

MODIFIED GLUCOCEREBROSIDASE POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 63/608,088, filed December 8, 2023, and U.S. Provisional Application Serial No. 63/663,617, filed June 24, 2024. The entire content of the applications referenced above are hereby incorporated by reference herein.

BACKGROUND

Glucocerebrosidase (GCase) is a lysosomal enzyme that has glucosylceramidase activity and catalyzes the breakdown of glucosylceramide to ceramide and glucose. Mutations in glucocerebrosidase are known to cause or be associated with a number of diseases including Gaucher's disease (Brady et al., *J. Clin. Invest.* 45(7):1112- 1115, 1966), Parkinson's disease (Balestrino et al., *Neuroscientist* 24(5):540-559, 2018), Parkinsonism (Lwin et al., *Mol. Genet. Metab.* 81(1):70-73, 2004), and dementia with Lewy Bodies (Nalls et al., *JAMA Neurol.* 70(6):727-735, 2013; Guerreiro et al., *Lancet Neurol* 17(1):64-74, 2018). While enzyme replacement therapies are available to patients with GCase deficiencies (e.g., imiglucerase, also known as Cerezyme™), these recombinant forms suffer from low serum stability and enzyme inactivation at physiological conditions. Accordingly, there is a need for more stable forms of recombinant GCase that maintain activity under physiological conditions.

SUMMARY

Thus, certain embodiments provide a GCase polypeptide comprising two or more modifications with respect to SEQ ID NO:1, wherein the two or more modifications provide increased stability (e.g., in the presence of serum), and wherein the increased stability is indicated by (i) greater enzymatic activity (e.g., at least 2-fold greater; or at least 5-fold greater), and/or (ii) greater conformational stability (e.g., at least 2-fold greater; or at least 5-fold greater), relative to that of SEQ ID NO:1.

Certain embodiments provide a GCase polypeptide comprising two or more modifications, wherein at least two modifications with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425, and 451; or wherein at least two modifications with respect to SEQ ID NO:1, are at

positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 316, 386, 387, 388, 425 and 451.

For example, certain embodiments provide a GCase polypeptide comprising two or more modifications, wherein at least two of the modifications, with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 316, 425, and 451. In some embodiments, the GCase polypeptide further comprises a modification, with respect to SEQ ID NO:1, at position 233.

Certain embodiments also provide a GCase polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 104, wherein:

- 10 a) X_{1d} is selected from the group consisting of: G, E, R, and Q;
- b) X_{2d} is selected from the group consisting of: A, N, T, S, H, D, R, and G;
- c) X_{3d} is selected from the group consisting of: V, Y, F, L, M, S, G, and W;
- d) X_{4d} is selected from the group consisting of: N, V, I, T, A, M, E, G, D, and S;
- e) X_{5d} is G;
- 15 f) X_{6d} is selected from the group consisting of: P, S, K, Q, and T;
- g) X_{7d} is S;
- h) X_{8d} is selected from the group consisting of: V, T, A, and N;
- i) X_{9d} is selected from the group consisting of: S, F, T, and I;
- j) X_{10d} is selected from the group consisting of: K, L, Q, F, N, E, and V;
- 20 k) X_{11d} is selected from the group consisting of: F, P, S, and T;
- l) X_{12d} is R; and
- m) X_{13d} is R.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 171, wherein:

- 25 a) X_{1e} is selected from the group consisting of: G, E, R, and Q;
- b) X_{2e} is selected from the group consisting of: A, N, T, S, H, D, R, and G;
- c) X_{3e} is selected from the group consisting of: V, Y, F, L, M, S, G, and W;
- d) X_{4e} is selected from the group consisting of: N, V, I, T, A, M, E, G, D, and S;
- e) X_{5e} is G or E;
- 30 f) X_{6e} is selected from the group consisting of: P, S, K, Q, and T;
- g) X_{7e} is S or F;
- h) X_{8e} is selected from the group consisting of: V, T, A, and N;
- i) X_{9e} is selected from the group consisting of: S, F, T, and I;
- j) X_{10e} is selected from the group consisting of: K, L, Q, F, N, E, and V;

- k) X_{11e} is selected from the group consisting of: F, P, S, and T;
- l) X_{12e} is K or R; and
- m) X_{13e} is H or R; and

wherein in the GCase polypeptide comprises two or more modifications at positions
 5 described herein (e.g., at positions X_{1e} - X_{13e}), with respect to SEQ ID NO:1.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:80, wherein:

- a) X_{1c} is selected from the group consisting of: S and A;
- b) X_{2c} is selected from the group consisting of: C, S and T;
- 10 c) X_{3c} is selected from the group consisting of: A and S;
- d) X_{4c} is selected from the group consisting of: L and V;
- e) X_{5c} is selected from the group consisting of: S, T, and A;
- f) X_{6c} is selected from the group consisting of: G, Q and E;
- g) X_{7c} is selected from the group consisting of: S, L, I, and T;
- 15 h) X_{8c} is selected from the group consisting of: A and N;
- i) X_{9c} is selected from the group consisting of: L, M, Q and Y;
- j) X_{10c} is S;
- k) X_{11c} is selected from the group consisting of: D and N;
- l) X_{12c} is selected from the group consisting of: P, M, T, and L;
- 20 m) X_{13c} is selected from the group consisting of: E and K;
- n) X_{14c} is R; and
- o) X_{15c} is R.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:5, wherein:

- 25 a) X_{1b} is selected from the group consisting of: G and E;
- b) X_{2b} is selected from the group consisting of: L and S;
- c) X_{3b} is S;
- d) X_{4b} is selected from the group consisting of Y and H;
- e) X_{5b} is selected from the group consisting of V and A;
- 30 f) X_{6b} is selected from the group consisting of K and R; and
- g) X_{7b} is selected from the group consisting of H and R.

Certain embodiments provide a pharmaceutical composition comprising a GCase polypeptide as described herein and a pharmaceutically acceptable excipient.

Certain embodiments provide a polynucleotide comprising a nucleic acid sequence encoding a GCase polypeptide as described herein.

Certain embodiments provide a vector comprising a polynucleotide as described herein.

Certain embodiments provide a host cell comprising a polynucleotide as described herein
5 or a vector as described herein.

Certain embodiments provide a method for producing a GCase polypeptide, comprising culturing a host cell under conditions in which the GCase polypeptide encoded by a polynucleotide as described herein is expressed.

Certain embodiments provide a method of treating a GCase deficiency in a patient in
10 need thereof, the method comprising administering a GCase polypeptide as described herein to the patient.

Certain embodiments provide a GCase polypeptide as described herein for use in treating a GCase deficiency in a patient in need thereof.

Certain embodiments provide the use of a GCase polypeptide as described herein in the
15 preparation of a medicament for treating a GCase deficiency in a patient in need thereof.

Certain embodiments provide a method of decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency, the method comprising administering a GCase polypeptide as described herein to the patient.

Certain embodiments provide a GCase polypeptide as described herein for use in
20 decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency.

Certain embodiments provide the use of a GCase polypeptide as described herein in the preparation of a medicament for decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency.

Certain embodiments provide a method of making a modified GCase polypeptide,
25 wherein the method comprises:

- (i) modifying a nucleic acid sequence encoding SEQ ID NO:1 to change a residue at two or more positions in regions of SEQ ID NO:1 selected from the group consisting of positions: 119-127, 130-134, 177-184, 187-197, 230-240, 243-249, 284-296, 310-312,
30 315-336, 343-347, 350-362, 386-400, and 414-417;
- (ii) transforming a host cell with the modified nucleic acid sequence;
- (iii) culturing the host cell to express the polypeptide encoded by the modified nucleic acid sequence; and
- (iv) isolating the polypeptide from the host cell culture;

thereby making the modified GCCase polypeptide.

Certain embodiments provide a modified GCCase polypeptide produced by a method as described herein.

5 Certain embodiments provide a method of generating a library of nucleic acid sequences encoding modified GCCase polypeptides, comprising modifying a plurality of nucleic acids encoding SEQ ID NO:1 to change a residue at two or more positions in regions of SEQ ID NO:1 that undergo structural alteration upon binding of SEQ ID NO:1 to isofagomine.

10 Certain embodiments provide a method of screening a library of nucleic acid sequences to identify modified GCCase polypeptides having improved conformational stability and maintained or greater activity relative to that of SEQ ID NO:1, wherein the method comprises:

- a) generating a library of nucleic acid sequences encoding modified GCCase polypeptides according to a method as described herein;
- b) transforming a plurality of host cells with the library of nucleic acids;
- c) culturing the transformed cells to express the polypeptides encoded by the library of
15 nucleic acid sequences;
- d) subjecting the transformed cells to serum stress by incubating the cells in serum from a mammalian host for at least 30 minutes at temperatures ranging from room temperature to 37°C;
- e) labeling the cells with (i) a fluorescent cyclophellitol-type activity-based probe which
20 covalently reacts with a residue in the active site of GCCase, and (ii) an anti-GCCase antibody; and
- f) sorting the cells to isolate a population enriched for the fluorescent activity-based probe and the anti-GCCase antibody.

25 Certain embodiments provide a modified GCCase polypeptide identified by a method as described herein.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic of the enrichment of a yeast-displayed DNA shuffled GCCase library for variants that maintain GCCase expression post-serum stress. A double-labeling strategy
30 (anti-V5 antibody + biotinylated anti-GCCase antibody) was used for enrichment using FACS.

FIG. 2 shows FACS histograms for selected variant GCCase clones with improved expression (anti-V5 antibody + biotinylated anti-GCCase antibody) relative to wild-type (WT) GCCase. Data presented is with respect to anti-GCCase antibody pre- and post-serum stress. Wild-type GCCase data not shown due to non-detection of expression above background levels.

FIG. 3 shows FACS results for wild-type (WT) GCase and two GCase variants (SH2-20, SH2-88). The cell populations expressing WT GCase, SH2-20, and SH2-88 were labeled with a probe cap (MDW933) and anti-GCase antibody for detection of enzymatic activity and GCase expression in the cells.

5 **FIG. 4** illustrates the conditions of cell sorting and selection for enrichment of GCase variants exhibiting improved enzymatic activity and GCase expression from GCase Variant Library 1. The successive rounds of FACS narrowed the library size of about 3×10^7 variants to an enriched pool of about 10^2 candidates.

10 **FIG. 5** illustrates the conditions of cell sorting and selection for enrichment of GCase variants exhibiting improved enzymatic activity and GCase expression from GCase Variant Library 2. The successive rounds of FACS narrowed the library size of about 2×10^8 variants to an enriched pool of about 10^2 candidates.

DETAILED DESCRIPTION

15 As described herein, certain embodiments provide GCase polypeptides having increased stability relative to that of a reference GCase polypeptide (e.g., relative to a wild-type GCase, such as SEQ ID NO:1). For example, as described in the Examples, a variety of approaches were used to generate a series of modified GCase polypeptides having increased conformational stability in the presence of serum (e.g., certain clones from libraries SH2, SH3, Library 1 and
20 Library 2), wherein a subset of these clones showed increased conformational stability and enhanced enzymatic activity as compared to a wild-type GCase polypeptide in the presence of serum (see, e.g., certain clones from Libraries 1 and 2; and Tables 7b and 9b).

MODIFIED GCASE POLYPEPTIDES

25 Accordingly, certain embodiments provide a GCase polypeptide comprising two or more modifications at positions described herein, with respect to SEQ ID NO:1. Examples of various modifications are described below and in the Examples, wherein certain combinations may result in a GCase polypeptide having improved qualities.

30 In certain embodiments, the GCase polypeptide comprises two modifications, with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises more than two modifications, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more modifications, with respect to SEQ ID NO:1.

For example, certain embodiments provide a GCase polypeptide comprising two or more modifications,

wherein at least two modifications with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425, and 451; or

wherein at least two modifications with respect to SEQ ID NO:1, are at positions
5 selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 316, 386, 387, 388, 425 and 451.

In particular, as described below, a certain exemplary embodiment provides a GCase polypeptide comprising two or more modifications, wherein at least two modifications, with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 316, 425, and
10 451. In certain embodiments, at least two of the modifications are selected from the group consisting of: F316S, K425R, and H451R. In certain embodiments, the GCase polypeptide comprises modifications at positions 316, 425, and 451, with respect to SEQ ID NO:1. In certain embodiments, the modifications comprise F316S, K425R, and H451R.

In certain embodiments, the modified GCase polypeptide has increased stability
15 wherein the increased stability is indicated by (i) greater enzymatic activity, and/or (ii) greater conformational stability, relative to that of SEQ ID NO:1. For example, certain embodiments provide a GCase polypeptide comprising two or more modifications with respect to SEQ ID NO:1, wherein the two or more modifications provide increased stability, wherein the increased stability is indicated by (i) greater enzymatic activity (e.g., at least 2-fold greater), and (ii)
20 greater conformational stability (e.g., at least 2-fold greater), relative to that of SEQ ID NO:1. In certain embodiments, the increased stability is in the presence of serum. In certain embodiments, at least two of the modifications are at positions selected from the group consisting of: 316, 425, and 451, wherein the positions are with respect to SEQ ID NO:1. In certain embodiments, at least two of the modifications are selected from the group consisting of:
25 F316S, K425R, and H451R.

Other exemplary embodiments of modified GCase polypeptides are also described below.

**Certain Embodiments Associated with Two or More of Positions 189, 190, 191, 192, 233,
30 245, 316, 343, 345, 346, 347, 425 and 451**

In certain embodiments, a GCase polypeptide as described herein comprises two or more modifications, wherein at least two modifications with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425, and 451. In certain embodiments, the GCase polypeptide comprises a modification at

three, four, five, six, seven, eight, nine, ten, eleven, twelve or thirteen positions selected from the group consisting of: 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425, and 451. In certain embodiments, the GCase polypeptide comprises a modification at three, four, five, six, seven, eight, nine, ten, eleven, or twelve positions selected from the group consisting of: 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425, and 451.

In certain embodiments, the GCase polypeptide comprises two or more modifications, wherein at least two modifications, with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 316, 425, and 451 (e.g., selected from F316S, K425R, and H451R). In certain embodiments, the GCase polypeptide comprises a modification at positions 316 and 425.

10 In certain embodiments, the GCase polypeptide comprises a modification at positions 316 and 451. In certain embodiments, the GCase polypeptide comprises a modification at positions 425 and 451. In certain embodiments, the GCase polypeptide comprises a modification at positions 316, 425, and 451 (e.g., F316S, K425R, and H451R). In certain embodiments, the GCase polypeptide further comprises a modification at position 233 with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide further comprises a modification, with respect to SEQ ID NO:1, at one or more positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. For example, in certain embodiments, the GCase polypeptide comprises modifications at positions 233, 316, 425, and 451; and comprises a modification at one or more position(s) selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises two modifications at positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. In certain embodiments, the GCase polypeptide comprises three modifications at positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. In certain 25 embodiments, the GCase polypeptide comprises four modifications at positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. In certain embodiments, the GCase polypeptide comprises five modifications at positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. In certain 30 embodiments, the GCase polypeptide comprises six modifications at positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. In certain embodiments, the GCase polypeptide comprises seven modifications at positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. In certain embodiments, the GCase polypeptide comprises eight modifications at positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347. In certain embodiments, the GCase

polypeptide comprises modifications at positions 189, 190, 191, 192, 245, 343, 345, 346, and 347.

In certain other embodiments, a GCase polypeptide as described herein comprises two or more modifications, wherein at least two modifications, with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 233, 343, and 347. In certain embodiments, the GCase polypeptide comprises a modification at positions 233 and 343. In certain embodiments, the GCase polypeptide comprises a modification at positions 233 and 347. In certain embodiments, the GCase polypeptide comprises a modification at positions 343 and 347. In certain embodiments, the GCase polypeptide comprises a modification at positions 233, 343, and 347. In certain embodiments, the GCase polypeptide further comprises a modification at position 191 and/or 192. In certain embodiments, the GCase polypeptide further comprises a modification at position 191. In certain embodiments, the GCase polypeptide further comprises a modification at position 192. In certain embodiments, the GCase polypeptide comprises a modification at the following positions: 191, 192, 233, 343, and 347. In certain embodiments, the GCase polypeptide may further comprise a modification at one, two, three, four, five, six, seven, or eight positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at one, two, three, four, five, six, or seven positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at one position selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at two positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at three positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at four positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at five positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at six positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at seven positions selected from the group consisting of: 189, 190, 245, 316, 345, 346, 425, and 451. In certain embodiments, the GCase polypeptide further comprises a modification at each of the following positions: 189, 190, 245, 316, 345, 346, 425, and 451.

In certain embodiments, the GCase polypeptide comprises a modification at position 189. In certain embodiments, the GCase polypeptide comprises a modification at position 189 selected from the group consisting of: G189E, G189R, and G189Q.

5 In certain embodiments, the GCase polypeptide comprises a modification at position 190. In certain embodiments, the GCase polypeptide comprises a modification at position 190 selected from the group consisting of: A190N, A190T, A190S, A190H, A190D, A190R, and A190G.

10 In certain embodiments, the GCase polypeptide comprises a modification at position 191. In certain embodiments, the GCase polypeptide comprises a modification at position 191 selected from the group consisting of: V191Y, V191F, V191L, V191M, V191S, V191G, and V191W.

In certain embodiments, the GCase polypeptide comprises a modification at position 192. In certain embodiments, the GCase polypeptide comprises a modification at position 192 selected from the group consisting of: N192V, N192I, N192T, N192A, N192M, 15 N192E, N192G, N192D, and N192S.

In certain embodiments, the GCase polypeptide comprises a modification at position 233. In certain embodiments, the modification at position 233 is E233G.

20 In certain embodiments, the GCase polypeptide comprises a modification at position 245. In certain embodiments, the GCase polypeptide comprises a modification at position 245 selected from the group consisting of: P245S, P245K, P245Q, and P245T.

In certain embodiments, the GCase polypeptide comprises a modification at position 316. In certain embodiments, the modification at position 316 is F316S.

25 In certain embodiments, the GCase polypeptide comprises a modification at position 343. In certain embodiments, the GCase polypeptide comprises a modification at position 343 selected from the group consisting of: V343T, V343A, and V343N.

In certain embodiments, the GCase polypeptide comprises a modification at position 345. In certain embodiments, the GCase polypeptide comprises a modification at position 345 selected from the group consisting of: S345F, S345T, and S345I.

30 In certain embodiments, the GCase polypeptide comprises a modification at position 346. In certain embodiments, the GCase polypeptide comprises a modification at position 346 selected from the group consisting of: K346L, K346Q, K346F, K346N, K346E, and K346V.

In certain embodiments, the GCase polypeptide comprises a modification at position 347. In certain embodiments, the GCase polypeptide comprises a modification at

position 347 selected from the group consisting of: F347P, F347S, and F347T.

In certain embodiments, the GCase polypeptide comprises a modification at position 425. In certain embodiments, the modification at position 425 is K425R.

In certain embodiments, the GCase polypeptide comprises a modification at position 451. In certain embodiments, the modification at position 451 is H451R.

In certain embodiments, the GCase polypeptide comprises a combination of modifications as indicated in a clone from Library 2 or as indicated in Table 9a.

For example, in certain embodiments, the GCase polypeptide comprises modifications at positions 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1.

In certain embodiments, the GCase polypeptide comprises G189E, A190N, V191Y, N192V, E233G, P245S, F316S, V343T, S345T, K346L, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 105, wherein the sequence comprises with respect to SEQ ID NO:105: Val at position 192; Gly at position 233; Ser at position 245; Ser at position 316; Thr at position 343; Thr at position 345; Leu at position 346; Pro at position 347; Arg at position 425; and Arg at position 451. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 105, wherein the sequence comprises with respect to SEQ ID NO:105: Glu at position 189; Asn at position 190; Tyr at position 191; Val at position 192; Gly at position 233; Ser at position 245; Ser at position 316; Thr at position 343; Thr at position 345; Leu at position 346; Pro at position 347; Arg at position 425; and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189E, A190S, V191L, N192T, E233G, P245K, F316S, V343T, S345I, K346F, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 107, wherein the sequence comprises with respect to SEQ ID NO:107: Glu at position 189, Ser at position 190, Leu at position 191, Thr at position 192, Gly at position 233, Lys at position 245, Ser at position 316, Thr at position 343, Ile at position 345, Phe at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189Q, A190T, V191Y, N192T, E233G, P245Q, F316S, V343T, S345F, K346E, F347T, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity

(*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 118, wherein the sequence comprises with respect to SEQ ID NO:118: Gln at position 189, Thr at position 190, Tyr at position 191, Thr at position 192, Gly at position 233, Gln at position 245, Ser at position 316, Thr at position 343, Phe at position 345, Glu at position 346, Thr at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189Q, A190T, V191Y, N192M, E233G, P245T, F316S, V343A, S345F, K346E, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 123, wherein the sequence comprises with respect to SEQ ID NO:123: Gln at position 189, Thr at position 190, Tyr at position 191, Met at position 192, Gly at position 233, Thr at position 245, Ser at position 316, Ala at position 343, Phe at position 345, Glu at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189R, A190G, V191F, N192E, E233G, P245Q, F316S, V343T, S345F, K346Q, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 124, wherein the sequence comprises with respect to SEQ ID NO:124: Arg at position 189, Gly at position 190, Phe at position 191, Glu at position 192, Gly at position 233, Gln at position 245, Ser at position 316, Thr at position 343, Phe at position 345, Gln at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain other embodiments, the GCase polypeptide comprises modifications at positions 189, 190, 191, 192, 233, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1.

In certain embodiments, the GCase polypeptide comprises G189Q, A190T, V191F, N192I, E233G, F316S, V343T, S345F, K346Q, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 106, wherein the sequence comprises with respect to SEQ ID NO:106: Gln at position 189, Thr at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Gln at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189R, A190D, V191F, N192V, E233G, F316S, V343T, S345F, K346N, F347S, K425R, and H451R. In some

embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 109, wherein the sequence comprises with respect to SEQ ID NO:109: Arg at position 189, Asp at position 190, Phe at position 191, Val at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Asn at position 346, Ser at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189E, A190N, V191M, N192T, E233G, F316S, V343A, S345F, K346E, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 111, wherein the sequence comprises with respect to SEQ ID NO:111: Glu at position 189, Asn at position 190, Met at position 191, Thr at position 192, Gly at position 233, Ser at position 316, Ala at position 343, Phe at position 345, Glu at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189E, A190R, V191L, N192E, E233G, F316S, V343T, S345F, K346E, F347T, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 119, wherein the sequence comprises with respect to SEQ ID NO:119: Glu at position 189, Arg at position 190, Leu at position 191, Glu at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Glu at position 346, Thr at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises G189E, A190D, V191W, N192D, E233G, F316S, V343T, S345F, K346E, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 126, wherein the sequence comprises with respect to SEQ ID NO:126: Glu at position 189, Asp at position 190, Trp at position 191, Asp at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Glu at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1.

In certain embodiments, the GCase polypeptide comprises A190S, V191Y, N192V,

E233G, P245K, F316S, V343A, S345F, K346Q, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 108, wherein the sequence comprises with respect to SEQ ID NO:108: Ser at position 190, Tyr at position 191, Val at position 192, Gly at position 233, Lys at position 245, Ser at position 316, Ala at position 343, Phe at position 345, Gln at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises A190N, V191F, N192I, E233G, P245K, F316S, V343T, S345F, K346Q, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 125, wherein the sequence comprises with respect to SEQ ID NO:125: Asn at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Lys at position 245, Ser at position 316, Thr at position 343, Phe at position 345, Gln at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1.

In certain embodiments, the GCase polypeptide comprises A190H, V191Y, N192A, E233G, F316S, V343T, S345F, K346E, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 110, wherein the sequence comprises with respect to SEQ ID NO:110: His at position 190, Tyr at position 191, Ala at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Glu at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises A190T, V191L, N192M, E233G, F316S, V343N, S345F, K346L, F347S, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 115, wherein the sequence comprises with respect to SEQ ID NO:115: Thr at position 190, Leu at position 191, Met at position 192, Gly at position 233, Ser at position 316, Asn at position 343, Phe at position 345, Leu at position 346, Ser at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises A190D, V191F, N192T, E233G, F316S, V343N, S345F, K346Q, F347T, K425R, and H451R. In some embodiments,

the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 116, wherein the sequence comprises with respect to SEQ ID NO:116: Asp at position 190, Phe at position 191, Thr at position 192, Gly at position 233, Ser at position 316, Asn at position 343, Phe at position 345, Gln at position 346, Thr at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises A190N, V191F, N192I, E233G, F316S, V343T, S345F, K346Q, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 117, wherein the sequence comprises with respect to SEQ ID NO:117: Asn at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Gln at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises A190N, V191S, N192S, E233G, F316S, V343T, S345F, K346E, F347S, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 120, wherein the sequence comprises with respect to SEQ ID NO:120: Asn at position 190, Ser at position 191, Ser at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Glu at position 346, Ser at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises A190G, V191F, N192T, E233G, F316S, V343A, S345F, K346Q, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 129, wherein the sequence comprises with respect to SEQ ID NO:129: Gly at position 190, Phe at position 191, Thr at position 192, Gly at position 233, Ser at position 316, Ala at position 343, Phe at position 345, Gln at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises A190H, V191Y, N192E, E233G, F316S, V343N, S345F, K346Q, F347S, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 131, wherein the sequence comprises with respect to SEQ ID NO:131: His at position 190, Tyr at position 191, Glu at position 192, Gly at position 233, Ser at position 316, Asn at position 343, Phe at position 345, Gln at position 346, Ser at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions

189, 191, 192, 233, 245, 316, 343, 345, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCCase polypeptide comprises G189Q, V191Y, N192M, E233G, P245Q, F316S, V343T, S345F, F347P, K425R, and H451R. In some embodiments, the GCCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 112, wherein the sequence comprises with respect to SEQ ID NO:112: Gln at position 189, Tyr at position 191, Met at position 192, Gly at position 233, Gln at position 245, Ser at position 316, Thr at position 343, Phe at position 345, Pro at position 347, Arg at position 425, and Arg at position 451.

10 In certain embodiments, the GCCase polypeptide comprises modifications at positions 189, 191, 192, 233, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCCase polypeptide comprises G189Q, V191F, N192E, E233G, F316S, V343N, S345F, K346E, F347S, K425R, and H451R. In some embodiments, the GCCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 113, wherein the sequence comprises with respect to SEQ ID NO:113: Gln at position 189, Phe at position 191, Glu at position 192, Gly at position 233, Ser at position 316, Asn at position 343, Phe at position 345, Glu at position 346, Ser at position 347, Arg at position 425, and Arg at position 451.

20 In certain embodiments, the GCCase polypeptide comprises modifications at positions 190, 191, 192, 233, 245, 316, 343, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCCase polypeptide comprises A190H, V191F, N192G, E233G, P245K, F316S, V343T, K346L, F347P, K425R, and H451R. In some embodiments, the GCCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 114, wherein the sequence comprises with respect to SEQ ID NO:114: His at position 190, Phe at position 191, Gly at position 192, Gly at position 233, Lys at position 245, Ser at position 316, Thr at position 343, Lys at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

30 In certain embodiments, the GCCase polypeptide comprises modifications at positions 189, 190, 191, 192, 233, 245, 316, 343, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCCase polypeptide comprises G189E, A190R, V191G, N192S, E233G, P245Q, F316S, V343T, K346Q, F347P, K425R, and H451R. In some embodiments, the GCCase polypeptide comprises a sequence

having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 121, wherein the sequence comprises with respect to SEQ ID NO:121: Glu at position 189, Arg at position 190, Gly at position 191, Ser at position 192, Gly at position 233, Gln at position 245, Ser at position 316, Thr at position 343, Gln at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 189, 190, 191, 192, 233, 316, 343, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises G189E, A190R, V191G, N192S, E233G, F316S, V343T, K346V, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 122, wherein the sequence comprises with respect to SEQ ID NO:122: Glu at position 189, Arg at position 190, Gly at position 191, Ser at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Val at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 345, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190D, V191F, N192I, E233G, F316S, V343A, S345F, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 127, wherein the sequence comprises with respect to SEQ ID NO:127: Asp at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Ala at position 343, Phe at position 345, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190N, V191F, N192I, E233G, F316S, V343T, K346L, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 128, wherein the sequence comprises with respect to SEQ ID NO:128: Asn at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Leu at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions

189, 190, 192, 233, 316, 343, 345, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises G189E, A190D, N192T, E233G, F316S, V343T, S345F, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 130, wherein the sequence comprises with respect to SEQ ID NO:130: Glu at position 189, Asp at position 190, Thr at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Phe at position 345, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 343, 345, 346, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190H, V191Y, N192E, E233G, V343N, S345F, K346Q, and F347S. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 138, wherein the sequence comprises with respect to SEQ ID NO:138: His at position 190, Tyr at position 191, Glu at position 192, Gly at position 233, Asn at position 343, Phe at position 345, Gln at position 346, and Ser at position 347. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 144, wherein the sequence comprises with respect to SEQ ID NO:144: His at position 190, Tyr at position 191, Glu at position 192, Gly at position 233, Asn at position 343, Phe at position 345, Gln at position 346, and Ser at position 347.

In certain embodiments, the GCase polypeptide comprises modifications at positions 191, 192, 233, 343, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises V191F, N192I, E233G, V343T, and F347P. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 139, wherein the sequence comprises with respect to SEQ ID NO:139: Phe at position 191, Ile at position 192, Gly at position 233, Thr at position 343, and Pro at position 347.

In certain embodiments, the GCase polypeptide comprises modifications at positions 233, 343, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises E233G, V343T, and F347P. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 139,

wherein the sequence comprises with respect to SEQ ID NO:139: Gly at position 233, Thr at position 343, and Pro at position 347. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 159, wherein the sequence comprises with respect to SEQ ID NO:159: Gly at position 233, Thr at position 343, and Pro at position 347.

In certain embodiments, the GCase polypeptide comprises modifications at positions 191, 192, 233, 343, 346, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises V191F, N192I, E233G, V343T, K346L, and F347P. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 139, wherein the sequence comprises with respect to SEQ ID NO:139: Phe at position 191, Ile at position 192, Gly at position 233, Thr at position 343, Leu at position 346; and Pro at position 347. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 140, wherein the sequence comprises with respect to SEQ ID NO:140: Phe at position 191, Ile at position 192, Gly at position 233, Thr at position 343, Leu at position 346; and Pro at position 347.

In certain embodiments, the GCase polypeptide comprises modifications at positions 191, 192, 233, 316, 343, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises V191F, N192I, E233G, F316S, V343T, K346L, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 154, wherein the sequence comprises with respect to SEQ ID NO:154: Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Leu at position 346, Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 343, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190N, V191F, N192I, E233G, V343T, K346L, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 155, wherein the sequence comprises with respect to SEQ ID NO:155: Asn at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Thr at position 343, Leu at position 346,

Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises

5 A190N, V191F, N192I, E233G, F316S, V343T, F347P, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 156, wherein the sequence comprises with respect to SEQ ID NO:156: Asn at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Thr at position 343,
10 Pro at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 346, 347, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190N, V191F, N192I, E233G, F316S, V343T, K346L, F347P, and H451R. In some
15 embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 157, wherein the sequence comprises with respect to SEQ ID NO:157: Asn at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Leu at position 346, Pro at position 347, and Arg at position 451.

20 In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 346, 347, and 425, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190N, V191F, N192I, E233G, F316S, V343T, K346L, F347P, and K425R. In some
25 embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 158, wherein the sequence comprises with respect to SEQ ID NO:158: Asn at position 190, Phe at position 191, Ile at position 192, Gly at position 233, Ser at position 316, Thr at position 343, Leu at position 346, Pro at position 347, and Arg at position 425.

In certain embodiments, the GCase polypeptide comprises modifications at positions
30 190, 191, 192, 233, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190H, V191Y, N192E, E233G, V343N, S345F, K346Q, F347S, K425R, and H451R. In some
embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 144,

wherein the sequence comprises with respect to SEQ ID NO:144: His at position 190, Tyr at position 191, Glu at position 192, Gly at position 233, Asn at position 343, Phe at position 345, Gln at position 346, Ser at position 347, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 5 190, 191, 192, 233, 316, 343, 345, 346, 347, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190H, V191Y, N192E, E233G, F316S, V343N, S345F, K346Q, F347S, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 148, 10 wherein the sequence comprises with respect to SEQ ID NO:148: His at position 190, Tyr at position 191, Glu at position 192, Gly at position 233, Ser at position 316, Asn at position 343, Phe at position 345, Gln at position 346, Ser at position 347, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 345, 346, 347, and 425, wherein the modifications and positions 15 are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises A190H, V191Y, N192E, E233G, F316S, V343N, S345F, K346Q, F347S, and H425R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 149, wherein the sequence comprises with respect to SEQ ID NO:149: His at position 190, Tyr at 20 position 191, Glu at position 192, Gly at position 233, Ser at position 316, Asn at position 343, Phe at position 345, Gln at position 346, Ser at position 347, and Arg at position 425.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 192, 233, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises 25 the residues specified at positions 190, 192, 233, 316, 343, 345, 346, 347, 425, and 451 in SEQ ID NO:141. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 141, wherein the sequence comprises the residues specified at positions 190, 192, 233, 316, 343, 345, 346, 347, 425, and 451 in SEQ ID NO:141.

30 In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 233, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 233, 316, 343, 345, 346, 347, 425, and 451 in SEQ ID NO:142. In some embodiments, the GCase polypeptide comprises a sequence having at least

about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 142, wherein the sequence comprises the residues specified at positions 190, 191, 233, 316, 343, 345, 346, 347, 425, and 451 in SEQ ID NO:142.

5 In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 316, 343, 345, 346, 347, 425, and 451 in SEQ ID NO:143. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to
10 SEQ ID NO: 143, wherein the sequence comprises the residues specified at positions 190, 191, 192, 316, 343, 345, 346, 347, 425, and 451 in SEQ ID NO:143.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises
15 the residues specified at positions 190, 191, 192, 233, 316, 345, 346, 347, 425, and 451 in SEQ ID NO:145. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 145, wherein the sequence comprises the residues specified at positions 190, 191, 192, 233, 316, 345, 346, 347, 425, and 451 in SEQ ID NO:145.

20 In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 345, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 233, 316, 343, 345, 347, 425, and 451 in SEQ ID NO:146. In some embodiments, the GCase polypeptide comprises a sequence having at least
25 about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 146, wherein the sequence comprises the residues specified at positions 190, 191, 192, 233, 316, 343, 345, 347, 425, and 451 in SEQ ID NO:146.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 345, 346, 425, and 451, wherein the modifications and positions
30 are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 233, 316, 343, 345, 346, 425, and 451 in SEQ ID NO:147. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 147, wherein the sequence comprises the residues specified at positions 190, 191,

192, 233, 316, 343, 345, 346, 425, and 451 in SEQ ID NO:147.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 343, 345, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 233, 343, 345, and 347 in SEQ ID NO:150. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 150, wherein the sequence comprises the residues specified at positions 190, 191, 192, 233, 343, 345, and 347 in SEQ ID NO:150.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 343, 345, 347, and 425, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 233, 343, 345, 347, and 425 in SEQ ID NO:151. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 151, wherein the sequence comprises the residues specified at positions 190, 191, 192, 233, 343, 345, 347, and 425 in SEQ ID NO:151.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 316, 343, 345, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 233, 316, 343, 345, and 347 in SEQ ID NO:152. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 152, wherein the sequence comprises the residues specified at positions 190, 191, 192, 233, 316, 343, 345, and 347 in SEQ ID NO:152.

In certain embodiments, the GCase polypeptide comprises modifications at positions 190, 191, 192, 233, 343, 345, 347, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 233, 343, 345, 347, and 451 in SEQ ID NO:153. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 153, wherein the sequence comprises the residues specified at positions 190, 191, 192, 233, 343, 345, 347, and 451 in SEQ ID NO:153.

In certain embodiments, the GCase polypeptide comprises modifications at positions

191, 192, 233, 316, 343, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 191, 192, 233, 316, 343, and 347 in SEQ ID NO:160. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 5 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 160, wherein the sequence comprises the residues specified at positions 191, 192, 233, 316, 343, and 347 in SEQ ID NO:160.

In certain embodiments, the GCase polypeptide comprises modifications at positions 191, 192, 233, 343, 347, and 425, wherein the modifications and positions are with respect to 10 SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 191, 192, 233, 343, 347, and 425 in SEQ ID NO:161. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 161, wherein the sequence comprises the residues specified at positions 191, 192, 233, 343, 347, and 425 in SEQ ID 15 NO:161.

In certain embodiments, the GCase polypeptide comprises modifications at positions 191, 192, 233, 343, 347, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 191, 192, 233, 343, 347, and 451 in SEQ ID NO:162. In some embodiments, the 20 GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 162, wherein the sequence comprises the residues specified at positions 191, 192, 233, 343, 347, and 451 in SEQ ID NO:162.

In certain embodiments, the GCase polypeptide comprises modifications at positions 25 190, 191, 192, 233, 343, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises the residues specified at positions 190, 191, 192, 233, 343, and 347 in SEQ ID NO:163. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 163, wherein the sequence 30 comprises the residues specified at positions 190, 191, 192, 233, 343, and 347 in SEQ ID NO:163.

In certain embodiments, the GCase polypeptide comprises modifications at positions 233, 343, 345, 346, and 347, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises E233G, V343N, S345F,

K346Q, and F347T. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 172, wherein the sequence comprises with respect to SEQ ID NO:172: Gly at position 233, Asn at position 343, Phe at position 345, Gln at position 346, and Thr at position 347.

In certain embodiments, the GCase polypeptide comprises modifications at positions 233, 316, 343, 345, 346, 347, 425, and 451, wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises E233G, F316S, V343N, S345F, K346Q, F347T, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 173, wherein the sequence comprises with respect to SEQ ID NO:173: Gly at position 233, Ser at position 316, Asn at position 343, Phe at position 345, Gln at position 346, Thr at position 347, Arg at position 425, and Arg at position 451.

Certain embodiments also provide a GCase polypeptide comprising an amino acid sequence having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 104, wherein:

- X_{1d} is selected from the group consisting of: G, E, R, and Q;
- X_{2d} is selected from the group consisting of: A, N, T, S, H, D, R, and G;
- X_{3d} is selected from the group consisting of: V, Y, F, L, M, S, G, and W;
- X_{4d} is selected from the group consisting of: N, V, I, T, A, M, E, G, D, and S;
- X_{5d} is G;
- X_{6d} is selected from the group consisting of: P, S, K, Q, and T;
- X_{7d} is S;
- X_{8d} is selected from the group consisting of: V, T, A, and N;
- X_{9d} is selected from the group consisting of: S, F, T, and I;
- X_{10d} is selected from the group consisting of: K, L, Q, F, N, E, and V;
- X_{11d} is selected from the group consisting of: F, P, S, and T;
- X_{12d} is R; and
- X_{13d} is R. Accordingly, such a GCase polypeptide would comprise a sequence having 1) the specified sequence identity to SEQ ID NO:104; and 2) a specified residue at each of positions X_{1d}-X_{13d}.

In certain embodiments, the amino acid sequence has at least about 80% identity to SEQ ID NO:104. In certain embodiments, the amino acid sequence has at least about 85% identity to

SEQ ID NO:104. In certain embodiments, the amino acid sequence has at least about 90% identity to SEQ ID NO: 104. In certain embodiments, the amino acid sequence has at least about 95% identity to SEQ ID NO: 104. In certain embodiments, the amino acid sequence has at least about 96% identity to SEQ ID NO: 104. In certain embodiments, the amino acid sequence has at least about 97% identity to SEQ ID NO: 104. In certain embodiments, the amino acid sequence has at least about 98% identity to SEQ ID NO: 104. In certain embodiments, the amino acid sequence has at least about 99% identity to SEQ ID NO: 104. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 104. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 104.

10 In certain embodiments, X_{1d} is G. In certain embodiments, X_{1d} is E. In certain embodiments, X_{1d} is R. In certain embodiments, X_{1d} is Q.

In certain embodiments, X_{2d} is A. In certain embodiments, X_{2d} is N. In certain embodiments, X_{2d} is T. In certain embodiments, X_{2d} is S. In certain embodiments, X_{2d} is H. In certain embodiments, X_{2d} is D. In certain embodiments, X_{2d} is R. In certain embodiments, X_{2d} is G.

15 In certain embodiments, X_{3d} is V. In certain embodiments, X_{3d} is Y. In certain embodiments, X_{3d} is F. In certain embodiments, X_{3d} is L. In certain embodiments, X_{3d} is M. In certain embodiments, X_{3d} is S. In certain embodiments, X_{3d} is G. In certain embodiments, X_{3d} is W.

20 In certain embodiments, X_{4d} is N. In certain embodiments, X_{4d} is V. In certain embodiments, X_{4d} is I. In certain embodiments, X_{4d} is T. In certain embodiments, X_{4d} is A. In certain embodiments, X_{4d} is M. In certain embodiments, X_{4d} is E. In certain embodiments, X_{4d} is G. In certain embodiments, X_{4d} is D. In certain embodiments, X_{4d} is S.

In certain embodiments, X_{6d} is P. In certain embodiments, X_{6d} is S. In certain embodiments, X_{6d} is K. In certain embodiments, X_{6d} is Q. In certain embodiments, X_{6d} is T.

25 In certain embodiments, X_{8d} is V. In certain embodiments, X_{8d} is T. In certain embodiments, X_{8d} is A. In certain embodiments, X_{8d} is N.

In certain embodiments, X_{9d} is S. In certain embodiments, X_{9d} is F. In certain embodiments, X_{9d} is T. In certain embodiments, X_{9d} is I.

30 In certain embodiments, X_{10d} is K. In certain embodiments, X_{10d} is L. In certain embodiments, X_{10d} is Q. In certain embodiments, X_{10d} is F. In certain embodiments, X_{10d} is N. In certain embodiments, X_{10d} is E. In certain embodiments, X_{10d} is V.

In certain embodiments, X_{11d} is F. In certain embodiments, X_{11d} is P. In certain embodiments, X_{11d} is S. In certain embodiments, X_{11d} is T.

In certain embodiments, X_{1d} is E, or Q; X_{2d} is N, T, or S; X_{3d} is Y, or L; X_{4d} is V, T, or M; X_{5d} is G; X_{6d} is S, K, Q, or T; X_{7d} is S; X_{8d} is T, or A; X_{9d} is F, T, or I; X_{10d} is L, F, or E; X_{11d} is P, or T; X_{12d} is R; and X_{13d} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 104, wherein the variables are defined as indicated above.

In some embodiments, the GCase polypeptide comprises SEQ ID NO: 104, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 104, wherein the variables are defined as indicated above.

In certain embodiments, X_{1d} is E, R, or Q; X_{2d} is N, T, D, or R; X_{3d} is F, L, M, or W; X_{4d} is V, I, T, E, or D; X_{5d} is G; X_{6d} is P; X_{7d} is S; X_{8d} is T, or A; X_{9d} is F; X_{10d} is Q, N, or E; X_{11d} is P, S, or T; X_{12d} is R; and X_{13d} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 104, wherein the variables are defined as indicated above.

In some embodiments, the GCase polypeptide comprises SEQ ID NO: 104, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 104, wherein the variables are defined as indicated above.

In certain embodiments, X_{1d} is G; X_{2d} is N, or S; X_{3d} is Y, or F; X_{4d} is V or I; X_{5d} is G; X_{6d} is K; X_{7d} is S; X_{8d} is T, or A; X_{9d} is F; X_{10d} is Q; X_{11d} is P, or S; X_{12d} is R; and X_{13d} is R.

In certain embodiments, X_{1d} is G; X_{2d} is N, T, H, D, or G; X_{3d} is Y, F, L, or S; X_{4d} is I, T, A, M, E, or S; X_{5d} is G; X_{6d} is P; X_{7d} is S; X_{8d} is T, A, or N; X_{9d} is F; X_{10d} is L, Q, or E; X_{11d} is P, S, or T; X_{12d} is R; and X_{13d} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 104, wherein the variables are defined as indicated above.

In some embodiments, the GCase polypeptide comprises SEQ ID NO: 104, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 104, wherein the variables are defined as indicated above.

In certain embodiments, X_{1d} is G; X_{2d} is N, or H; X_{3d} is Y, or F; X_{4d} is I, or E; X_{5d} is G; X_{6d} is P; X_{7d} is S; X_{8d} is T, or N; X_{9d} is S, or F; X_{10d} is L, or Q; X_{11d} is P, or S; X_{12d} is R; and X_{13d} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 104, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 104, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 104, wherein the variables are defined as indicated above.

Certain embodiments also provide a GCase polypeptide comprising an amino acid sequence having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 171, wherein:

- X_{1e} is selected from the group consisting of: G, E, R, and Q;
- 5 X_{2e} is selected from the group consisting of: A, N, T, S, H, D, R, and G;
- X_{3e} is selected from the group consisting of: V, Y, F, L, M, S, G, and W;
- X_{4e} is selected from the group consisting of: N, V, I, T, A, M, E, G, D, and S;
- X_{5e} is G or E;
- X_{6e} is selected from the group consisting of: P, S, K, Q, and T;
- 10 X_{7e} is S or F;
- X_{8e} is selected from the group consisting of: V, T, A, and N;
- X_{9e} is selected from the group consisting of: S, F, T, and I;
- X_{10e} is selected from the group consisting of: K, L, Q, F, N, E, and V;
- X_{11e} is selected from the group consisting of: F, P, S, and T;
- 15 X_{12e} is K or R; and
- X_{13e} is H or R; and

wherein in the GCase polypeptide comprises two or more modifications at positions described herein, with respect to SEQ ID NO:1. Accordingly, such a GCase polypeptide would comprise a sequence having 1) the specified sequence identity to SEQ ID NO:171; and 2) a
20 specified residue at each of positions X_{1e}-X_{13e}.

In certain embodiments, the amino acid sequence has at least about 80% identity to SEQ ID NO:171. In certain embodiments, the amino acid sequence has at least about 85% identity to SEQ ID NO: 171. In certain embodiments, the amino acid sequence has at least about 90% identity to SEQ ID NO: 171. In certain embodiments, the amino acid sequence has at least about
25 95% identity to SEQ ID NO: 171. In certain embodiments, the amino acid sequence has at least about 96% identity to SEQ ID NO: 171. In certain embodiments, the amino acid sequence has at least about 97% identity to SEQ ID NO: 171. In certain embodiments, the amino acid sequence has at least about 98% identity to SEQ ID NO: 171. In certain embodiments, the amino acid sequence has at least about 99% identity to SEQ ID NO: 171. In certain embodiments, the
30 GCase polypeptide comprises SEQ ID NO: 171. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 171.

In certain embodiments, X_{1e} is G. In certain embodiments, X_{1e} is E. In certain embodiments, X_{1e} is R. In certain embodiments, X_{1e} is Q.

In certain embodiments, X_{2e} is A. In certain embodiments, X_{2e} is N. In certain embodiments, X_{2e} is T. In certain embodiments, X_{2e} is S. In certain embodiments, X_{2e} is H. In certain embodiments, X_{2e} is D. In certain embodiments, X_{2e} is R. In certain embodiments, X_{2e} is G.

5 In certain embodiments, X_{3e} is V. In certain embodiments, X_{3e} is Y. In certain embodiments, X_{3e} is F. In certain embodiments, X_{3e} is L. In certain embodiments, X_{3e} is M. In certain embodiments, X_{3e} is S. In certain embodiments, X_{3e} is G. In certain embodiments, X_{3e} is W.

10 In certain embodiments, X_{4e} is N. In certain embodiments, X_{4e} is V. In certain embodiments, X_{4e} is I. In certain embodiments, X_{4e} is T. In certain embodiments, X_{4e} is A. In certain embodiments, X_{4e} is M. In certain embodiments, X_{4e} is E. In certain embodiments, X_{4e} is G. In certain embodiments, X_{4e} is D. In certain embodiments, X_{4e} is S.

In certain embodiments, X_{5e} is G. In certain embodiments, X_{5e} is E.

15 In certain embodiments, X_{6e} is P. In certain embodiments, X_{6e} is S. In certain embodiments, X_{6e} is K. In certain embodiments, X_{6e} is Q. In certain embodiments, X_{6e} is T.

In certain embodiments, X_{7e} is S. In certain embodiments, X_{7e} is F;

In certain embodiments, X_{8e} is V. In certain embodiments, X_{8e} is T. In certain embodiments, X_{8e} is A. In certain embodiments, X_{8e} is N.

20 In certain embodiments, X_{9e} is S. In certain embodiments, X_{9e} is F. In certain embodiments, X_{9e} is T. In certain embodiments, X_{9e} is I.

In certain embodiments, X_{10e} is K. In certain embodiments, X_{10e} is L. In certain embodiments, X_{10e} is Q. In certain embodiments, X_{10e} is F. In certain embodiments, X_{10e} is N. In certain embodiments, X_{10e} is E. In certain embodiments, X_{10e} is V.

25 In certain embodiments, X_{11e} is F. In certain embodiments, X_{11e} is P. In certain embodiments, X_{11e} is S. In certain embodiments, X_{11e} is T.

In certain embodiments, X_{12e} is K. In certain embodiments, X_{12e} is R.

In certain embodiments, X_{13e} is H. In certain embodiments, X_{13e} is R.

30 In certain embodiments, X_{1e} is G; X_{2e} is A, N, or H; X_{3e} is Y, F, or V; X_{4e} is E, I, or N; X_{5e} is G or E; X_{6e} is P; X_{7e} is S or F; X_{8e} is T, V, or N; X_{9e} is F or S; X_{10e} is K, L, or Q; X_{11e} is P, S, or F; X_{12e} is R or K; and X_{13e} is R or H. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase

polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is A, N, or H; X_{3e} is Y, F, or V; X_{4e} is E, I, or N; X_{5e} is G; X_{6e} is P; X_{7e} is S or F; X_{8e} is T or N; X_{9e} is F or S; X_{10e} is K, L, or Q; X_{11e} is P or S; X_{12e} is R or K; and X_{13e} is R or H. In some embodiments, the GCase polypeptide comprises a
 5 sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

10 In certain embodiments, X_{1e} is G; X_{2e} is H; X_{3e} is Y; X_{4e} is E; X_{5e} is G; X_{6e} is P; X_{7e} is F; X_{8e} is N; X_{9e} is F; X_{10e} is Q; X_{11e} is S; X_{12e} is K; and X_{13e} is H. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID
 15 NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is A; X_{3e} is F; X_{4e} is I; X_{5e} is G; X_{6e} is P; X_{7e} is F; X_{8e} is T; X_{9e} is S; X_{10e} is K; X_{11e} is P; X_{12e} is K; and X_{13e} is H. In some embodiments, the GCase
 20 polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated
 25 above.

In certain embodiments, X_{1e} is G; X_{2e} is A; X_{3e} is V; X_{4e} is N; X_{5e} is G; X_{6e} is P; X_{7e} is F; X_{8e} is T; X_{9e} is S; X_{10e} is K; X_{11e} is P; X_{12e} is K; and X_{13e} is H. In some embodiments, the GCase
 30 polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is A; X_{3e} is F; X_{4e} is I; X_{5e} is G; X_{6e} is P; X_{7e} is F;

X_{8e} is T; X_{9e} is S; X_{10e} is L; X_{11e} is P; X_{12e} is K; and X_{13e} is H. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is A; X_{3e} is F; X_{4e} is I; X_{5e} is G; X_{6e} is P; X_{7e} is S; X_{8e} is T; X_{9e} is S; X_{10e} is L; X_{11e} is P; X_{12e} is R; and X_{13e} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is N; X_{3e} is F; X_{4e} is I; X_{5e} is G; X_{6e} is P; X_{7e} is F; X_{8e} is T; X_{9e} is S; X_{10e} is L; X_{11e} is P; X_{12e} is R; and X_{13e} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is N; X_{3e} is F; X_{4e} is I; X_{5e} is G; X_{6e} is P; X_{7e} is S; X_{8e} is T; X_{9e} is S; X_{10e} is K; X_{11e} is P; X_{12e} is R; and X_{13e} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is N; X_{3e} is F; X_{4e} is I; X_{5e} is G; X_{6e} is P; X_{7e} is S; X_{8e} is T; X_{9e} is S; X_{10e} is L; X_{11e} is P; X_{12e} is K; and X_{13e} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%,

90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is N; X_{3e} is F; X_{4e} is I; X_{5e} is G; X_{6e} is P; X_{7e} is S; X_{8e} is T; X_{9e} is S; X_{10e} is L; X_{11e} is P; X_{12e} is R; and X_{13e} is H. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is H; X_{3e} is Y; X_{4e} is E; X_{5e} is G; X_{6e} is P; X_{7e} is F; X_{8e} is N; X_{9e} is F; X_{10e} is Q; X_{11e} is S; X_{12e} is R; and X_{13e} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is H; X_{3e} is Y; X_{4e} is E; X_{5e} is G; X_{6e} is P; X_{7e} is S; X_{8e} is N; X_{9e} is F; X_{10e} is Q; X_{11e} is S; X_{12e} is K; and X_{13e} is R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is H; X_{3e} is Y; X_{4e} is E; X_{5e} is G; X_{6e} is P; X_{7e} is S; X_{8e} is N; X_{9e} is F; X_{10e} is Q; X_{11e} is S; X_{12e} is R; and X_{13e} is H. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID

NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is A; X_{3e} is V; X_{4e} is N; X_{5e} is G; X_{6e} is P; X_{7e} is F; X_{8e} is N; X_{9e} is F; X_{10e} is Q; X_{11e} is T; X_{12e} is K; and X_{13e} is H. In some embodiments, the GCCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, X_{1e} is G; X_{2e} is A; X_{3e} is V; X_{4e} is N; X_{5e} is G; X_{6e} is P; X_{7e} is S; X_{8e} is N; X_{9e} is F; X_{10e} is Q; X_{11e} is T; X_{12e} is R; and X_{13e} is R. In some embodiments, the GCCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCCase polypeptide comprises SEQ ID NO: 171, wherein the variables are defined as indicated above. In some embodiments, the GCCase polypeptide consists of SEQ ID NO: 171, wherein the variables are defined as indicated above.

In certain embodiments, a GCCase polypeptide comprises a sequence having a combination of residues at positions 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425 and 451 as recited in any one of SEQ ID NOs: 138-163. In some embodiments, the GCCase polypeptide comprises a sequence having: 1) at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to any one of SEQ ID NOs: 138-163; and 2) the recited residues of the specified sequence at each of positions 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425 and 451.

In certain embodiments, a GCCase polypeptide comprises a sequence having a combination of residues at positions 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425 and 451 as recited in any one of SEQ ID NOs: 138-163 and 172-173. In some embodiments, the GCCase polypeptide comprises a sequence having: 1) at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to any one of SEQ ID NOs: 138-163 and 172-173; and 2) the recited residues of the specified sequence at each of positions 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425 and 451.

In certain embodiments, a GCase polypeptide comprises a sequence having a combination of residues at positions 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425 and 451 as recited in any one of SEQ ID NOs: 141-143, 145-147, 150-153, and 160-163. In some embodiments, the GCase polypeptide comprises a sequence having: 1) at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to any one of SEQ ID NOs: 141-143, 145-147, 150-153, and 160-163; and 2) the recited residues of the specified sequence at each of positions 189, 190, 191, 192, 233, 245, 316, 343, 345, 346, 347, 425 and 451.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO: 172, and SEQ ID NO: 173.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID

NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, and SEQ ID NO:163.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, and SEQ ID NO:131.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:105. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:105. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:105.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:106. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:106. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:106.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:107. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:107. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:107.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:108. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:108. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:108.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:109. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:109. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:109.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:110. In certain embodiments, the GCase polypeptide comprises SEQ

ID NO:110. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:110.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:111. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:111. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:111.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:112. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:112. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:112.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:113. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:113. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:113.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:114. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:114. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:114.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:115. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:115. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:115.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:116. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:116. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:116.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:117. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:117. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:117.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:118. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:118. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:118.

In embodiments the GCase polypeptide comprises an amino acid sequence having at

least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:119. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:119. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:119.

5 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:120. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:120. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:120.

10 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:121. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:121. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:121.

15 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:122. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:122. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:122.

20 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:123. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:123. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:123.

25 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:124. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:124. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:124.

30 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:125. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:125. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:125.

35 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:126. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:126. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:126.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:127. In certain embodiments, the GCase polypeptide comprises SEQ

ID NO:127. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:127.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:128. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:128. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:128.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:129. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:129. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:129.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:130. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:130. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:130.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:131. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:131. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:131.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, SEQ ID NO:172, and SEQ ID NO: 173.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, and SEQ ID NO:163.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:138. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:138. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:138.

5 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:139. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:139. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:139.

10 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:159. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:159. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:159.

15 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:140. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:140. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:140.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:154. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:154. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:154.

20 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:155. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:155. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:155.

25 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:156. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:156. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:156.

30 In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:157. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:157. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:157.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%

identity) to SEQ ID NO:158. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:158. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:158.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:144. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:144. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:144.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:148. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:148. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:148.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:149. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:149. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:149.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:172. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:172. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:172.

In embodiments the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:173. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:173. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:173.

In certain embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to any one of SEQ ID NOs: 141-143, 145-147, 150-153, and 160-163. In certain embodiments, the GCase polypeptide comprises any one of SEQ ID NOs: 141-143, 145-147, 150-153, and 160-163. In certain embodiments, the GCase polypeptide consists of any one of SEQ ID NOs: 141-143, 145-147, 150-153, and 160-163.

30 **Certain Embodiments Associated with Two or More of Positions 125, 126, 181, 230, 232, 233, 237, 238, 240, 316, 386, 387, 388, 425, and 451**

Certain embodiments also provide a GCase polypeptide comprising two or more modifications, wherein at least two modifications with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 316, 386,

387, 388, 425 and 451.

In certain embodiments, the GCase polypeptide comprises two or more modifications, wherein at least two modifications, with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 316, 425, and 451. In certain embodiments, the GCase polypeptide
5 comprises a modification at positions 316 and 425. In certain embodiments, the GCase polypeptide comprises a modification at positions 316 and 451. In certain embodiments, the GCase polypeptide comprises a modification at positions 425 and 451. In certain embodiments, the GCase polypeptide comprises a modification at positions 316, 425, and 451.

In certain embodiments, the GCase polypeptide comprises a modification at position
10 316. In certain embodiments, the GCase polypeptide comprises a modification at position 316 that is F316S.

In certain embodiments, the GCase polypeptide comprises a modification at position 425. In certain embodiments, the GCase polypeptide comprises a modification at position 425 that is K425R.

15 In certain embodiments, the GCase polypeptide comprises a modification at position 451. In certain embodiments, the GCase polypeptide comprises a modification at position 451 that is H451R.

In certain embodiments, at least two of the modifications are selected from the group consisting of: F316S, K425R, and H451R. In certain embodiments, the GCase polypeptide
20 comprises modifications at positions 316, 425, and 451, with respect to SEQ ID NO:1. In certain embodiments, the modifications comprise F316S, K425R, and H451R.

In certain embodiments, the GCase polypeptide further comprises a modification, with respect to SEQ ID NO:1, at one or more positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388.

25 In certain embodiments, the GCase polypeptide comprises a modification at position 125. In certain embodiments, the GCase polypeptide comprises a modification at position 125 that is S125A.

In certain embodiments, the GCase polypeptide comprises a modification at position 126. In certain embodiments, the GCase polypeptide comprises a modification at position 126
30 selected from the group consisting of: C126T and C126S.

In certain embodiments, the GCase polypeptide comprises a modification at position 181. In certain embodiments, the GCase polypeptide comprises a modification at position 181 that is S181A.

In certain embodiments, the GCase polypeptide comprises a modification at

position 230. In certain embodiments, the GCase polypeptide comprises a modification at position 230 that is V230L.

In certain embodiments, the GCase polypeptide comprises a modification at position 232. In certain embodiments, the GCase polypeptide comprises a modification at position 232 selected from the group consisting of: A232S and A232T.

In certain embodiments, the GCase polypeptide comprises a modification at position 233. In certain embodiments, the GCase polypeptide comprises a modification at position 233 selected from the group consisting of: E233G and E233Q.

In certain embodiments, the GCase polypeptide comprises a modification at position 237. In certain embodiments, the GCase polypeptide comprises a modification at position 237 selected from the group consisting of: S237L, S237I, and S237T.

In certain embodiments, the GCase polypeptide comprises a modification at position 238. In certain embodiments, the GCase polypeptide comprises a modification at position 238 that is A238N.

In certain embodiments, the GCase polypeptide comprises a modification at position 240. In certain embodiments, the GCase polypeptide comprises a modification at position 240 selected from the group consisting of: L240M, L240Q, and L240Y.

In certain embodiments, the GCase polypeptide comprises a modification at position 386. In certain embodiments, the GCase polypeptide comprises a modification at position 386 that is N386D.

In certain embodiments, the GCase polypeptide comprises a modification at position 387. In certain embodiments, the GCase polypeptide comprises a modification at position 387 selected from the group consisting of: P387M, P387T, and P387L.

In certain embodiments, the GCase polypeptide comprises a modification at position 388. In certain embodiments, the GCase polypeptide comprises a modification at position 388 that is E388K.

In certain embodiments, the GCase polypeptide comprises modifications at positions 316, 425, and 451; and comprises a modification at one or more position(s) selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388, wherein the modifications and positions are with respect to SEQ ID NO:1 (e.g., comprises modifications at positions 233, 316, 425, and 451, and optionally, comprises a modification at one or more position(s) selected from the group consisting of: 125, 126, 181, 230, 232, 237, 238, 240, 386, 387, and 388). In certain embodiments, the GCase polypeptide comprises two modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240,

386, 387, and 388. In certain embodiments, the GCase polypeptide comprises three modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises four modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises five modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises six modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises seven modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises eight modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises nine modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises ten modifications at positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388. In certain embodiments, the GCase polypeptide comprises modifications at positions 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388.

In certain embodiments, the GCase polypeptide comprises a combination of modifications as indicated in a clone from Library 1 or as indicated in Table 7a.

For example, in certain embodiments, the GCase polypeptide comprises modifications at positions 126, 230, 232, 233, 240, 316, 386, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, V230L, A232S, E233G, L240M, F316S, N386D, P387L, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 81, wherein the sequence comprises with respect to SEQ ID NO:81: Thr at position 126, Leu at position 230, Ser at position 232, Gly at position 233, Met at position 240, Ser at position 316, Asp at position 386, Leu at position 387, Arg at position 425, and Arg at position 451.

In certain other embodiments, the GCase polypeptide comprises modifications at positions 126, 181, 230, 232, 233, 237, 238, 316, 386, 387, 388, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1.

In certain embodiments, the GCase polypeptide comprises C126T, S181A, V230L, A232S, E233Q, S237L, A238N, F316S, N386D, P387L, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 82,

5 wherein the sequence comprises with respect to SEQ ID NO:82: Thr at position 126, Ala at position 181, Leu at position 230, Ser at position 232, Gln at position 233, Leu at position 237, Asn at position 238, Ser at position 316, Asp at position 386, Leu at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises C126T, S181A, V230L, 10 A232T, E233Q, S237I, A238N, F316S, N386D, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 87, wherein the sequence comprises with respect to SEQ ID NO:87: Thr at position 126, Ala at position 181, Leu at position 230, Thr at position 232, Gln at position 233, Ile at position 237, 15 Asn at position 238, Ser at position 316, Asp at position 386, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 230, 232, 233, 237, 316, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises 20 C126T, V230L, A232T, E233Q, S237I, F316S, P387L, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 83, wherein the sequence comprises with respect to SEQ ID NO:83: Thr at position 126, Leu at position 230, Thr at position 232, Gln at position 233, Ile at position 237, Ser at position 316, 25 Leu at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 181, 230, 232, 233, 237, 238, 316, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, V230L, A232T, E233G, S237I, A238N, F316S, P387T, K425R, and 30 H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 84, wherein the sequence comprises with respect to SEQ ID NO:84: Thr at position 126, Ala at position 181, Leu at position 230, Thr at position 232, Gly at position 233, Ile at position 237, Asn at position 238, Ser at position 316, Thr at position 387, Arg at position 425,

and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 230, 232, 233, 237, 238, 240, 316, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide
5 comprises C126T, V230L, A232S, E233Q, S237I, A238N, L240M, F316S, P387T, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 85, wherein the sequence comprises with respect to SEQ ID NO:85: Thr at position 126, Leu at position 230, Ser at position 232, Gln at position 233, Ile at position 237, Asn at
10 position 238, Met at position 240, Ser at position 316, Thr at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 181, 232, 233, 237, 238, 316, 387, 388, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide
15 comprises C126T, S181A, A232T, E233Q, S237I, A238N, F316S, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 86, wherein the sequence comprises with respect to SEQ ID NO:86: Thr at position 126, Ala at position 181, Thr at position 232, Gln at position 233, Ile at position 237, Asn at
20 position 238, Ser at position 316, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 232, 233, 237, 240, 316, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises
25 C126T, A232S, E233Q, S237I, L240Q, F316S, P387L, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 88, wherein the sequence comprises with respect to SEQ ID NO:88: Thr at position 126, Ser at position 232, Gln at position 233, Ile at position 237, Gln at position 240, Ser at position 316,
30 Leu at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 232, 233, 237, 240, 316, 386, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, A232S, E233Q, S237T, L240Y, F316S, N386D, P387T, K425R, and H451R. In some

embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 89, wherein the sequence comprises with respect to SEQ ID NO:89: Thr at position 126, Ser at position 232, Gln at position 233, Thr at position 237, Tyr at position 240, Ser at position 316, Asp at position 386, Thr at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 230, 232, 233, 237, 238, 316, 387, 388, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1.

In certain embodiments, the GCase polypeptide comprises C126T, V230L, A232T, E233G, S237I, A238N, F316S, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 90, wherein the sequence comprises with respect to SEQ ID NO:90: Thr at position 126, Leu at position 230, Thr at position 232, Gly at position 233, Ile at position 237, Asn at position 238, Ser at position 316, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises C126T, V230L, A232T, E233Q, S237I, A238N, F316S, P387T, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 103, wherein the sequence comprises with respect to SEQ ID NO:103: Thr at position 126, Leu at position 230, Thr at position 232, Gln at position 233, Ile at position 237, Asn at position 238, Ser at position 316, Thr at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 181, 230, 232, 233, 237, 238, 240, 316, 386, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, V230L, A232T, E233Q, S237I, A238N, L240M, F316S, N386D, P387T, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 91, wherein the sequence comprises with respect to SEQ ID NO:91: Thr at position 126, Ala at position 181, Leu at position 230, Thr at position 232, Gln at position 233, Ile at position 237, Asn at position 238, Met at position 240, Ser at position 316, Asp at position 386, Thr at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 181, 232, 233, 237, 238, 316, 386, 387, 388, 425, and 451 wherein the modifications and

positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, A232T, E233G, S237I, A238N, F316S, N386D, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99%

5 identity) to SEQ ID NO: 92, wherein the sequence comprises with respect to SEQ ID NO:92: Thr at position 126, Ala at position 181, Thr at position 232, Gly at position 233, Ile at position 237, Asn at position 238, Ser at position 316, Asp at position 386, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions
10 126, 181, 232, 233, 237, 316, 386, 387, 388, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, A232S, E233Q, S237T, F316S, N386D, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to
15 SEQ ID NO: 93, wherein the sequence comprises with respect to SEQ ID NO:93: Thr at position 126, Ala at position 181, Ser at position 232, Gln at position 233, Thr at position 237, Ser at position 316, Asp at position 386, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions
20 126, 181, 230, 232, 233, 237, 238, 316, 387, 388, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, V230L, A232S, E233Q, S237I, A238N, F316S, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99%
25 identity) to SEQ ID NO: 94, wherein the sequence comprises with respect to SEQ ID NO:94: Thr at position 126, Ala at position 181, Leu at position 230, Ser at position 232, Gln at position 233, Ile at position 237, Asn at position 238, Ser at position 316, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions
30 126, 181, 232, 233, 237, 316, 387, 388, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126S, S181A, A232S, E233Q, S237T, F316S, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 95,

wherein the sequence comprises with respect to SEQ ID NO:95: Ser at position 126, Ala at position 181, Ser at position 232, Gln at position 233, Thr at position 237, Ser at position 316, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 5 126, 181, 230, 232, 233, 237, 238, 316, 386, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, V230L, A232S, E233Q, S237L, A238N, F316S, N386D, P387L, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% 10 identity) to SEQ ID NO: 96, wherein the sequence comprises with respect to SEQ ID NO:96: Thr at position 126, Ala at position 181, Leu at position 230, Ser at position 232, Gln at position 233, Leu at position 237, Asn at position 238, Ser at position 316, Asp at position 386, Leu at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 15 126, 181, 232, 233, 237, 316, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, A232S, E233Q, S237I, F316S, P387T, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 97, 20 wherein the sequence comprises with respect to SEQ ID NO:97: Thr at position 126, Ala at position 181, Ser at position 232, Gln at position 233, Ile at position 237, Ser at position 316, Thr at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 232, 233, 237, 316, 387, 425, and 451 wherein the modifications and positions are with 25 respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, A232S, E233G, S237T, F316S, P387T, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 98, wherein the sequence comprises with respect to SEQ ID NO:98: Thr at position 126, Ser at position 232, Gly at 30 position 233, Thr at position 237, Ser at position 316, Thr at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 232, 233, 237, 240, 316, 387, 388, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises

C126T, A232S, E233Q, S237T, L240Y, F316S, P387M, E388K, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 99, wherein the sequence comprises with respect to SEQ ID NO:99: Thr at position 126, Ser at position 232, Gln at position 233, Thr at position 237, Tyr at position 240, Ser at position 316, Met at position 387, Lys at position 388, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 181, 232, 233, 237, 316, 386, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises

S181A, A232S, E233Q, S237T, F316S, N386D, P387M, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 100, wherein the sequence comprises with respect to SEQ ID NO:100: Ala at position 181, Ser at position 232, Gln at position 233, Thr at position 237, Ser at position 316, Asp at position 386, Met at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 125, 126, 230, 232, 233, 237, 316, 386, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises S125A, C126T, V230L, A232T, E233G, S237T, F316S, N386D, P387L, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 101, wherein the sequence comprises with respect to SEQ ID NO:101: Ala at position 125, Thr at position 126, Leu at position 230, Thr at position 232, Gly at position 233, Thr at position 237, Ser at position 316, Asp at position 386, Leu at position 387, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises modifications at positions 126, 181, 233, 316, 386, 387, 425, and 451 wherein the modifications and positions are with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises C126T, S181A, E233G, F316S, N386D, P387L, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 102, wherein the sequence comprises with respect to SEQ ID NO:102: Thr at position 126, Ala at position 181, Gly at position 233, Ser at position 316, Asp at position 386, Leu at position 387, Arg at position 425, and Arg at position 451.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 80, wherein:

- X_{1c} is selected from the group consisting of: S and A;
- 5 X_{2c} is selected from the group consisting of: C, S and T;
- X_{3c} is selected from the group consisting of: A and S;
- X_{4c} is selected from the group consisting of: L and V;
- X_{5c} is selected from the group consisting of: S, T, and A;
- X_{6c} is selected from the group consisting of: G, Q and E;
- 10 X_{7c} is selected from the group consisting of: S, L, I, and T;
- X_{8c} is selected from the group consisting of: A and N;
- X_{9c} is selected from the group consisting of: L, M, Q and Y;
- X_{10c} is S;
- X_{11c} is selected from the group consisting of: D and N;
- 15 X_{12c} is selected from the group consisting of: P, M, T, and L; and
- X_{13c} is selected from the group consisting of: E and K;
- X_{14c} = R; and
- X_{15c} = R.

- In certain embodiments, the amino acid sequence has at least about 80% identity to SEQ ID NO:80. In certain embodiments, the amino acid sequence has at least about 85% identity to SEQ ID NO: 80. In certain embodiments, the amino acid sequence has at least about 90% identity to SEQ ID NO: 80. In certain embodiments, the amino acid sequence has at least about 95% identity to SEQ ID NO: 80. In certain embodiments, the amino acid sequence has at least about 96% identity to SEQ ID NO: 80. In certain embodiments, the amino acid sequence has at least about 97% identity to SEQ ID NO: 80. In certain embodiments, the amino acid sequence has at least about 98% identity to SEQ ID NO: 80. In certain embodiments, the amino acid sequence has at least about 99% identity to SEQ ID NO: 80. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 80. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 80.

- 30 In certain embodiments, X_{1c} is S. In certain embodiments, X_{1c} is A.
- In certain embodiments, X_{2c} is C. In certain embodiments, X_{2c} is S. In certain embodiments, X_{2c} is T.
- In certain embodiments, X_{3c} is A. In certain embodiments, X_{3c} is S.
- In certain embodiments, X_{4c} is L. In certain embodiments, X_{4c} is V.

In certain embodiments, X_{5c} is S. In certain embodiments, X_{5c} is T. In certain embodiments, X_{5c} is A.

In certain embodiments, X_{6c} is G. In certain embodiments, X_{6c} is Q. In certain embodiments, X_{6c} is E.

5 In certain embodiments, X_{7c} is S. In certain embodiments, X_{7c} is L. In certain embodiments, X_{7c} is I. In certain embodiments, X_{7c} is T.

In certain embodiments, X_{8c} is A. In certain embodiments, X_{8c} is N.

In certain embodiments, X_{9c} is L. In certain embodiments, X_{9c} is M. In certain embodiments, X_{9c} is Q. In certain embodiments, X_{9c} is Y.

10 In certain embodiments, X_{11c} is D. In certain embodiments, X_{11c} is N.

In certain embodiments, X_{12c} is P. In certain embodiments, X_{12c} is M. In certain embodiments, X_{12c} is T. In certain embodiments, X_{12c} is L.

In certain embodiments, X_{13c} is E. In certain embodiments, X_{13c} is K.

In certain embodiments, X_{1c} is S; X_{2c} is T; X_{3c} is A; X_{4c} is L; X_{5c} is S or T; X_{6c} is Q; X_{7c} is L, or I; X_{8c} is N; X_{9c} is L; X_{10c} is S; X_{11c} is D; X_{12c} is M, or L; X_{13c} is K; X_{14c} = R; and X_{15c} = R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 80, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 80, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 80, wherein the variables are defined as indicated above.

20 In certain embodiments, X_{1c} is S; X_{2c} is T; X_{3c} is S; X_{4c} is L; X_{5c} is T; X_{6c} is G or Q; X_{7c} is I; X_{8c} is N; X_{9c} is L; X_{10c} is S; X_{11c} is N; X_{12c} is M, or T; X_{13c} is K; X_{14c} = R; and X_{15c} = R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 80, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 80, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 80, wherein the variables are defined as indicated above.

30 In certain embodiments, X_{1c} is S; X_{2c} is T; X_{3c} is A or S; X_{4c} is V or L; X_{5c} is S or T; X_{6c} is Q; X_{7c} is I; X_{8c} is A or N; X_{9c} is L; X_{10c} is S; X_{11c} is N; X_{12c} is T; X_{13c} is E or K; X_{14c} = R; and X_{15c} = R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 80, wherein the variables are defined as indicated above. In some embodiments,

the GCase polypeptide comprises SEQ ID NO: 80, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 80, wherein the variables are defined as indicated above.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, and SEQ ID NO:103.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:81. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:81. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:81.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:82. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:82. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:82.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:83. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:83. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:83.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:84. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:84. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:84.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:85. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:85. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:85.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:86. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:86. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:86.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:87. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:87. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:87.

5 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:88. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:88. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:88.

10 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:89. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:89. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:89.

15 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:90. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:90. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:90.

20 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:91. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:91. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:91.

25 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:92. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:92. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:92.

30 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:93. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:93. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:93.

35 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO:94. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:94. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:94.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or

100% identity) to SEQ ID NO:95. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:95. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:95.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
5 100% identity) to SEQ ID NO:96. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:96. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:96.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
10 100% identity) to SEQ ID NO:97. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:97. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:97.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
100% identity) to SEQ ID NO:98. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:98. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:98.

15 In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
100% identity) to SEQ ID NO:99. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:99. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:99.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence
20 having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
100% identity) to SEQ ID NO:100. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:100. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:100.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence
25 having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
100% identity) to SEQ ID NO:101. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:101. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:101.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence
30 having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
100% identity) to SEQ ID NO:102. In certain embodiments, the GCase polypeptide comprises SEQ ID NO:102. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:102.

In certain embodiments, the GCase polypeptide comprises an amino acid sequence
having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or
100% identity) to SEQ ID NO:103. In certain embodiments, the GCase polypeptide comprises
SEQ ID NO:103. In certain embodiments, the GCase polypeptide consists of SEQ ID NO:103.

CERTAIN OTHER MODIFIED GCASE POLYPEPTIDES

Certain embodiments are directed to a GCASE polypeptide comprising one or more modifications with respect to SEQ ID NO: 1, wherein the one or more modifications provide increased stability relative to that of SEQ ID NO:1, wherein the increased stability is indicated
5 by greater conformational stability, relative to that of SEQ ID NO:1. In certain embodiments, the increased stability is in the presence of serum. In certain embodiments, the one or more modifications also provide increased enzymatic activity relative to SEQ ID NO:1.

In certain embodiments, the GCASE polypeptide comprises two modifications, with respect to SEQ ID NO:1. In certain embodiments, the GCASE polypeptide comprises more than
10 two modifications, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more modifications, with respect to SEQ ID NO:1.

In certain embodiments, the GCASE polypeptide comprises one or more modifications with respect to SEQ ID NO:1, wherein at least one modification is selected from the group consisting of: 26, 27, 31, 102, 233, 286, 313, 316, 347, 348, 351, 373, 376, 392, 394, 398, 420,
15 425, and 451.

In certain embodiments, the GCASE polypeptide comprises a modification at position 26 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 26 is F26L. In certain embodiments, the modification at position 26 is F26S.

In certain embodiments, the GCASE polypeptide comprises a modification at position 27
20 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 27 is D27G.

In certain embodiments, the GCASE polypeptide comprises a modification at position 31 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 31 is F31L.

In certain embodiments, the GCASE polypeptide comprises a modification at position 102 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 102 is
25 N102D.

In certain embodiments, the GCASE polypeptide comprises a modification at position 233 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 233 is E233G.

In certain embodiments, the GCASE polypeptide comprises a modification at position 286
30 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 286 is L286S.

In certain embodiments, the GCASE polypeptide comprises a modification at position 313 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 313 is Y313H. In certain embodiments, the modification at position 313 is Y313D. In certain

embodiments, the modification at position 313 is Y313C.

In certain embodiments, the GCase polypeptide comprises a modification at position 316 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 316 is F316S. In certain embodiments, the modification at position 316 is F316L.

5 In certain embodiments, the GCase polypeptide comprises a modification at position 347 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 347 is F347S.

In certain embodiments, the GCase polypeptide comprises a modification at position 348 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 348 is W348G. In certain embodiments, the modification at position 348 is W348R.

In certain embodiments, the GCase polypeptide comprises a modification at position 351 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 351 is S351G. In certain embodiments, the modification at position 351 is S351N.

15 In certain embodiments, the GCase polypeptide comprises a modification at position 373 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 373 is Y373H.

In certain embodiments, the GCase polypeptide comprises a modification at position 376 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 376 is V376A.

20 In certain embodiments, the GCase polypeptide comprises a modification at position 392 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 392 is N392S.

In certain embodiments, the GCase polypeptide comprises a modification at position 394 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 394 is V394A.

25 In certain embodiments, the GCase polypeptide comprises a modification at position 398 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 398 is V398A.

In certain embodiments, the GCase polypeptide comprises a modification at position 420 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 420 is L420I.

30 In certain embodiments, the GCase polypeptide comprises a modification at position 425 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 425 is K425R.

In certain embodiments, the GCase polypeptide comprises a modification at position 451 with respect to SEQ ID NO:1. In certain embodiments, the modification at position 451 is H451R.

In certain embodiments, the GCase polypeptide comprises two modifications selected from the group consisting of: 26, 27, 31, 102, 233, 286, 313, 316, 347, 348, 351, 373, 376, 392, 394, 398, 420, 425, and 451. In certain embodiments, the GCase polypeptide comprises three modifications selected from the group consisting of: 26, 27, 31, 102, 233, 286, 313, 316, 347, 348, 351, 373, 376, 392, 394, 398, 420, 425, and 451. In certain embodiments, the GCase polypeptide comprises four modifications selected from the group consisting of: 26, 27, 31, 102, 233, 286, 313, 316, 347, 348, 351, 373, 376, 392, 394, 398, 420, 425, and 451. In certain embodiments, the GCase polypeptide comprises five modifications selected from the group consisting of: 26, 27, 31, 102, 233, 286, 313, 316, 347, 348, 351, 373, 376, 392, 394, 398, 420, 425, and 451. In certain embodiments, the GCase polypeptide comprises six modifications selected from the group consisting of: 26, 27, 31, 102, 233, 286, 313, 316, 347, 348, 351, 373, 376, 392, 394, 398, 420, 425, and 451. In certain embodiments, the GCase polypeptide comprises seven modifications selected from the group consisting of: 26, 27, 31, 102, 233, 286, 313, 316, 347, 348, 351, 373, 376, 392, 394, 398, 420, 425, and 451.

In certain embodiments, the GCase polypeptide comprises a combination of modifications as indicated in a clone from Library SH2 or SH3 or as indicated in Table 1 or 2.

For example, certain embodiments provide a GCase polypeptide comprising two or more modifications with respect to SEQ ID NO:1, wherein the two or more modifications provide increased stability (e.g., in the presence of serum), wherein the increased stability is indicated by greater conformational stability (e.g., at least 2-fold or 5-fold greater conformational stability), relative to that of SEQ ID NO:1, and wherein at least one of the modifications is at a position selected from the group consisting of: 233, 286, 316, 373, 398, 425, and 451. In certain embodiments, at least one of the modifications is at a position selected from the group consisting of: 286, 316, 373, 398, 425, and 451.

In certain embodiments, the GCase polypeptide comprises at least two modifications, with respect to SEQ ID NO:1, selected from the group consisting of: 233, 286, 316, 373, 398, 425, and 451. In certain embodiments, the GCase polypeptide comprises at least three modifications, with respect to SEQ ID NO:1, selected from the group consisting of: 233, 286, 316, 373, 398, 425, and 451. In certain embodiments, the GCase polypeptide comprises at least four modifications, with respect to SEQ ID NO:1, selected from the group consisting of: 233, 286, 316, 373, 398, 425, and 451.

In certain embodiments, the GCase polypeptide comprises a modification at position 233 (e.g., E233G) with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises a modification at position 286 (e.g., L286S) with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises a modification at position 316 (e.g., F316S) with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises a modification at position 373 (e.g., Y373H) with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises a modification at position 398 (e.g., V398A) with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises a modification at position 425 (e.g., K425R) with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises a modification at position 451 (e.g., H451R) with respect to SEQ ID NO:1.

In certain embodiments, the GCase polypeptide comprises at least two modifications selected from the group consisting of: E233G, L286S, F316S, Y373H, V398A, K425R, and H451R. In certain embodiments, the GCase polypeptide comprises at least three modifications selected from the group consisting of: E233G, L286S, F316S, Y373H, V398A, K425R, and H451R. In certain embodiments, the GCase polypeptide comprises at least four modifications selected from the group consisting of: E233G, L286S, F316S, Y373H, V398A, K425R, and H451R.

In certain embodiments, the GCase polypeptide comprises a modification at position 316 and at least one modification selected from the group consisting of: 233, 286, 373, 398, 425, and 451, wherein the positions are with respect to SEQ ID NO:1. For example, in certain embodiments, the GCase polypeptide comprises F316S and at least one modification selected from the group consisting of: E233G, L286S, Y373H, V398A, K425R, and H451R. In certain embodiments, the GCase polypeptide comprises F316S and Y373H.

In certain embodiments, the GCase polypeptide comprises modifications at positions 316 and 373 and at least one modification selected from the group consisting of: 233, 286, 398, 425, and 451, wherein the positions are with respect to SEQ ID NO:1. For example, in certain embodiments, the GCase polypeptide comprises F316S, Y373H and at least one modification selected from the group consisting of: E233G, L286S, V398A, K425R, and H451R.

In certain embodiments, the GCase polypeptide comprises E233G, F316S, and Y373H. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (e.g., at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 19, wherein the sequence comprises with respect to SEQ ID NO:19: Gly at position 233, Ser at position 316, and His at position 373.

In certain embodiments, the GCase polypeptide comprises E233G, F316S, K425R, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:20, wherein the sequence comprises with respect to SEQ ID NO:20: Gly at position 233, Ser at position 316, Arg at position 425, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises L286S, F316S, Y373H, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:27, wherein the sequence comprises with respect to SEQ ID NO:27: Ser at position 286, Ser at position 316, His at position 373, and Arg at position 451.

In certain embodiments, the GCase polypeptide comprises E233G, L286S, F316S, and Y373H.

In certain embodiments, the GCase polypeptide comprises L286S, F316S, and Y373H. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:28, wherein the sequence comprises with respect to SEQ ID NO:28: Ser at position 286, Ser at position 316, and His at position 373.

In certain embodiments, the GCase polypeptide comprises F316S, Y373H, and V398A. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:36, wherein the sequence comprises with respect to SEQ ID NO:36: Ser at position 316, His at position 373, and Ala at position 398.

In certain embodiments, the GCase polypeptide comprises F316S, Y373H, and H451R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:39, wherein the sequence comprises with respect to SEQ ID NO:39: Ser at position 316, His at position 373, and Arg at position 451.

In certain embodiments, the GCase polypeptide does not comprise a F246S modification. In certain embodiments, the GCase polypeptide does not comprise a W312R modification. In certain embodiments, the GCase polypeptide does not comprise a L314P modification. In certain embodiments, the GCase polypeptide does not comprise F246S, W312R, and L314P modifications.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID

NO: 4, wherein:

- a) X_{1a} is F, L, or S;
- b) X_{2a} is D or G;
- c) X_{3a} is F or L;
- 5 d) X_{4a} is N or D;
- e) X_{5a} is E or G;
- f) X_{6a} is L or S;
- g) X_{7a} is Y, H, D, or C;
- h) X_{8a} is F, L, or S;
- 10 i) X_{9a} is F or S;
- j) X_{10a} is W, G, or R;
- k) X_{11a} is S, G, or N;
- l) X_{12a} is Y or H;
- m) X_{13a} is V or A;
- 15 n) X_{14a} is N or S;
- o) X_{15a} is V or A;
- p) X_{16a} is V or A;
- q) X_{17a} is L or I;
- r) X_{18a} is K or R; and
- 20 s) X_{19a} is H or R;

wherein the amino acid sequence is not SEQ ID NO:1. For example, in certain embodiments, the GCase polypeptide comprises at least one modification with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises at least two modifications with respect to SEQ ID NO:1. In certain embodiments, the GCase polypeptide comprises more than two
 25 modifications, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or more modifications, with respect to SEQ ID NO:1.

In certain embodiments, the amino acid sequence has at least about 80% identity to SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1. In certain embodiments, the amino acid sequence has at least about 85% identity to SEQ ID NO:4, wherein the amino acid
 30 sequence is not SEQ ID NO:1. In certain embodiments, the amino acid sequence has at least about 90% identity to SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1. In certain embodiments, the amino acid sequence has at least about 95% identity to SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1. In certain embodiments, the amino acid sequence has at least about 96% identity to SEQ ID NO:4, wherein the amino acid sequence is

not SEQ ID NO:1. In certain embodiments, the amino acid sequence has at least about 97% identity to SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1. In certain embodiments, the amino acid sequence has at least about 98% identity to SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1. In certain embodiments, the amino acid sequence has at least about 99% identity to SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1. In certain embodiments, the GCaase polypeptide comprises SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1. In certain embodiments, the GCaase polypeptide consists of SEQ ID NO:4, wherein the amino acid sequence is not SEQ ID NO:1.

10 In certain embodiments, X_{1a} is F. In certain embodiments, X_{1a} is L. In certain embodiments, X_{1a} is S.

In certain embodiments, X_{2a} is D. In certain embodiments, X_{2a} is G.

In certain embodiments, X_{3a} is F. In certain embodiments, X_{3a} is L.

In certain embodiments, X_{4a} is N. In certain embodiments, X_{4a} is D.

In certain embodiments, X_{5a} is E. In certain embodiments, X_{5a} is G.

15 In certain embodiments, X_{6a} is L. In certain embodiments, X_{6a} is S.

In certain embodiments, X_{7a} is Y. In certain embodiments, X_{7a} is H. In certain embodiments, X_{7a} is D. In certain embodiments, X_{7a} is C.

In certain embodiments, X_{8a} is F. In certain embodiments, X_{8a} is L. In certain embodiments, X_{8a} is S.

20 In certain embodiments, X_{9a} is F. In certain embodiments, X_{9a} is S.

In certain embodiments, X_{10a} is W. In certain embodiments, X_{10a} is G. In certain embodiments, X_{10a} is R.

In certain embodiments, X_{11a} is S. In certain embodiments, X_{11a} is G. In certain embodiments, X_{11a} is N.

25 In certain embodiments, X_{12a} is Y. In certain embodiments, X_{12a} is H.

In certain embodiments, X_{13a} is V. In certain embodiments, X_{13a} is A.

In certain embodiments, X_{14a} is N. In certain embodiments, X_{14a} is S.

In certain embodiments, X_{15a} is V. In certain embodiments, X_{15a} is A.

In certain embodiments, X_{16a} is V. In certain embodiments, X_{16a} is A.

30 In certain embodiments, X_{17a} is L. In certain embodiments, X_{17a} is I.

In certain embodiments, X_{18a} is K. In certain embodiments, X_{18a} is R.

In certain embodiments, X_{19a} is H. In certain embodiments, X_{19a} is R.

In certain embodiments, X_{1a} is F, L, or S; X_{2a} is D or G; X_{3a} is F or L; X_{4a} is N or D; X_{5a} is E or G; X_{6a} is L or S; X_{7a} is Y or H; X_{8a} is F or S; X_{9a} is F or S; X_{10a} is W, G, or R; X_{11a} is S,

G, or N; X_{12a} is Y or H; X_{13a} is V; X_{14a} is N or S; X_{15a} is V; X_{16a} is V or A; X_{17a} is L or I; X_{18a} is K or R; and X_{19a} is H or R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 4, wherein the variables are defined as indicated above. In some
 5 embodiments, the GCase polypeptide comprises SEQ ID NO: 4, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 4, wherein the variables are defined as indicated above.

In certain embodiments, X_{1a} is F; X_{2a} is D; X_{3a} is F; X_{4a} is N; X_{5a} is E or G; X_{6a} is L or S; X_{7a} is Y; X_{8a} is S; X_{9a} is F; X_{10a} is W; X_{11a} is S; X_{12a} is Y or H; X_{13a} is V; X_{14a} is N; X_{15a} is
 10 V; X_{16a} is V or A; X_{17a} is L; X_{18a} is K or R; and X_{19a} is H or R. In some embodiments, the GCase polypeptide comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 4, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 4, wherein the variables are defined as indicated above. In some embodiments, the GCase
 15 polypeptide consists of SEQ ID NO: 4, wherein the variables are defined as indicated above.

In certain embodiments, X_{1a} is F, L, or S; X_{2a} is D or G; X_{3a} is F or L; X_{4a} is N or D; X_{5a} is E or G; X_{6a} is L or S; X_{7a} is Y, H, D, or C; X_{8a} is F, L, or S; X_{9a} is F or S; X_{10a} is W, G, or R; X_{11a} is S or G; X_{12a} is Y or H; X_{13a} is V or A; X_{14a} is N or S; X_{15a} is V or A; X_{16a} is V or A; X_{17a} is L or I; X_{18a} is K or R; and X_{19a} is H or R. In some embodiments, the GCase polypeptide
 20 comprises a sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO: 4, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide comprises SEQ ID NO: 4, wherein the variables are defined as indicated above. In some embodiments, the GCase polypeptide consists of SEQ ID NO: 4, wherein the variables are defined as indicated above.

25 Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to SEQ ID NO: 5, wherein:

- a) X_{1b} is selected from the group consisting of: G and E;
- b) X_{2b} is selected from the group consisting of: L and S;
- 30 c) X_{3b} is S;
- d) X_{4b} is selected from the group consisting of Y and H;
- e) X_{5b} is selected from the group consisting of V and A;
- f) X_{6b} is selected from the group consisting of K and R; and
- g) X_{7b} is selected from the group consisting of H and R.

In certain embodiments, the amino acid sequence has at least about 80% identity to SEQ ID NO:5. In certain embodiments, the amino acid sequence has at least about 85% identity to SEQ ID NO:5. In certain embodiments, the amino acid sequence has at least about 90% identity to SEQ ID NO:5. In certain embodiments, the amino acid sequence has at least about 95% identity to SEQ ID NO:5. In certain embodiments, the amino acid sequence has at least about 96% identity to SEQ ID NO:5. In certain embodiments, the amino acid sequence has at least about 97% identity to SEQ ID NO:5. In certain embodiments, the amino acid sequence has at least about 98% identity to SEQ ID NO:5. In certain embodiments, the amino acid sequence has at least about 99% identity to SEQ ID NO:5. In certain embodiments, the GCCase polypeptide comprises SEQ ID NO:5. In certain embodiments, the GCCase polypeptide consists of SEQ ID NO:5.

In certain embodiments, X_{1b} is G. In certain embodiments, X_{1b} is E. In certain embodiments, X_{2b} is L. In certain embodiments, X_{2b} is S. In certain embodiments, X_{4b} is Y. In certain embodiments, X_{4b} is H. In certain embodiments, X_{5b} is V. In certain embodiments, X_{5b} is A. In certain embodiments, X_{6b} is K. In certain embodiments, X_{6b} is R. In certain embodiments, X_{7b} is H. In certain embodiments, X_{7b} is R.

In certain embodiments, the GCCase polypeptide comprises an amino acid sequence having X_{1b} - X_{7b} as defined above and having at least about at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to a sequence selected from the group consisting of: SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:36, and SEQ ID NO:39.

In certain embodiments, X_{1b} is G; X_{2b} is L; X_{3b} is S; X_{4b} is H; X_{5b} is V; X_{6b} is K; and X_{7b} is H. In certain embodiments, the GCCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:19.

In certain embodiments, X_{1b} is G; X_{2b} is L; X_{3b} is S; X_{4b} is Y; X_{5b} is V; X_{6b} is R; and X_{7b} is R. In certain embodiments, the GCCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:20.

In certain embodiments, X_{1b} is E; X_{2b} is S; X_{3b} is S; X_{4b} is H; X_{5b} is V; X_{6b} is K; and X_{7b} is R. In certain embodiments, the GCCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:27.

In certain embodiments, X_{1b} is E; X_{2b} is S; X_{3b} is S; X_{4b} is H; X_{5b} is V; X_{6b} is K; and X_{7b} is H. In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:28.

5 In certain embodiments, X_{1b} is E; X_{2b} is L; X_{3b} is S; X_{4b} is H; X_{5b} is A; X_{6b} is K; and X_{7b} is H. In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:36.

10 In certain embodiments, X_{1b} is E; X_{2b} is L; X_{3b} is S; X_{4b} is H; X_{5b} is V; X_{6b} is K; and X_{7b} is R. In certain embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity) to SEQ ID NO:39.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 15 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:6; SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, and SEQ ID NO:45. In certain embodiments, the GCase polypeptide comprises a sequence recited above. In certain embodiments, the GCase polypeptide consists of a sequence recited above.

25 In embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a sequence selected from the group consisting of: SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:36, and SEQ ID NO:39.

In embodiments, the GCase polypeptide comprises an amino acid sequence having at 30 least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO: 19. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 19. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 19.

In embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%

identity) to SEQ ID NO: 20. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 20. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 20.

In embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO: 27. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 27. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 27.

In embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO: 28. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 28. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 28.

In embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO: 36. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 36. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 36.

In embodiments, the GCase polypeptide comprises an amino acid sequence having at least about 80% identity (*e.g.*, at least about 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity) to SEQ ID NO: 39. In certain embodiments, the GCase polypeptide comprises SEQ ID NO: 39. In certain embodiments, the GCase polypeptide consists of SEQ ID NO: 39.

Certain embodiments provide a GCase polypeptide comprising an amino acid sequence having at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity to a sequence selected from the group consisting of: SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78. In certain embodiments, the amino acid sequence has at least about 80% identity to a sequence recited above. In certain embodiments, the amino acid sequence has at least about 85% identity to a sequence recited above. In certain embodiments, the amino acid sequence has at least about 90% identity to a sequence recited above. In certain embodiments, the amino acid sequence has at least about 95% identity to a sequence recited above. In certain embodiments, the amino acid sequence has at least about 96% identity to a sequence recited above. In certain embodiments, the amino acid sequence has at least about 97% identity to a sequence recited above. In certain embodiments, the amino acid sequence has at

least about 98% identity to a sequence recited above. In certain embodiments, the amino acid sequence has at least about 99% identity to a sequence recited above. In certain embodiments, the GCase polypeptide comprises a sequence recited above. In certain embodiments, the GCase polypeptide consists of a sequence recited above.

5

IMPROVED STABILITY OF MODIFIED GCASE POLYPEPTIDES

As described herein, modified GCase polypeptides having improved stability relative to a reference GCase polypeptide (e.g., a wild-type GCase, such as SEQ ID NO:1, or to a baseline reference GCase variant, such as SEQ ID NO:20) are provided. As used herein, “increased stability” may be indicated by greater enzymatic activity relative to a reference GCase polypeptide, greater conformational stability (*i.e.*, the ability to maintain its native folded conformation) relative to a reference GCase polypeptide, or both. Methods for evaluating enzymatic activity and conformational stability are known in the art and described herein (*see, e.g.*, the Examples). In certain embodiments, a modified GCase polypeptide as described herein has improved/greater stability in the presence of serum. In certain embodiments, a modified GCase polypeptide as described herein has improved/greater stability at physiological conditions. Physiological conditions are typically at neutral pH (*e.g.*, about pH 6.8 to 7.4, or about pH 7 to 7.2) and temperatures of about 37°C. In some embodiments, physiological conditions include the presence of serum. Exposures to physiological conditions to assess GCase polypeptide stability can range from, e.g., about 30 minutes to about 48 hours in duration, as described herein.

Accordingly, in certain embodiments, a modified GCase polypeptide has equivalent or improved conformational stability relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide has equivalent conformational stability relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide has improved conformational stability (e.g., by at least about 1.1-fold, 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold or more) relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide has at least about 75%, 80%, 85%, 87%, 90%, 95%, 96%, 98%, or 99% enzymatic activity, relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide has at least about 75%, 80%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, or 99% enzymatic activity, relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide as described herein has equivalent

enzymatic activity relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide as described herein has increased enzymatic activity (e.g., by at least about 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 25-fold, 50-fold, 100-fold or more) relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20).

In some embodiments, the modified GCase polypeptides have increased enzymatic activity relative to that of a reference GCase polypeptide (SEQ ID NO:1 or SEQ ID NO:20) under physiological conditions. In some embodiments, the modified GCase polypeptides have equivalent or improved conformational stability (*i.e.* are able to maintain their native folded conformation to the same or greater extent) relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20) under physiological conditions. In some embodiments, under physiological conditions, the modified GCase polypeptides (i) have increased enzymatic activity relative to that of a reference GCase polypeptide (SEQ ID NO:1), and (ii) have improved conformational stability relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:1). In some embodiments, under physiological conditions, the modified GCase polypeptides (i) have increased enzymatic activity relative to that of a reference GCase polypeptide (SEQ ID NO:20), and (ii) have equivalent or improved conformational stability relative to that of a reference GCase variant (e.g., SEQ ID NO:20).

To assess enzymatic activity or conformational stability, an assay described herein or known in the art may be used. For example, assays for evaluating enzymatic activity include, *e.g.*, detection with a probe cap surrogate for GCase activity, ELISA methods involving a fluorogenic substrate, and GCase substrate correction assays (*e.g.*, *in vitro* glucosylceramide or glucosylsphingosine assays). For example, a fluorescent cyclophellitol-type activity-based probe (e.g., MDW933 or MDW941) which covalently reacts with a residue in the active site of GCase may be used in conjunction with FACs analysis to evaluate GCase activity (*see, e.g.*, Examples 4, 6, and 8). Alternatively, assays that measure activity *in vitro* using a fluorogenic artificial substrate, such as those described in the Examples section (e.g., 4-Methylumbelliferyl β -D-glucopyranoside as described in Example 6), may also be used. In some embodiments, GCase substrate correction assays can be used, *e.g.*, by detecting a correction in the levels of one or both of glucosylsphingosine and glucosylceramide in a *GBA* knockout cell or cell line (*e.g.* *GBA* knock-out HEK293 cells). Mass spectrometry-based methods for measuring glucosylsphingosine and glucosylceramide are described in, *e.g.*, Merrill, Jr. *et al.* 2005. *Methods* 36(2):207-224. Additional methods for measuring glucosylsphingosine and glucosylceramide are known in the art. Thus, in certain embodiments, enzymatic activity is

measured by detection with a fluorescent cyclophellitol-type activity-based probe and evaluation with FACS. In certain embodiments, the fluorescent cyclophellitol-type activity-based probe is selected from the group consisting of: MDW933, MDW941, and 2-deoxy-2-fluoro- β -d-glucopyranosyl-*N*-phenyltrifluoroacetimidate. In certain embodiments, enzymatic activity is measured by an *in vitro* reaction with 4-MUG (4-Methylumbelliferyl β -D-glucopyranoside) substrate. Exemplary assays for evaluating conformational stability include, e.g., ELISA detection methods, FACS analysis, circular dichroism, differential scanning calorimetry, and differential scanning fluorimetry. For example, the use of a GCase antibody to detect a test modified polypeptide (e.g., assayed by fluorescence-based assay, such as FACS or ELISA) may be used as an indicator of proper protein conformation or folding (*see, e.g.*, Examples 3, 6, 8). Thus, in certain embodiments, conformational stability is measured using a fluorescence-based assay using an anti-GCase antibody, circular dichroism, or differential scanning calorimetry. In certain embodiments, the fluorescence-based assay is an ELISA assay, a FACS assay, or a differential scanning fluorimetry assay. When comparing conformational stability and/or enzymatic activity levels, a modified GCase polypeptide being tested and a reference GCase (e.g., SEQ ID NO:1 or SEQ ID NO:20) are assayed under identical conditions. The Examples provided herein are not limiting; other methods for measuring conformational stability and enzymatic activity are known in the art and can be used for the purposes described herein.

In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum from a mammal, such as mouse, rabbit, monkey, or human serum. In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of mouse serum. In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of human serum.

In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, a serum concentration ranging from about 10% to about 100% (v/v) in a suitable buffer at neutral pH (*e.g.*, PBST). In certain embodiments, the serum concentration (v/v) is about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%. In certain embodiments, the serum concentration is about 50% (v/v). In certain embodiments, the serum concentration is about 80% (v/v). In certain embodiments, the serum concentration is about 100% (v/v).

In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum for at least about 30 minutes, such as for between about 30 minutes to about 48 hours (e.g., 30, 60, 90, or 120 minutes; or 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, 24, 36, or 48 hours). In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 30 minutes or longer before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 60 minutes or longer before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 90 minutes or longer before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 4 hours or longer before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 6 hours or longer before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 12 hours before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 18 hours before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 24 hours before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 36 hours before the stability of the modified GCase polypeptide is evaluated. In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum for about 48 hours before the stability of the modified GCase polypeptide is evaluated.

In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum at a temperature of about room temperature to about 37°C. For example, in certain embodiments, a modified GCase polypeptide as described herein is contacted with serum at a temperature ranging from about 20°C to about 37°C (e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36 or 37°C). In certain embodiments, a modified GCase polypeptide as described herein is contacted with serum at room temperature (*i.e.*, about 20°C to about 22°C). In certain embodiments, a modified GCase

polypeptide as described herein is contacted with serum at about 30°C. In certain embodiments, a modified GCCase polypeptide as described herein is contacted with serum at about 37°C.

In certain embodiments, a modified GCCase polypeptide as described herein has improved stability relative to a reference GCCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum at physiological conditions (e.g., at about 37°C, about pH 7-7.2).

In certain embodiments, a modified GCCase polypeptide as described herein has improved stability relative to a reference GCCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum having a concentration of about 10% (v/v) for about 30-90 minutes (e.g., 30, 60 or 90 min) at room temperature. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

In certain embodiments, a modified GCCase polypeptide as described herein has improved stability relative to a reference GCCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum having a concentration of about 10% (v/v) for about 30-90 minutes (e.g., 30, 60 or 90 min) at about 37°C. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

In certain embodiments, a modified GCCase polypeptide as described herein has improved stability relative to a reference GCCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum having a concentration of about 25% (v/v) for about 30-90 minutes (e.g., 30, 60 or 90 min) at room temperature. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

In certain embodiments, a modified GCCase polypeptide as described herein has improved stability relative to a reference GCCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum having a concentration of about 25% (v/v) for about 30-90 minutes (e.g., 30, 60 or 90 min) at about 37°C. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

In certain embodiments, a modified GCCase polypeptide as described herein has improved stability relative to a reference GCCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum having a concentration of about 50% (v/v) for about 30-90 minutes (e.g., 30, 60 or 90 min) at room temperature. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

In certain embodiments, a modified GCCase polypeptide as described herein has improved stability relative to a reference GCCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in

the presence of, or after being contacted with, serum having a concentration of about 50% (v/v) for about 30-90 minutes (e.g., 30, 60 or 90 min) at about 37°C. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

5 In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum having a concentration of about 50% (v/v) for about 4-6 hours (e.g., 6 hours) at room temperature. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

10 In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, serum having a concentration of about 50% (v/v) for about 4-6 hours (e.g., 6 hours) at about 37°C. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

15 In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, about 80% to 100% (v/v) serum (e.g., 100% (v/v) serum) for about 4-6 hours (e.g., 6 hours) at about 37°C. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

20 In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, about 80% to 100% (v/v) serum (e.g., 100% (v/v) serum) for about 12-24 hours (e.g., 12, 18 or 24 hours) at about 30°C. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

25 In certain embodiments, a modified GCase polypeptide as described herein has improved stability relative to a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20), in the presence of, or after being contacted with, about 80% to 100% (v/v) serum (e.g., 100% (v/v) serum) for about 12-24 hours (e.g., 12, 18 or 24 hours) hours at about 37°C. In some embodiments, the serum is mouse serum. In some embodiments, the serum is human serum.

30 In certain embodiments, a modified GCase polypeptide as described herein has at least about 75%, 80%, 85%, 87%, 90%, 95%, 96%, 98%, or 99% enzymatic activity, relative to a reference GCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide as described herein has at least about 75%, 80%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, or 99% enzymatic activity, relative to a reference GCase polypeptide (e.g., SEQ ID

NO:20). In certain embodiments, a modified GCase polypeptide as described herein has equivalent enzymatic activity relative to that of a reference GCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCase polypeptide as described herein has increased enzymatic activity relative to that of a reference GCase polypeptide (SEQ ID NO:1 or SEQ ID NO:20). In certain embodiments, the enzymatic activity is increased by at least about 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 25-fold, 50-fold, 100-fold or more, relative to that of SEQ ID NO:1. In certain embodiments, the enzymatic activity is increased by at least about 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold or more, relative to that of SEQ ID NO:20. In certain embodiments, enzymatic activity is measured at neutral pH and room temperature. In certain embodiments, enzymatic activity is measured under physiological conditions (*e.g.* neutral pH and about 37°C). In certain embodiments, enzymatic activity is measured in the presence of, or after the polypeptide is contacted with, serum (*e.g.*, a set of serum conditions described above). In certain embodiments, enzymatic activity is measured in the presence of, or after the polypeptide is contacted with, serum at physiological conditions. In certain embodiments, the modified GCase polypeptides retains enzymatic activity better than a reference GCase polypeptide (e.g., SEQ ID NO:1 or SEQ ID NO:20) under physiological conditions. For example, the modified GCase polypeptides are able to retain at least about 25% of their baseline enzymatic activity under physiological conditions. In certain embodiments, a modified GCase polypeptide as described herein retains at least about 25%, 30%, 35%, 40%, 45%, or 50% of its baseline enzymatic activity under physiological conditions (*e.g.*, neutral pH and about 37°C). In certain embodiments, a modified GCase polypeptide as described herein retains at least about 25%, 30%, 35%, 40%, 45%, or 50% of its baseline enzymatic activity in the presence of, or after the polypeptide is contacted with, serum at physiological conditions. In the foregoing embodiments, baseline enzymatic activity is measured at t=0 (at initial exposure) or immediately prior to exposure to physiological conditions or to serum at physiological conditions.

In certain embodiments, a modified GCase polypeptide as described herein has improved conformational stability of at least about 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold or more, relative to that of a reference GCase (e.g., SEQ ID NO:1), at physiological conditions. In certain embodiments, a modified GCase polypeptide as described herein has equivalent or improved conformational stability of at least about 1-fold, 1.1-fold, 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold or more, relative to that of a baseline reference GCase variant (e.g., SEQ ID NO:20), at physiological

conditions. The conformational stability can be measured, *e.g.* via detection with an antibody-based assay such as ELISA or FACS.

In certain embodiments, a modified GCCase polypeptide as described herein has improved conformational stability of at least about 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold or more, relative to that of a reference GCCase polypeptide (e.g., SEQ ID NO:1) and has increased enzymatic activity relative to that of SEQ ID NO:1. In certain embodiments, the enzymatic activity is increased by at least about 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 25-fold, 50-fold, 100-fold or more, relative to that of SEQ ID NO:1. In certain embodiments, the improved conformational stability and increased enzymatic activity are observed at neutral pH and room temperature. In certain embodiments, the improved conformational stability and increased enzymatic activity are observed under physiological conditions, in the presence or absence of serum (*e.g.*, in the presence of serum, such as under serum conditions described herein).

In certain embodiments, a modified GCCase polypeptide as described herein has equivalent or improved conformational stability of at least about 1-fold, 1.1-fold, 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold or more, relative to that of a reference GCCase polypeptide (e.g., SEQ ID NO:20) and has at least about 75%, 80%, 85%, 87%, 90%, 95%, 96%, 98%, or 99% enzymatic activity, relative to a reference GCCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCCase polypeptide as described herein has equivalent or improved conformational stability of at least about 1-fold, 1.1-fold, 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold or more, relative to that of a reference GCCase polypeptide (e.g., SEQ ID NO:20) and has at least about 75%, 80%, 85%, 87%, 90%, 95%, 96%, 97%, 98%, or 99% enzymatic activity, relative to a reference GCCase polypeptide (e.g., SEQ ID NO:20). In certain embodiments, a modified GCCase polypeptide as described herein has equivalent or improved conformational stability of at least about 1-fold, 1.1-fold, 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold or more, relative to that of a reference GCCase polypeptide (e.g., SEQ ID NO:20) and has equivalent enzymatic activity relative to that of a reference GCCase variant (e.g., SEQ ID NO:20). In certain embodiments, a modified GCCase polypeptide as described herein has equivalent or improved conformational stability of at least about 1-fold, 1.1-fold, 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold or more, relative to that of a reference GCCase (e.g., SEQ ID NO:20) and has increased enzymatic activity relative to that of SEQ ID NO:20. In certain embodiments, the enzymatic activity is increased by at least about 1.25-fold, 1.5-fold, 1.75-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold,

9-fold, 10-fold, 20-fold or more, relative to that of SEQ ID NO:20. In certain embodiments, the maintained or improved conformational stability and increased enzymatic activity are observed at neutral pH and room temperature. In certain embodiments, the maintained or improved conformational stability and increased enzymatic activity are observed under physiological
5 conditions, in the presence or absence of serum (*e.g.*, in the presence of serum, such as under serum conditions described herein).

PHARMACEUTICAL COMPOSITIONS AND KITS

In other aspects, pharmaceutical compositions and kits comprising a modified GCase
10 polypeptide described herein are provided.

Pharmaceutical Compositions

Guidance for preparing formulations for use in the present disclosure can be found in any number of handbooks for pharmaceutical preparation and formulation that are known to those of skill in the art.

15 In some embodiments, a pharmaceutical composition comprises a GCase polypeptide as described herein and further comprises one or more pharmaceutically acceptable carriers and/or one or more pharmaceutically acceptable excipients. In certain embodiments the pharmaceutical composition comprises one or more pharmaceutically acceptable carriers. In certain embodiments the pharmaceutical composition comprises one or more pharmaceutically
20 acceptable excipients. In certain embodiments the pharmaceutical composition comprises one or more pharmaceutically acceptable carriers and one or more pharmaceutically acceptable excipients. A pharmaceutically acceptable carrier includes any solvents, dispersion media, or coatings that are physiologically compatible and that do not interfere with or otherwise inhibit the activity of the active agent.

25 Dosages and desired drug concentration of pharmaceutical compositions described herein may vary depending on the particular use envisioned.

Kits

In some embodiments, a kit for use in treating a GCase deficiency, comprising a GCase polypeptide as described herein, is provided.

30 In some embodiments, the kit further comprises one or more additional therapeutic agents. For example, in some embodiments, the kit comprises a GCase polypeptide as described herein and further comprises one or more additional therapeutic agents for use in the treatment of one or more symptoms of GCase deficiency (*e.g.*, one or more symptoms associated with Gaucher's disease, Parkinson's disease, Parkinsonism, or dementia with Lewy Bodies). In some

embodiments, the kit further comprises instructional materials containing directions (*i.e.*, protocols) for the practice of the methods described herein (*e.g.*, instructions for using the kit for administering a GCase polypeptide). While the instructional materials typically comprise written or printed materials, they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to, electronic storage media (*e.g.*, magnetic discs, tapes, cartridges, chips), optical media (*e.g.*, CD-ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

10 NUCLEIC ACIDS, VECTORS, AND HOST CELLS

A GCase polypeptide as described herein is typically prepared using recombinant methods. Accordingly, in some aspects, the present disclosure provides an isolated polynucleotide comprising a nucleic acid sequence encoding a GCase polypeptide as described herein, and a host cell into which the polynucleotide is introduced that are used to replicate the polypeptide-encoding nucleic acid and/or to express the polypeptide. In some embodiments, the host cell is eukaryotic, *e.g.*, a human cell.

The polynucleotide may be single-stranded or double-stranded. In some embodiments, the polynucleotide is DNA. In particular embodiments, the polynucleotide is cDNA. In some embodiments, the polynucleotide is RNA.

20 In some embodiments, the polynucleotide is included within a nucleic acid construct. In some embodiments, the construct is a replicable vector. In some embodiments, the vector is selected from a plasmid, a viral vector, a phagemid, a yeast chromosomal vector, and a non-episomal mammalian vector.

In some embodiments, the polynucleotide is operably linked to one or more regulatory nucleotide sequences in an expression construct. In one series of embodiments, nucleic acid expression constructs are adapted for use as a surface expression library. In some embodiments, the library is adapted for surface expression in yeast. In some embodiments, the library is adapted for surface expression in phage. In another series of embodiments, the nucleic acid expression construct is adapted for expression of the polypeptide in a system that permits isolation of the polypeptide in milligram or gram quantities. In some embodiments, the system is a mammalian cell expression system. In some embodiments, the system is a yeast cell expression system.

Expression vehicles for production of a recombinant polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the following types: pBR322-

derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids, and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo, and pHyg-derived vectors are examples of mammalian expression
5 vectors suitable for transfection of eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived, and p205) can be used for transient expression of polypeptides in eukaryotic cells. In some embodiments, it may be desirable to express the recombinant polypeptide by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived
10 vectors (such as pVL1392, pVL1393, and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors. Additional expression systems include adenoviral, adeno-associated virus, and other viral expression systems.

Vectors may be transformed into any suitable host cell. In some embodiments, the host cells, *e.g.*, bacteria or yeast cells, may be adapted for use as a surface expression library. In
15 some cells, the vectors are expressed in host cells to express relatively large quantities of the polypeptide. Such host cells include mammalian cells, yeast cells, insect cells, and prokaryotic cells. In some embodiments, the cells are mammalian cells, such as Chinese Hamster Ovary (CHO) cell, baby hamster kidney (BHK) cell, NS0 cell, Y0 cell, HEK293 cell, COS cell, Vero cell, or HeLa cell.

20 Thus, certain embodiments provide a method for producing a GCase polypeptide, comprising culturing a host cell under conditions in which a GCase polypeptide encoded by a polynucleotide as described herein is expressed. For example, a host cell transfected with an expression vector encoding a GCase polypeptide as described herein can be cultured under appropriate conditions to allow expression of the polypeptide to occur. The polypeptide may be
25 secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, the polypeptide may be retained in the cytoplasm or in a membrane fraction and the cells harvested, lysed, and the polypeptide isolated using a desired method.

CERTAIN METHODS FOR GENERATING MODIFIED GCASE POLYPEPTIDES

30 As described in the Examples, modified GCase polypeptides may be made by identifying regions within the GCase polypeptide having structural differences between unbound- and isofagomine- (IFG-bound) GCase and then modifying position(s) present within those regions. The regions exhibiting structural differences may maintain direct contact (*e.g.*, have hydrogen-bonding interactions) with IFG during binding, or they may be sequentially or conformationally

adjacent to those regions in direct contact with IFG during binding. For example, regions that undergo: 1) strong perturbations upon IFG binding ($\geq 9\%$ decrease in average deuteration level as measured by H/D exchange protocol) that also make direct contact with IFG (amino acid numbers 119-127, 177-184, 230-240, and 386-400 of SEQ ID NO:1); 2) strong perturbations upon IFG binding that are sequentially or conformationally adjacent to regions that are hydrogen bound to IFG (amino acid numbers 187-197, 243-249, 310-312, 343-347, and 414-417 of SEQ ID NO:1); and/or 3) intermediate perturbations upon IFG binding (about 5-8% decrease in average deuteration level) as measured by H/D exchange protocol that are sequentially or conformationally adjacent to regions that are hydrogen bound to IFG (amino acid numbers 130-134, 284-296, 315-336, and 350-362 of SEQ ID NO:1), may be selected (*see*, Kornhaber, et al. 2008. *Chembiochem* 9(16):2643-2649; and Hamuro et al. 2003. *J Biomol Tech* 14:171).

Accordingly, certain embodiments provide a method of modifying a GCase polypeptide, wherein the method comprises modifying SEQ ID NO:1 to include a residue change at two or more positions (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more positions) in regions of SEQ ID NO:1 that undergo structural alteration upon binding of SEQ ID NO:1 to isofagomine. Certain embodiments also provide a method of generating a library of nucleic acid sequences encoding modified GCase polypeptides, comprising modifying a plurality of nucleic acids encoding SEQ ID NO:1 to change a residue at two or more positions (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more positions) in regions of SEQ ID NO:1 that undergo structural alteration upon binding of SEQ ID NO:1 to isofagomine. As such, the nucleic acid sequences in the plurality are each independently modified. In certain embodiments, the two or more positions are in regions of SEQ ID NO:1 selected from the group consisting of positions: 119-127, 130-134, 177-184, 187-197, 230-240, 243-249, 284-296, 310-312, 315-336, 343-347, 350-362, 386-400, and 414-417.

Other embodiments provide a method of making a modified GCase polypeptide, wherein the method comprises:

- (i) modifying a nucleic acid sequence encoding SEQ ID NO:1 to change a residue at two or more positions in regions of SEQ ID NO:1 selected from the group consisting of positions: 119-127, 130-134, 177-184, 187-197, 230-240, 243-249, 284-296, 310-312, 315-336, 343-347, 350-362, 386-400, and 414-417;
 - (ii) transforming a host cell with the modified nucleic acid sequence;
 - (iii) culturing the host cell to express the polypeptide encoded by the modified nucleic acid sequence; and
 - (iv) isolating the polypeptide from the host cell culture;
- thereby making the modified GCase polypeptide.

In certain embodiments, the method comprises a residue change at two positions in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at more than two positions in SEQ ID NO:1, such as 3, 4, 5, 6, 7, 8, 9, 10 or more residue changes in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at three or more
5 positions in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at four or more positions in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at five or more positions in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at six or more positions in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at seven or more positions in SEQ ID NO:1. In certain
10 embodiments, the method comprises a residue change at eight or more positions in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at nine or more positions in SEQ ID NO:1. In certain embodiments, the method comprises a residue change at ten or more positions in SEQ ID NO:1.

In certain embodiments, one or more residue(s) are changed within the region of
15 positions 119-127 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 119, 120, 121, 122, 123, 124, 125, 126, and/or 127).

In certain embodiments, one or more residue(s) are changed within the region of positions 130-134 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 130, 131, 132, 133, and/or 134).

20 In certain embodiments, one or more residue(s) are changed within the region of positions 177-184 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 177, 178, 179, 180, 181, 182, 183, and/or 184).

In certain embodiments, one or more residue(s) are changed within the region of positions 187-197 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 187, 188,
25 189, 190, 191, 192, 193, 194, 195, 196, and/or 197).

In certain embodiments, one or more residue(s) are changed within the region of positions 230-240 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, and/or 240).

In certain embodiments, one or more residue(s) are changed within the region of
30 positions 243-249 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 243, 244, 245, 246, 247, 248, and/or 249).

In certain embodiments, one or more residue(s) are changed within the region of positions 284-296 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, and/or 296).

In certain embodiments, one or more residue(s) are changed within the region of positions 310-312 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 310, 311, and/or 312).

5 In certain embodiments, one or more residue(s) are changed within the region of positions 315-336 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335 and/or 336).

10 In certain embodiments, one or more residue(s) are changed within the region of positions 343-347 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 343, 344, 345, 346, and/or 347).

In certain embodiments, one or more residue(s) are changed within the region of positions 350-362 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, and/or 362).

15 In certain embodiments, one or more residue(s) are changed within the region of positions 386-400 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, and/or 400).

In certain embodiments, one or more residue(s) are changed within the region of positions 414-417 (e.g., a residue is changed with respect to SEQ ID NO:1 at position 414, 415, 416, and/or 417).

20 In certain embodiments, evaluation of one or more GCase orthologs may be used to select positions within the regions. For example, the sequence of wild-type human GCase (SEQ ID NO:1) may be compared to one or more ortholog sequences (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more sequences) to identify positions having non-conserved residues. In certain
25 embodiments, more than one ortholog sequence is selected, wherein the sequences are from more than one species. In certain embodiments, the GCase ortholog sequence(s) are mammalian in origin. In certain embodiments, the GCase ortholog sequence(s) are non-mammalian in origin. In certain embodiments, the GCase ortholog sequences are mammalian and non-mammalian in origin. Non-limiting examples from which GCase ortholog sequences may be
30 selected include: non-human primates (e.g., monkeys), rodents (e.g., mice), rabbits, collared flycatchers, coelacanths, alligators, finches, lampreys, foxes, bottlenose dolphins, sperm whales, sheep, yaks, and sea otters. In certain embodiments, an ortholog sequence selected for comparison has less than about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91% or 90% identity to SEQ ID NO:1. In certain embodiments, an ortholog sequence selected for comparison has greater than about 45%, 50%, 55%, 60%, 65% or 70% identity to SEQ ID NO:1.

In certain embodiments, an ortholog sequence selected for comparison has less than about 98% identity to SEQ ID NO:1 and greater than about 60% identity to SEQ ID NO:1.

Secondary structure may also be evaluated when selecting the two or more positions. For example, positions found in loop and alpha helical regions may be selected. In certain
5 embodiments, at least one position of the two or more positions is present in a loop or alpha helical region. In certain embodiments, at least one position of the two or more positions is present in a loop region. In certain embodiments, at least one position of the two or more positions is present in an alpha helical region. In certain embodiments, the two or more positions are located in loop or alpha helical regions.

10 Accordingly, in certain embodiments, the two or more positions in SEQ ID NO:1 are selected by:

(i) carrying out one or more sequence alignments between SEQ ID NO:1 and a sequence ortholog(s) to identify a set of positions within the defined regions described above (*e.g.*, regions of SEQ ID NO:1 consisting of positions: 119-127, 130-134, 177-184, 187-197, 230-240, 243-
15 249, 284-296, 310-312, 315-336, 343-347, 350-362, 386-400, and 414-417) at locations where residues are not conserved; and

(ii) selecting two or more positions from the identified set of positions.

In certain embodiments, the two or more positions in SEQ ID NO:1 1) are within the defined regions at locations where residues are not conserved; and 2) are present in loop or alpha
20 helical regions.

In certain embodiments, at least one residue change is a substitution with an ortholog sequence residue that is non-conserved at that position, or a conservative substitution thereof. In certain embodiments, the residue change at the two or more positions is a substitution with an ortholog sequence residue that is non-conserved at that position, or a conservative substitution
25 thereof.

In certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is in a region that undergoes strong perturbations upon IFG binding ($\geq 9\%$ decrease in average deuteration level as measured by H/D exchange protocol) that also makes direct contact with IFG. In certain embodiments, the two or more positions in SEQ ID NO:1 are in regions that
30 undergo strong perturbations upon IFG binding ($\geq 9\%$ decrease in average deuteration level as measured by H/D exchange protocol) that also make direct contact with IFG.

Thus, in certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is in a region of SEQ ID NO:1 selected from the group consisting of: positions 119-127, 177-184, 230-240, and 386-400 of SEQ ID NO:1. In certain embodiments, the two or more positions

in SEQ ID NO:1 are in regions of SEQ ID NO:1 selected from the group consisting of: positions 119-127, 177-184, 230-240, and 386-400 of SEQ ID NO:1.

In certain embodiments, at least one of the two or more positions in SEQ ID NO:1 are selected from the group consisting of: 124, 125, 126, 181, 230, 232, 233, 237, 238, 239, 240, 386, 387, and 388. In certain embodiments, the two or more positions in SEQ ID NO:1 are selected from the group consisting of: 124, 125, 126, 181, 230, 232, 233, 237, 238, 239, 240, 386, 387, and 388.

In certain embodiments, the position(s) and residue change(s) are based on the Library 1 design described in Table 5 of Example 5. In particular, in certain embodiments, the residue changes at the two or more positions are selected from the group consisting of:

- (i) substitution at position 124 with Thr;
- (ii) substitution at position 125 with Cys, Gly, or Ala;
- (iii) substitution at position 126 with Ser or Thr;
- (iv) substitution at position 181 with Ala;
- (v) substitution at position 230 with Leu;
- (vi) substitution at position 232 with Ser, Thr, or Gly;
- (vii) substitution at position 233 with Gly, Gln, or Arg;
- (viii) substitution at position 237 with Arg, Thr, Leu, Pro, or Ile;
- (ix) substitution at position 238 with Asp, Thr, or Asn;
- (x) substitution at position 239 with Arg;
- (xi) substitution at position 240 with Glu, Phe, Met, Asn, Gln, Val, Tyr, Lys, Ile, His, or Asp;
- (xii) substitution at position 386 with Asp;
- (xiii) substitution at position 387 with Leu, Met, or Thr; and
- (xiv) substitution at position 388 with Lys.

In certain embodiments, the method comprises a residue change at three, four, five, six, seven, eight, or nine or more positions. In certain embodiments, the method comprises a residue change at three, four, five, six, seven, eight, nine, or ten or more positions.

In certain embodiments, the method further comprises a residue change at position 316, 425 and/or 451. In certain embodiments, the method further comprises residue change F316S, K425R and/or H451R. In certain embodiments, the method comprises a residue change at positions 316, 425 and 451 (e.g., F316S, K425R and H451R).

In certain embodiments the modified GCase polypeptide produced by a method described herein comprises a set of residues changes, with respect to SEQ ID NO:1, as described herein, such as in Table 7a.

5 In certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is in a region that undergoes strong perturbations upon IFG binding that are sequentially or conformationally adjacent to regions that are hydrogen bound to IFG. In certain embodiments, the two or more positions in SEQ ID NO:1 are in regions that undergo strong perturbations upon IFG binding that are sequentially or conformationally adjacent to regions that are hydrogen bound to IFG.

10 Thus, in certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is in a region of SEQ ID NO:1 selected from the group consisting of: positions 187-197, 243-249, 310-312, 343-347, and 414-417 of SEQ ID NO:1. In certain embodiments, the two or more positions in SEQ ID NO:1 are in regions of SEQ ID NO:1 selected from the group consisting of: positions 187-197, 243-249, 310-312, 343-347, and 414-417 of SEQ ID NO:1.

15 In certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is selected from the group consisting of: 189, 190, 191, 192, 343, 345, 346, and 347. In certain embodiments, the two or more positions in SEQ ID NO:1 are selected from the group consisting of: 189, 190, 191, 192, 343, 345, 346, and 347.

20 In certain embodiments, the position(s) and residue change(s) are based on the Library 2 design described in Table 8 of Example 7. In particular, in certain embodiments, the residue changes at the two or more positions are selected from the group consisting of:

- (i) substitution at position 189 with Glu, Arg, or Gln;
- (ii) substitution at position 190 with Asp, Ser, Arg, His, Asn, Gly, Pro, or Thr;
- (iii) substitution at position 191 with Ala, Phe, Gly, Leu, Met, Val, Tyr, Arg, Asn, Asp, Cys, Glu, Ile, Lys, Ser, Thr, or Trp;
- (iv) substitution at position 192 with Ile, Lys, Arg, Thr, Val, Ala, Asp, Glu, Gly, Met, or Ser;
- (v) substitution at position 343 with Ala, Ile, Leu, Asn, Thr, His, Pro, or Asp;
- (vi) substitution at position 345 with Phe, Thr, or Ile;
- (vii) substitution at position 346 with Glu, Phe, His, Leu, Asn, Tyr, Val, Ile, Gln, or Asp;
- 30 and
- (viii) substitution at position 347 with Met, Ser, Pro, Thr, Ile, or Leu.

In certain embodiments, the method comprises a residue change at three, four, five, six, seven, or eight, or more positions. In certain embodiments, the method comprises a residue

change at three, four, five, six, seven, eight, or nine, or more positions.

In certain embodiments, the method further comprises a residue change at position 233, 316, 425 and/or 451. In certain embodiments, the method further comprises residue change E233G, F316S, K425R and/or H451R. In certain embodiments, the method further comprises a
5 residue change at positions 233, 316, 425 and 451 (e.g., E233G, F316S, K425R and H451R).

In certain other embodiments, the method comprises a residue change at position 343 and/or 347, and optionally further comprises a residue change at position 233. In certain embodiments, the method comprises a residue change of V343T/A/N and/or F347P/S/T, and optionally further comprises E233G. In certain embodiments, the method comprises a residue
10 change at positions 233, 343, and 347 (e.g., E233G, V343T/A/N, and/or F347P/S/T).

In certain embodiments the modified GCase polypeptide produced by a method described herein comprises a set of residues changes, with respect to SEQ ID NO:1, as described herein, such as in Table 9a.

In certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is in a
15 region that undergoes intermediate perturbations upon IFG binding (about 5-8% decrease in average deuteration level) as measured by H/D exchange protocol that are sequentially or conformationally adjacent to regions that are hydrogen bound to IFG. In certain embodiments, the two or more positions in SEQ ID NO:1 are in regions that undergo intermediate perturbations upon IFG binding (about 5-8% decrease in average deuteration level) as measured
20 by H/D exchange protocol that are sequentially or conformationally adjacent to regions that are hydrogen bound to IFG.

Thus, in certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is in a region of SEQ ID NO:1 selected from the group consisting of: positions 130-134, 284-296, 315-336, and 350-362 of SEQ ID NO:1. In certain embodiments, the two or more positions
25 in SEQ ID NO:1 are in regions of SEQ ID NO:1 selected from the group consisting of: positions 130-134, 284-296, 315-336, and 350-362 of SEQ ID NO:1.

In certain embodiments, at least one of the two or more positions in SEQ ID NO:1 is selected from the group consisting of: 316, 317, 318, 319, 320, 321, 322, 325, 329, 350, 351, 353, 361, and 362. In certain embodiments, the two or more positions in SEQ ID NO:1 is
30 selected from the group consisting of: 316, 317, 318, 319, 320, 321, 322, 325, 329, 350, 351, 353, 361, and 362.

In certain embodiments, the position(s) and residue change(s) are based on the Library 3 design described in Table 10 of Example 9. In particular, in certain embodiments, the residue changes at the two or more positions are selected from the group consisting of:

- (i) substitution at position 316 with Gly, Leu, Cys, Trp, or Val;
- (ii) substitution at position 317 with Ile or Phe;
- (iii) substitution at position 318 with Val, Gly, Ile, Ser, or Thr;
- (iv) substitution at position 319 with Ser;
- 5 (v) substitution at position 320 with Ile, Pro, Leu, Thr, or Val;
- (vi) substitution at position 321 with Asp, Glu, Gly, Asn, Gln, Arg, Thr, Pro, Ser, His, or Ala;
- (vii) substitution at position 322 with Cys, Asp, Leu, Pro, Arg, Ser, Gly, His, Phe, Tyr, or Val;
- 10 (viii) substitution at position 325 with Glu, Leu, Ser, Val, Trp, or Ala;
- (ix) substitution at position 329 with Glu, His, Lys, Asn, Gln, Asp, Gly, or Ser;
- (x) substitution at position 350 with Phe, Lys, Asn, Pro, Arg, Cys, His, Ile, Leu, Met, Ser, Thr, Trp, or Tyr;
- (xi) substitution at position 351 with Ala, Cys, Asp, Gly, Arg, His, or Pro;
- 15 (xii) substitution at position 353 with Ala, Ile, Leu, Gln, Ser, Val, Asn, Asp, Glu, Gly, His, Pro, or Thr;
- (xiii) substitution at position 361 with Cys, Asp, Glu, Ile, Asn, Gln, Ser, Thr, Val, Ala, Arg, Gly, His, Leu, Lys, Phe, Pro, Trp or Tyr; and
- (xiv) substitution at position 362 with Ala, Glu, His, Lys, Pro, Gln, Arg, Asn, Gly, Ile, Leu, Thr, or Val.
- 20

In certain embodiments, the method comprises a residue change at three, four, five, six, seven, eight, or nine or more positions.

- In certain embodiments, the method further comprises a residue change at position 233, 425 and/or 451. In certain embodiments, the method further comprises residue change E233G, K425R and/or H451R. In certain embodiments, the method further comprises a residue change at positions 233, 425 and 451 (e.g., E233G, K425R and H451R).
- 25

- In certain embodiments, the modified GCase polypeptide has greater conformational stability relative to that of SEQ ID NO:1. In certain embodiments, the modified GCase polypeptide has greater conformational stability by at least about 2-fold relative to that of SEQ ID NO:1. In certain embodiments, the modified GCase polypeptide has greater enzymatic activity relative to that of SEQ ID NO:1. In certain embodiments, the modified GCase polypeptide has greater enzymatic activity by at least about 2 fold relative to that of SEQ ID NO:1.
- 30

Certain embodiments provide a modified GCase polypeptide produced by a method as

described herein.

CERTAIN EMBODIMENTS FOR IDENTIFYING MODIFIED GCASE POLYPEPTIDES HAVING IMPROVED PROPERTIES

5 As described in the Examples, libraries encoding modified GCase polypeptides may be screened/enriched for polypeptides having increased conformational stability and maintained (or increased) enzymatic activity, relative to a reference GCase (e.g., SEQ ID NO:1) (*see, e.g.*, Examples 5 and 7). Such a method utilizes 1) a fluorescent cyclophellitol-type activity-based probe that covalently reacts with a residue in the active site of GCase and acts as a surrogate for
10 enzymatic activity; and 2) a GCase antibody that detects conformationally-folded GCase protein and may act as an indicator of GCase stability after serum stress conditions are imposed.

Accordingly, certain embodiments provide a method of screening a library of nucleic acid sequences to identify modified GCase polypeptides having improved conformational stability and maintained (or increased) activity relative to that of SEQ ID NO:1, wherein the
15 method comprises:

- a) generating a library of nucleic acid sequences encoding modified GCase polypeptides according to a method described herein;
- b) transforming a plurality of host cells (e.g., yeast cells) with the library of nucleic acids;
- c) culturing the transformed cells to express the polypeptides encoded by the library of
20 nucleic acid sequences;
- d) subjecting the transformed cells to serum stress by incubating the cells in serum from a mammalian host for at least 30 minutes at temperatures ranging from room temperature to 37°C;
- e) labeling the cells with (i) a fluorescent cyclophellitol-type activity-based probe which
25 covalently reacts with a residue in the active site of GCase, and (ii) an anti-GCase antibody; and
- f) sorting the cells to isolate a population enriched for the fluorescent activity-based probe and the anti-GCase antibody.

In certain embodiments, such a method further comprises:

- g) propagating the population of enriched cells. Such propagated enriched cells may be
30 used for further rounds of sorting to select for higher activity and/or conformational stability (*see, e.g.*, Examples 5 and 7 describing multiple rounds of selection). Accordingly, in certain embodiments, the method further comprises:

h) subjecting the propagated enriched cells to serum stress by incubating the cells in serum from a mammalian host for at least 30 minutes at temperatures ranging from room temperature to 37°C;

5 i) labeling the propagated enriched cells with (i) a fluorescent cyclophellitol-type activity-based probe which covalently reacts with a residue in the active site of GCCase, and (ii) an anti-GCCase antibody; and

j) sorting the cells to isolate a second population enriched for the fluorescent activity-based probe and the anti-GCCase antibody. Steps g) through j) may be further repeated one or more times (e.g., 1, 2, 3, 4 or more times) using propagated cells from the previous round to
10 continue to select for polypeptides having higher activity and/or conformational stability.

In some embodiments, a method of screening a library of nucleic acid sequences encoding modified GCCase polypeptides is provided, wherein the method comprises:

- a) generating a library of nucleic acid sequences encoding modified GCCase polypeptides according to a method described herein;
- 15 b) transforming a plurality of host cells (e.g., yeast cells) with the library of nucleic acids;
- c) culturing the transformed cells to express the polypeptides encoded by the library of nucleic acid sequences;
- d) labeling the cells with (i) a fluorescent cyclophellitol-type activity-based probe which covalently reacts with a residue in the active site of GCCase, and (ii) an anti-GCCase
20 antibody; and
- e) sorting the cells to isolate a population enriched for the fluorescent activity-based probe and the anti-GCCase antibody.

In certain embodiments, the method further comprises:

- f) propagating the population of enriched cells;
- 25 g) subjecting the propagated enriched cells to serum stress by incubating the cells in serum from a mammalian host for at least 30 minutes at temperatures ranging from room temperature to 37°C;

h) labeling the propagated enriched cells with (i) a fluorescent cyclophellitol-type activity-based probe which covalently reacts with a residue in the active site of GCCase, and (ii)
30 an anti-GCCase antibody;

i) sorting the cells to isolate a second population enriched for the fluorescent activity-based probe and the anti-GCCase antibody; and

j) optionally repeating steps f) through i) one or more times (e.g., 1, 2, 3, 4 or more times).

In certain embodiments, the concentration of the fluorescent cyclophellitol-type activity-based probe is decreased each round to select for higher enzymatic activity. For example, the concentration of the probe in an initial round may be about 500nM, about 250nM in a second round, about 150nM in a third round, about 75nM in a fourth round, etc.

5 In certain embodiments, the serum is mouse, rabbit, monkey, or human serum. In certain embodiments, the serum is mouse serum. In certain embodiments, the serum is human serum. In certain embodiments, the serum concentration ranges from about 10% to about 100% (v/v) in a suitable buffer at neutral pH (*e.g.*, PBST). In certain embodiments, the serum concentration (v/v) is about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%,
10 85%, 90%, 95%, or 100%.

In certain embodiments, the cells are incubated in serum for at least about 30 minutes, for example, from about 30 minutes to about 48 hours (*e.g.*, 30, 60, 90, or 120 minutes; or 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, 24, 36, or 48 hours).

In certain embodiments, the cells are incubated with serum at a temperature range of
15 about room temperature to about 37°C. For example, in certain embodiments, a modified GCcase polypeptide as described herein is contacted with serum at a temperature ranging from about 20°C to about 37°C (*e.g.*, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36 or 37°C).

In certain embodiments, the fluorescent cyclophellitol-type activity-based probe is a
20 fluorescent form of 8-deoxy-8-azidocyclophellitol (KY170). In certain embodiments, the fluorescent cyclophellitol-type activity-based probe is a fluorescent form of conduritol β -epoxide (CBE). In certain embodiments, the fluorescent cyclophellitol-type activity-based probe is a 2-deoxy-2-fluoroglycoside.

In certain embodiments, the fluorescent cyclophellitol-type activity-based probe is
25 selected from the group consisting of: MDW933, MDW941, and 2-deoxy-2-fluoro- β -d-glucopyranosyl-*N*-phenyltrifluoroacetimidate.

In certain embodiments, the anti-GCcase antibody comprises:

- (i) a CDR-L1 sequence comprising the sequence of RASQGISSYLA (SEQ ID NO:132);
- 30 (ii) a CDR-L2 sequence comprising the sequence of AASSLQS (SEQ ID NO:133);
- (iii) a CDR-L3 sequence comprising the sequence of QQYYSYPFT (SEQ ID NO:134);
- (iv) a CDR-H1 sequence comprising the sequence of GFTFSSYH (SEQ ID NO:135);
- (v) a CDR-H2 sequence comprising the sequence of SINPSNGGTYTADSVKG

(SEQ ID NO:136); and

- (vi) a CDR-H3 sequence comprising the sequence of SSYYYYEMDY (SEQ ID NO:137).

Certain embodiments also provide a modified GCase polypeptide having one or more improved properties (e.g., improved conformational stability and maintained enzymatic activity) that is identified by a method as described herein.

THERAPEUTIC METHODS

A GCase polypeptide as described herein may be used therapeutically to treat a GCase deficiency in a patient in need thereof. In certain embodiments, the GCase deficiency is associated with a disease or disorder, such as Gaucher's disease, Parkinson's disease, Parkinsonism, or dementia with Lewy Bodies. As such, a GCase polypeptide described herein may be used to treat these diseases/disorders.

Accordingly, certain embodiments provide a method of treating a GCase deficiency in a patient in need thereof, the method comprising administering a GCase polypeptide as described herein to the patient.

Certain embodiments provide a GCase polypeptide as described herein for use in treating a GCase deficiency in a patient in need thereof.

Certain embodiments provide the use of a GCase polypeptide as described herein in the preparation of a medicament for treating a GCase deficiency in a patient in need thereof.

Certain embodiments also provide a method of decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency, the method comprising administering a GCase polypeptide as described herein to the patient.

Certain embodiments provide a GCase polypeptide as described herein for use in decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency.

Certain embodiments provide the use of a GCase polypeptide as described herein in the preparation of a medicament for decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency.

In certain embodiments, the toxic metabolic product comprises glucosylceramide. In certain embodiments, the toxic metabolic product comprises glucosylsphingosine.

A GCase polypeptide described herein is administered to a subject at a therapeutically effective amount or dose.

In various embodiments, a GCase polypeptide described herein is administered parenterally. In some embodiments, the protein is administered intravenously.

In some parenteral embodiments, a GCase polypeptide as described herein is administered intraperitoneally, intradermally, or intramuscularly. In some embodiments, the GCase polypeptide as described herein is administered intrathecally, such as by epidural administration, or intracerebroventricularly.

Certain Definitions

As used herein, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a polypeptide” may include two or more such molecules, and the like.

As used herein, the terms “about” and “approximately,” when used to modify an amount specified in a numeric value or range, indicate that the numeric value as well as reasonable deviations from the value known to the skilled person in the art, for example $\pm 20\%$, $\pm 10\%$, or $\pm 5\%$, are within the intended meaning of the recited value.

The term “plurality” as used herein refers to two or more. For example, the term plurality may be used to refer to 10, 50, 100, 500, 1,000, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 10,000,000, 100,000,000 or more.

The term “subject,” “individual,” and “patient,” as used interchangeably herein, refer to a mammal, including but not limited to humans, non-human primates, rodents (*e.g.*, rats, mice, and guinea pigs), rabbits, cows, pigs, horses, and other mammalian species. In one embodiment, the patient is a human. In some embodiments, the human is a patient in need of treatment for a disease or disorder associated with GCase deficiency (*e.g.*, Gaucher’s disease, Parkinson’s disease, Parkinsonism, or dementia with Lewy Bodies). In some embodiments, the patient has one or more signs or symptoms associated with GCase deficiency (*e.g.*, Gaucher’s disease, Parkinson’s disease, Parkinsonism, or dementia with Lewy Bodies).

The term “pharmaceutically acceptable excipient” refers to a non-active pharmaceutical ingredient that is biologically or pharmacologically compatible for use in humans or animals, such as but not limited to a buffer, carrier, or preservative.

The term “administer” refers to a method of delivering agents (*e.g.*, a therapeutic agent, such as a GCase polypeptide described herein), compounds, or compositions (*e.g.*, pharmaceutical composition) to the desired site of biological action. These methods include, but are not limited to, parenteral delivery, intravenous delivery, intradermal delivery, intramuscular

delivery, intrathecal delivery, or intraperitoneal delivery. In one embodiment, the polypeptides described herein are administered intravenously.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis.

The phrase “effective amount” means an amount of a compound described herein that (i) treats or prevents the particular disease, condition, or disorder, (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease, condition, or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease, condition, or disorder described herein.

A “therapeutically effective amount” of a substance/molecule disclosed herein may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the substance/molecule are outweighed by the therapeutically beneficial effects. A

“prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount would be less than the therapeutically effective amount.

“Glucocerebrosidase” or “GCase” is also known as “ β -glucocerebrosidase”, “GBA” or “glucosylceramidase” (EC 3.2.1.45). The term as used herein refers to a lysosomal enzyme that has glucosylceramidase activity and catalyzes the breakdown of glucosylceramide to ceramide and glucose. Deficiency of GCase is associated with Gaucher’s disease, Parkinson’s disease, Parkinsonism, or dementia with Lewy Bodies. The term “GCase” as used herein is catalytically active and encompasses functional variants, including allelic and splice variants, and catalytically active fragments of wild-type GCase and its functional variants. A catalytically active GCase fragment has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% of the enzymatic activity of the corresponding full-length GCase or variant thereof, *e.g.*, when assayed under identical conditions. Enzymatic activity can be measured according to methods known in the art,

including those described herein. The sequence of human GCase, long isoform, which is designated as the canonical sequence, is available under UniProt entry P04062-1 and is encoded by the human GBA gene at 1q22. The full-length sequence is provided as SEQ ID NO:2. A “mature” GCase sequence as used herein refers to a form of a polypeptide chain that lacks the signal and propeptide sequences of the naturally occurring full-length polypeptide chain. The amino acid sequence of a mature human GCase polypeptide is provided as SEQ ID NO:1, which corresponds to amino acids 40-536 of the full-length human sequence. Commercially available forms of recombinant GCase include imiglucerase (CEREZYME®), velaglucerase alfa (VPRIV®), and taliglucerase alfa (ELELYSO®).

As used herein, a “loop region” refers to a patternless region, which connects two regular secondary structures. An “alpha-helical region” refers a portion of a protein that forms a right-handed helix, wherein the side-chains point out from the central coil. Identifying such regions in a GCase polypeptide is within the skill of art; the structure of human GCase has been well-characterized and nearly 20 crystal structures of GCase are available. Additionally, publicly available software may be used to identify varying regions of secondary structure (e.g., loop regions, alpha-helical regions, etc.) within a given amino acid sequence. Such programs include PyMOL (Schrödinger, Inc.), Maestro (Schrödinger, Inc.), and Molecular Operating Environment (MOE, Chemical Computing Group).

The term “ortholog” as used herein refers to genes separated in evolution by speciation, as well as encoded polypeptides thereof. For example, non-human primate GCase sequences have been described, including chimpanzee (UniProt entry Q9BDT0) and orangutan (UniProt entry Q5R8E3) and a mouse GCase sequence is available under UniProt entry P17439. Ortholog GCase sequences from species including, but not limited to, bottlenose dolphin, sperm whale, rabbit, sheep, yak, sea otter, fox, lamprey, finch, alligator, coelacanth, and collard flycatcher, may also be considered as described herein.

Unless specified otherwise, amino acid numbers or positions are with respect to SEQ ID NO:1, unless an alternative sequence is referenced. Thus, position 316 would be the 316th amino acid occurring in the reference sequence (e.g., the 316th amino acid in SEQ ID NO:1).

The term “GCase deficiency” as used herein refers to a condition wherein a subject expresses levels of GCase that are less than that of a control or population of control subjects and/or wherein the enzymatic activity level of the subject’s expressed GCase is less than that of a control or population of control subjects.

The term “serum” refers to the liquid fraction of clotted blood, which is generally depleted of cells, fibrin and clotting factors. For example, serum may be prepared by

centrifuging until the clot and remaining blood cells are separated from the liquid phase. Modified GCase polypeptides described herein may be contacted with serum to assess the impact that exposure to serum has on the stability of the polypeptide. Thus, to determine if a modified GCase has improved stability in the presence of serum, a modified GCase polypeptide and a reference polypeptide may be contacted with serum for a period of time (e.g., under conditions described herein) and subsequently assessed for stability using an assay described herein or known in the art.

“Naturally occurring,” “native” or “wild type” is used to describe an object that can be found in nature as distinct from being artificially produced. For example, a nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified in the laboratory, is naturally occurring. Furthermore, “wild-type” refers to the normal gene, or organism found in nature without any known mutation. For example, the terms “wild-type,” “native,” and “naturally occurring” with respect to GCase are used herein to refer to a GCase polypeptide that has a sequence that occurs in nature. In some embodiments, the wild-type GCase polypeptide is a wild-type human GCase polypeptide.

As used herein, the term “mutant” with respect to a mutant polypeptide or mutant polynucleotide is used interchangeably with “variant.” A variant with respect to a given wild-type GCase reference sequence can include naturally occurring allelic variants. A “non-naturally” occurring GCase refers to a variant or mutant domain that is not present in a cell in nature and that is produced by genetic modification, *e.g.*, using genetic engineering technology or mutagenesis techniques, of a native GCase polynucleotide or polypeptide. A “variant” includes any domain comprising at least one amino acid mutation with respect to wild-type. Mutations may include substitutions, insertions, and deletions.

The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids.

Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate and O-phosphoserine. “Amino acid analogs” refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. “Amino acid mimetics” refers to chemical compounds that have

a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

Naturally occurring α -amino acids include, without limitation, alanine (Ala), cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), phenylalanine (Phe), glycine (Gly), histidine (His), isoleucine (Ile), arginine (Arg), lysine (Lys), leucine (Leu), methionine (Met), asparagine (Asn), proline (Pro), glutamine (Gln), serine (Ser), threonine (Thr), valine (Val), tryptophan (Trp), tyrosine (Tyr), and combinations thereof. Stereoisomers of a naturally-occurring α -amino acids include, without limitation, D-alanine (D-Ala), D-cysteine (D-Cys), D-aspartic acid (D-Asp), D-glutamic acid (D-Glu), D-phenylalanine (D-Phe), D-histidine (D-His), D-isoleucine (D-Ile), D-arginine (D-Arg), D-lysine (D-Lys), D-leucine (D-Leu), D-methionine (D-Met), D-asparagine (D-Asn), D-proline (D-Pro), D-glutamine (D-Gln), D-serine (D-Ser), D-threonine (D-Thr), D-valine (D-Val), D-tryptophan (D-Trp), D-tyrosine (D-Tyr), and combinations thereof.

Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

The terms “polypeptide” and “peptide” are used interchangeably herein to refer to a polymer of amino acid residues in a single chain. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. Amino acid polymers may comprise entirely L-amino acids, entirely D-amino acids, or a mixture of L and D amino acids.

The term “protein” as used herein refers to either a polypeptide or a dimer (*i.e.*, two) or multimer (*i.e.*, three or more) of single chain polypeptides. The single chain polypeptides of a protein may be joined by a covalent bond, *e.g.*, a disulfide bond, or non-covalent interactions.

The term “conservative substitution,” “conservative mutation,” or “conservatively modified variant” refers to an alteration that results in the substitution of an amino acid with another amino acid that can be categorized as having a similar feature. Examples of categories of conservative amino acid groups defined in this manner can include: a “charged/polar group” including Glu (Glutamic acid or E), Asp (Aspartic acid or D), Asn (Asparagine or N), Gln (Glutamine or Q), Lys (Lysine or K), Arg (Arginine or R), and His (Histidine or H); an “aromatic group” including Phe (Phenylalanine or F), Tyr (Tyrosine or Y), Trp (Tryptophan or W), and (Histidine or H); and an “aliphatic group” including Gly (Glycine or G), Ala (Alanine or A), Val (Valine or V), Leu (Leucine or L), Ile (Isoleucine or I), Met (Methionine or M), Ser

(Serine or S), Thr (Threonine or T), and Cys (Cysteine or C). Within each group, subgroups can also be identified. For example, the group of charged or polar amino acids can be sub-divided into sub-groups including: a “positively-charged sub-group” comprising Lys, Arg and His; a “negatively-charged sub-group” comprising Glu and Asp; and a “polar sub-group” comprising Asn and Gln. In another example, the aromatic or cyclic group can be sub-divided into sub-groups including: a “nitrogen ring sub-group” comprising Pro, His and Trp; and a “phenyl sub-group” comprising Phe and Tyr. In another further example, the aliphatic group can be sub-divided into sub-groups, *e.g.*, an “aliphatic non-polar sub-group” comprising Val, Leu, Gly, and Ala; and an “aliphatic slightly-polar sub-group” comprising Met, Ser, Thr, and Cys. Examples of categories of conservative mutations include amino acid substitutions of amino acids within the sub-groups above, such as, but not limited to: Lys for Arg or vice versa, such that a positive charge can be maintained; Glu for Asp or vice versa, such that a negative charge can be maintained; Ser for Thr or vice versa, such that a free -OH can be maintained; and Gln for Asn or vice versa, such that a free -NH₂ can be maintained. In some embodiments, hydrophobic amino acids are substituted for naturally occurring hydrophobic amino acid, *e.g.*, in the active site, to preserve hydrophobicity.

The terms “identical” or percent “identity,” in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues, *e.g.*, at least 60% identity, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% or greater, that are identical over a specified region when compared and aligned for maximum correspondence over a comparison window, or designated region, as measured using a sequence comparison algorithm or by manual alignment and visual inspection. In some embodiments, a sequence that has a specified percent identity relative to a reference sequence differs from the reference sequence by one or more conservative substitutions.

For sequence comparison of polypeptides, typically one amino acid sequence acts as a reference sequence, to which a candidate sequence is compared. Alignment can be performed using various methods available to one of skill in the art, *e.g.*, visual alignment or using publicly available software using known algorithms to achieve maximal alignment. Such programs include the BLAST programs, ALIGN, ALIGN-2 (Genentech, South San Francisco, Calif.) or Megalign (DNASTAR). The parameters employed for an alignment to achieve maximal alignment can be determined by one of skill in the art. For sequence comparison of polypeptide sequences for purposes of this application, the BLASTP algorithm standard protein BLAST for aligning two proteins sequence with the default parameters is used.

The terms “corresponding to,” “determined with reference to,” or “numbered with reference to” when used in the context of the identification of a given amino acid residue in a polypeptide sequence, refers to the position of the residue of a specified reference sequence when the given amino acid sequence is maximally aligned and compared to the reference sequence. Thus, for example, an amino acid residue in a modified GCase polypeptide “corresponds to” an amino acid in SEQ ID NO:1, when the residue aligns with the amino acid in SEQ ID NO:1 when optimally aligned to SEQ ID NO:1. The polypeptide that is aligned to the reference sequence need not be the same length as the reference sequence.

The term “polynucleotide” and “nucleic acid” interchangeably refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. Examples of polynucleotides contemplated herein include single- and double-stranded DNA, single- and double-stranded RNA, and hybrid molecules having mixtures of single- and double-stranded DNA and RNA.

The following Examples are intended to be non-limiting.

EXAMPLE 1: GENERATION AND SCREENING OF ERROR-PRONE GCASE LIBRARY

A library of GCase polypeptide variants was generated by error-prone PCR. Briefly, the wild-type GCase DNA sequence was used as the template for PCR containing low-fidelity TAQ polymerase and nucleotide analogs, resulting in random incorporation of mutations across the entire length of the gene.

Synthesis of GCase mutant library. An error-prone library was built using the wild-type GCase DNA sequence (SEQ ID NO:3) as the template, with mutations incorporated using low-fidelity TAQ polymerase (Thermo Fisher Scientific, Catalog No. 4398790) and the nucleotide analogs, 8-oxo-dGTP (TriLink Biotechnologies, Catalog No. N-2034) and dPTP (TriLink Biotechnologies, Catalog No. N-2037). Six individual PCR reactions were performed in which the number of amplification cycles (8, 12 or 16) and the concentration of nucleotide analogs (2 μ M or 20 μ M) were varied to achieve a range of mutational frequencies. The products of these reactions were purified by agarose gel electrophoresis and amplified by PCR using forward and reverse primers containing 50 base pairs of homology to a yeast display vector in the absence of nucleotide

analogs. Amplified library DNA was purified by agarose gel electrophoresis, ethanol precipitated, and resuspended in water to a final concentration of 1mg/mL.

5 *Generation of Yeast-Displayed GCase of Library.* Amplified library DNA from the six mutagenesis reactions were pooled, mixed with linearized yeast display vector in a 6:1 ratio by weight, and electroporated into freshly made electrocompetent EBY100 yeast according to established protocols (Chao et al. 2006. *Nat Protocols* 1:755-768). After assembly by homologous recombination, the resulting library encoded variants of GCase expressed as fusions to the C-terminus of the yeast coat protein, Aga2p, with a C-terminal V5 epitope tag. The yeast library was
10 outgrown in SD-CAA medium (Teknova, Catalog No. 2S0540) at 30°C in a shaker (250 RPM) to select for transformed cells, and library size was estimated to be $>1 \times 10^8$ by dilution plating. The library was then grown in SG-CAA medium (Teknova, Catalog No. 2S0542) overnight at 30°C with shaking (250 RPM) to induce expression of the enzyme on the yeast cell surface.

15 *Selection of highly expressed and serum stable GCase variants.* Yeast displaying GCase variants with improved levels of expression (a measure of conformational stability) relative to wild-type GCase were enriched within the library using fluorescence activated cell sorting (FACS). For FACS round 1, yeast cells were immunolabeled to detect GCase expression with a combination of an anti-V5 antibody (1:1000, Sigma Aldrich, Catalog No. V8012) to select for full-length
20 clones and a biotinylated anti-GCase antibody (having the CDRs of SEQ ID NOs.:132-137, or similar, 100nM final concentration) to bias against in-frame truncations and deletions. Primary labeling was carried out at room temperature in PBST (1xPBS, 0.1% BSA, 0.02% Tween-20, pH7.4). Cells were pelleted, washed twice with PBST and secondary labeling was performed at 4°C with a 1:100 dilution of anti-mouse AlexaFluor488® antibody (Invitrogen, Catalog No.
25 A11001) and streptavidin AlexaFluor647® (Invitrogen, Catalog No. S32357). Clones displaying strong binding to both expression handles were selected by FACS (FACS Aria III, BD Biosciences) and propagated for subsequent sorting.

For FACS round 2, a similar approach was taken. A first sample from the outputs of FACS round 1
30 was subjected to primary and secondary labeling as described above, and sorting was conducted to select for those clones showing the highest levels of binding to both expression handles (“S2” products). In parallel, a second sample from the outputs of FACS round 1 was incubated at 37°C for 90 minutes in a 1:1 mixture of PBST and mouse serum (Rockland, Cat# D108-00-0050, or similar) to impart selective pressure on the library for clones showing enhanced serum stability.

After serum incubation, primary and secondary labeling was carried out as described above, and variants showing strong binding to both expression handles on the yeast cell surface were selected by FACS (“S2 serum” products).

- 5 All sorting was conducted such that the top 1 – 2% of the clones displaying the highest levels of double positive staining were selected, thereby enriching the library for GCase sequences with improved expression in the presence of serum. Sorted clones were propagated in SD-CAA medium, induced for expression in SG-CAA medium, and subjected to additional rounds of FACS. After FACS round 2, plasmid DNA was isolated using a Zymoprep yeast plasmid
10 miniprepII kit (Zymo Research), transformed into One Shot® Top10 electrocompetent *E.coli* bacteria (Invitrogen), and sequenced.

EXAMPLE 2: GENERATION AND SCREENING OF SHUFFLED GCASE LIBRARY

- 15 *Synthesis of a shuffled GCase library.* GCase DNA from S2 and S2 serum products (Example 1) were amplified by PCR and purified by agarose gel electrophoresis. After purification, 2.5µg and 5µg from S2 and S2 serum products, respectively, were pooled and treated with DNase I (NEB, Catalog No. M0303) for 3 minutes at 15°C. Fragmented DNA without purification was self-assembled by PCR, and products from the self-assembly reaction were subsequently amplified by
20 PCR using forward and reverse primers with 50 base pairs of homology to the yeast-display vector. Library DNA was purified by agarose gel electrophoresis, ethanol precipitated, and resuspended in water to a final concentration of 1mg/mL.

- Generation and screening of a yeast-displayed shuffled GCase library.* Amplified DNA from the
25 shuffled reactions were used to transform yeast as described above (Example 1), resulting in a library of shuffled GCase variants. Yeast displaying shuffled GCase variants with high levels of expression (a measure of conformational stability) were enriched within the library by FACS using the double labeling strategy with anti-V5 antibody and biotinylated anti-GCase antibody as described (Example 1, Figure 1). Clones displaying strong binding to both expression handles
30 were selected and propagated for subsequent sorting.

In FACS round 1 (“SH1”), yeast were incubated at room temperature for 30 minutes in a 1:1 mixture of PBST and mouse serum (Rockland, Cat# D108-00-0050, or similar) to impart selective pressure on the library for clones showing enhanced stability in the presence of serum. After

incubation with serum, the double labeling strategy to detect GCase expression was carried out as described (Example 1), and variants showing strong binding to both expression handles on the yeast cell surface were selected by FACS. In FACS rounds 2 (“SH2”) and 3 (“SH3”), incubation in serum (50% v/v in PBST) was carried out as described for FACS round 1 with the following changes: incubation temperature was increased to 37°C and incubation times were 30 minutes (round 2) and 6 hours (round 3). Double labeling and FACS selections were then carried out as described (Example 1).

All sorting was conducted as outlined above, and plasmid DNA was isolated after SH2 and SH3 using a Zymoprep yeast plasmid miniprepII kit (Zymo Research), transformed into One Shot® Top10 electrocompetent *E.coli* bacteria (Invitrogen), and sequenced to identify GCase variants with enhanced expression in the presence of serum.

Consensus sequences of stable GCase variants. Tables 1 and 2 summarize the set of residues and their corresponding positions that appeared with high frequency in the SH2 and SH3 pools. The positions in Tables 1 and 2 are numbered with respect to positions in SEQ ID NO:1. “SH2” in the clone name indicates the clones are from FACS round 2 of the shuffled library; “SH3” in the clone name indicates the clones are from FACS round 3 of the shuffled library. Clones from SH2 and SH3 demonstrating improved stability on yeast contain the consensus sequence of SEQ ID NO:4:

```

1   ARPCIPKSFYSSVVCNATYCDSX1aX2aPPTX3aPALGTFSTRYESTRSGRRMELSMGPIQANH
20  61   TGTGLLLTLQPEQKFKVKGFGGAMTDAAALNIALSPPAQX4aLLKSYFSEEGIGYNIIR
    121  VPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQRVSLASPWT
    181  SPTWLKTNGAVNGKSLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAX5aNEPSAGL
    241  LSGYPFQCLGFTPEHQRFIARDLGPITLANSTHHNVRLMLDDQRX6aLLPHWAKVVLTDPE
    301  AAKYVHGIHAVHXX7aLDX8aLAPAKATLGETHRLFPNTMLFASEACVGSKX9aX10aEQX11aVRLGSWDRG
25  361  MQYSHSIITNLLX12aHVX13aGWTDWNLALNPEGGPX14aWX15aRNFXX16aDSPIIVDITKDTFYKQPMFYHX17a
    421  GHFSX18aFIPEGSQVRVGLVASQKNDLDAVALMX19aPDGSAVVVVLNRSSKDVPLTIKDPVAVGFL
    481  ETISPGYSIHTYLWRRQ (SEQ ID NO:4)

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wherein X_{1a} is F, L, or S; X_{2a} is D or G; X_{3a} is F or L; X_{4a} is N or D; X_{5a} is E or G; X_{6a} is L or S; X_{7a} is Y, H, D, or C; X_{8a} is F, L or S; X_{9a} is F or S; X_{10a} is W, G, or R; X_{11a} is S, G, or N; X_{12a} is Y or H; X_{13a} is V or A; X_{14a} is N or S; X_{15a} is V or A; X_{16a} is V or A; X_{17a} is L or I; X_{18a} is K or R; and X_{19a} is H or R. In clones from the SH2 library, X_{7a} is Y or H; X_{8a} is F, or S; X_{13a} is V; and X_{15a} is V. In clones from the SH3 library, X_{11a} is S or G.

Clones that included one or more of modifications F246S, W312R, and L314P were excluded from Tables 1 and 2 due to the observation that F246S, W312R, and L314P negatively impacted enzymatic activity (data not shown). However, the possibility remains that such

modifications may have a different impact on conformational stability and/or enzymatic activity in an alternative combination of modifications.

Table 1. Consensus Positions From SH2 Clones

SEQ ID NO.	Position #	26	27	31	102	233	286	313	316	347	348	351	373	392	398	420	425	451
	WT	F	D	F	N	E	L	Y	F	F	W	S	Y	N	V	L	K	H
6	SH2-72	F	D	F	N	E	L	Y	F	F	W	S	H	N	V	L	K	R
7	SH2-81	F	D	F	N	G	L	Y	F	F	W	G	H	S	V	L	K	H
8	SH2-60	F	D	F	N	E	L	Y	F	F	G	S	H	N	A	L	K	H
9	SH2-14	F	D	F	N	E	L	Y	F	F	W	S	H	N	V	L	K	H
10	SH2-37	F	D	F	N	G	L	Y	F	F	R	S	H	N	V	L	R	H
11	SH2-83	F	D	F	D	G	L	Y	F	F	W	S	H	N	V	L	R	H
12	SH2-25	F	D	F	N	E	S	Y	F	F	W	S	H	N	V	L	K	H
13	SH2-59	F	D	F	N	E	S	Y	F	F	W	S	H	N	V	I	K	H
14	SH2-28	S	G	F	D	E	S	Y	F	S	W	S	H	N	V	L	K	H
15	SH2-93	F	D	F	D	G	S	Y	F	F	W	S	H	N	V	L	R	H
16	SH2-1	F	D	F	N	G	L	Y	S	F	W	S	H	N	V	L	R	H
17	SH2-31	F	D	F	N	G	L	Y	S	F	W	S	H	S	V	L	K	R
18	SH2-44	F	D	F	N	G	L	Y	S	F	W	S	H	N	A	L	K	H
19	SH2-30	F	D	F	N	G	L	Y	S	F	W	S	H	N	V	L	K	H
20	SH2-20	F	D	F	N	G	L	Y	S	F	W	S	Y	N	V	L	R	R
21	SH2-71	F	D	F	N	G	L	Y	S	F	W	S	Y	N	V	I	K	H
22	SH2-2	F	D	F	N	E	S	H	S	S	W	S	H	N	A	L	K	H
23	SH2-48	F	D	F	N	E	S	Y	S	S	W	S	Y	N	A	L	R	H
24	SH2-27	F	D	F	N	E	S	Y	S	F	W	S	H	N	V	L	R	H
25	SH2-95	F	D	F	N	E	S	Y	S	F	W	G	H	N	V	L	K	H
26	SH2-23	F	D	F	N	E	S	Y	S	F	W	S	H	N	A	L	K	H
27	SH2-18	F	D	F	N	E	S	Y	S	F	W	S	H	N	V	L	K	R
28	SH2-88	F	D	F	N	E	S	Y	S	F	W	S	H	N	V	L	K	H
29	SH2-85	F	D	L	N	E	S	Y	S	F	W	S	H	S	V	L	R	H
30	SH2-70	L	D	F	N	G	S	Y	S	F	G	N	Y	N	V	L	K	H
31	SH2-69	F	D	F	N	E	S	Y	S	F	W	S	H	N	A	I	K	H
32	SH2-61	F	D	F	N	E	S	Y	S	F	W	S	H	N	V	L	K	H
33	SH2-38	F	D	F	N	E	L	Y	S	F	W	S	H	N	V	L	R	H
34	SH2-26	F	D	F	N	E	L	Y	S	F	W	S	H	S	V	L	K	R
35	SH2-79	F	D	F	N	E	L	Y	S	S	W	S	H	S	V	L	K	H
36	SH2-65	F	D	F	N	E	L	Y	S	F	W	S	H	N	A	L	K	H
37	SH2-56	F	D	F	N	E	L	Y	S	F	W	S	H	S	V	L	K	H
38	SH2-53	F	D	L	N	E	L	Y	S	F	W	S	H	S	V	L	K	H

SEQ ID NO.	Position #	26	27	31	102	233	286	313	316	347	348	351	373	392	398	420	425	451
	WT	F	D	F	N	E	L	Y	F	F	W	S	Y	N	V	L	K	H
39	SH2-9	F	D	F	N	E	L	Y	S	F	W	S	H	N	V	L	K	R
40	SH2-82	F	D	F	N	E	L	Y	S	F	W	S	H	S	V	L	K	H
41	SH2-8	F	D	F	N	E	L	Y	S	F	W	S	H	N	V	L	K	H
42	SH2-16	F	D	F	N	E	L	Y	S	S	W	S	Y	N	V	L	R	H
43	SH2-94	F	D	F	N	E	L	H	S	F	W	S	Y	N	V	I	K	H
44	SH2-17	F	D	F	D	E	L	Y	S	F	W	S	Y	N	V	I	K	R
45	SH2-66	F	D	F	N	E	L	Y	S	F	W	S	Y	N	V	I	K	H
--	SH2-73	F	G	F	N	E	S	Y	S	F	W	S	H	N	V	L	K	H

WT = wild-type GCase

Table 2. Consensus Positions from SH3 Clones

SEQ ID NO.	Position #	26	27	31	102	233	286	313	316	347	348	351	373	376	392	394	398	420	425	451
	WT	F	D	F	N	E	L	Y	F	F	W	S	Y	V	N	V	V	L	K	H
46	SH3-65	F	D	F	N	E	S	Y	S	F	R	G	Y	V	N	V	A	I	R	H
47	SH3-40	F	D	F	N	E	L	Y	S	F	W	S	H	V	N	A	A	L	K	H
48	SH3-67	F	D	F	N	E	S	Y	S	F	W	S	H	V	S	A	V	L	K	H
49	SH3-31	F	D	F	N	E	L	Y	S	F	W	S	H	V	N	V	V	I	K	H
50	SH3-57	F	D	F	N	E	S	Y	S	F	W	S	H	V	N	V	V	I	K	H
51	SH3-86	F	D	F	N	E	L	Y	F	F	W	S	H	V	S	V	V	I	K	H
52	SH3-80	F	D	F	N	E	S	Y	S	F	W	S	H	V	N	V	A	L	K	H
53	SH3-17	F	D	F	N	E	L	Y	S	S	W	S	H	A	N	V	A	L	K	H
54	SH3-6	F	D	F	N	G	S	Y	S	S	W	S	H	V	N	V	V	L	K	H
55	SH3-54	F	D	F	N	G	S	Y	F	F	W	G	H	V	N	V	V	L	K	R
56	SH3-90	F	D	F	D	E	S	Y	S	S	W	S	H	V	S	V	V	L	K	H
57	SH3-11	S	G	F	N	E	S	Y	F	S	W	S	H	A	N	V	V	L	K	H
58	SH3-10	S	G	F	N	E	S	Y	S	F	W	S	H	V	N	V	V	I	K	R
59	SH3-59	S	G	F	N	E	L	H	S	F	W	S	H	V	N	V	V	L	K	R
60	SH3-43	S	D	F	N	E	S	Y	S	S	W	S	H	V	N	V	V	L	K	H
61	SH3-49	L	D	F	N	G	L	D	F	F	W	S	H	V	N	V	V	L	K	H
62	SH3-25	L	D	F	N	E	L	D	S	F	W	S	H	V	S	V	V	L	K	H
63	SH3-24	L	D	F	N	E	L	Y	S	F	W	S	H	V	N	V	A	L	K	H
64	SH3-91	F	D	L	N	G	L	Y	S	F	W	S	H	V	N	V	V	I	K	H
65	SH3-69	F	D	L	N	G	S	Y	S	F	W	S	H	V	N	V	V	L	K	H
66	SH3-68	F	D	L	N	G	L	Y	S	F	W	S	H	V	N	V	V	L	R	H
67	SH3-87	F	D	L	N	G	S	Y	S	F	R	G	H	V	N	V	V	L	K	H
68	SH3-93	F	D	F	D	E	S	Y	S	F	W	S	H	V	S	V	V	L	K	H

SEQ ID NO.	Position #	26	27	31	102	233	286	313	316	347	348	351	373	376	392	394	398	420	425	451
69	SH3-75	L	D	L	D	G	L	Y	S	F	W	S	H	V	N	V	V	L	K	R
70	SH3-81	F	D	F	N	E	S	C	S	F	W	S	H	V	N	V	V	I	K	H
71	SH3-9	F	D	F	N	E	S	H	S	S	W	S	H	V	N	V	A	L	K	H
72	SH3-88	F	D	F	N	E	L	Y	L	F	G	S	H	V	S	V	A	L	R	H
73	SH3-63	F	D	F	N	G	S	Y	S	F	W	S	H	V	N	V	V	L	K	H
74	SH3-56	F	D	F	N	G	L	Y	S	F	W	S	H	V	N	V	V	L	R	H
75	SH3-85	F	D	F	N	E	S	Y	S	F	W	S	H	A	N	V	V	L	R	H
76	SH3-13	F	D	F	N	E	L	Y	S	S	W	S	H	V	S	V	V	L	K	H
77	SH3-45	F	D	F	N	E	L	Y	S	F	W	S	H	V	N	A	V	L	R	H
78	SH3-64	F	D	F	N	E	L	H	S	F	W	S	Y	V	N	V	V	I	K	R
--	SH3-77	L	G	F	N		L	Y	S	F	W	S	H	V	S	V	V	L	K	R
--	SH3-72	F	D	F	N		S	Y	S	F	G	S	H	A	N	V	A	L	K	H

WT = wild-type GCCase

EXAMPLE 3: CLONAL ANALYSIS OF GCASE VARIANTS ON YEAST

A set of variants was selected for further analysis from the SH2 and SH3 pools that only contained mutations identified in the consensus set (Table 1, Table 2) and had at most four changes relative to wild-type GCCase (SEQ ID NO:1). The variants that were analyzed were: SH2-30, SH2-20, SH2-18, SH2-88, SH2-65, and SH2-9. Yeast displaying one of these variants or wild-type GCCase were double labeled with anti-V5 antibody and biotinylated anti-GCCase antibody as described (Example 1) and analyzed by flow cytometry to assess cell surface expression of each GCCase clone. The yeast were analyzed pre- and post-serum stress. Pre-serum stress yeast were immunolabeled as described (Example 1) and analyzed by flow cytometry. Serum-stressed yeast were labeled and analyzed identically with the following change. Prior to immunolabeling, yeast were incubated at room temperature for 6 hours in a 1:1 mixture of PBST and mouse serum (Rockland, Cat# D108-00-0050, or similar). All variants, as measured by flow cytometry, showed improved expression relative to the wild-type GCCase enzyme (Figure 2, Table 3). This improvement in expression relative to SEQ ID NO:1 (a measure of conformational stability) was observed both in the presence and absence of serum. In Figure 2, data is shown for biotinylated anti-GCCase antibody expression for simplicity even though the yeast were double labeled. Histogram data for wild-type GCCase is not shown in Figure 2 due to non-detection of expression above background levels.

Table 3. GCCase expression (anti-GCCase antibody) in variants and wild-type (WT) GCCase as measured by FACS

Variant	GCCase Expression (anti-GCCase antibody)		
	PBST (Fluorescence a.u.)	Post-Serum Stress (Fluorescence a.u.)	Retained Expression (%)
WT GCCase	ND	ND	NA
SH2-9	1052	637	61%
SH2-18	2026	1850	91%
SH2-20	1668	1664	100%
SH2-30	1481	1437	97%
SH2-65	1372	1349	98%
SH2-88	1783	1443	81%

ND = not detected
NA = not applicable
a.u. = arbitrary units

EXAMPLE 4: VALIDATION OF GCASE ACTIVITY PROBE ON YEAST

An activity-based assay compatible with yeast displayed GCCase and analysis by flow cytometry was developed to facilitate further engineering efforts. SH2-20 and SH2-88 were chosen as representative clones for this work as both showed improved expression but contained different sets of consensus mutations, overlapping at a single position.

Yeast displaying wild-type, SH2-20, or SH2-88 were washed twice with GCCase staining buffer (GSB; 500mM sodium citrate, 50mM phosphate, 120 mM NaCl, 1% BSA, pH 5.2) and resuspended in GSB containing 250nM of MDW933, a fluorescent cyclophellitol-type activity-based probe which covalently reacts with residue E340 in the active site of GCCase (Witte, et al. 2010. *Nat Chem Biol* 6:907-913) and allowed to incubate at room temperature for 1 hour. After probe capping with MDW933, cells were washed twice with PBSA (1x PBS, 1% BSA, pH 7.4) and stained with 1 µg/mL of human anti-GCCase antibody (having the CDRs of SEQ ID NOs: 132-137, or similar) for 30 minutes at room temperature. After primary immunolabeling, cells were washed with PBSA and stained with 1:500 of anti-human IgG AlexaFluor®647 (Jackson ImmunoResearch, Catalog No. 109-605-006) on ice for 30 minutes.

Analysis of MDW933 probe capping, a surrogate for enzymatic activity, and GCCase expression (by anti-GCCase antibody detection) was assessed by flow cytometry (FACS Aria III, BD Biosciences). SH2-88 was well-expressed on yeast but showed minimal probe capping (low enzymatic activity), whereas SH2-20 displayed high expression and was noticeably labeled by the activity-based probe relative to wild-type GCCase (Figure 3, Table 4). These data established that

MDW933 can be used with yeast display and flow cytometry to assess the activity of GCase variants, and that SH2-20 has high expression and enzymatic activity.

Table 4. GCase expression (anti-GCase antibody) and enzymatic activity in wild-type (WT)

5 GCase and GCase variants as measured by FACS

GCase Clone	GCase Expression (Fluorescence a.u.)	Probe capping (Fluorescence a.u.)
SH-20	5577	253
SH2-88	5550	91
WT GCase	ND	ND

ND = not detected

a.u. = arbitrary units

EXAMPLE 5: GENERATION OF GCASE VARIANT LIBRARY 1

10 To further improve GCase stability, clone SH2-20 (Example 4, SEQ ID NO:20) was used as the parental sequence for the design of a first GCase variant library (“GCase Variant Library 1” or “GCase Library 1”) given its high expression and probe capping on yeast. The library was designed by selecting amino acids for randomization based off structural differences between unbound (PDB ID: 2NT1) and isofagomine (IFG)-bound (PDB ID: 3GXF) GCase. Briefly, IFG

15 is known to stabilize the overall structure of GCase, and comparison of unbound and IFG-bound GCase structures revealed areas on GCase perturbed upon binding to IFG (Kornhaber, et al. 2008. *Chembiochem* 9(16):2643-2649). Regions containing strong perturbations upon IFG binding ($\geq 9\%$ decrease in average deuteration level as measured by H/D exchange protocol; Hamuro et al. 2003. *J Biomol Tech* 14:171) that also make direct contact with IFG were identified (amino

20 acid numbers 119-127, 177-184, 230-240, and 386-400 of SEQ ID NO:1; Kornhaber, et al. 2008. *supra*). To enable combinatorial exploration of a manageable number of positions, a multiple sequence alignment of GCase orthologs was used to identify positions within these regions that were non-conserved. Briefly, GCase ortholog sequences were downloaded from the National Center for Biotechnology Information (NCBI) via a BLAST query of the human GCase sequence

25 (SEQ ID NO:1), and a multiple sequence alignment was performed. To reduce redundancy, any sequences with $>98\%$ identity to any other sequence in the alignment (aside from human GCase sequence) were removed. Sequences with $<60\%$ identity with human GCase sequence were also removed to provide a floor for diversity. Using this alignment of ortholog GCase sequences, non-conserved residues within the regions were identified. This set of non-conserved residues was

30 further narrowed by selecting positions found in loop and alpha helical regions. The final set of positions chosen for the GCase Variant Library 1 are provided in Table 5. Diversification of the

library positions was accomplished by allowing each position to be mutated to amino acids found at that position in the multiple sequence alignment of GCCase orthologs described. The final library contained additional diversity beyond the ortholog set (Table 5, italicized and bolded amino acids) as an artifact of the degenerate codons used to build it.

5

Table 5 provides a summary of the library design, including positions which were mutated, and the restricted set of amino acids interrogated within the library at each of these positions. Three of four mutations in SH2-20 (F316S, K425R, and H451R) were present in the library but are not included in Table 5 because they were not varied. Italicized and bolded amino acids represent the non-ortholog amino acids incorporated in the library because of the degenerate codons used in library synthesis.

10

Table 5. Design of GCCase Variant Library 1

Position #	124	125	126	181	230	232	233	237	238	239	240	386	387	388
WT Residue	A	S	C	S	V	A	E	S	A	G	L	N	P	E
	T	C	C	A	V	A	E	S	A	R	E	N	P	E
	A	G	S	S	L	S	G	R	D	G	F	D	L	K
		S	T			T	Q	T	T		L		M	
		<i>A</i>				G	R	L	<i>N</i>		M		T	
								<i>P</i>			N			
								<i>I</i>			Q			
											<i>V</i>			
											<i>Y</i>			
											<i>K</i>			
											<i>I</i>			
											<i>H</i>			
											<i>D</i>			

WT = wild-type

Synthesis of GCCase Variant Library 1. GCCase Variant Library 1 was built by PCR using the SH2-20 DNA sequence (SEQ ID NO:79) as the template, with the positions indicated in Table 5 mutated to the specified subset of amino acids using oligos with degenerate codons. Amplified library DNA was purified by agarose gel electrophoresis, ethanol precipitated, and resuspended in water to a final concentration of 1 mg/mL.

20

Generation and screening of yeast-displayed GCCase Variant Library 1. Mutated GCCase DNA and linearized yeast display vector was used to transform yeast as described above (Example 1),

resulting in a library of GCase variants with diversity incorporated as outlined in Table 5. The size of GCase Variant Library 1 was estimated to be 3×10^7 by dilution plating. Following induction of yeast for GCase expression, 3×10^8 cells were stained with MDW933 probe in GCase staining buffer (GSB; 500mM sodium citrate, 50mM phosphate, 120 mM NaCl, 1% BSA, pH 5.2) and allowed to incubate at room temperature for 1 hour. After probe capping with MDW933, cells were washed twice with PBSA and stained with 1 μ g/mL of human anti-GCase antibody (having the CDRs of SEQ ID NOs.: 132-137, or similar) for 30 minutes at room temperature. After primary immunolabeling, cells were washed with PBSA and stained with 1:500 of anti-human IgG AlexaFluor®647 (Jackson ImmunoResearch, Catalog No. 109-605-006) on ice for 30 minutes. Yeast displaying GCase variants with high levels of expression and probe capping were selected by FACS and propagated for subsequent sorting.

All sorting rounds included primary and secondary labeling for GCase expression as described above. For FACS rounds 1 and 2, 500nM and 250nM MDW933 was used to stain the library, respectively. For FACS round 3, the library was split into two conditions. For round 3A, 150nM MDW933 was used to label the library as described above. For round 3S, the library was first subjected to serum stress by incubation in 100% mouse serum (Rockland, Cat# D108-00-0050, or similar) at 37°C for 4 hours with shaking at 150RPM. Following serum stress, the library was washed, and labeled with 150nM of MDW933 in GSB. For FACS round 4, the outputs from round 3 were again divided into multiple conditions. In round 4A, outputs from round 3A were labeled with 150nM MDW933 as described above. For rounds 4AS and 4SS, outputs from round 3A and round 3S were separately subjected to serum stress prior to MDW933 labeling as described above with the following modifications: serum incubation was carried out for 18 hours at 30°C. Figure 4 shows a summary of the serum treatment and labeling strategy across four rounds of FACS.

All sorting was conducted such that the top 0.5% to 1.5% of the clones displaying the highest fluorescence for expression and probe capping were selected, thereby enriching the library for GCase sequences with improved expression and enzymatic activity in the presence of serum. Sorted clones were propagated in SD-CAA, induced for expression in SG-CAA, and subjected to additional rounds of FACS. After FACS rounds 4A, 4SS, and 4SA, plasmid DNA was isolated using a Zymoprep yeast plasmid miniprepII kit (Zymo Research), transformed into One Shot® Top10 electrocompetent *E. coli* (Invitrogen), and sequenced.

EXAMPLE 6: GENERATION AND SCREENING OF GCASE VARIANT LIBRARY 1 SECRETION LIBRARY

Synthesis of GCase variant secretion library. DNA encoding variants from FACS rounds 4A, 4SS, and 4SA (Example 5) were amplified by PCR and purified by agarose gel electrophoresis. This DNA was used to generate a yeast secretion library following the same protocol described above for the yeast display libraries (Example 1) with the following change: the vector used for the secretion library was modified to excise Aga2p, which physically links the yeast cell surface to the display cassette. The modified cassette used for generation of the yeast secretion library encoded a yeast secretion signal peptide upstream of the GCase sequence with a C-terminal V5 epitope tag. Accordingly, the GCase polypeptide expressed from the modified cassette was secreted into the culture medium/supernatant rather than tethered to the cells. Transformed yeast were plated on SD-CAA agar (Teknova, Catalog No. S0551) and incubated at 30°C for two days. Single colonies were used to inoculate individual wells of deep well 96-well plates containing 500 µL of SD-CAA medium, and plates were incubated overnight in a 30°C shaker (250RPM). To induce expression of GCase variants, 100 µL from each well of the overnight SD-CAA culture was transferred to a fresh 96-well plate, the plate was centrifuged, and the medium was replaced with SG-CAA before incubating the plate overnight in a 30°C shaker (250RPM).

Analysis of GCase expression of GCase Library 1 variants. Expression of GCase Library 1 variants was assessed by ELISA to measure of conformational stability of the variants. Briefly, high-binding 384-well ELISA plates (Thermo Scientific, Catalog No. 464718 or 460518) were adsorption coated using PBS with 1µg/mL anti-V5 antibody (Sigma Aldrich, Catalog No. V8012). Coating was carried out overnight at 4°C after which plates were washed with PBST (1xPBS, pH 7.4, 0.1% Tween-20®) to remove excess antibody and blocked with PBSA (1xPBS, 1% BSA) for one hour at room temperature. All subsequent incubations were carried out at room temperature for one hour unless otherwise specified. After blocking, plates were washed with PBST, and supernatants from overnight SG-CAA secretion cultures, diluted 1:5 in PBSA, were added. Plates were then washed with PBST, and a human anti-GCase antibody (having the CDRs of SEQ ID NOs.: 132-137, or similar) at 1µg/mL in PBSA was added. After primary immunolabeling, plates were washed with PBST and secondary labeling was performed using a 1:10,000 of anti-human kappa HRP (Thermo Fisher Scientific, Catalog No. A18853). Plates were washed with PBST and the ELISA was developed using TMB substrate (Thermo Fisher Scientific, Catalog No. 34021)

and quenched with 1N HCl prior to being read on a BioTek Synergy H1 Microplate Reader.

Analysis of GCase activity of GCase Library 1 clones. To assess the enzymatic activity of GCase Library 1 clones, a modified version of the expression ELISA was performed. Adsorption coating, blocking, and sample addition was performed as described above. After sample incubation, plates were washed and 5mM 4-Methylumbelliferyl β -D-glucopyranoside (4-MUG) substrate in activity buffer (100 mM phosphate citrate buffer, pH 5.2, 0.5% sodium taurocholate, 0.25% Triton X-100) was added to the plates. The reaction was allowed to proceed for two hours in the dark, after which plates were quenched with 0.5M sodium carbonate, pH 10.3, and fluorescence was measured using a BioTek Synergy H1 Microplate Reader.

Analysis of post-serum exposure activity of GCase Variant Library 1 clones. The enzymatic activity of GCase Library 1 clones was measured post-serum exposure using the method described above to measure GCase activity with the following modification: prior to being added to plates, supernatants from overnight SG-CAA secretion cultures were mixed 1:5 with mouse serum (Rockland, Cat# D108-00-0050, or similar) and incubated at 37°C for 18-24 hours. After incubation, samples were added to the ELISA plates and retained enzymatic activity of GCase Library 1 clones was measured as described above.

Data analysis of expression and activity of GCase Variant Library 1 clones. Signal from media-only controls on the activity and serum stability plates was subtracted as background from each plate. Background subtracted values were normalized to corresponding values from the expression ELISA. The activity (pre- and post-serum exposure) and expression data of recombinant, secreted wild-type GCase, clone SH2-20, and clone SH2-88 are shown in Table 6. These data highlight the high activity and expression, as well as high retention of activity post-serum exposure, of SH2-20. In comparison, wild-type GCase displays low activity, very little expression, and complete loss of activity after serum incubation. To assess further improvements in engineered clones, the stability of GCase Library 1 variants was compared to SH2-20; the data is summarized in Table 7b. Samples showing improved expression, activity, and post-serum exposure activity relative to SH2-20 (SEQ ID NO:20) were selected for sequencing.

Sequencing of clone candidates. Selected yeast cultures from 96-well plates were added to 20mM NaOH (10 μ L yeast, 30 μ L basic solution) and boiled for 30 minutes at 95°C. Boiled supernatants were used as template DNA for a PCR reaction to amplify the GCase sequence, and products of

the reactions were sequenced.

Results. Table 6 summarizes the activity, expression, and post-serum exposure activity of wild-type GCase, SH2-20, and SH2-88. Table 7a provides examples of library clones with wild-type (WT) or non-wild-type residues at certain positions and Table 7b shows the activity, expression, and post-serum exposure activity of these clones with respect to clone SH2-20 (SEQ ID NO:20). To compare the attributes of the library clones relative to wild-type GCase, the data in Table 7b can be compared with the data in Table 6, which illustrate the improvements in the activity, expression, and post-serum stress activity of SH2-20 relative to wild-type GCase. The listed clones generally had good expression on yeast and at least 25% retention of baseline enzymatic activity post-serum stress. The listed clones contained the consensus sequence of SEQ ID NO:80:

1 ARPCIPKSFYSSVVCNATYCDSFDPPTFPALGTFSRYESTRSGRRMELSMGPIQANH
61 TGTGLLLTLQPEQKFQKVGFGGAMTDAAALNILALSPPAQNLLKSYFSEEGIGYNIIR
15 121 VPMAX_{1c}X_{2c}DFSIRTYTYADTPDDFQLHNFSLEEDTKLKIPLIHRALQLAQRPVSLASPWT
181 X_{3c}PTWLKTNGAVNGKGSCLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAX_{4c}TX_{5c}X_{6c}NEPX_{7c}X_{8c}GX_{9c}
241 LSGYPFQCLGFTPEHQDFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDP
301 AAKYVHGIHAVHWYLDX_{10c}LAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRG
361 MQYSHSIITNLLYHVVGWTDWNLALX_{11c}X_{12c}X_{13c}GGPNWVRNFVDSPIIVDITKDTFYKQPMFYHL
20 421 GHFSX_{14c}FIPEGSRVGLVASQKNDLDAVALMX_{15c}PDGSAVVVVLNRSSKDVPLTIKDPVAVGFL
481 ETISPGYSIHTYLWRRQ

wherein X_{1c} = S or A; X_{2c} = C, S, or T; X_{3c} = A or S; X_{4c} = L or V; X_{5c} = S, T, or A; X_{6c} = G, Q, or E; X_{7c} = S, I, L, or T; X_{8c} = A or N; X_{9c} = L, M, Q or Y; X_{10c} = S; X_{11c} = D or N; X_{12c} = P, M, T, or L; X_{13c} = E or K; X_{14c} = R; and X_{15c} = R.

Table 6. Attributes of secreted wild-type (WT) GCase and GCase variant enzymes.

GCase Variant	ELISA Expression (A450, a.u.)	Enzymatic Activity (A445, a.u.)	Post-Serum Stress Enzymatic Activity (A445, a.u.)
SH2-20	0.496	195.5	202
SH2-88	0.299	4.5	14
WT GCase	0.075	15.5	0

a.u. = arbitrary units

Table 7a. Exemplary clones obtained from screening of GCase Variant Library 1

SEQ ID NO.	Clone	Position #																									
		125	126	181	230	231	232	233	234	235	236	237	238	239	240	315	316	317	386	387	388	389	424	425	426	450	451
1	WT	S	C	S	V	T	A	E	N	E	P	S	A	G	L	D	F	L	N	P	E	G	S	K	F	M	H
20	SH-20	S	C	S	V	T	A	G	N	E	P	S	A	G	L	D	S	L	N	P	E	G	S	R	F	M	R
81	CI_B4	S	T	S	L	T	S	G	N	E	P	S	A	G	M	D	S	L	D	L	E	G	S	R	F	M	R
82	CI_B5	S	T	A	L	T	S	Q	N	E	P	L	N	G	L	D	S	L	D	L	K	G	S	R	F	M	R
83	CI_B8	S	T	S	L	T	T	Q	N	E	P	I	A	G	L	D	S	L	N	L	E	G	S	R	F	M	R
84	CI_B9	S	T	A	L	T	T	G	N	E	P	I	N	G	L	D	S	L	N	T	E	G	S	R	F	M	R
85	CI_C10	S	T	S	L	T	S	Q	N	E	P	I	N	G	M	D	S	L	N	T	E	G	S	R	F	M	R
86	CI_C11	S	T	A	V	T	T	Q	N	E	P	I	N	G	L	D	S	L	N	M	K	G	S	R	F	M	R
87	CI_C12	S	T	A	L	T	T	Q	N	E	P	I	N	G	L	D	S	L	D	M	K	G	S	R	F	M	R
88	CI_C7	S	T	S	V	T	S	Q	N	E	P	I	A	G	Q	D	S	L	N	L	E	G	S	R	F	M	R
89	CI_C9	S	T	S	V	T	S	Q	N	E	P	T	A	G	Y	D	S	L	D	T	E	G	S	R	F	M	R
90	CI_D11	S	T	S	L	T	T	G	N	E	P	I	N	G	L	D	S	L	N	M	K	G	S	R	F	M	R
91	CI_E11	S	T	A	L	T	T	Q	N	E	P	I	N	G	M	D	S	L	D	T	E	G	S	R	F	M	R
92	CI_E4	S	T	A	V	T	T	G	N	E	P	I	N	G	L	D	S	L	D	M	K	G	S	R	F	M	R
93	CI_F10	S	T	A	V	T	S	Q	N	E	P	T	A	G	L	D	S	L	D	M	K	G	S	R	F	M	R
94	CI_F11	S	T	A	L	T	S	Q	N	E	P	I	N	G	L	D	S	L	N	M	K	G	S	R	F	M	R
95	CI_F12	S	S	A	V	T	S	Q	N	E	P	T	A	G	L	D	S	L	N	M	K	G	S	R	F	M	R
96	CI_F4	S	T	A	L	T	S	Q	N	E	P	L	N	G	L	D	S	L	D	L	E	G	S	R	F	M	R
97	CI_F7	S	T	A	V	T	S	Q	N	E	P	I	A	G	L	D	S	L	N	T	E	G	S	R	F	M	R
98	CI_F9	S	T	S	V	T	S	G	N	E	P	T	A	G	L	D	S	L	N	T	E	G	S	R	F	M	R
99	CI_G10	S	T	S	V	T	S	Q	N	E	P	T	A	G	Y	D	S	L	N	M	K	G	S	R	F	M	R
100	CI_G12	S	C	A	V	T	S	Q	N	E	P	T	A	G	L	D	S	L	D	M	E	G	S	R	F	M	R
101	CI_G8	A	T	S	L	T	T	G	N	E	P	T	A	G	L	D	S	L	D	L	E	G	S	R	F	M	R
102	CI_H5	S	T	A	V	T	A	G	N	E	P	S	A	G	L	D	S	L	D	L	E	G	S	R	F	M	R
103	CI_H6	S	T	S	L	T	T	Q	N	E	P	I	N	G	L	D	S	L	N	T	K	G	S	R	F	M	R

WT = wild-type GCase

a.u. = arbitrary units

Table 7b. Activity, Expression, and Post-Serum Exposure Activity of GCase Library 1 Variants

SEQ ID NO.	Clone	ELISA (A450, a.u.)	Enzymatic Activity (A445, a.u.)	Post-Serum Stress Enz. Activity (A445, a.u.)	SEQ ID NO.	Clone	ELISA (A450, a.u.)	Enzymatic Activity (A445, a.u.)	Post-Serum Stress Enz. Activity (A445, a.u.)
1	WT				1	WT			
20	SH-20	0.177	175	110	92	CI_E4	0.283	1491	207
81	CI_B4	0.309	362	188	93	CI_F10	0.417	545	500
82	CI_B5	0.579	1125	254	94	CI_F11	0.339	1602	652
83	CI_B8	0.43	629	185	95	CI_F12	0.52	627	449
84	CI_B9	0.49	1461	258	96	CI_F4	0.279	1090	251
85	CI_C10	0.189	931	218	97	CI_F7	0.369	1455	531
86	CI_C11	0.444	1320	531	98	CI_F9	0.352	779	435
87	CI_C12	0.536	1620	545	99	CI_G10	0.321	403	151
88	CI_C7	0.309	249	119	100	CI_G12	0.202	490	311
89	CI_C9	0.283	428	149	101	CI_G8	0.32	278	152
90	CI_D11	0.24	710	99	102	CI_H5	0.281	369	216
91	CI_E11	0.18	495	115	103	CI_H6	0.209	985	277

WT = wild-type GCase
a.u. = arbitrary units

EXAMPLE 7: GENERATION OF GCASE VARIANT LIBRARY 2

SH2-20 (Example 4, SEQ ID NO:20) was used as the parental sequence for the design of a second GCase variant library (“GCase Variant Library 2” or “GCase Library 2”) given its high expression and probe capping on yeast. The library was designed by selecting amino acids for randomization based off structural differences between unbound (PDB ID: 2NT1) and isofagomine (IFG)-bound (PDB ID: 3GXF) GCase, as described for the generation of GCase Variant Library 1 (Example 5). Regions containing strong perturbations upon IFG binding ($\geq 9\%$ decrease in average deuteration level as measured by H/D exchange protocol (Hamuro et al. 2003. *J Biomol Tech* 14:171) that were sequentially or conformationally adjacent to regions that are hydrogen bound to IFG (as described by Kornhaber, et al. 2008. *Chembiochem* 9(16):2643-2649) were identified (amino acid numbers 187-197, 243-249, 310-312, 343-347, and 414-417 of SEQ ID NO:1). To enable combinatorial exploration of a manageable number of positions, a multiple sequence alignment of GCase orthologs, as described previously (Example 5), was used to identify positions within these regions that were non-conserved. This set of non-conserved residues was further narrowed by selecting positions found in loop and alpha helical regions. The final set of positions

chosen for the GCase Variant Library 2 are provided in Table 8. Diversification of the library positions was accomplished by allowing each position to be mutated to amino acids found at that position in the multiple sequence alignment of GCase orthologs described. The final library contained additional diversity beyond the ortholog set (Table 8, italicized amino acids) as an artifact of the degenerate codons used to build it.

Table 8 provides a summary of the library design, including positions which were mutated, and the restricted set of amino acids interrogated within the library at each of these positions. Four mutations in SH2-20 (E233G, F316S, K425R, and H451R) were present in the library but are not included in Table 8 because they were not varied. Italicized and bolded amino acids represent the non-ortholog amino acids incorporated in the library because of the degenerate codons used in library synthesis.

Table 8. Design of GCase Variant Library 2

Position #	189	190	191	192	343	345	346	347
WT residue	G	A	V	N	V	S	K	F
	G	A	V	N	V	S	K	F
	R	D	A	I	A	F	E	M
	E	S	F	K	I	T	F	S
	<i>Q</i>	R	G	R	L	I	H	P
		<i>H</i>	L	T	N		L	<i>T</i>
		<i>N</i>	M	V	T		N	<i>I</i>
		<i>G</i>	V	<i>A</i>	<i>H</i>		Y	<i>L</i>
		<i>P</i>	Y	<i>D</i>	<i>P</i>		<i>V</i>	
		<i>T</i>	<i>R</i>	<i>E</i>	<i>D</i>		<i>I</i>	
			<i>N</i>	<i>G</i>			<i>Q</i>	
			<i>D</i>	<i>M</i>			<i>D</i>	
			<i>C</i>	<i>S</i>				
			<i>E</i>					
			<i>I</i>					
			<i>K</i>					
			<i>S</i>					
			<i>T</i>					
			<i>W</i>					

WT = wild-type

Synthesis of GCase Variant Library 2. The GCase Variant Library 2 was built by PCR using the SH2-20 DNA sequence as the template, with the positions indicated in Table 8 mutated to the specified subset of amino acids using oligos with degenerate codons. Amplified library DNA was purified by agarose gel electrophoresis, ethanol precipitated, and resuspended in water to a final concentration of 1 mg/mL.

Generation and screening of a yeast-displayed GCase Variant Library 2. Mutated GCase DNA and linearized yeast display vector was used to transform yeast as described above (Example 1), resulting in a library of GCase variants with diversity incorporated as outlined in Table 8. Library size was estimated to be 2×10^8 by dilution plating. Following induction of yeast for GCase expression, cells were stained with MDW933 probe in GCase staining buffer (GSB; 500mM sodium citrate, 50mM phosphate, 120 mM NaCl, 1% BSA, pH 5.2) and subsequently labeled with primary human anti-GCase antibody followed by secondary labeling with anti-human IgG AlexaFluor®647 as described previously (Example 5). Yeast displaying GCase variants with high levels of expression and probe capping were enriched within the library by FACS and propagated for subsequent sorting.

All sorting rounds included primary and secondary labeling for GCase expression and staining with MDW933 using protocols similar to that described in Example 5. For FACS rounds 1 and 2, 500nM and 250nM MDW933 was used to stain the library, respectively. For FACS round 3, the library was split into three conditions. For round 3A and 3C, 150 nM MDW933 was used to label the library, and differential stringency was applied when sorting these two samples; 0.5% (round 3A) or 3% (round 3C) of clones displaying the highest levels of GCase expression and probe capping were collected. For round 3B, the library was first incubated in 100% mouse serum (Rockland, Cat# D108-00-0050, or similar) at 30°C overnight with shaking at 150RPM, followed by labeling with 125 nM of MDW933. All serum treatments for subsequent rounds of sorting were performed in the same manner unless otherwise noted. For FACS round 4A, outputs from round 3A were incubated with serum followed by labeling with 100nM MDW933. The outputs from round 3C were subjected to two additional rounds of sorting, rounds 4C and 5C, which were serum stressed and subsequently stained with MDW933 at 100nM and 75nM, respectively. Figure 5 shows a summary of the serum treatment and labeling strategy across rounds of FACS.

All sorting was conducted such that the top 1% of clones displaying the highest fluorescence for expression and probe capping were selected, unless otherwise noted, thereby enriching the library

for GCase sequences with improved expression and enzymatic activity in the presence of serum. Sorted clones were propagated in SD-CAA, induced for expression in SG-CAA, and subjected to additional rounds of FACS. After FACS rounds 4A, 3B, and 5C, plasmid DNA was isolated using a Zymoprep yeast plasmid miniprepII kit (Zymo Research), transformed into One Shot® Top10 electrocompetent *E. coli* (Invitrogen), and sequenced.

EXAMPLE 8: GENERATION AND SCREENING OF A GCASE VARIANT LIBRARY 2 SECRETION LIBRARY

10 *Synthesis of a GCase Variant Library 2 secretion library.* Yeast secretion libraries were generated from FACS rounds 4A, 3B, and 5C of GCase Variant Library 2 using the same methods described for synthesis of the GCase Variant Library 1 secretion library (Example 6). Single colonies from each of the resulting libraries were grown in liquid culture and induced to drive expression of secreted GCase variants as described (Example 6).

15 *Analysis of GCase expression, activity, and post-serum exposure activity of GCase Variant Library 2 secretion variants.* The expression, activity, and post-serum exposure activity of GCase Variant Library 2 variants was assessed by ELISA methods as described in Example 6. The activity, expression, and post-serum stress activity data of GCase Library 2 variants is summarized in Table 9b. Samples displaying improved expression, activity, and post-serum exposure activity relative to SH2-20 (SEQ ID NO:20) were selected for sequencing.

25 *Sequencing of clone candidates.* Selected yeast cultures from 96-well plates were added to 20mM NaOH (10µL yeast, 30µL basic solution) and boiled for 30 minutes at 95°C. Boiled supernatants were used as template DNA for a PCR to amplify the GCase sequence, and products of the reactions were sequenced.

30 *Results.* Table 9a provides examples of library clones with wild-type (WT) or non-wild-type residues at certain positions and Table 9b shows the activity, expression, and post-serum exposure activity of these clones with respect to SEQ ID NO:20. To compare the attributes of the library clones relative to wild-type GCase, the data in Table 9b can be compared with the data in Table 6, which illustrate the improvements in the activity, expression, and post-serum stress activity of SH2-20 relative to wild-type GCase. The listed clones generally had good expression on yeast and at least 25% retention of enzymatic activity post-serum stress. The listed clones contained the

consensus sequence of SEQ ID NO:104:

1 ARPCIPKSFYSSVVCNATYCDSEDPPTFPALGTFSRYESTRSGRRMELSMGPIQANH
 61 TGTGLLLTLQPEQKFQKVGFGGAMTDAAALNILALSPPAQNLLLKSIFSEEGIGYNIIR
 121 VPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQRPVSLLASPWT
 5 181 SPTWLKTNX_{1d}X_{2d}X_{3d}X_{4d}GKGS LKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAX_{5d}NEPSAGL
 241 LSGYX_{6d}FQCLGFTPEHQRFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDP
 301 AAKYVHGI AVHWYLDX_{7d}LAPAKATLGETHRLFPNTMLFASEACX_{8d}GX_{9d}X_{10d}X_{11d}WEQSVRLGSWDRG
 361 MQYSHSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPMFYHL
 421 GHFSX_{12d}FIPEGSQVRGLVASQKNDLDAVALMX_{13d}PDGSAVVVVLNRSSKDVPLTIKDPVAVGFL
 10 481 ETISPGYSIHTYLWRRQ

wherein X_{1d} = G, E, R, or Q; X_{2d} = A, N, T, S, H, D, R, or G; X_{3d} = V, Y, F, L, M, S,
 G, or W; X_{4d} = N, V, I, T, A, M, E, G, D, or S; X_{5d} = G; X_{6d} = P, S, K, Q, or T; X_{7d} = S;
 X_{8d} = V, T, A, or N; X_{9d} = S, F, T, or I; X_{10d} = K, L, Q, F, N, E, or V; X_{11d} = F, P, S, or T; X_{12d} =
 R; and X_{13d} = R.

15

Table 9a. Exemplary clones obtained from screening of GCase Variant Library 2

SEQ ID NO.	Clone	Position #																			
		189	190	191	192	232	233	234	244	245	246	315	316	317	342	343	344	345	346	347	348
1	WT	G	A	V	N	A	E	N	Y	P	F	D	F	L	C	V	G	S	K	F	W
20	SH-20	G	A	V	N	A	G	N	Y	P	F	D	S	L	C	V	G	S	K	F	W
105	mI3-A9	E	N	Y	V	A	G	N	Y	S	F	D	S	L	C	T	G	T	L	P	W
106	mI3-B7	Q	T	F	I	A	G	N	Y	P	F	D	S	L	C	T	G	F	Q	P	W
107	mI3-F111	E	S	L	T	A	G	N	Y	K	F	D	S	L	C	T	G	I	F	P	W
108	mI3-F113	G	S	Y	V	A	G	N	Y	K	F	D	S	L	C	A	G	F	Q	P	W
109	mI3-F12	R	D	F	V	A	G	N	Y	P	F	D	S	L	C	T	G	F	N	S	W
110	mI3-G11	G	H	Y	A	A	G	N	Y	P	F	D	S	L	C	T	G	F	E	P	W
111	mI3-H2	E	N	M	T	A	G	N	Y	P	F	D	S	L	C	A	G	F	E	P	W
112	mI3-H3	Q	A	Y	M	A	G	N	Y	Q	F	D	S	L	C	T	G	F	K	P	W
113	mI4-A10	Q	A	F	E	A	G	N	Y	P	F	D	S	L	C	N	G	F	E	S	W
114	mI4-A11	G	H	F	G	A	G	N	Y	K	F	D	S	L	C	T	G	S	L	P	W
115	mI4-B2	G	T	L	M	A	G	N	Y	P	F	D	S	L	C	N	G	F	L	S	W
116	mI4-B5	G	D	F	T	A	G	N	Y	P	F	D	S	L	C	N	G	F	Q	T	W
117	mI4-F2	G	N	F	I	A	G	N	Y	P	F	D	S	L	C	T	G	F	Q	P	W
118	mI4-F31	Q	T	Y	T	A	G	N	Y	Q	F	D	S	L	C	T	G	F	E	T	W
119	mI4-F32	E	R	L	E	A	G	N	Y	P	F	D	S	L	C	T	G	F	E	T	W
120	mI4-G4	G	N	S	S	A	G	N	Y	P	F	D	S	L	C	T	G	F	E	S	W
121	mI4-G71	E	R	G	S	A	G	N	Y	Q	F	D	S	L	C	T	G	S	Q	P	W
122	mI4-G73	E	R	G	S	A	G	N	Y	P	F	D	S	L	C	T	G	S	V	P	W
123	mI4-H2	Q	T	Y	M	A	G	N	Y	T	F	D	S	L	C	A	G	F	E	P	W
124	mI5-A11	R	G	F	E	A	G	N	Y	Q	F	D	S	L	C	T	G	F	Q	P	W
125	mI5-D10	G	N	F	I	A	G	N	Y	K	F	D	S	L	C	T	G	F	Q	P	W
126	mI5-D12	E	D	W	D	A	G	N	Y	P	F	D	S	L	C	T	G	F	E	P	W
127	mI5-F11	G	D	F	I	A	G	N	Y	P	F	D	S	L	C	A	G	F	K	P	W
128	mI5-F3	G	N	F	I	A	G	N	Y	P	F	D	S	L	C	T	G	S	L	P	W
129	mI5-G11	G	G	F	T	A	G	N	Y	P	F	D	S	L	C	A	G	F	Q	P	W
130	mI5-G2	E	D	V	T	A	G	N	Y	P	F	D	S	L	C	T	G	F	K	P	W
131	mI5-H2	G	H	Y	E	A	G	N	Y	P	F	D	S	L	C	N	G	F	Q	S	W

WT = wild-type GCase

Table 9b. Activity, Expression, and Post-Serum Exposure Activity of GCase Library 2 Variants

SEQ ID NO.	Clone	ELISA (A450, a.u.)	Enzymatic Activity (A445, a.u.)	Post-Serum Stress Enz. Activity (A445, a.u.)		SEQ ID NO.	Clone	ELISA (A450, a.u.)	Enzymatic Activity (A445, a.u.)	Post-Serum Stress Enz. Activity (A445, a.u.)
1	WT					1	WT			
20	SH-20	0.696	375	128		118	mI4-F31	0.43	3271	833
105	mI3-A9	0.754	1860	491		119	mI4-F32	0.43	3271	833
106	mI3-B7	0.798	1370	220		120	mI4-G4	0.619	3847	925
107	mI3-F111	1.081	2284	671		121	mI4-G71	0.779	2554	654
108	mI3-F113	1.081	2284	671		122	mI4-G73	0.779	2554	654
109	mI3-F12	1.037	3156	1145		123	mI4-H2	0.193	2901	1148
110	mI3-G11	1.269	3760	1125		124	mI5-A11	0.727	1189	368
111	mI3-H2	1.118	5680	2375		125	mI5-D10	0.233	742	184
112	mI3-H3	0.669	1494	440		126	mI5-D12	0.674	1202	346
113	mI4-A10	0.693	3314	836		127	mI5-F11	1.004	3209	757
114	mI4-A11	0.834	1519	596		128	mI5-F3	0.783	4543	844
115	mI4-B2	0.53	3230	1141		129	mI5-G11	1.053	3212	903
116	mI4-B5	0.686	3151	858		130	mI5-G2	0.839	2025	457
117	mI4-F2	0.287	877	238		131	mI5-H2	0.809	4571	1073

WT = wild-type GCase
a.u. = arbitrary units

EXAMPLE 9. GENERATION OF GCASE VARIANT LIBRARY 3

SH2-20 (Example 4, SEQ ID NO:20) is used as the parental sequence for the design of a third GCase variant library (“GCase Variant Library 3” or “GCase Library 3”) given its high expression and probe capping on yeast. The library is designed by selecting amino acids for randomization based off structural differences between unbound (PDB ID: 2NT1) and isofagomine (IFG)-bound (PDB ID: 3GXF) GCase, as described previously for the generation of GCase Variant Library 1 (Example 5) and GCase Variant Library 2 (Example 7). Regions containing intermediate perturbations upon IFG binding (about 5-8% decrease in average deuteration level) as measured by H/D exchange protocol (Hamuro et al. 2003. *J Biomol Tech* 14:171) that are sequentially or conformationally adjacent to regions that are hydrogen bound to IFG (as described by Kornhaber, et al. 2008. *Chembiochem* 9(16):2643-2649) are identified (amino acid numbers 130-134, 284-296, 315-336, and 350-362 of SEQ ID NO:1). To enable combinatorial exploration of a manageable number of positions, a multiple sequence alignment of

GCase orthologs, as described previously (Example 5), is used to identify positions within this highlighted set that are non-conserved. This set of non-conserved residues is further narrowed by selecting positions found in loop and alpha helical regions. The final set of positions chosen for the GCase Variant Library 3 are provided in Table 10. Diversification of the GCase Variant Library 3 positions is accomplished by allowing each position to be mutated to amino acids found at that position in the multiple sequence alignment of GCase orthologs described. Due to large diversity, the library is split up into two libraries generated independently: Library 3-1 (316-322, 325, 329), and Library 3-2 (350, 351, 353, 361, 362). These libraries are then sorted to select for clones with improved GCase expression and stability using the dual labeling strategy of probe capping and anti-GCase expression as described previously (*e.g.*, as described in Examples 5 or 7). The two libraries are then combined by DNA shuffling after diversity is reduced via FACS sorting, as described in Example 2. Secretion libraries of the post-sorted and post-DNA shuffled variants are then generated, and expression, activity, and stability of secretion library variants are assessed by ELISA methods as described in Example 6.

Table 10 provides a summary of the library design, including positions which are mutated, and the restricted set of amino acids that is interrogated within the library at each of these positions. Three of four mutations in SH2-20 (E233G, K425R, and H451R) are present in the library but are not included in Table 10 because they are not varied. *Italicized and bolded* amino acids represent the non-ortholog amino acids incorporated in the library because of the degenerate codons used in library synthesis.

Table 10. Design of GCase Variant Library 3

Position #	316	317	318	319	320	321	322	325	329	350	351	353	361	362
WT Residue	F	L	A	P	A	K	A	G	R	Q	S	R	M	Q
	F	L	A	P	A	K	A	G	R	Q	S	R	M	Q
	G	I	V	S	I	D	C	E	E	F	A	A	C	A
	L	<i>F</i>	G		P	E	D	L	H	K	C	I	D	E
	<i>C</i>		I		<i>L</i>	G	L	S	K	N	D	L	E	H
	<i>W</i>		<i>S</i>		<i>T</i>	N	P	V	N	P	G	Q	I	K
	<i>V</i>		<i>T</i>		<i>V</i>	Q	R	W	<i>Q</i>	R	R	S	N	P
						R	S	<i>A</i>	<i>D</i>	<i>C</i>	<i>H</i>	V	Q	Q
						T	<i>G</i>		<i>G</i>	<i>H</i>	<i>P</i>	N	S	R
						<i>P</i>	<i>H</i>		<i>S</i>	<i>I</i>		<i>D</i>	T	<i>N</i>
						<i>S</i>	<i>F</i>			<i>L</i>		<i>E</i>	V	<i>G</i>
						<i>H</i>	<i>Y</i>			<i>M</i>		<i>G</i>	<i>A</i>	<i>I</i>
						<i>A</i>	<i>V</i>			<i>S</i>		<i>H</i>	<i>R</i>	<i>L</i>
										<i>T</i>		<i>P</i>	<i>G</i>	<i>T</i>
										<i>W</i>		<i>T</i>	<i>H</i>	<i>V</i>
										<i>Y</i>			<i>L</i>	
													<i>K</i>	
													<i>F</i>	
													<i>P</i>	
													<i>W</i>	
													<i>Y</i>	

WT = wild-type

EXAMPLE 10: COMBINATORIAL ANALYSIS OF GCASE VARIANTS FOR FURTHER IMPROVEMENTS IN EXPRESSION, ACTIVITY, AND STABILITY

A set of improved variants from GCase Variant Library 2 (Examples 7, 8) were selected for further engineering to explore additional combinatorial sequence space for GCase variants. These additional combinatorial libraries are designed such that each position in the GCase sequence encoded either the wild-type residue (from SEQ ID NO: 1) or any other amino acid observed at that specific residue in the improved GCase variants (Table 9a). In a particular embodiment, two sequences were selected from Table 9a (SEQ ID NOs:128 and 131) which contained improved serum stability, expression, and activity relative to SH2-20 (SEQ ID NO: 20). The wild-type sequence was used as template DNA sequence, and each residue at non-conserved positions was allowed to toggle between wild-type (from SEQ ID NO: 1) or the amino acid contained in SEQ ID NOs: 128 or 131. Table 11 provides a summary of the library design,

including positions which were mutated and the restricted set of amino acids interrogated within the library, at each of these positions. The final library contains additional diversity beyond the ortholog set (Table 11, italicized and bolded amino acids) as an artifact of the degenerate codons used to build it. The combinatorial library is then transformed into yeast, as described previously (Examples 1, 7) and selected for improved probe capping (MDW933) and expression (anti-GCase antibody) after treatment with mouse serum at 37°C.

Table 11. Design of Additional Combinatorial Library for Further GCase Engineering

Position #	190	191	192	233	316	343	345	346	347	425	451
WT Residue	A	V	N	E	F	V	S	K	F	K	H
	A	V	N	E	F	V	S	K	F	K	H
	N	Y	E	G	S	N	F	Q	S	R	R
	H	F	I			T		L	P		
	D	D	K			D		M	L		
	P		V			I					
	T					A					

WT = wild-type

10

Synthesis of GCase combinatorial analysis library. The combinatorial analysis library is built by PCR using the wild-type DNA sequence as the template, with the positions indicated in Table 11 mutated to the specified subset of amino acids using oligonucleotides with degenerate codons. Amplified library DNA is purified by agarose gel electrophoresis, ethanol precipitated, and resuspended in water to a final concentration of 1 mg/mL.

15

Generation and screening of a yeast-displayed combinatorial analysis library. Mutated GCase DNA and linearized yeast display vector is used to transform yeast as described previously (Example 1), resulting in a library of GCase variants with diversity encompassing the sequence space illustrated in Table 11. Following induction of yeast for GCase expression, cells are stained with MDW933 probe in GCase staining buffer (GSB; 500mM sodium citrate, 50mM phosphate, 120 mM NaCl, 1% BSA, pH 5.2) and subsequently labeled with primary human anti-GCase antibody followed by secondary labeling with anti-human IgG AlexaFluor®647 as described previously (Example 5).

25

Rounds of cell sorting using GCase immunodetection and probe capping are carried out to enrich the library for GCase sequences with improved expression and activity in a manner similar to that

previously described (Examples 5, 7). Following the final round of FACS, plasmid DNA is isolated using a Zymoprep yeast plasmid miniprepII kit (Zymo Research), transformed into One Shot® Top10 electrocompetent E.coli (Invitrogen), and sequenced.

- 5 *Analysis of GCase expression, activity, and stability of combinatorial analysis secretion variants.* Yeast secretion libraries are produced from the GCase library enriched for sequences with improved expression and activity as described previously (Example 6). Single colonies from each of the resulting libraries are grown in liquid culture and induced to drive expression of secreted GCase variants as described. The expression, activity, and post-serum exposure activity of single
10 clones of GCase variants is assessed by ELISA methods as described in Example 6. Samples displaying improved expression, activity, and post-serum exposure activity relative to SH2-20 (SEQ ID NO:20) are selected for sequencing.

- Sequencing of clone candidates.* Selected yeast cultures from 96-well plates are added to 20mM
15 NaOH (10µL yeast, 30µL basic solution) and boiled for 30 minutes at 95°C. Boiled supernatants are used as template DNA for a PCR to amplify the GCase sequence, and products of the reactions are sequenced.

- Results.* The resulting clones contain certain combinations of SEQ ID NO:104, as well as the
20 sequences of SEQ ID NOs: 138-170.

EXAMPLE 11: EVALUATION OF SELECT GCASE VARIANTS SECRETED FROM CHO CELLS

- Certain sequences were interrogated for expression in Chinese Hamster Ovary (CHO) cells
25 and evaluated for their protein titer, baseline activity, and post-serum exposure activity relative to wild-type GCase enzyme (SEQ ID NO:1), imiglucerase (Cerezyme™), and a comparator variant from WO 2022/023761.

CHO cell expression of GCase enzyme and enzyme variants.

- Sequences were cloned into a derived pRK5 vector containing a mouse kappa light chain signal
30 sequence at its N-terminal and an uncleavable C-terminal 10x histidine tag. Protein was transiently transfected and expressed in ExpiCHO cells for 5 days following the High Titer Protocol from the manufacturer (ThermoFischer Scientific™).

Purification of GCCase variants.

Cells were harvested by centrifugation at 3000rpm for 10 minutes, and supernatant was filtered through a 0.22 μ M membrane and loaded onto a Ni-NTA Xcel resin (Cytiva) pre-equilibrated with 25mM HEPES pH 7.5, 150mM NaCl (Buffer A). After loading of the sample, the resin was
5 washed with Buffer A supplemented with 15mM imidazole, and histidine-tagged protein was eluted with Buffer A with 300mM imidazole. Elution fractions were concentrated down to a volume of about 0.8 mL and delipidated by addition of 20% (v/v) butanol. The delipidation reaction was incubated for one hour at room temperature and subsequently centrifuged at 10k rpm for 10 minutes. The aqueous fraction was loaded onto a Superdex S200 10/300 size exclusion
10 column pre-equilibrated with 20mM sodium acetate pH 5.5 and 150 mM NaCl. Fractions containing protein were analyzed by SDS-PAGE, pooled and concentrated down to about 1 mg/mL. Post-purification yield was assessed by NanoDrop™ spectrophotometry measurement.

Evaluation of GCCase activity, and stability of combinatorial analysis secretion variants

The baseline activity and post-serum exposure activity of the purified GCCase variant proteins were
15 assessed using the 4-Methylumbelliferyl β -D-glucopyranoside (4-MUG) assay as described in Example 6. Background subtracted values for each protein were normalized to expression titer measured for the corresponding protein. Serum exposure was conducted at both 4°C and 37°C for a 24-hour period prior to activity measurement.

Results. Table 12 summarizes the post-purification yield, baseline enzymatic activity, and post-
20 serum exposure activity (at 4°C and 37°C) of wild-type GCCase, certain GCCase variants disclosed herein, and a comparator GCCase variant. The baseline activity of imiglucerase (Cerezyme™) was also measured for comparison.

The GCCase variants exhibited better baseline activity than wild-type GCCase, imiglucerase, and the
25 comparator variant. Relative to wild-type GCCase, imiglucerase, and the comparator variant, the GCCase variants also exhibited better retention of activity levels ($\geq 40\%$ of baseline activity) after 24 hours of exposure to serum at both 4°C and 37°C.

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Table 12. Post-Purification Yield, Baseline Activity, and Post-Serum Exposure Activity of GCASE Enzymes and Enzyme Variants

SEQ ID NO.	Clone	Yield [mg/L]	Enzymatic Activity (pmol min ⁻¹ pmol ⁻¹)	Post-Serum Stress (4°C) Enz. Activity (pmol min ⁻¹ pmol ⁻¹)	Post-Serum Stress (37°C) Enz. Activity (pmol min ⁻¹ pmol ⁻¹)
--	Cerezyme™	N/A	648.53	--	--
1	WT	8.6	565.75	242.64	29.99
131	mI5-H2	120	2244.7	2273.3	1525.9
128	mI5-F3	67.5	2137.1	2751.9	2113.6
173	mI6-C2	86	2451.9	3415.5	1008.3
139	mI6-B2	89.2	929.67	1258.9	928.85
174	Comparator	17.6	202.17	283.85	125.01

WT = wild-type GCASE

5

EXAMPLE 12: COMBINATORIAL ANALYSIS OF GCASE VARIANTS FOR IMPROVEMENTS IN EXPRESSION, ACTIVITY, AND STABILITY

Additional exploration of the combinatorial sequence space across the GCASE variant libraries (e.g., Examples 5, 7, and 9) can be carried out. These combinatorial libraries are designed such that each position selected for mutation in the GCASE sequence encodes: (i) the wild-type residue (from SEQ ID NO: 1), (ii) a non-conserved ortholog residue, or (iii) an amino acid that is an artifact of the degenerate codons used to build the library GCASE sequences. The wild-type sequence is used as template DNA sequence, and each selected position is allowed to toggle between (i), (ii) and (iii). The combinatorial library is then transformed into yeast as described previously (Examples 1, 5, 7, 9), and selected for improved probe capping (MDW933) and expression (anti-GCASE antibody) after treatment with mouse serum at 37°C.

Synthesis of GCASE combinatorial analysis library. The combinatorial analysis library is built by PCR using the wild-type DNA sequence as the template, with the selected positions (e.g., two or more positions from Tables 5, 8, and 10) mutated to the specified subset of amino acids using oligos with degenerate codons. Amplified library DNA is purified by agarose gel electrophoresis, ethanol precipitated, and resuspended in water to a final concentration of 1 mg/mL.

Generation and screening of a yeast-displayed combinatorial analysis library. Mutated GCASE DNA and linearized yeast display vector is used to transform yeast as described above (Example 1), resulting in a library of GCASE variants with diversity. In a particular embodiment,

25

the generated library encompasses the sequence space provided in Tables 5, 8, and 10. Following induction of yeast for GCase expression, cells are stained with MDW933 probe in GCase staining buffer (GSB; 500mM sodium citrate, 50mM phosphate, 120 mM NaCl, 1% BSA, pH 5.2) and subsequently labeled with primary human anti-GCase antibody followed by secondary labeling with anti-human IgG AlexaFluor®647 as described previously (Example 5). Yeast displaying GCase variants with high levels of expression and probe capping are enriched within the library by FACS and propagated for subsequent sorting.

All sorting rounds include primary and secondary labeling for GCase expression and staining with MDW933 using protocols similar to that described in Examples 5 and 7. MDW933 concentration is decreased for each round of sorting to select for higher enzymatic activity, and serum stress is applied at some point during sorting and carried out for all subsequent rounds of sorting after it is applied. For example, for FACS round 1, 500nM MDW933 is used to stain the library, respectively. For FACS round 2, the library is first incubated in 100% mouse serum (Rockland, Cat# D108-00-0050, or similar) at 30°C or 37°C overnight shaking at 150RPM, followed by labeling with 250nM of MDW933. All serum treatments for subsequent rounds of sorting are performed in the same manner unless otherwise noted. For FACS round 3, cells are treated with serum followed by labeling with 150nM MDW933. For optional FACS round 4, cells are treated with serum followed by labeling with 75nM MDW933.

All sorting is conducted such that the top 0.5% to 1.5% of clones displaying the highest fluorescence for expression and probe capping are selected, thereby enriching the library for GCase sequences with improved expression and activity. Sorted clones are propagated in SD-CAA, induced for expression in SG-CAA, and subjected to additional rounds of FACS. Following final rounds of FACS for library enrichment, plasmid DNA is isolated using a Zymoprep yeast plasmid miniprepII kit (Zymo Research), transformed into One Shot® Top10 electrocompetent *E.coli* (Invitrogen), and optionally sequenced.

Analysis of GCase expression, activity, and stability of combinatorial analysis secretion variants.

Secretion libraries of the post-sorted variants are generated, and expression, activity, and stability of secretion library variants are assessed by ELISA methods as described in Example 6. Samples displaying improved expression, activity or serum stability relative to SH2-20 (SEQ ID NO:20) are selected for sequencing.

Sequencing of clone candidates. Selected yeast cultures from 96-well plates are added to 20mM NaOH (10µL yeast, 30µL basic solution) and boiled for 30 minutes at 95°C. Boiled supernatants are used as template DNA for a PCR to amplify the GCcase sequence, and products of the reactions were sequenced.

5

EXAMPLE 13: FURTHER ENGINEERING OF IDENTIFIED GCASE VARIANTS FOR IMPROVEMENTS IN EXPRESSION, ACTIVITY, AND STABILITY

Additional exploration of the combinatorial sequence space across the GCcase variants to generate improved variants from GCcase Variant Libraries 1, 2, and 3, including, but not limited to, those outlined in Tables 7 and 9, are carried out. These combinatorial libraries are designed such that each position selected for mutation in the GCcase sequence encodes: (i) the wild-type residue (from SEQ ID NO: 1), (ii) an amino acid observed at that specific position in two or more improved GCcase variants (*e.g.*, from Tables 7 and 9), or (iii) an amino acid that is an artifact of the degenerate codons used to build the library GCcase sequences. The wild-type sequence is used as template DNA sequence, and each selected position is allowed to toggle between (i), (ii), and (iii). The combinatorial library is then transformed into yeast as described previously (Examples 1, 5, 7, 9), and selected for improved probe capping (MDW933) and expression (anti-GCcase antibody) after treatment with mouse serum at 37°C.

Synthesis of GCcase combinatorial analysis library. The combinatorial analysis library is built by PCR using the wild-type DNA sequence as the template, with the selected positions that are non-conserved between the two or more improved variants (*e.g.*, from Tables 7 and 9) mutated to the specified subset of amino acids using oligos with degenerate codons. Amplified library DNA is purified by agarose gel electrophoresis, ethanol precipitated, and resuspended in water to a final concentration of 1 mg/mL.

Generation and screening of a yeast-displayed combinatorial analysis library. Mutated GCcase DNA and linearized yeast display vector is used to transform yeast as described above (Example 1), resulting in a library of GCcase variants with diversity. In a particular embodiment, the generated library encompasses the sequence space provided from two or more variants from Tables 7 and 9. Following induction of yeast for GCcase expression, cells are stained with MDW933 probe in GCcase staining buffer (GSB; 500mM sodium citrate, 50mM phosphate, 120 mM NaCl, 1% BSA, pH 5.2) and subsequently labeled with primary human anti-GCcase antibody followed by secondary labeling with anti-human IgG AlexaFluor®647 as described previously

(Example 5). Yeast displaying GCase variants with high levels of expression and probe capping are enriched within the library by FACS and propagated for subsequent sorting.

5 All sorting rounds include primary and secondary labeling for GCase expression and staining with MDW933 using protocols similar to that described in Examples 5 and 7. MDW933 concentration is decreased for each round of sorting to select for higher enzymatic activity, and serum stress is applied at some point during sorting and carried out for all subsequent rounds of sorting after it is applied. For example, for FACS round 1, 500nM MDW933 is used to stain the library, respectively. For FACS round 2, the library is first incubated in 100% mouse serum (Rockland,
10 Cat# D108-00-0050, or similar) at 30°C overnight shaking at 150RPM, followed by labeling with 250nM of MDW933. All serum treatments for subsequent rounds of sorting are performed in the same manner unless otherwise noted. For FACS round 3, cells are treated with serum followed by labeling with 150nM MDW933. For optional FACS round 4, cells are treated with serum followed by labeling with 75nM MDW933.

15

All sorting is conducted such that the top 0.5% to 1.5% of clones displaying the highest fluorescence for expression and probe capping are selected, thereby enriching the library for GCase sequences with improved expression and enzymatic activity in the presence of serum. Sorted clones are propagated in SD-CAA, induced for expression in SG-CAA, and subjected to
20 additional rounds of FACS. Following final rounds of FACS for library enrichment, plasmid DNA is isolated using a Zymoprep yeast plasmid miniprepII kit (Zymo Research), transformed into One Shot® Top10 electrocompetent *E.coli* (Invitrogen), and optionally sequenced.

*Analysis of GCase expression, activity, and post-serum exposure activity of combinatorial
25 analysis secretion variants.*

Secretion libraries of the post-sorted variants are generated, and expression, activity, and post-serum exposure activity of secretion library variants are assessed by ELISA methods as described in Example 6. Samples displaying improved expression, activity or post-serum stress activity relative to SH2-20 (SEQ ID NO:20) are selected for sequencing.

30

Sequencing of clone candidates. Selected yeast cultures from 96-well plates are added to 20mM NaOH (10μL yeast, 30μL basic solution) and boiled for 30 minutes at 95°C. Boiled supernatants are used as template DNA for a PCR to amplify the GCase sequence, and products of the reactions were sequenced.

Results. The resulting clones contain any of the potential combinations of residues identified at positions listed in Tables 5, 7a, 8, 9a, 10, and 11.

5 Informal Sequence Listing

SEQ ID NO:	Sequence	Description
1	ARPCIPKSFYSSVVCVCNATYCDSFDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRALQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDPAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQVRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISP GYSIHTYLWRRQ	Wild-type human GCase sequence (mature)
2	MEFSSPSREEC PKPLSRVSIMAGSLTG LLLLQAVSWASGARPCIPKSFYSS VVCVCNATYCDSFDPPTFPALGTFSTRYESTRSGRRMELSMGPIQANHTGTGL LLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYN IIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQ RPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQTWARYFVKFLDAYA EHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQDFIARDLGPTLANSTH HNVRLMLDDQRLLLPHWAKVVLTDPAAKYVHGI AVHWYLDLAPAKATLG ETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVG WTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPE GSQVRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDVPLTIKDPAVGFLE TISP GYSIHTYLWRRQ	Wild-type human GCase sequence (full-length)

3	GCCCGCCCCGTCATCCCTAAAAGCTTCGGCTACAGCTCGGTGGTGTGTCT GCAATGCCACATACTGTGACTCCTTTGACCCCCGACCTTTCTGCCCCTGG TACCTTCAGCCGCTATGAGAGTACACGCAGTGGGCGACGGATGGAGCTGAGT ATGGGGCCCCATCCAGGCTAATCACACGGGCACAGGCCTGCTACTGACCCTGC AGCCAGAACAGAAGTTCCAGAAAGTGAAGGGATTTGGAGGGGCCATGACAGA TGCTGCTGCTCTCAACATCCTTGCCCTGTCACCCCTGCCCAAAATTTGCTA CTTAAATCGTACTTCTCTGAAGAAGGAATCGGATATAACATCATCCGGGTAC CCATGGCCAGCTGTGACTTCTCCATCCGCACCTACACCTATGCAGACACCCC TGATGATTTCCAGTTGCACAACCTTCAGCCTCCCAGAGGAAGATACCAAGCTC AAGATACCCCTGATTACCGAGCCCTGCAGTTGGCCAGCGTCCCGTTTCAC TCCTTGCCAGCCCCTGGACATCACCCACTTGGCTCAGACCAATGGAGCGGT GAATGGGAAGGGGTCACTCAAGGGACAGCCCGGAGACATCTACCACCAGACC TGGGCCAGATACTTTGTGAAGTTCTTGGATGCCTATGCTGAGCACAAGTTAC AGTTCTGGGCAGTGACAGCTGAAAATGAGCCTTCTGCTGGGCTGTTGAGTGG ATACCCCTTCCAGTGCCTGGGCTTCACCCCTGAACATCAGCGAGACTTCATT GCCCGTGACCTAGGTCTACCCCTCGCCAACAGTACTACCACAATGTCCGCC TACTCATGCTGGATGACCAACGCTTGCTGCTGCCCCACTGGGCAAGGTGGT ACTGACAGACCCAGAAGCAGCTAAATATGTTTCATGGCATTGCTGTACATTGG TACCTGGACTTTCTGGCTCCAGCCAAAGCCACCCTAGGGGAGACACACCGCC TGTTCCCCAACACCATGCTCTTTGCCTCAGAGGCCTGTGTGGGCTCCAAGTT CTGGGAGCAGAGTGTGCGGCTAGGCTCCTGGGATCGAGGGATGCAGTACAGC CACAGCATCATCACGAACCTCCTGTACCATGTGGTTCGGCTGGACCGACTGGA ACCTTGCCCTGAACCCCGAAGGAGGACCCAATTGGGTGCGTAACTTTGTGCGA CAGTCCCATCATTGTAGACATCACCAGGACACGTTTTACAAACAGCCCATG TTCTACCACCTTGGCCACTTCAGCAAGTTCATTCTGAGGGCTCCAGAGAG TGGGGCTGGTTGCCAGTCAGAAGAACGACCTGGACGCAGTGGCACTGATGCA TCCCGATGGCTCTGCTGTTGTGGTCTGCTAAACCGCTCCTCTAAGGATGTG CCTCTTACCATCAAGGATCCTGCTGTGGGCTTCTTGAGACAATCTCACCTG GCTACTCCATTCACACCTACCTGTGGCGTCGCCAG	Wild-type GCase DNA sequence
4	ARPCIPKSFYSSVVCVNATYCDST_{1a}X_{2a}PPT_{3a}PALGTFSTRYESTRSG RRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILAL SPPAQ_{4a}LLLKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLH NFSLPEEDTKLKIPLIHRALQLAQRPVSLASPTWLTNGAVNGKG SLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAX_{5a}NEPSAGLLSGY PFQCLGFTPEHQRFIARDLGPTLANSTHHNVRLMLDDQR_{6a}LLPHWAK VVLTDPEAAKYVHGIAVHW_{7a}L_{8a}LAPAKATLGETHRLFPNTMLFASEA CVGSK_{9a}X_{10a}EQ_{11a}VRLGSWDRGMQYSHSIITNLL_{12a}HV_{13a}GWTDWN LALNPEGGP_{14a}W_{15a}RNF_{16a}DSPIIVDITKDTFYKQPMFYH_{17a}GHFS_{18a} FIPEGSQVRVGLVASQKNDLDAVALM_{19a}PDGSVAVVVLNRSSKDVPLT IKDPAVGFLETISPGLSIHTYLWRRQ	Consensus sequence for SH2 and SH3 library [X _{1a} = F, L, or S; X _{2a} = D or G; X _{3a} = F or L; X _{4a} = N or D; X _{5a} = E or G; X _{6a} = L or S; X _{7a} = Y, H, D, or C; X _{8a} = F, L, or S; X _{9a} = F or S; X _{10a} = W, G, or R; X _{11a} = S, G, or N; X _{12a} = Y or H; X _{13a} = V or A; X _{14a} = N or S; X _{15a} = V or A; X _{16a} = V or A; X _{17a} = L or I; X _{18a} = K or R; and X _{19a} = H or R]

5	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAX _{1b} NEPSAGLLSGYPFQCLGFTPEHQRD FIARDLGPTLANSTHHNVRLMLDDQRX _{2b} LLPHWAKVVLTDPEAAKYVHGI AVHWYLDX _{3b} LAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWD RGMQYSHSIITNLLX _{4b} HVVGWTDWNLALNPEGGPNWVRNFX _{5b} DSPIIVDIT KDTFYKQPMFYHLGHFSX _{6b} FIPEGSQVRGLVASQKNDLDAVALMX _{7b} PDGSA VVVVLNRSSKDVPLTIKDPVGFLETISPGYSIHTYLWRRQ	Consensus sequence 2 for SH2 library [X _{1b} = G or E; X _{2b} = L or S; X _{3b} = S; X _{4b} = Y or H; X _{5b} = V or A; X _{6b} = K or R; and X _{7b} = H or R
6	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRD FIARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDPEAAKYVHGI AVHWYLDLFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWD RGMQYSHSIITNLLHHVVGWTDWNLALNPEGGPNWVRNFX _{5b} DSPIIVDIT KDTFYKQPMFYHLGHFSKFIPEGSQVRGLVASQKNDLDAVALMRPDGSA VVVVLNRSSKDVPLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone SH2-72
7	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRD FIARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDPEAAKYVHGI AVHWYLDLFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWD RGMQYSHSIITNLLHHVVGWTDWNLALNPEGGPNWVRNFX _{5b} DSPIIVDIT KDTFYKQPMFYHLGHFSKFIPEGSQVRGLVASQKNDLDAVALMHPDGSA VVVVLNRSSKDVPLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone SH2-81
8	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRD FIARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDPEAAKYVHGI AVHWYLDLFLAPAKATLGETHRLFPNTMLFASEACVGSKFGEQSVRLGSWD RGMQYSHSIITNLLHHVVGWTDWNLALNPEGGPNWVRNFX _{5b} DSPIIVDTT KDTFYKQPMFYHLGHFSKFIPEGSQVRGLVASQKNDLDAVALMHPDGSA VVVVLNRSSKDVPLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone SH2-60
9	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRD FIARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDPEAAKYVHGI AVHWYLDLFLAPAKATLGETHRLFPNTTLFASEACVGSKFWEQSVRLGSWD RGIQYSHSIITNLLHHVVGWTDWNLALNPEGGPNWVRNFX _{5b} DSPIIVDTT KDTFYKQPMFYHLGHFSKFIPEGSQVRGLVASQKNDLDAVALMHPDGSA VVVVLNRSSKDVPLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone SH2-14

10	ARPCIPKSFHGSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFREQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-37
11	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQDLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDFPAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-83
12	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSVRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLARRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWARNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-25
13	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITSLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-59
14	ARPCIPKSFYSSVVCVNATYCDSSGPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQDLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDFLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-28

15	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQDLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAEATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-93
16	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGTGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-1
17	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LRGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-31
18	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNWRNFADSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-44
19	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-30

20	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-20
21	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-71
22	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW HLDLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFADSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-2
23	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCGFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIRRALQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFADSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-48
24	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVDGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDRRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-27

25	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASGACVGSKFWEQGVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-95
26	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFAEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFADSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-23
27	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFAEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-18
28	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFAEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-88
29	ARPCIPKSFYSSVVCVCNATYCDSDPPTLPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFAEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPSWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-85

30	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARPLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFGEQNVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQENDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-70
31	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFGEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFADSPIIVDITKDTFYKQPM FYHIGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-69
32	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSEYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFGEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-61
33	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFGEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-38
34	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFGEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-26

35	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPSWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGQSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-79
36	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGNWVRNFADSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGQSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-65
37	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPSWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGQSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-56
38	ARPCIPKSFYSSVVCVCNATYCDSDPPTLPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPSWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGQSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-53
39	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGQSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-9

40	ARPCIPKSFHGSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLRFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPSWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-82
41	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLPLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASGACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-8
42	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-16
43	ARPCIPKSFHCSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW HLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-94
44	TRPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQDLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-17

45	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH2-66
46	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDSSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFADSPIIVDITKDTFYKQPM FYHIGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-65
47	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNWRNFADSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-40
48	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNWRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-67
49	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-31

50	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-57
51	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-86
52	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFADSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-80
53	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVAGWTDWNLALNPEGPNWVRNFADSPPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-17
54	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGEGLKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-6

55	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTS PAWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMRY HSIITNLLHHVVGWTDWNLALNPEGPNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIEDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-54
56	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQDLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTTLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPSWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-90
57	ARPCIPKSFYSSVVCVCNATYCDSSGPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTTLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVAGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-11
58	ARPCIPKSFYSSVVCVCNATYCDSSGPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPRWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTTLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-10
59	ARPCIPKSFYSSVVCVCNATYCDSSGPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPWAKVVLTDEAAKYVHGIAVHW HLDLTPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-59

60	ARPCIPKSFYSSVVCVCNATYCDSSDPPTFPALGTFSRYESTRSGRRMELS VGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSESEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNSVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-43
61	ARPCIPKSFYSSVVCVCNATYCDSLDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGI AVHW DLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-49
62	ARPCIPKSFYSSVVCVCNATYCDSLDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGI AVHW DLDFLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-25
63	ARPCIPKSFYSSVVCVCNATYCDSLDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFADSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-24
64	ARPCIPKSFYSSVVCVCNATYCDSDPPTLPALGTFSRYESTRSGRRMELS MGPIQANRTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHRLQFWAVAAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSKFIPEGSRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-91

65	ARPCIPKSFYSSVVCVNATYCDSDPPTLPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-69
66	ARPCIPKSFYSSVVCVNATYCDSDPPTLPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PFTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-68
67	ARPCIPKSFYSSVVCVNATYCDSDPPTLPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-87
68	ARPCIPKSFYSSVVCVNATYCDSDPLTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQDLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-93
69	ARPCIPKSFYSSVVCVNATYCDSLDPPTLPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPTQDLL LKSYFSEEGIGYNIIRVPMASDSDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-75

70	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHRLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW CLDSLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-81
71	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHRLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW HLDSLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPWVRNFADSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-9
72	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHRLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLPAPAKATLGETHRLFPNTMLFASEACVGSKFGEQSVRLGSWDRGMQYS HSIITDLLHHVVGWTDWNLALNPEGGPWVRNFADSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-88
73	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHRLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-63
74	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHRLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-56

75	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRSLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVAGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-85
76	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKSWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-13
77	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLHHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-45
78	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLPHWAKVVLADPEAAKYVHGIAVHW HLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHIGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone SH3-64

79	GCCCGCCCCCTGCATCCCTAAAAGCTTCGGCTACAGCTCGGTGGTGTGTGTCT GCAATGCCACATACTGTGACTCCTTTGACCCCCGACCTTTCCTGCCCTTGG TACCTTCAGCCGCTATGAGAGTACACGCAGTGGGCGACGGATGGAGCTGAGT ATGGGGCCCCATCCAGGCTAATCACACGGGCACAGGCCTGCTACTGACCCTGC AGCCAGAACAGAAAGTTCCAGAAAGTGAAGGGATTTGGAGGGGCCATGACAGA TGCTGCTGCTCTCAACATCCTTGCCCTGTCACCCCTGCCCAAAATTTGCTA CTTAAATCGTACTTCTCTGAAGAAGGAATCGGATATAACATCATCCGGGTAC CCATGGCCAGCTGTGACTTCTCCATCCGCACCTACACCTATGCAGACACCCC TGATGATTTCCAGTTGCACAACCTTCAGCCTCCCAGAGGAAGATACCAAGCTC AAGATAACCCCTGATTACCCGAGCCCTGCAGTTGGCCAGCGTCCCGTTTCAC TCCTTGCCAGCCCCCTGGACATCACCCACTTGGCTCAAGACCAATGGAGCGGT GAATGGGAAGGGGTCACTCAAGGGACAGCCCGGAGACATCTACCACCAGACC TGGGCCAGATACTTTGTGAAGTTCTTGGATGCCTATGCTGAGCACAAAGTTAC AGTTCTGGGCAGTGACAGCTGGAAATGAGCCTTCTGCTGGGCTGTTGAGTGG ATACCCCTTCCAGTGCCTGGGCTTCACCCCTGAACATCAGCGAGACTTCATT GCCCCGTGACCTAGGTCTACCCCTCGCCAACAGTACTCACCACAATGTCCGCC TACTCATGCTGGATGACCAACGCTTGCTGCTGCCCCACTGGGCAAAGGTGGT ACTGACAGACCCAGAAGCAGCTAAATATGTTTCATGGCATTGCTGTACATTGG TACCTGGACTCTCTGGCTCCAGCCAAAGCCACCCTAGGGGAGACACACCGCC TGTTCCCCAACACCATGCTCTTTGCCTCAGAGGCCTGTGTGGGCTCCAAGTT CTGGGAGCAGAGTGTGCGGCTAGGCTCCTGGGATCGAGGGATGCAGTACAGC CACAGCATCATCACGAACCTCCTGTACCATGTGGTTCGGCTGGACCGACTGGA ACCTTGCCCTGAACCCCGAAGGAGGACCCAATTGGGTGCGTAACTTTGTCTGA CAGTCCCATCATTGTAGACATCACCAAGGACACGTTTACAAACAGCCCATG TTCTACCACCTTGGCCACTTCAGCAGGTTTATTCTGAGGGCTCCAGAGAG TGGGGCTGGTTGCCAGTCAGAAGAACGACCTGGACGCAGTGGCACTGATGCG TCCCGATGGCTCTGCTGTTGTGGTCTGTCTAAACCGCTCCTCTAAGGATGTG CCTCTTACCATCAAGGATCCTGCTGTGGGCTTCTGGAGACAATCTCACCTG GCTACTCCATTACACCTACCTGTGGCGTCGCCAG	Clone SH2-20 DNA sequence
80	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMAX_{1c}X_{2c}DFSIRTYTYADTPDDFQLHNFSLPEED TKLKIPLIHRALQLAQRPVSLASPWTX_{3c}PTWLKTNGAVNGKSLKGQPGD IYHQTWARYFVKFLDAYAEHKLQFWAX_{4c}TX_{5c}X_{6c}NEFX_{7c}X_{8c}GX_{9c}LSGYPFQ CLGFTPEHQRFIARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTD EAAKYVHGIADVHWYLDX_{10c}LAPAKATLGETHRLFPNTMLFAEACVGSKFWE QSVRLGSWDRGMQYSHSIIITNLLYHVVGWTDWNLALX_{11c}X_{12c}X_{13c}GGPNWVR NEVDSPIIVDITKDTFYKQPMFYHLGHFSX_{14c}FIPSGSQRVGLVASQKNDLD AVALMX_{15c}PDGSVVVVVNLNRSSKDVPLTIKDPVGFLETISPGYSIHTYLWR RQ	GCase Library 1 consensus sequence [X_{1c} = S or A; X_{2c} = C, S, or T; X_{3c} = A or S; X_{4c} = L or V; X_{5c} = S, T, or A; X_{6c} = G, Q, or E; X_{7c} = S, I, L, or T; X_{8c} = A or N; X_{9c} = L, M, Q or Y; X_{10c} = S; X_{11c} = D or N; X_{12c} = P, M, T, or L; X_{13c} = E or K; X_{14c} = R; and X_{15c} = R

81	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTSGNEPSAGMLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_B4
82	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTSGNEPSAGMLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_B5
83	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTSGNEPSAGMLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_B8
84	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTSGNEPSAGMLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_B9
85	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTSGNEPSAGMLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_C10

86	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTTQNEPINGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNMKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_C11
87	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTQNEPINGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDMKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_C12
88	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPIAGQLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_C7
89	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPTAGYLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDTEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_C9
90	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTGNEPINGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNMKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_D11

91	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTAPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTQTQNEPINGMLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDTEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_E11
92	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTAPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTTGNEPINGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDMKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_E4
93	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTAPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPTAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDMKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_F10
94	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTAPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTSQNEPINGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNMKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_F11
95	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTAPTWLKTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPTAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNMKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_F12

96	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTSQNEPLNGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_F4
97	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPIAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNTEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_F7
98	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPTAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNTEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_F9
99	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPTAGYLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNMKGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_G10
100	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTSQNEPTAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDMEGGNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_G12

101	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMATDFSIPTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTTGNEPTAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVSGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_G8
102	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMATDFSIPTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVSGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALDLEGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_H5
103	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMATDFSIPTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGAVNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWALTTQNEPINGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVSGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNTKGGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone CI_H6
104	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIPTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPTWLTN $X_{1d}X_{2d}X_{3d}X_{4d}$ GKGS X_{6d} KGQPG DIYHQTWARYFVKFLDAYAEHKLQFWAVTA X_{5d} NEPSAGLLSGY X_{6d} FQCLGF TPEHQRDFIARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAK YVHGIAVHWYLD X_{7d} LAPAKATLGETHRLFPNTMLFASEAC $X_{8d}GX_{9d}X_{10d}X_{11d}$ WEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVD SPIIVDITKDTFYKQPMFYHLGHFS X_{12d} FIPEGSQRVGLVASQKNDLDAVAL M X_{13d} PDGSAVVVVLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ	GCase Library 2 consensus sequence [X_{1d} = G, E, R, or Q; X_{2d} = A, N, T, S, H, D, R, or G; X_{3d} = V, Y, F, L, M, S, G, or W; X_{4d} = N, V, I, T, A, M, E, G, D, or S; X_{5d} = G; X_{6d} = P, S, K, Q, or T; X_{7d} = S; X_{8d} = V, T, A, or N; X_{9d} = S, F, T, or I; X_{10d} = K, L, Q, F, N, E, or V; X_{11d} = F, P, S, or T; X_{12d} = R; and X_{13d} = R]

105	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNENYVGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYSFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGTLPEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-A9
106	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNQTFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFQPEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-B7
107	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNESLTGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYKFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGIFPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-F111
108	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNNGSYVGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYKFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACAGFQPEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-F113
109	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNRDFVVGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFNSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-F12

110	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYAGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFEPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-G11
111	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNNMTGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACAGFEPEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-H2
112	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNQAYMGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI3-H3
113	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNQAFEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFESWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI4-A10
114	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHFGGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYKFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI4-A11

115	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGTLMGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFLSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGYQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI4-B2
116	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGDFTGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQTWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI4-B5
117	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGNFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFQWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI4-F2
118	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNQTYYTGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYQFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFETWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI4-F31
119	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNERLEGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFETWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI4-F32

120	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGNSSGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFESWEQSVRLGSWDRGMQYS HSIIANLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI4-G4
121	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNERGSGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSQPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI4-G71
122	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNERGSGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSVPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI4-G73
123	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNTQTYMGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYTFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACAGFEPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI4-H2
124	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNRGFEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYQFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFQPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI5-A11

125	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGNFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYKFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFQPEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI5-D10
126	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNEDWDGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFEPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI5-D12
127	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGDFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACAGFKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI5-F11
128	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGNFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI5-F3
129	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGGFTGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACAGFPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSVVVVVLRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI5-G11

130	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPWLKTNEDVTGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGFKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI5-G2
131	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI5-H2
132	RASQGISSYLA	CDR-L1 of anti- GCase antibody
133	AASSLQS	CDR-L2 of anti- GCase antibody
134	QQYYSYPFT	CDR-L3 of anti- GCase antibody
135	GFTFSSYH	CDR-H1 of anti- GCase antibody
136	SINPSNGGTYADSVKG	CDR-H2 of anti- GCase antibody
137	SSYYYYEMDY	CDR-H3 of anti- GCase antibody
138	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-A2
139	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKL KIPLIHRAQLAQRPVSLASPWTSPWLKTNGAFI GKS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPNNVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B2

140	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAFIKGSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-B3
141	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHVEGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A5
142	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYNGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A6
143	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A7
144	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A8

145	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A9
146	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFKSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A11
147	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A12
148	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A13
149	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNILALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A14

150	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFKSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A15
151	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFKSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A16
152	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFKSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A17
153	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGHYEGKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFKSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A18
154	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAFI GKGS LKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-B4

155	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGNFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B8
156	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGNFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B10
157	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGNFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B12
158	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGNFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B13
159	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAVNGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B14

160	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B15
161	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B16
162	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B17
163	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGNFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSKPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-B18
164	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPWTSPTWLKTNGAYEGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGI AVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGGPWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGS AVVVV LNRSSKDV PLTIKDP AVGFLETISPGYSIHTYLWRRQ	Clone mI6-A4

165	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGHYEGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGSQSWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-A10
166	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGNFGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-B6
167	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGNVIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-B5
168	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGNFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-B7
169	ARPCIPKSFYSSVVCVCNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNIALSPPAQNLL LKSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEDTKL KIPLIHRAQLAQRPVSLASPTWLTNGNFIGKSLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHHNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACVGLPWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRFIPEGSQRVGLVASQKNDLDAVALMRPDGSAVVVVLRSSKDV PLTIKDPAVGFLETISPGYSIHTYLWRRQ	Clone mI6-B9

170	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLEEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNGNFIGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACTGSLFWEQSVRLGSDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQVRGLVASQKNDLDAVALMRPDGSVVVVLNRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI6-B11
171	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLEEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTN $X_{1e}X_{2e}X_{3e}X_{4e}$ GKGSCLKGQPG DIYHQTWARYFVKFLDAYAEHKLQFWAVTA X_{5e} NEPSAGLLSGY X_{6e} FQCLGF TPEHQDFIARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAK YVHGIAVHWYLD X_{7e} LAPAKATLGETHRLFPNTMLFASEAC $X_{8e}GX_{9e}X_{10e}X_{11e}$ WEQSVRLGSDRGMQYSHSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVD SPIIVDITKDTFYKQPMFYHLGHFS X_{12e} FIPEGSQVRGLVASQKNDLDAVAL M X_{13e} PDGSVVVVLNRSSKDVPLTIKDPVGFLETISPGYSIHTYLWRRQ	Consensus Sequence Embodiment [X_{1e} = G, E, R, or Q; X_{2e} = A, N, T, S, H, D, R, or G; X_{3e} = V, Y, F, L, M, S, G, or W; X_{4e} = N, V, I, T, A, M, E, G, D, or S; X_{5e} = G or E; X_{6e} = P, S, K, Q, or T; X_{7e} = S or F; X_{8e} = V, T, A, or N; X_{9e} = S, F, T, or I; X_{10e} = K, L, Q, F, N, E, or V; X_{11e} = F, P, S, or T; X_{12e} = R or K; and X_{13e} = R or H]
172	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLEEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNGAVNGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQTWEQSVRLGSDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSKFIPEGSQVRGLVASQKNDLDAVALMHPDGSVVVVLNRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI6-C1
173	ARPCIPKSFYSSVVCVNATYCDSDPPTFPALGTFSTRYESTRSGRRMELS MGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLL LKSIFYSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLEEDTKL KIPLIHRAQLAQRPVSLLASPWTSPWLKTNGAVNGKGSCLKGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAGNEPSAGLLSGYPFQCLGFTPEHQDFI ARDLGPTLANSTHNNVRLMLDDQRLLLPHWAKVVLTDEAAKYVHGIAVHW YLDLAPAKATLGETHRLFPNTMLFASEACNGFQTWEQSVRLGSDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGPNWVRNFVDSPIIVDITKDTFYKQPM FYHLGHFSRIFIPEGSQVRGLVASQKNDLDAVALMRPDGSVVVVLNRSSKDV PLTIKDPVGFLETISPGYSIHTYLWRRQ	Clone mI6-C2

174	ARPCIPKSFgyssvvcnatycdsfdpptfpalgtfsryestrsgrrmels mgpiqanhtgtgllltlqpeqkfQkvkgfGGAMTDAAALNILALSPPAQNLL LksyFseEGIGYNIIRVPMASCDfSIRTYTYADTPDDFQLHNfSLPEEDTKL KIPLIHRAQLAQRPVSLLASPWTSPTWLKTNGAVNGKGS�KGQPGDIYHQT WARYFVKFLDAYAEHKLQFWAVTAQNEPSAGLLSGYPFQCLGFTPEHQRDFI ARDLGPTLANSTHhNVRLMLDDQRLLLPHWAKVVLTdPEAAKYVHGIAVHW YLDfLAPAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYS HSIITNLLYHVVGWTDWNLALNPEGgPNWVRNFVDSPIIVDITKDTfYKQPM FYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSavvvVLNRSSKDV PLTIKDPavGFLETISPGYSIHtyLWRRQGS	Comparator
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All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The present disclosure has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

CLAIMS

What is claimed is:

1. A GCase polypeptide comprising two or more modifications with respect to SEQ ID NO:1, wherein the two or more modifications provide increased stability in the presence of serum, wherein the increased stability is indicated by (i) at least 2-fold greater enzymatic activity, and (ii) at least 2-fold greater conformational stability, relative to that of SEQ ID NO:1.
2. The GCase polypeptide of claim 1, wherein the conformational stability is at least 5-fold greater than that of SEQ ID NO:1.
3. The GCase polypeptide of claim 1 or 2, wherein the conformational stability is measured using a fluorescence-based assay using an anti-GCase antibody, circular dichroism, or differential scanning calorimetry.
4. The GCase polypeptide of claim 3, wherein the fluorescence-based assay is an ELISA assay, a FACS assay, or a differential scanning fluorimetry assay.
5. The GCase polypeptide of any one of claims 1 to 4, wherein the enzymatic activity is at least 5-fold greater than that of SEQ ID NO:1.
6. The GCase polypeptide of any one of claims 1 to 5, wherein the enzymatic activity is measured by detection with a fluorescent cyclophellitol-type activity-based probe and evaluation with FACS.
7. The GCase polypeptide of claim 6, wherein the fluorescent cyclophellitol-type activity-based probe is selected from the group consisting of: MDW933, MDW941, and 2-deoxy-2-fluoro- β -d-glucopyranosyl-N-phenyltrifluoroacetimidate.
8. The GCase polypeptide of any one of claims 1 to 5, wherein the enzymatic activity is measured by an *in vitro* reaction with 4-MUG (4-Methylumbelliferyl β -D-glucopyranoside) substrate.

9. The GCase polypeptide of any one of claims 1 to 8, wherein at least two of the modifications, with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 316, 425, and 451.
10. The GCase polypeptide of any one of claims 1 to 9, wherein at least two of the modifications are selected from the group consisting of: F316S, K425R, and H451R.
11. The GCase polypeptide of any one of claims 1 to 9, wherein the two or more modifications comprise a modification at positions 316, 425, and 451, with respect to SEQ ID NO:1.
12. The GCase polypeptide of claim 11, wherein the two or more modifications comprise F316S, K425R, and H451R.
13. The GCase polypeptide of any one of claims 9 to 12, further comprising a modification at position 233 with respect to SEQ ID NO:1.
14. The GCase polypeptide of claim 13, wherein the modification at position 233 is E233G.
15. The GCase polypeptide of any one of claims 9 to 14, further comprising a modification, with respect to SEQ ID NO:1, at one or more positions selected from the group consisting of: 189, 190, 191, 192, 245, 343, 345, 346, and 347.
16. The GCase polypeptide of claim 15, comprising a modification at position 189 selected from the group consisting of: G189E, G189R, and G189Q.
17. The GCase polypeptide of claim 15 or 16, comprising a modification at position 190 selected from the group consisting of: A190N, A190T, A190S, A190H, A190D, A190R, and A190G.
18. The GCase polypeptide of any one of claims 15 to 17, comprising a modification at position 191 selected from the group consisting of: V191Y, V191F, V191L, V191M, V191S, V191G, and V191W.

19. The GCase polypeptide of any one of claims 15 to 18, comprising a modification at position 192 selected from the group consisting of: N192V, N192I, N192T, N192A, N192M, N192E, N192G, N192D, and N192S.
20. The GCase polypeptide of any one of claims 15 to 19, comprising a modification at position 343 selected from the group consisting of: V343T, V343A, and V343N.
21. The GCase polypeptide of any one of claims 15 to 20, comprising a modification at position 345 selected from the group consisting of: S345F, S345T, and S345I.
22. The GCase polypeptide of any one of claims 15 to 21, comprising a modification at position 346 selected from the group consisting of: K346L, K346Q, K346F, K346N, K346E, and K346V.
23. The GCase polypeptide of any one of claims 15 to 22, comprising a modification at position 347 selected from the group consisting of: F347P, F347S, and F347T.
24. The GCase polypeptide of any one of claims 15 to 23, comprising a modification at position 245 selected from the group consisting of: P245S, P245K, P245Q, and P245T.
25. The GCase polypeptide of any one of claims 9 to 12, further comprising a modification, with respect to SEQ ID NO:1, at one or more positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388.
26. The GCase polypeptide of claim 25, comprising a modification at position 125 that is S125A.
27. The GCase polypeptide of claim 25 or 26, comprising a modification at position 126 that is C126T or C126S.
28. The GCase polypeptide of any one of claims 25 to 27, comprising a modification at position 181 that is S181A.

29. The GCase polypeptide of any one of claims 25 to 28, comprising a modification at position 230 that is V230L.
30. The GCase polypeptide of any of claims 25 to 29, comprising a modification at position 232 selected from the group consisting of: A232S and A232T.
31. The GCase polypeptide of any of claims 25 to 30, comprising a modification at position 233 selected from the group consisting of: E233G and E233Q.
32. The GCase polypeptide of any of claims 25 to 31, comprising a modification at position 237 selected from the group consisting of: S237L, S237I, and S237T.
33. The GCase polypeptide of any of claims 25 to 32, comprising a modification at position 238 that is A238N.
34. The GCase polypeptide of any of claims 25 to 33, comprising a modification at position 240 selected from the group consisting of: L240M, L240Q, and L240Y.
35. The GCase polypeptide of any of claims 25 to 34, comprising a modification at position 386 that is N386D.
36. The GCase polypeptide of any of claims 25 to 35, comprising a modification at position 387 selected from the group consisting of: P387M, P387T, and P387L.
37. The GCase polypeptide of any of claims 25 to 36, comprising a modification at position 388 that is E388K.
38. A GCase polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO: 104, wherein:
- a) X_{1d} is selected from the group consisting of: G, E, R, and Q;
 - b) X_{2d} is selected from the group consisting of: A, N, T, S, H, D, R, and G;
 - c) X_{3d} is selected from the group consisting of: V, Y, F, L, M, S, G, and W;

- d) X_{4d} is selected from the group consisting of: N, V, I, T, A, M, E, G, D, and S;
- e) X_{5d} is G;
- f) X_{6d} is selected from the group consisting of: P, S, K, Q, and T;
- g) X_{7d} is S;
- h) X_{8d} is selected from the group consisting of: V, T, A, and N;
- i) X_{9d} is selected from the group consisting of: S, F, T, and I;
- j) X_{10d} is selected from the group consisting of: K, L, Q, F, N, E, and V;
- k) X_{11d} is selected from the group consisting of: F, P, S, and T;
- l) X_{12d} is R; and
- m) X_{13d} is R.

39. The GCase polypeptide of claim 38, wherein the amino acid sequence has at least 95% identity to SEQ ID NO:104.

40. The GCase polypeptide of claim 38, wherein the amino acid sequence has at least 96% identity to SEQ ID NO: 104.

41. The GCase polypeptide of claim 38, wherein the amino acid sequence has at least 97% identity to SEQ ID NO: 104.

42. The GCase polypeptide of claim 38, wherein the amino acid sequence has at least 98% identity to SEQ ID NO: 104.

43. The GCase polypeptide of claim 38, wherein the amino acid sequence has at least 99% identity to SEQ ID NO: 104.

44. The GCase polypeptide of claim 38, comprising SEQ ID NO: 104.

45. The GCase polypeptide of any one of claims 38 to 44, wherein:

- a) X_{1d} is G;
- b) X_{2d} is selected from the group consisting of: N and H;
- c) X_{3d} is selected from the group consisting of: F and Y;
- d) X_{4d} is selected from the group consisting of: I and E;

- e) X_{5d} is G;
- f) X_{6d} is P;
- g) X_{7d} is S;
- h) X_{8d} is selected from the group consisting of: T and N;
- i) X_{9d} is selected from the group consisting of: S and F;
- j) X_{10d} is selected from the group consisting of: L and Q;
- k) X_{11d} is selected from the group consisting of: P and S;
- l) X_{12d} is R; and
- m) X_{13d} is R.

46. The GCase polypeptide of claim 38, comprising a sequence selected from the group consisting of: SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, and SEQ ID NO:131.

47. The GCase polypeptide of claim 46, comprising SEQ ID NO: 128.

48. The GCase polypeptide of claim 46, comprising SEQ ID NO: 131.

49. A GCase polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:80, wherein:

- a) X_{1c} is selected from the group consisting of: S and A;
- b) X_{2c} is selected from the group consisting of: C, S and T;
- c) X_{3c} is selected from the group consisting of: A and S;
- d) X_{4c} is selected from the group consisting of: L and V;
- e) X_{5c} is selected from the group consisting of: S, T, and A;
- f) X_{6c} is selected from the group consisting of: G, Q and E;
- g) X_{7c} is selected from the group consisting of: S, L, I, and T;
- h) X_{8c} is selected from the group consisting of: A and N;
- i) X_{9c} is selected from the group consisting of: L, M, Q and Y;

- j) X_{10c} is S;
- k) X_{11c} is selected from the group consisting of: D and N;
- l) X_{12c} is selected from the group consisting of: P, M, T, and L;
- m) X_{13c} is selected from the group consisting of: E and K;
- n) X_{14c} is R; and
- o) X_{15c} is R.

50. The GCase polypeptide of claim 49, wherein the amino acid sequence has at least 95% identity to SEQ ID NO:80.

51. The GCase polypeptide of claim 49, wherein the amino acid sequence has at least 96% identity to SEQ ID NO:80.

52. The GCase polypeptide of claim 49, wherein the amino acid sequence has at least 97% identity to SEQ ID NO:80.

53. The GCase polypeptide of claim 49, wherein the amino acid sequence has at least 98% identity to SEQ ID NO:80.

54. The GCase polypeptide of claim 49, wherein the amino acid sequence has at least 99% identity to SEQ ID NO:80.

55. The GCase polypeptide of claim 49, comprising SEQ ID NO:80.

56. The GCase polypeptide of any one of claims 49 to 55, wherein:

- a) X_{1c} is S;
- b) X_{2c} is T;
- c) X_{3c} is A or S;
- d) X_{4c} is L or V;
- e) X_{5c} is S or T;
- f) X_{6c} is Q;
- g) X_{7c} is I;
- h) X_{8c} is A or N;

- i) X_{9c} is L;
- j) X_{10c} is S;
- k) X_{11c} is N;
- l) X_{12c} is T;
- m) X_{13c} is E;
- n) X_{14c} is R; and
- o) X_{15c} is R.

57. The GCase polypeptide of claim 49, comprising a sequence selected from the group consisting of: SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, and SEQ ID NO:103.

58. The GCase polypeptide of claim 57, comprising SEQ ID NO: 103.

59. The GCase polypeptide of claim 57, comprising SEQ ID NO: 97.

60. A GCase polypeptide comprising two or more modifications, wherein at least two of the modifications, with respect to SEQ ID NO:1, are at positions selected from the group consisting of: 316, 425, and 451.

61. The GCase polypeptide of claim 60, wherein the two or more modifications comprise F316S, K425R, and H451R.

62. The GCase polypeptide of claim 60 or 61, further comprising a modification at position 233 with respect to SEQ ID NO:1.

63. The GCase polypeptide of claim 62, wherein the modification at position 233 is E233G.

64. The GCase polypeptide of any one of claims 60 to 63, further comprising a modification, with respect to SEQ ID NO:1, at one or more positions selected from the group consisting of:

189, 190, 191, 192, 245, 343, 345, 346, and 347.

65. The GCase polypeptide of claim 64, comprising a modification at position 189 selected from the group consisting of: G189E, G189R, and G189Q.

66. The GCase polypeptide of claim 64 or 65, comprising a modification at position 190 selected from the group consisting of: A190N, A190T, A190S, A190H, A190D, A190R, and A190G.

67. The GCase polypeptide of any one of claims 64 to 66, comprising a modification at position 191 selected from the group consisting of: V191Y, V191F, V191L, V191M, V191S, V191G, and V191W.

68. The GCase polypeptide of any one of claims 64 to 67, comprising a modification at position 192 selected from the group consisting of: N192V, N192I, N192T, N192A, N192M, N192E, N192G, N192D, and N192S.

69. The GCase polypeptide of any one of claims 64 to 68, comprising a modification at position 245 selected from the group consisting of: P245S, P245K, P245Q, and P245T.

70. The GCase polypeptide of any one of claims 64 to 69, comprising a modification at position 343 selected from the group consisting of: V343T, V343A, and V343N.

71. The GCase polypeptide of any one of claims 64 to 70, comprising a modification at position 345 selected from the group consisting of: S345F, S345T, and S345I.

72. The GCase polypeptide of any one of claims 64 to 71, comprising a modification at position 346 selected from the group consisting of: K346L, K346Q, K346F, K346N, K346E, and K346V.

73. The GCase polypeptide of any one of claims 64 to 72, comprising a modification at position 347 selected from the group consisting of: F347P, F347S, and F347T.

74. The GCase polypeptide of claim 60 or 61, further comprising a modification, with respect to SEQ ID NO:1, at one or more positions selected from the group consisting of: 125, 126, 181, 230, 232, 233, 237, 238, 240, 386, 387, and 388.
75. The GCase polypeptide of claim 74, comprising a modification at position 125 that is S125A.
76. The GCase polypeptide of claim 74 or 75, comprising a modification at position 126 that is C126T or C126S.
77. The GCase polypeptide of any one of claims 74 to 76, comprising a modification at position 181 is that is S181A.
78. The GCase polypeptide of any one of claims 74 to 77, comprising a modification at position 230 that is V230L.
79. The GCase polypeptide of any of claims 74 to 78, comprising a modification at position 232 selected from the group consisting of: A232S and A232T.
80. The GCase polypeptide of any of claims 74 to 79, comprising a modification at position 233 selected from the group consisting of: E233G and E233Q.
81. The GCase polypeptide of any of claims 74 to 80, comprising a modification at position 237 selected from the group consisting of: S237L, S237I, and S237T.
82. The GCase polypeptide of any of claims 74 to 81, comprising a modification at position 238 that is A238N.
83. The GCase polypeptide of any of claims 74 to 82, comprising a modification at position 240 selected from the group consisting of: L240M, L240Q, and L240Y.
84. The GCase polypeptide of any of claims 74 to 83, comprising a modification at position 386 that is N386D.

85. The GCase polypeptide of any of claims 74 to 84, comprising a modification at position 387 selected from the group consisting of: P387M, P387T, and P387L.
86. The GCase polypeptide of any of claims 74 to 85, comprising a modification at position 388 that is E388K.
87. A GCase polypeptide comprising an amino acid sequence having at least 90% identity to SEQ ID NO:5, wherein:
- a) X_{1b} is selected from the group consisting of: G and E;
 - b) X_{2b} is selected from the group consisting of: L and S;
 - c) X_{3b} is S;
 - d) X_{4b} is selected from the group consisting of Y and H;
 - e) X_{5b} is selected from the group consisting of V and A;
 - f) X_{6b} is selected from the group consisting of K and R; and
 - g) X_{7b} is selected from the group consisting of H and R.
88. The GCase polypeptide of claim 87, comprising a sequence selected from the group consisting of: SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:36, and SEQ ID NO:39.
89. The GCase polypeptide of claim 87, comprising the sequence of SEQ ID NO:20.
90. The GCase polypeptide of any one of claims 38-89, which has increased stability in the presence of serum, wherein the increased stability is indicated by (i) greater enzymatic activity and/or (ii) greater conformational stability, relative to that of SEQ ID NO:1.
91. A pharmaceutical composition comprising the GCase polypeptide of any one of claims 1 to 90 and a pharmaceutically acceptable excipient.
92. A polynucleotide comprising a nucleic acid sequence encoding the GCase polypeptide of any one of claims 1 to 90.

93. A vector comprising the polynucleotide of claim 92.
94. A host cell comprising the polynucleotide of claim 92 or the vector of claim 93.
95. A method for producing a GCase polypeptide, comprising culturing a host cell under conditions in which the GCase polypeptide encoded by the polynucleotide of claim 92 is expressed.
96. A method of treating a GCase deficiency in a patient in need thereof, the method comprising administering a GCase polypeptide of any one of claims 1 to 90 to the patient.
97. A GCase polypeptide as described in any one of claims 1 to 90 for use in treating a GCase deficiency in a patient in need thereof.
98. The use of a GCase polypeptide as described in any one of claims 1 to 90 in the preparation of a medicament for treating a GCase deficiency in a patient in need thereof.
99. A method of decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency, the method comprising administering a GCase polypeptide as described in any one of claims 1 to 90 to the patient.
100. A GCase polypeptide as described in any one of claims 1 to 90 for use in decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency.
101. The use of a GCase polypeptide as described in any one of claims 1 to 90 in the preparation of a medicament for decreasing the accumulation of a toxic metabolic product in a patient having a GCase deficiency.
102. The method, polypeptide or use of any one of claims 99 to 101, wherein the toxic metabolic product comprises glucosylceramide or glucosylsphingosine.
103. A method of making a modified GCase polypeptide, wherein the method comprises:

- (i) modifying a nucleic acid sequence encoding SEQ ID NO:1 to change a residue at two or more positions in regions of SEQ ID NO:1 selected from the group consisting of positions: 119-127, 130-134, 177-184, 187-197, 230-240, 243-249, 284-296, 310-312, 315-336, 343-347, 350-362, 386-400, and 414-417;
 - (ii) transforming a host cell with the modified nucleic acid sequence;
 - (iii) culturing the host cell to express the polypeptide encoded by the modified nucleic acid sequence; and
 - (iv) isolating the polypeptide from the host cell culture;
- thereby making the modified GCase polypeptide.

104. The method of claim 103, wherein the two or more positions in SEQ ID NO:1 are selected by:

- (i) carrying out one or more sequence alignments between SEQ ID NO:1 and its sequence orthologs to identify a set of positions within the defined regions at locations where residues are not conserved; and
- (ii) selecting two or more positions from the identified set of positions.

105. The method of claim 104, wherein the two or more positions are located in loop or alpha helical regions.

106. The method of any one of claims 103 to 105, wherein the residue change at the two or more positions is a substitution with an ortholog sequence residue that is non-conserved at that position, or a conservative substitution thereof.

107. The method of any one of claims 103 to 106, comprising a residue change at three, four, five, six, seven, eight, nine, or ten or more positions in SEQ ID NO:1.

108. The method of any one of claims 103 to 106, wherein the two or more positions in SEQ ID NO:1 are in regions of SEQ ID NO:1 selected from the group consisting of: positions 119-127, 177-184, 230-240, and 386-400.

109. The method of claim 108, wherein the two or more positions in SEQ ID NO:1 are selected from the group consisting of: 124, 125, 126, 181, 230, 232, 233, 237, 238, 239, 240,

386, 387, and 388.

110. The method of claim 109, wherein the residue change at the two or more positions is selected from the group consisting of:

- (i) substitution at position 124 with Thr;
- (ii) substitution at position 125 with Cys, Gly, or Ala;
- (iii) substitution at position 126 with Ser or Thr;
- (iv) substitution at position 181 with Ala;
- (v) substitution at position 230 with Leu;
- (vi) substitution at position 232 with Ser, Thr, or Gly;
- (vii) substitution at position 233 with Gly, Gln, or Arg;
- (viii) substitution at position 237 with Arg, Thr, Leu, Pro, or Ile;
- (ix) substitution at position 238 with Asp, Thr, or Asn;
- (x) substitution at position 239 with Arg;
- (xi) substitution at position 240 with Glu, Phe, Met, Asn, Gln, Val, Tyr, Lys, Ile, His, or Asp;
- (xii) substitution at position 386 with Asp;
- (xiii) substitution at position 387 with Leu, Met, or Thr; and
- (xiv) substitution at position 388 with Lys.

111. The method of any one of claims 108 to 110, comprising a residue change at three, four, five, six, seven, eight, nine, or ten or more positions in SEQ ID NO:1.

112. The method of any one of claims 103 to 106, wherein the two or more positions in SEQ ID NO:1 are in regions of SEQ ID NO:1 selected from the group consisting of: positions 187-197, 243-249, 310-312, 343-347, and 414-417.

113. The method of claim 112, wherein the two or more positions in SEQ ID NO:1 is selected from the group consisting of: 189, 190, 191, 192, 343, 345, 346, and 347.

114. The method of claim 113, wherein the residue change at the two or more positions is selected from the group consisting of:

- (i) substitution at position 189 with Glu, Arg, or Gln;

- (ii) substitution at position 190 with Asp, Ser, Arg, His, Asn, Gly, Pro, or Thr;
 - (iii) substitution at position 191 with Ala, Phe, Gly, Leu, Met, Val, Tyr, Arg, Asn, Asp, Cys, Glu, Ile, Lys, Ser, Thr, or Trp;
 - (iv) substitution at position 192 with Ile, Lys, Arg, Thr, Val, Ala, Asp, Glu, Gly, Met, or Ser;
 - (v) substitution at position 343 with Ala, Ile, Leu, Asn, Thr, His, Pro, or Asp;
 - (vi) substitution at position 345 with Phe, Thr, or Ile;
 - (vii) substitution at position 346 with Glu, Phe, His, Leu, Asn, Tyr, Val, Ile, Gln, or Asp;
- and
- (viii) substitution at position 347 with Met, Ser, Pro, Thr, Ile, or Leu.

115. The method of any one of claims 112 to 114, comprising a residue change at three, four, five, six, seven, eight, or nine or more positions in SEQ ID NO:1.

116. The method of any one of claims 103 to 106, wherein the two or more positions in SEQ ID NO:1 are in regions of SEQ ID NO:1 selected from the group consisting of: positions 130-134, 284-296, 315-336, and 350-362.

117. The method of claim 116, wherein the two or more positions in SEQ ID NO:1 is selected from the group consisting of: 316, 317, 318, 319, 320, 321, 322, 325, 329, 350, 351, 353, 361, and 362.

118. The method of claim 117, wherein the residue change at the two or more positions is selected from the group consisting of:

- (i) substitution at position 316 with Gly, Leu, Cys, Trp, or Val;
- (ii) substitution at position 317 with Ile or Phe;
- (iii) substitution at position 318 with Val, Gly, Ile, Ser, or Thr;
- (iv) substitution at position 319 with Ser;
- (v) substitution at position 320 with Ile, Pro, Leu, Thr, or Val;
- (vi) substitution at position 321 with Asp, Glu, Gly, Asn, Gln, Arg, Thr, Pro, Ser, His, or Ala;
- (vii) substitution at position 322 with Cys, Asp, Leu, Pro, Arg, Ser, Gly, His, Phe, Tyr, or Val;

- (viii) substitution at position 325 with Glu, Leu, Ser, Val, Trp, or Ala;
- (ix) substitution at position 329 with Glu, His, Lys, Asn, Gln, Asp, Gly, or Ser;
- (x) substitution at position 350 with Phe, Lys, Asn, Pro, Arg, Cys, His, Ile, Leu, Met, Ser, Thr, Trp, or Tyr;
- (xi) substitution at position 351 with Ala, Cys, Asp, Gly, Arg, His, or Pro;
- (xii) substitution at position 353 with Ala, Ile, Leu, Gln, Ser, Val, Asn, Asp, Glu, Gly, His, Pro, or Thr;
- (xiii) substitution at position 361 with Cys, Asp, Glu, Ile, Asn, Gln, Ser, Thr, Val, Ala, Arg, Gly, His, Leu, Lys, Phe, Pro, Trp or Tyr; and
- (xiv) substitution at position 362 with Ala, Glu, His, Lys, Pro, Gln, Arg, Asn, Gly, Ile, Leu, Thr, or Val.

119. The method of any one of claims 116 to 118, comprising a residue change at three, four, five, six, seven, eight, or nine or more positions in SEQ ID NO:1.

120. The method of any one of claims 103 to 119, wherein the modified GCase polypeptide has greater conformational stability relative to that of SEQ ID NO:1.

121. The method of claim 120, wherein the modified GCase polypeptide has greater conformational stability by at least about 2-fold relative to that of SEQ ID NO:1.

122. The method of any one of claims 120 to 121, wherein the conformational stability is measured by a fluorescence-based assay using an anti-GCase antibody, circular dichroism, or differential scanning calorimetry.

123. The method of any one of claims 120 to 122, wherein the modified GCase polypeptide has greater enzymatic activity relative to that of SEQ ID NO:1.

124. The method of any one of claims 120 to 122, wherein the modified GCase polypeptide has greater enzymatic activity by at least about 2 fold relative to that of SEQ ID NO:1.

125. The method of any one of claims 123 to 124, wherein the enzymatic activity is measured by detection with a fluorescent cyclophellitol-type activity-based probe and evaluation with

FACS.

126. The method of claim 125, wherein the fluorescent cyclophellitol-type activity-based probe is selected from the group consisting of MDW933, MDW941, and 2-deoxy-2-fluoro- β -d-glucopyranosyl-*N*-phenyltrifluoroacetimidate.

127. The method of any one of claims 123 to 124, wherein the enzymatic activity is measured by an *in vitro* reaction with 4-MUG (4-Methylumbelliferyl β -D-glucopyranoside) substrate.

128. A modified GCase polypeptide produced by the method of any one of claims 103 to 127.

129. A method of generating a library of nucleic acid sequences encoding modified GCase polypeptides, comprising modifying a plurality of nucleic acids encoding SEQ ID NO:1 to change a residue at two or more positions in regions of SEQ ID NO:1 that undergo structural alteration upon binding of SEQ ID NO:1 to isofagomine.

130. The method of claim 129, wherein the two or more positions are in regions of SEQ ID NO:1 selected from the group consisting of positions: 119-127, 130-134, 177-184, 187-197, 230-240, 243-249, 284-296, 310-312, 315-336, 343-347, 350-362, 386-400, and 414-417.

131. The method of claim 129 or 130, wherein the two or more positions in SEQ ID NO:1 are selected by:

(i) carrying out one or more sequence alignments between SEQ ID NO:1 and its sequence orthologs to identify a set of positions within the defined regions at locations where residues are not conserved; and

(ii) selecting two or more positions from the identified set of positions.

132. The method of any one of claims 129 to 131, wherein the two or more positions are located in loop or alpha helical regions.

133. The method of any one of claims 129 to 132, wherein SEQ ID NO:1 is modified at the two or more positions by substituting the wild-type residue with an ortholog sequence residue that is non-conserved at that position, or a conservative substitution thereof.

134. The method of any one of claims 129 to 133, wherein the plurality of nucleic acids encoding SEQ ID NO:1 is modified to change a residue at three, four, five, six, seven, eight, nine, or ten or more positions in SEQ ID NO:1.

135. The method of any one of claims 129 to 133, wherein the two or more positions in SEQ ID NO:1 are selected from the group consisting of positions: 119-127, 177-184, 230-240, and 386-400 of SEQ ID NO:1.

136. The method of claim 135, wherein the two or more positions in SEQ ID NO:1 are selected from the group consisting of: 124, 125, 126, 181, 230, 232, 233, 237, 238, 239, 240, 386, 387, and 388.

137. The method of claim 136, wherein SEQ ID NO:1 is modified with two or more of:

- (i) substitution at position 124 with Thr;
- (ii) substitution at position 125 with Cys, Gly, or Ala;
- (iii) substitution at position 126 with Ser or Thr;
- (iv) substitution at position 181 with Ala;
- (v) substitution at position 230 with Leu;
- (vi) substitution at position 232 with Ser, Thr, or Gly;
- (vii) substitution at position 233 with Gly, Gln, or Arg;
- (viii) substitution at position 237 with Arg, Thr, Leu, Pro, or Ile;
- (ix) substitution at position 238 with Asp, Thr, or Asn;
- (x) substitution at position 239 with Arg;
- (xi) substitution at position 240 with Glu, Phe, Met, Asn, Gln, Val, Tyr, Lys, Ile, His, or Asp;
- (xii) substitution at position 386 with Asp;
- (xiii) substitution at position 387 with Leu, Met, or Thr; and
- (xiv) substitution at position 388 with Lys.

138. The method of any one of claims 135 to 137, wherein the plurality of nucleic acids encoding SEQ ID NO:1 is modified to change a residue at three, four, five, six, seven, eight, or nine or more positions in SEQ ID NO:1.

139. The method of any one of claims 129 to 133, wherein the two or more positions in SEQ ID NO:1 are selected from the group consisting of: positions 187-197, 243-249, 310-312, 343-347, and 414-417 of SEQ ID NO:1.

140. The method of claim 139, wherein the two or more positions in SEQ ID NO:1 are selected from the group consisting of: 189, 190, 191, 192, 343, 345, 346, and 347.

141. The method of claim 140, wherein SEQ ID NO:1 is modified with two or more of:

- (i) substitution at position 189 with Glu, Arg, or Gln;
 - (ii) substitution at position 190 with Asp, Ser, Arg, His, Asn, Gly, Pro, or Thr;
 - (iii) substitution at position 191 with Ala, Phe, Gly, Leu, Met, Val, Tyr, Arg, Asn, Asp, Cys, Glu, Ile, Lys, Ser, Thr, or Trp;
 - (iv) substitution at position 192 with Ile, Lys, Arg, Thr, Val, Ala, Asp, Glu, Gly, Met, or Ser;
 - (v) substitution at position 343 with Ala, Ile, Leu, Asn, Thr, His, Pro, or Asp;
 - (vi) substitution at position 345 with Phe, Thr, or Ile;
 - (vii) substitution at position 346 with Glu, Phe, His, Leu, Asn, Tyr, Val, Ile, Gln, or Asp;
- and
- (viii) substitution at position 347 with Met, Ser, Pro, Thr, Ile, or Leu.

142. The method of any one of claims 139 to 141, wherein the plurality of nucleic acids encoding SEQ ID NO:1 is modified to change a residue at three, four, five, six, seven, or eight or more positions in SEQ ID NO:1.

143. The method of any one of claims 129 to 133, wherein the two or more positions in SEQ ID NO:1 are selected from the group consisting of: positions 130-134, 284-296, 315-336, and 350-362 of SEQ ID NO:1.

144. The method of claim 143, wherein the two or more positions in SEQ ID NO:1 is selected from the group consisting of: 316, 317, 318, 319, 320, 321, 322, 325, 329, 350, 351, 353, 361, and 362.

145. The method of claim 144, wherein SEQ ID NO:1 is modified with two or more of:
- (i) substitution at position 316 with Gly, Leu, Cys, Trp, or Val;
 - (ii) substitution at position 317 with Ile or Phe;
 - (iii) substitution at position 318 with Val, Gly, Ile, Ser, or Thr;
 - (iv) substitution at position 319 with Ser;
 - (v) substitution at position 320 with Ile, Pro, Leu, Thr, or Val;
 - (vi) substitution at position 321 with Asp, Glu, Gly, Asn, Gln, Arg, Thr, Pro, Ser, His, or Ala;
 - (vii) substitution at position 322 with Cys, Asp, Leu, Pro, Arg, Ser, Gly, His, Phe, Tyr, or Val;
 - (viii) substitution at position 325 with Glu, Leu, Ser, Val, Trp, or Ala;
 - (ix) substitution at position 329 with Glu, His, Lys, Asn, Gln, Asp, Gly, or Ser;
 - (x) substitution at position 350 with Phe, Lys, Asn, Pro, Arg, Cys, His, Ile, Leu, Met, Ser, Thr, Trp, or Tyr;
 - (xi) substitution at position 351 with Ala, Cys, Asp, Gly, Arg, His, or Pro;
 - (xii) substitution at position 353 with Ala, Ile, Leu, Gln, Ser, Val, Asn, Asp, Glu, Gly, His, Pro, or Thr;
 - (xiii) substitution at position 361 with Cys, Asp, Glu, Ile, Asn, Gln, Ser, Thr, Val, Ala, Arg, Gly, His, Leu, Lys, Phe, Pro, Trp or Tyr; and
 - (xiv) substitution at position 362 with Ala, Glu, His, Lys, Pro, Gln, Arg, Asn, Gly, Ile, Leu, Thr, or Val.
146. The method of any one of claims 143 to 145, wherein the plurality of nucleic acids encoding SEQ ID NO:1 is modified to change a residue at three, four, five, six, seven, eight, or nine or more positions in SEQ ID NO:1.
147. A method of screening a library of nucleic acid sequences to identify modified GCase polypeptides having improved conformational stability and maintained or greater activity relative to that of SEQ ID NO:1, wherein the method comprises:
- a) generating a library of nucleic acid sequences encoding modified GCase polypeptides according to the method of any one of claims 129 to 146;
 - b) transforming a plurality of host cells with the library of nucleic acids;

- c) culturing the transformed cells to express the polypeptides encoded by the library of nucleic acid sequences;
- d) subjecting the transformed cells to serum stress by incubating the cells in serum from a mammalian host for at least 30 minutes at temperatures ranging from room temperature to 37°C;
- e) labeling the cells with (i) a fluorescent cyclophellitol-type activity-based probe which covalently reacts with a residue in the active site of GCCase, and (ii) an anti-GCCase antibody; and
- f) sorting the cells to isolate a population enriched for the fluorescent activity-based probe and the anti-GCCase antibody.

148. The method of claim 147, wherein the fluorescent cyclophellitol-type activity-based probe is a fluorescent form of 8-deoxy-8-azidocyclophellitol (KY170), is a fluorescent form of conduritol β -epoxide (CBE) or is a 2-deoxy-2-fluoroglycoside.

149. The method of claim 147 or 148, wherein the fluorescent cyclophellitol-type activity-based probe is selected from the group consisting of: MDW933, MDW941, and 2-deoxy-2-fluoro- β -d-glucopyranosyl-*N*-phenyltrifluoroacetimidate.

150. The method of any one of claims 147 to 149, wherein the serum is from a mouse, rabbit, monkey, or human.

151. The method of any one of claims 147 to 150, wherein the anti-GCCase antibody comprises:

- (i) a CDR-L1 sequence comprising the sequence of RASQGISSYLA (SEQ ID NO:132);
- (ii) a CDR-L2 sequence comprising the sequence of AASSLQS (SEQ ID NO:133);
- (iii) a CDR-L3 sequence comprising the sequence of QQYYSYPFT (SEQ ID NO:134);
- (iv) a CDR-H1 sequence comprising the sequence of GFTFSSYH (SEQ ID NO:135);
- (v) a CDR-H2 sequence comprising the sequence of SINPSNGGTYTADSVKG (SEQ ID NO:136); and
- (vi) a CDR-H3 sequence comprising the sequence of SSYYYYEMDY (SEQ ID

NO:137).

152. A modified GCase polypeptide identified by the method of any one of claims 147 to 151.