:169 and 229 (*e.g.*, SEQ ID NO:229) and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:231.

[0083] In some examples, the first polypeptide chain comprises the amino acid sequence of any one of SEQ ID NOS:205 and 228 (*e.g.*, SEQ ID NO:228) and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:235. In other examples, the first polypeptide chain comprises the amino acid sequence of any one of SEQ ID NOS:169 and 229 (*e.g.*, SEQ ID NO:229) and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO:235.

[0084] In some examples, the modified Fc polypeptide binds to the apical domain of TfR. In some examples, the binding of the protein to TfR does not substantially inhibit binding of transferrin to TfR.

[0085] In some examples, the modified Fc polypeptide has an amino acid sequence identity of at least 75%, or at least 80%, 85%, 90%, 92%, or 95%, as compared to the corresponding wild-type Fc polypeptide. In some examples, the corresponding wild-type Fc polypeptide is a human IgG1, IgG2, IgG3, or IgG4 Fc polypeptide.

[0086] In some examples, uptake of the ERT enzyme into the brain (*e.g.*, using an appropriate animal model such as those described herein) is greater than the uptake of the ERT enzyme in the absence of the Fc polypeptide or the uptake of the ERT enzyme without the modifications to the Fc polypeptide that result in TfR binding. In some examples, uptake of the ERT enzyme into the brain is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100-fold greater as compared to the uptake of the ERT enzyme in the absence of the Fc polypeptide or as compared to the uptake of the ERT enzyme without the modifications to the Fc polypeptide that result in TfR binding.

[0087] In some examples, provided herein is a method of treating an LSD, the method comprising administering a protein or polypeptide as described above to a patient in need

thereof. In some examples, the method decreases the accumulation of a toxic metabolic product in the patient, *e.g.*, a toxic metabolic product in the patient's brain and/or cerebrospinal fluid (CSF) is decreased.

[0088] In related examples, provided herein is a method of decreasing the accumulation of a toxic metabolic product in a patient having an LSD, the method comprising administering a protein or polypeptide as described above to the patient. In some examples, the method decreases the accumulation of a toxic metabolic product in the patient's brain and/or CSF.

[0089] In some examples, the LSD is Hunter syndrome, and the ERT enzyme is IDS. In some examples, the toxic metabolic product comprises heparan sulfate-derived disaccharides and/or dermatan sulfate-derived disaccharides.

[0090] In some examples, the LSD is Sanfilippo syndrome A, and the ERT enzyme is SGSH. In some examples, the toxic metabolic product comprises heparan sulfate-derived oligosaccharides (*e.g.*, hexasaccharides).

[0091] In some examples, the LSD is Niemann-Pick disease, and the ERT enzyme is ASM. In some examples, the toxic metabolic product comprises sphingomyelin.

[0092] In some examples, the LSD is Gaucher's disease or Parkinson's disease, and the ERT enzyme is GBA. In some examples, the toxic metabolic product comprises glucosylceramide.

[0093] In some examples, the total amount of a toxic metabolic product is reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% as compared to the total amount of the toxic metabolic product in the absence of the protein or polypeptide. Illustrative assays for measuring ERT enzyme activity and substrate accumulation are described herein.

[0094] In some examples, provided herein is a pharmaceutical composition comprising a protein or polypeptide as described above and a pharmaceutically acceptable carrier.

[0095] In some examples, provided herein is a method of monitoring substrate accumulation to assess IDS activity, the method comprising:

(a) disrupting cells in a cell or tissue sample or microvesicles in a fluid sample from a subject administered a protein or polypeptide as described above to break open microvesicles to obtain a glycosaminoglycan (GAG) solution to be analyzed;

(b) digesting the GAG solution with at least one heparinase (*e.g.*, heparinase I, heparinase II, and heparinase III) and chrondroitinase B to obtain GAG-derived disaccharides;

(c) analyzing the GAG-derived disaccharides by mass spectrometry (*e.g.*, LC-MS/MS); and

(d) determining the levels of heparan sulfate- and/or dermatan sulfate-derived disaccharides, wherein decreased levels of heparan sulfate- and/or dermatan sulfate-derived disaccharides compared to a control that lacks IDS activity is indicative of increased IDS activity in the sample compared to the control.

[0096] In some examples, the step of disrupting cells or microvesicles comprises at least one freeze-thaw cycle and/or at least one sonication step. In some examples, the cells are from a tissue sample and the method comprises at least three, four, or five freeze-thaw cycles. In some examples, the subject is a mouse deficient in IDS activity. In some examples, the subject is a non-human primate. In some examples, the subject is a human patient having Hunter syndrome.

[0097] In some examples, the levels of heparan sulfate- and/or dermatan sulfate-derived disaccharides are reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% as compared to the levels of heparan sulfate- and/or dermatan sulfate-derived disaccharides in a control that lacks IDS activity. In some examples, the control is a cell or tissue sample of the same cell of tissue type obtained from the subject prior to administration of the protein or polypeptide. In some examples, the control is a cell or tissue sample of the same cell of tissue type known to be deficient in IDS activity. In some examples, the protein or polypeptide increases IDS activity in the sample by at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100-fold as compared to the IDS activity in the control.

[0098] In other examples, provided herein is a method of monitoring substrate accumulation to assess SGSH activity, the method comprising:

(a) disrupting cells in a cell or tissue sample or microvesicles in a fluid sample from a subject administered a protein or polypeptide as described above to break open microvesicles to obtain a glycosaminoglycan (GAG) solution to be analyzed;

(b) digesting the GAG solution with at least one heparinase to obtain GAG-derived disaccharides;

(c) analyzing the GAG-derived disaccharides by mass spectrometry (*e.g.,* LC-MS/MS); and

(d) determining the levels of heparan sulfate-derived disaccharides, wherein decreased levels of heparan sulfate-derived disaccharides compared to a control that lacks SGSH activity is indicative of increased SGSH activity in the sample compared to the control.

[0099] In some examples, the step of disrupting cells or microvesicles comprises at least one freeze-thaw cycle and/or at least one sonication step. In some examples, the cells are from a tissue sample and the method comprises at least three, four, or five freeze-thaw cycles. In some examples, the subject is a mouse deficient in SGSH activity. In some examples, the subject is a non-human primate. In some examples, the subject is a human patient having Sanfilippo syndrome A.

[0100] In some examples, the levels of heparan sulfate-derived disaccharides are reduced by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% as compared to the levels of heparan sulfate-derived disaccharides in a control that lacks SGSH activity. In some examples, the control is a cell or tissue sample of the same cell of tissue type obtained from the subject prior to administration of the protein or polypeptide. In some examples, the control is a cell or tissue sample of the same cell of tissue type known to be deficient in SGSH activity. In some examples, the protein or polypeptide increases SGSH activity in the sample by at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100-fold as compared to the SGSH activity in the control.

[0101] In other examples, provided herein is a method for transporting an agent across the BBB of a mammal, the method comprising exposing the BBB to a protein that binds to a TfR with an affinity of from about 50 nM to about 250 nM, wherein the protein is linked to the agent and transports the linked agent across the BBB. In some examples, the maximum

concentration (C_{max}) of the agent in the brain of the mammal is improved. In some examples, the agent is useful for treating an LSD.

[0102] In still other examples, provided herein is a method for treating an LSD, the method comprising administering to a mammal a protein that binds to a TfR with an affinity of from about 50 nM to about 250 nM, wherein the protein is linked to an agent for treating the LSD, thereby exposing the brain of the mammal to the agent. In some examples, the protein improves the C_{max} of the agent in the brain as compared to the agent linked to a reference protein that binds to the TfR with a weaker affinity. In some examples, the reference protein binds to the TfR with an affinity of about 600 nM, or weaker.

[0103] In some examples, the TfR is a primate TfR. In some examples, the primate TfR is a human TfR. In some examples, the protein binds to the TfR apical domain.

[0104] In some examples, the protein binds to the TfR with an affinity of from about 100 nM to about 200 nM. In some examples, the protein binds to the TfR with an affinity of from about 110 nM to about 150 nM.

[0105] In some examples, the therapeutically effective concentration of the agent is a concentration that treats one or more symptoms of an LSD in the mammal. In some examples, the agent is a protein replacement therapeutic. In some examples, the agent or protein replacement therapeutic is an enzyme.

[0106] In some examples, the enzyme decreases the accumulation of a toxic metabolic product in the brain of the mammal having the LSD to a greater extent when linked to the protein as compared to when the enzyme is linked to the reference protein. In some examples, the enzyme is IDS and the LSD is Hunter syndrome. In some examples, the toxic metabolic product comprises heparin sulfate-derived disaccharides and/or dermatan sulfate-derived disaccharides. In some examples, the enzyme is SGSH and the LSD is Sanfilippo syndrome A. In some examples, the enzyme is ASM and the LSD is Niemann-Pick disease. In some examples, the enzyme is GBA and the LSD is Gaucher's disease.

[0107] In some examples, the agent comprises an antibody variable region. In some examples, the agent comprises an antibody fragment. In some examples, the agent comprises a Fab or scFv.

[0108] In some examples, the protein is a modified Fc polypeptide that contains a non-native binding site capable of binding TfR. In some examples, the protein comprises an antibody variable region that specifically binds TfR. In some examples, the protein comprises an antibody fragment. In some examples, the protein comprises a Fab or scFv.

[0109] In some examples, the protein linked to the agent is administered as part of a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

[0110] FIG. 1 shows the purification and analysis of an IDS-Fc fusion protein comprising an Fc polypeptide linked to an iduronate-2-sulfatase (IDS) enzyme and a modified Fc polypeptide that binds to transferrin receptor (TfR).

[0111] FIG. 2 shows the results of a binding affinity assay for IDS-Fc fusion proteins analyzed in FIG. 1 demonstrating that the fusion proteins bind to TfR.

[0112] FIG. 3 provides data demonstrating *in vitro* IDS activity of IDS-Fc fusion proteins analyzed in FIG. 1.

[0113] FIG. 4 provides data demonstrating that knockout cells that lack IDS have increased levels of heparan sulfate-derived disaccharides as assessed using an LC-MS/MS assay, and expression of IDS in the *IDS* knockout (KO) cells rescues the knockout phenotype.

[0114] FIG. 5A shows that IDS-Fc fusion proteins as either an N-terminal monozyme or a C-terminal monozyme comprising the same TfR-binding Fc polypeptide (*i.e.*, CH3C.35.21.17) reverse heparan sulfate and dermatan sulfate accumulation in *IDS* KO cells. FIG. 5B shows that the N-terminal monozyme ("ETV:IDS 35.21.17") has comparable cellular efficacy to IDS. FIG. 5C shows dose-dependent reduction of accumulated S^{35}-sulfate labeled proteins in MPS II patient fibroblasts treated with IDS-Fc fusion protein ("ETV:IDS") or IDS; n=8. FIG. 5D shows assessment of M6PR-dependent clearance of S^{35}-labeled proteins in MPS II patient fibroblasts treated with increasing doses of IDS-Fc fusion protein ("ETV:IDS") or IDS in the presence or absence of 5mM M6P; n=3. FIGS. 5C-5D: "ETV:IDS" = ETV:IDS 35.23.2; Graphs display mean values across experimental replicates ± SEM.