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(19) **United States**(12) **Patent Application Publication**
KALNIK et al.(10) **Pub. No.: US 2023/0416697 A1**(43) **Pub. Date: Dec. 28, 2023**(54) **NOVEL NICOTINE DEGRADING ENZYME
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System**, Austin, TX (US)(21) Appl. No.: **18/116,810**(22) Filed: **Mar. 2, 2023****Related U.S. Application Data**(62) Division of application No. 16/483,380, filed on Aug.
2, 2019, now Pat. No. 11,597,916, filed as application
No. PCT/US18/16664 on Feb. 2, 2018.(60) Provisional application No. 62/454,331, filed on Feb.
3, 2017, provisional application No. 62/535,507, filed
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CPC **C12N 9/0006** (2013.01); **A61K 47/60**
(2017.08); **A61P 43/00** (2018.01)(57) **ABSTRACT**Described are nicotine-degrading enzyme variants that
exhibit increased nicotine-degrading activity and/or
decreased immunogenicity relative to the wild-type NicA2
and NOX enzymes, compositions comprising the variants,
and methods using them.**Specification includes a Sequence Listing.****1****2****3**

62 kDa →

49 kDa →

38 kDa →

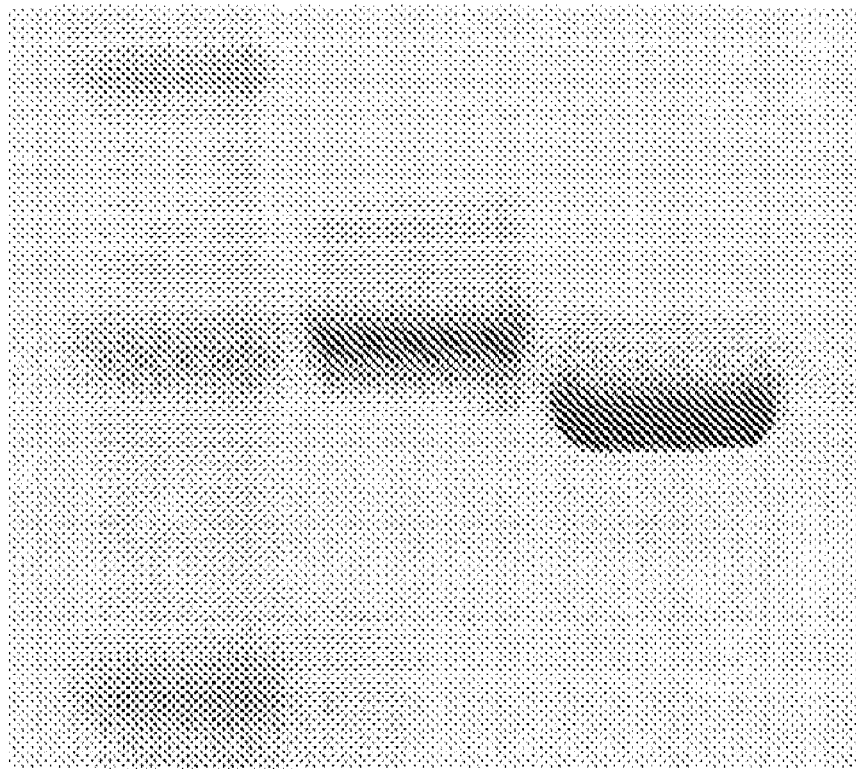
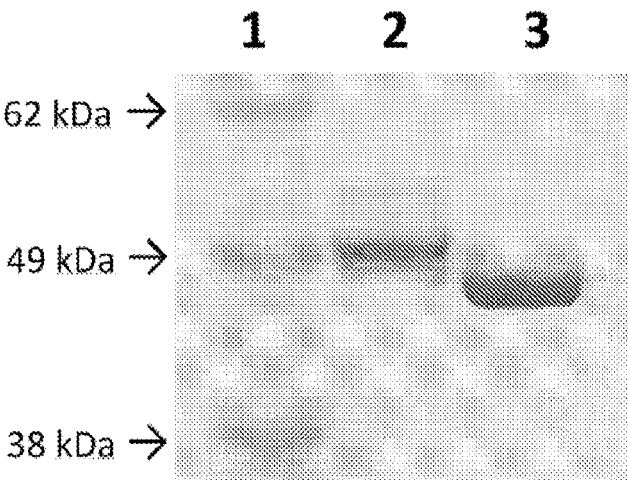


FIG 1



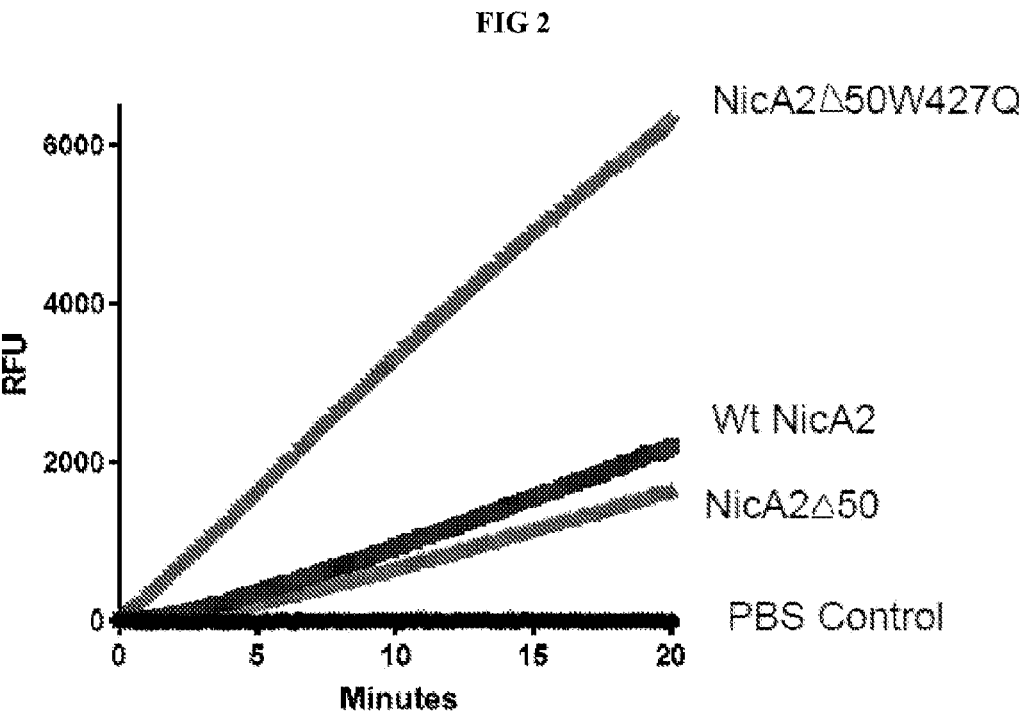


FIG 3

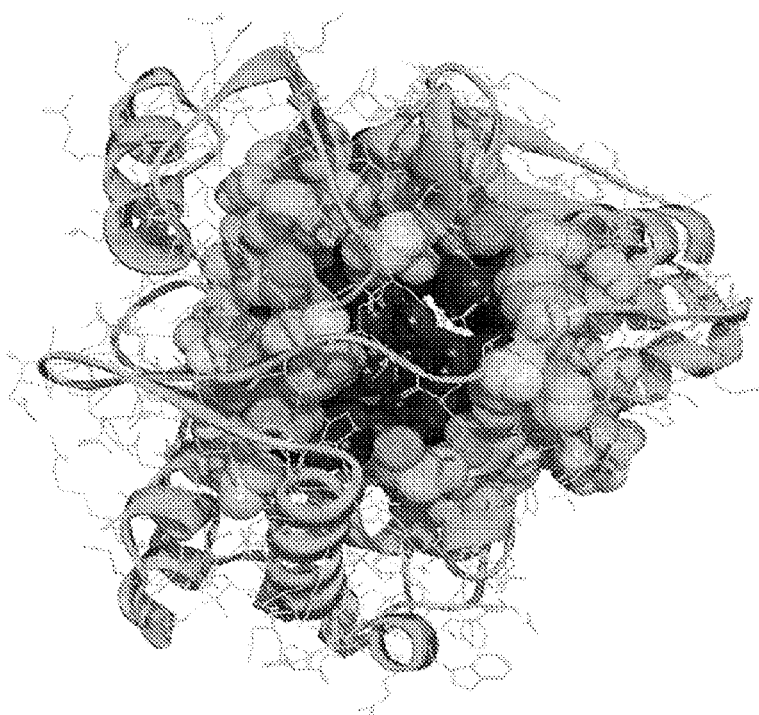


FIG 4A

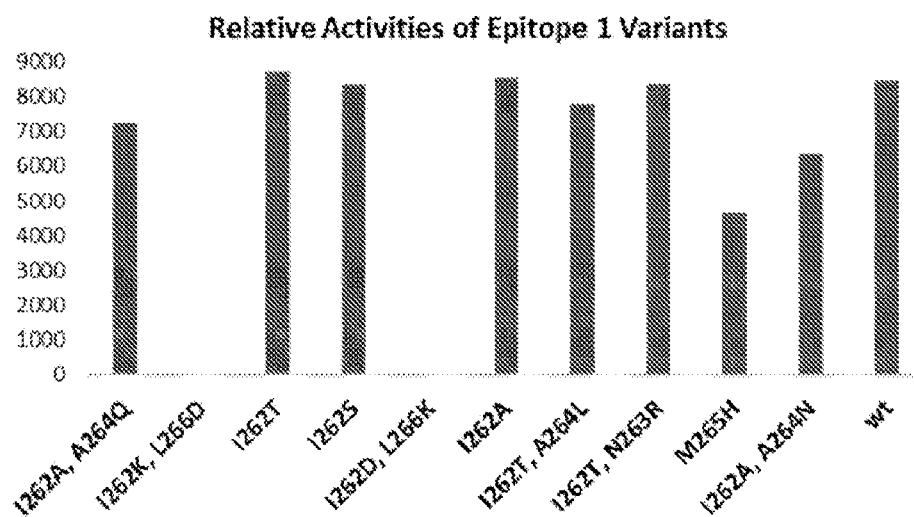


FIG 4B

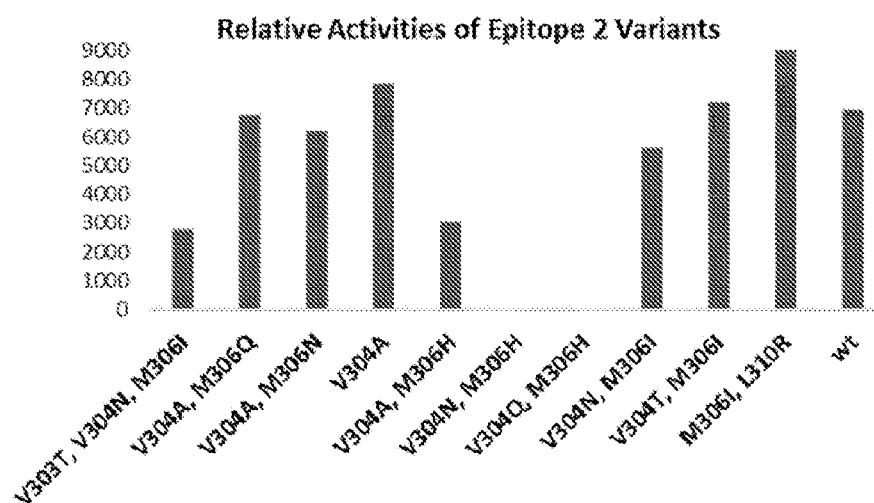


FIG 4C

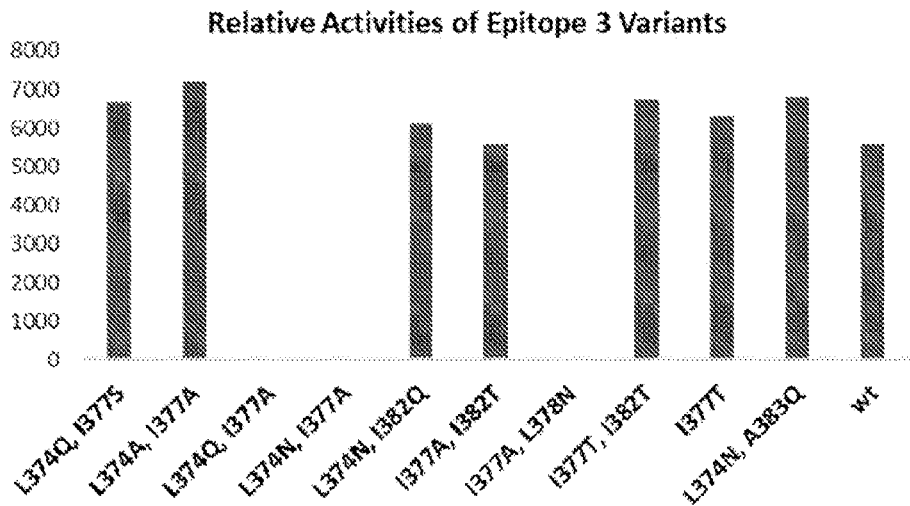


FIG 4D

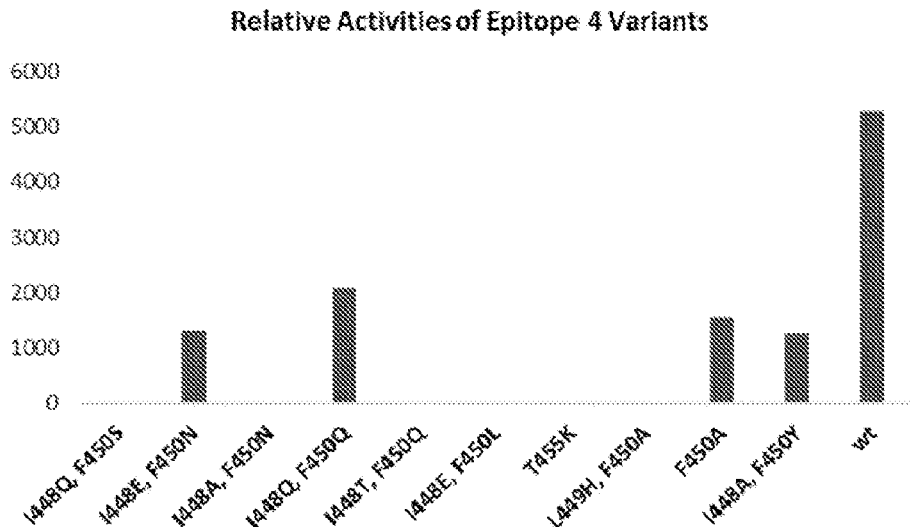


FIG 4E

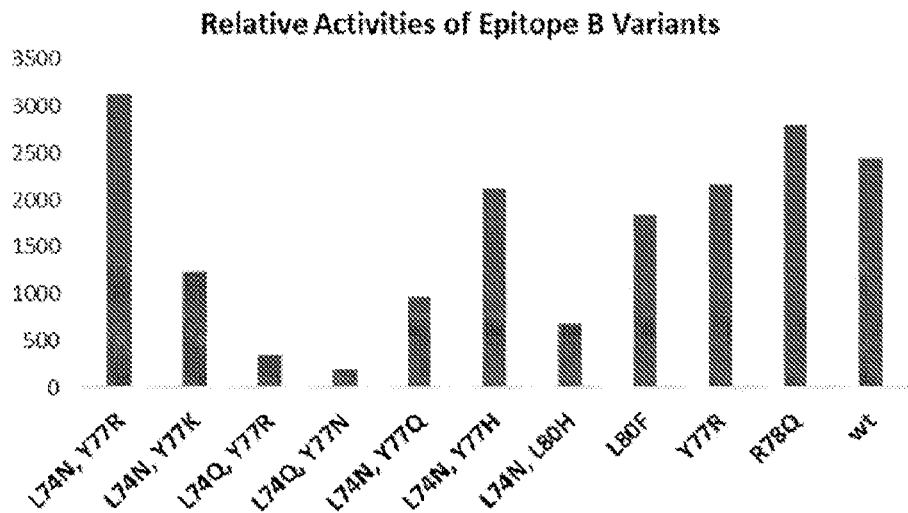


FIG 5

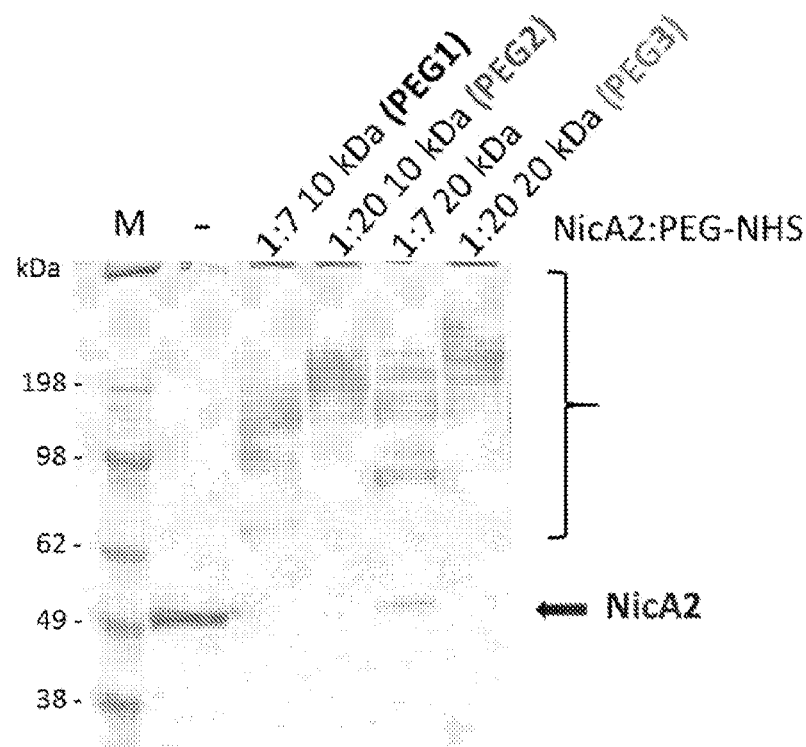


FIG 6

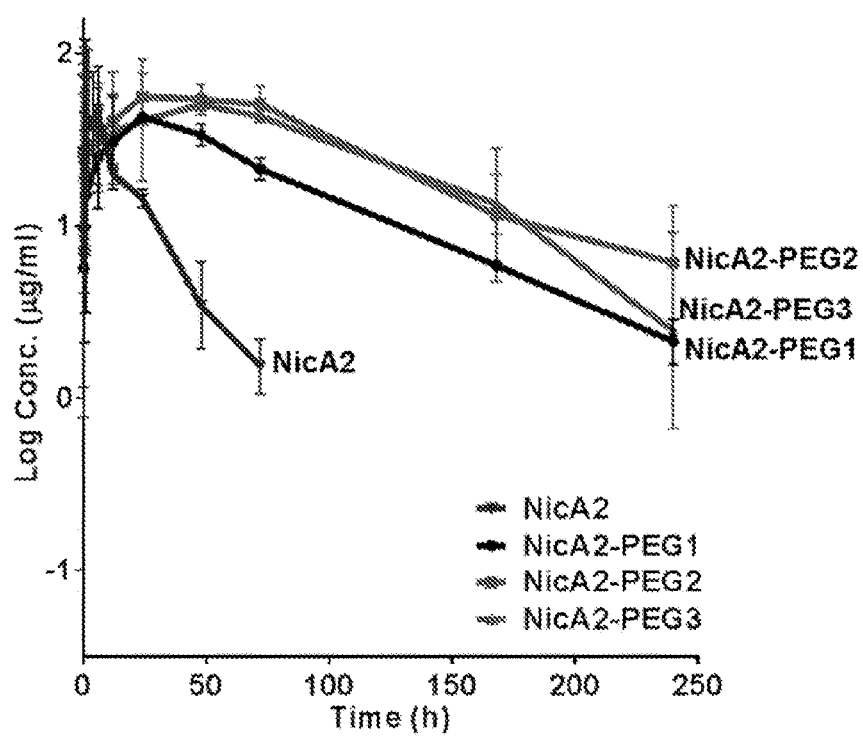


FIG 7

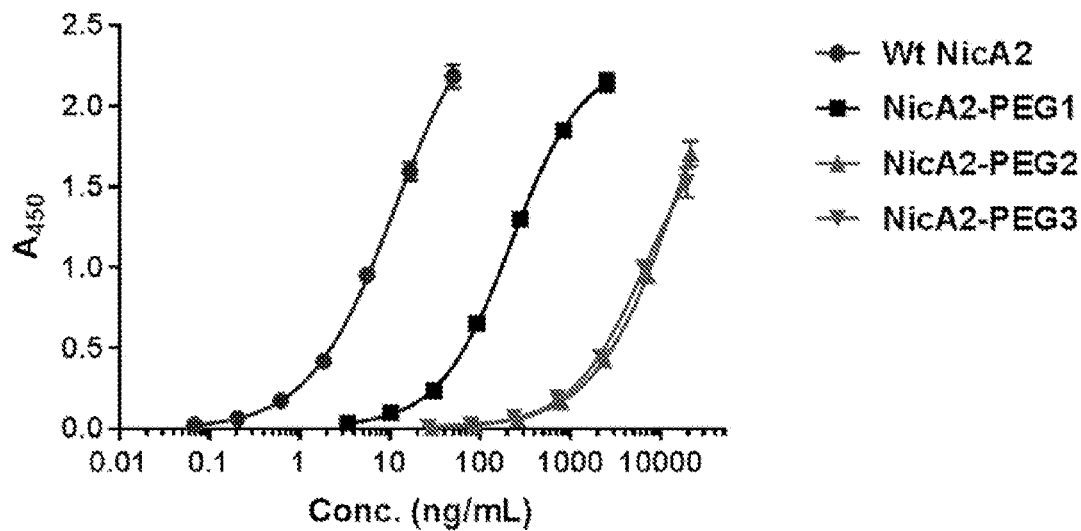


FIG 8

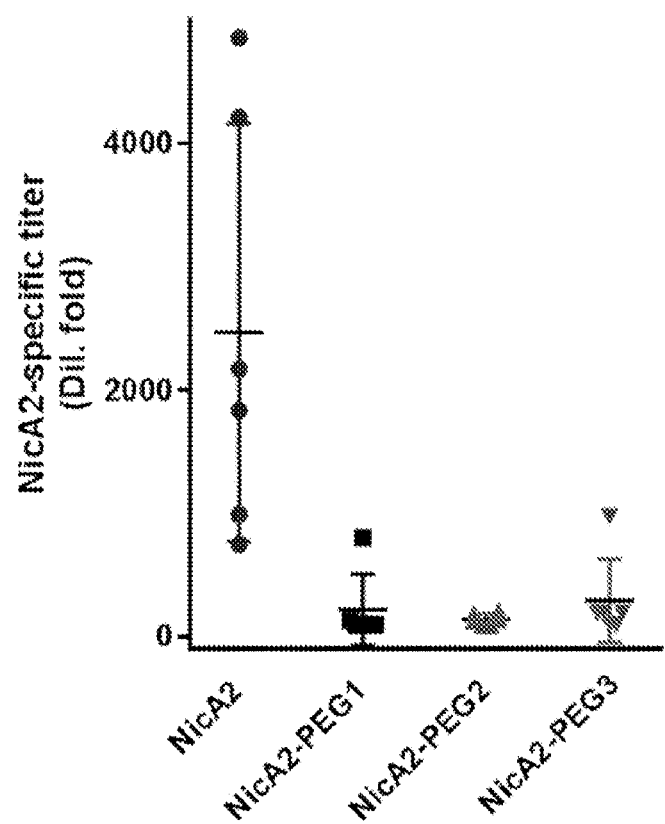


FIG 9

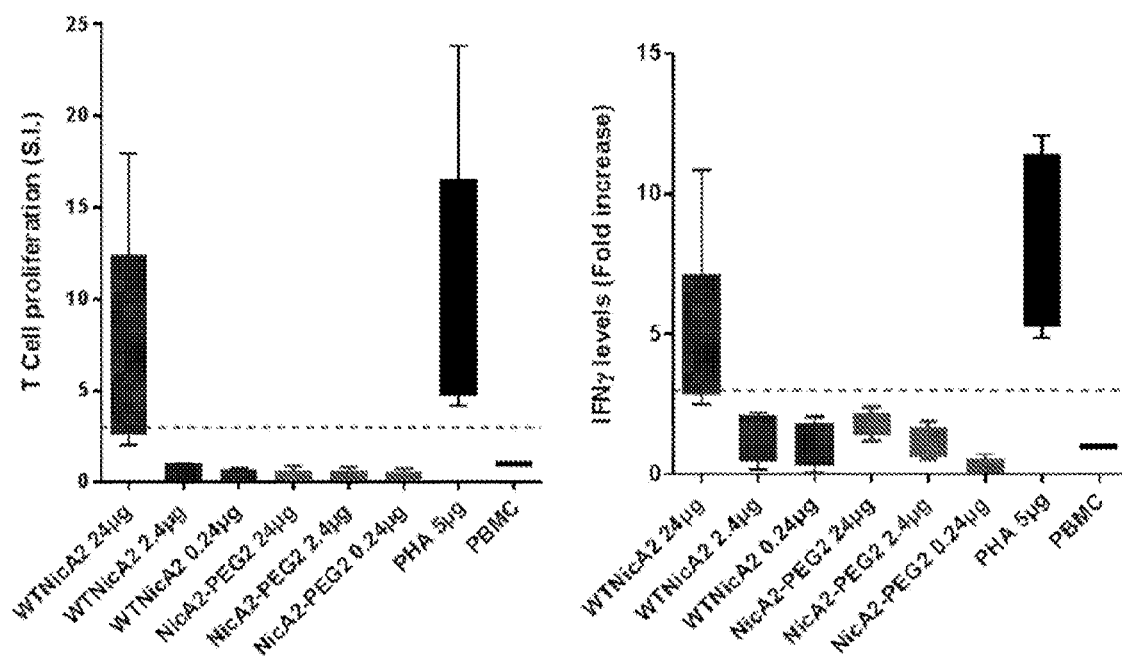


FIG 10

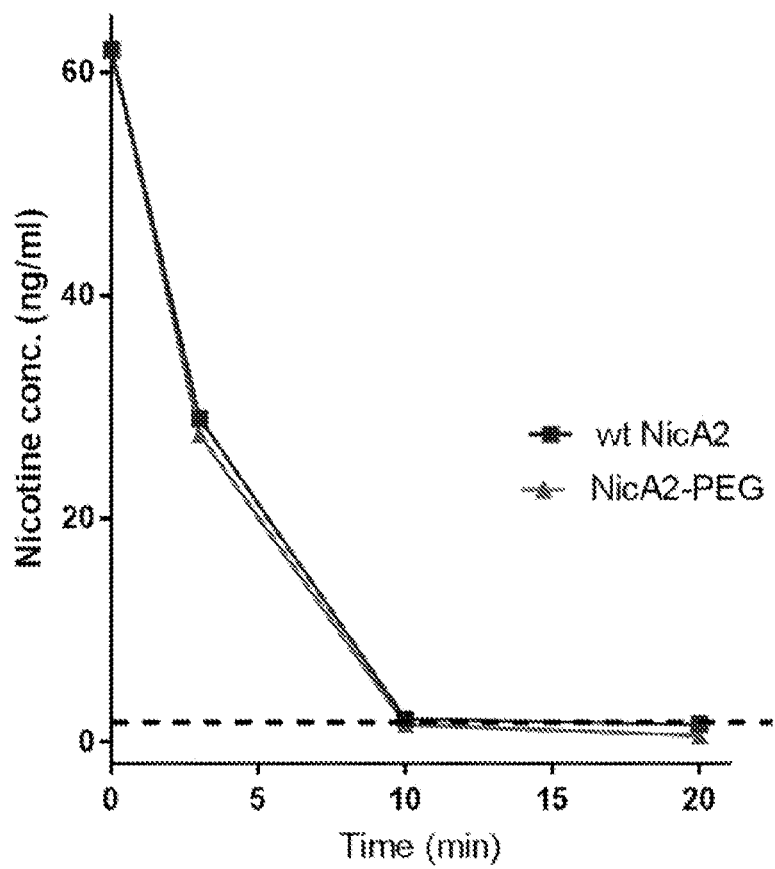
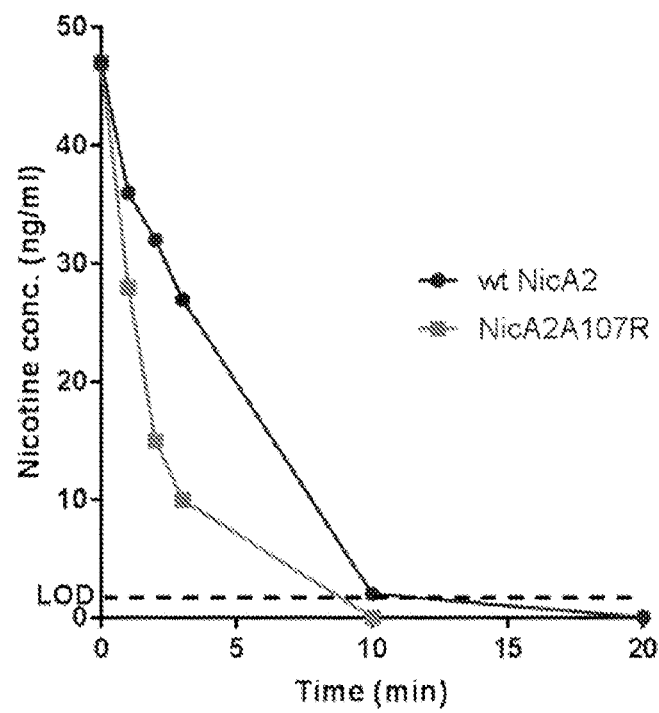


FIG 11



NOVEL NICOTINE DEGRADING ENZYME VARIANTS

RELATED APPLICATIONS

[0001] This application is a divisional of U.S. patent application Ser. No. 16/483,380 filed Aug. 2, 2019, which is the U.S. National Stage of International Application No. PCT/JS2018/016664, filed Feb. 2, 2018, and claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application 62/454,331 filed Feb. 3, 2017, and U.S. Provisional Application 62/535,507 filed Jul. 21, 2017, the entire contents of which are incorporated herein by reference.

GOVERNMENT LICENSE RIGHTS

[0002] This invention was made with Government support under National Institutes of Health grant R01 DA038877 awarded by the PHS. The Government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which is being submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML copy, was created on Jun. 16, 2023, is named 105894-0145_SL.xml and is 245,814 bytes in size.

FIELD

[0004] The present disclosure relates generally to the field of treating nicotine addiction or nicotine poisoning. Described are nicotine-degrading enzyme variants that exhibit increased nicotine-degrading activity and/or decreased immunogenicity relative to the wild-type NicA2 enzyme, compositions comprising them, and methods using them.

BACKGROUND

[0005] The following discussion is provided to aid the reader in understanding the disclosure and is not admitted to describe or constitute prior art thereto.

[0006] Smoking is a global healthcare problem. The World Health Organization estimates that there are 1.3 billion smokers worldwide today and nearly five million tobacco-related deaths each year. If current smoking patterns continue, smoking will cause some 10 million deaths each year by 2020. According to the U.S. Center for Disease Control (CDC), tobacco use is the single leading preventable cause of death in the U.S., responsible for approximately 438,000 deaths each year. In addition, it is estimated that smoking results in an annual health-related economic cost of approximately \$157 billion. The CDC estimates that, among the 45 million adult smokers in the U.S., 70% want to quit, but less than five percent of those who try to quit remain smoke-free after 12 months.

[0007] One reason it is difficult to quit smoking is addiction to the nicotine in cigarettes and other tobacco products. Nicotine is a small molecule that upon inhalation into the body quickly passes into the bloodstream and subsequently reaches the brain by crossing the blood-brain barrier. Once in the brain, the nicotine binds to nicotinic receptors, which results in the release of stimulants, such as dopamine,

activating the reward system and providing the smoker with a positive and pleasurable re-enforcing experience, which leads to addiction.

[0008] In addition to the detrimental health effects associated with smoking and other tobacco use, nicotine poisoning, which results from ingestion or inhalation of too much nicotine, is another nicotine-related health problem. The LD₅₀ of nicotine is 50 mg/kg for rats and 3 mg/kg for mice. A dose as low as 30-60 mg (0.5-1.0 mg/kg) may be lethal for adult humans, while children may become ill following ingestion of one cigarette, and ingestion of more than this may cause a child to become severely ill. On the other hand, some evidence suggests that a lethal dose may be as high as 500 mg or more (1.0-7.1 mg/kg) for a human adult. In either case, acute nicotine poisoning usually occurs in children who accidentally chew on nicotine gum or patches or ingest the “e-liquid” of electronic cigarettes. In rare instances, children have also been known to become ill after ingesting cigarettes. There are several hundred cases of acute nicotine poisoning reported every month in the United States alone. [0009] Typically, initial treatment of nicotine poisoning may include the administration of activated charcoal to try to reduce gastrointestinal absorption, while additional treatment may address the symptoms that result from nicotine poisoning.

[0010] The use of the wild-type NicA2 enzyme for smoking cessation has been proposed (see, e.g., Xue et al., *J. Am. Chem. Soc.* 137: 10136-39 (2015)). Nevertheless, there remains a need for additional agents, compositions and methods for treating nicotine addiction, as well as for agents, compositions, and methods for treating nicotine poisoning.

SUMMARY

[0011] Described herein are nicotine-degrading enzyme variants that exhibit increased nicotine-degrading activity and/or decreased immunogenicity relative to the wild-type NicA2 enzyme, compositions comprising them, and methods using them.

[0012] In some embodiments, the nicotine-degrading enzyme variant comprises an amino acid sequence that is a variant of the amino acid sequence of the wild-type NicA2 enzyme set forth in SEQ ID NO: 1, wherein the variant sequence has at least one substitution, addition, or deletion relative to SEQ ID NO: 1 that increases the nicotine-degrading activity and/or decreases the immunogenicity of the variant relative to the wild-type NicA2 enzyme.

[0013] In some embodiments, the variant exhibits increased nicotine-degrading activity relative to the wild-type NicA2 enzyme. In some embodiments, the variant of the wild-type NicA2 sequence comprises the substitution AF104L, G106S, A107H, A107P, 107R, A107K, A107T, F355C, F355V, W427Q, W427E, W427S, W427M, W427H, W427L, W427R, R91A, R91Q, R91F, R91G, R91T, R91L, R91S, R91N, T250G, T250L, T250R, T250V, T250P, K340P, K340I, K340V, K340D, K340E, Q366K, Q366E, Q366V, Q366L, Q366I, Q366Y, T381P, T381I, T381V, T381Q, T381N, T381L, T381M, N462L, N462Y, N462S, N462F, N462G, N462E, N462A, N462M, I463F, I463Y, I463A, I463V, I463L, L217Q, L217G, L217E, L217I, L217C, or L217S, or any two or more thereof at different positions. In some embodiments, the variant sequence comprises an amino acid sequence selected from any one of SEQ ID NOS: 5-56. In some embodiments, the nicotine-degrading activity of the variant is at least 200%, at least 300%, or at

least 400% of the nicotine-degrading activity of the wild-type NicA2 enzyme. In some embodiments, the variant sequence comprises at least one, at least two, or at least three substitution(s) at amino acid positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1. In some embodiments, the variant may have a deletion of 1-52 amino acids at the N-terminus of the peptide. For example, a variant derived from SEQ ID NO:1 may comprise a deletion of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 51, or 52 amino acids from the N-terminus of the peptide. In some embodiments, the variant additionally or alternatively may have a deletion of 1 or more amino acids from the C-terminus of the peptide, such as a deletion of the C-terminal residue.

[0014] Additionally or alternatively, in some embodiments, the NicA2 variant exhibits reduced immunogenicity relative to the wild-type NicA2 enzyme. In some embodiments, the immunogenicity relative to the wild-type NicA2 enzyme is reduced by 75% or more. In some embodiments, the variant sequence comprises at least one substitution, addition, or deletion in an immunogenic T-cell epitope within a region selected from amino acids 10-32, 68-94, 189-225, 248-285, 296-327, 336-391, or 435-459 of SEQ ID NO: 1. In some embodiments, the variant sequence comprises at least one substitution, addition, or deletion in an immunogenic T-cell epitope selected from amino acids 16-24, 73-81, 258-266, 302-310, 373-381, or 447-455 of SEQ ID NO: 1. In some embodiments, the variant sequence comprises at least one substitution, addition, or deletion at a position selected from (a) amino acid residues 74, 77, 78, or 80 of SEQ ID NO: 1; (b) amino acid residues 262, 263, 264, or 266 of SEQ ID NO: 1; (c) amino acid residues 303, 304, 306, or 310 of SEQ ID NO: 1; (d) amino acid residues 374, 377, 378, 382, or 383 of SEQ ID NO: 1; and/or (e) amino acid residues 450, 451, 452, or 457 of SEQ ID NO: 1. In some embodiments, the variant sequence comprises at least one substitution or substitution combination selected from those listed for Epitope B in Table 3. Additionally or alternatively, in some embodiments, the variant sequence comprises at least one substitution or substitution combination selected from those listed for Epitope 1 in Table 3. Additionally or alternatively, in some embodiments, the variant sequence comprises at least one substitution or substitution combination selected from those listed for Epitope 2 in Table 3. Additionally or alternatively, in some embodiments, the variant sequence comprises at least one substitution or substitution combination selected from those listed for Epitope 3 in Table 3. Additionally or alternatively, in some embodiments, the variant sequence comprises at least one substitution or substitution combination selected from those listed for Epitope 4 in Table 3. In some embodiments, the variant comprises at least one substitution or substitution combination selected from (a) I262T; (b) I262S; (c) I262A; (d) I262T and A264L; and/or (e) I262T and N263R.

[0015] Additionally or alternatively, in some embodiments, the variant may further comprise at least one substitution at amino acid positions 74, 77, 78, 80, 262-266, 303, 304, 306, 310, 374, 377, 378, 382, 383, 450-452, or 457. For example, the variant may comprise a substitution at amino acid position 262 or 263, or the variant may comprise substitutions at amino acid positions 262 and 263. In some embodiments, the substitution may be I262A, I262T, and/or

N263R. For example, in some embodiments, the variant sequence comprises an amino acid sequence of SEQ ID NO: 62 or 63.

[0016] In some embodiments, the nicotine-degrading enzyme variant comprises an amino acid sequence that is a variant of the amino acid sequence of the wild-type NOX enzyme set forth in SEQ ID NO: 57, wherein the variant sequence has at least one substitution, addition, or deletion relative to SEQ ID NO: 57 that increases the nicotine-degrading activity and/or decreases the immunogenicity of the variant relative to the wild-type NOX enzyme or the wild-type NicA2 enzyme.

[0017] In some embodiments, the variant of the wild-type NOX sequence comprises the substitution at position 423 of SEQ ID NO: 57. For example, the variant sequence may comprise the substitution W423A, W423S, W423E, or W423H. In some embodiments, the variant sequence comprises an amino acid sequence selected from any one of SEQ ID NOs: 58-61. In some embodiments, the nicotine-degrading activity of the NOX variant is at least 200% of the nicotine-degrading activity of the wild-type NicA2 enzyme. In some embodiments, the nicotine-degrading activity of the NOX variant is at least 200% of the nicotine-degrading activity of the wild-type NOX enzyme.

[0018] Additionally or alternatively, in some embodiments, the NOX variant exhibits reduced immunogenicity relative to the wild-type NOX enzyme or the wild-type NicA2 enzyme. In some embodiments, the immunogenicity relative to the wild-type NOX or NicA2 enzymes is reduced by 75% or more.

[0019] Additionally or alternatively, in some embodiments, the variant further comprises a deletion of at least amino acids 1-38 of SEQ ID NOs: 1 or 57, or of amino acids 1-50 of SEQ ID NOs: 1 or 57, or amino acids 1-51 of SEQ ID NOs: 1 or 57, or amino acids 1-52 of SEQ ID NOs: 1 or 57. In some embodiments, the variant may further comprise a His-tag, such as a His-tag comprising the amino acid sequence of SEQ ID NO: 139. Additionally or alternatively, in some embodiments, the variant comprises a deletion of one or more C-terminal amino acids, such as a deletion of the residue corresponding to S482 of the wild-type sequence.

[0020] In any of the embodiments, the variant may be a long-acting variant, such as wherein the variant is conjugated to an albumin-binding peptide, an albumin-binding protein domain, human serum albumin, or an inert polypeptide, such as recombinant PEG (XTEN®), a homo-amino acid polymer (HAP), a proline-alanine serine polymer (PAS), or an elastin-like peptide (ELP). In specific embodiments, the long-acting variant is conjugated to polyethylene glycol (i.e., the variant is PEGylated).

[0021] In some embodiments, there are provided pharmaceutical compositions comprising a nicotine-degrading enzyme variant described herein and a pharmaceutically acceptable carrier. In some embodiments, the composition is formulated for injection or infusion. In some embodiments the composition is formulated for oral administration.

[0022] In some embodiments, there are provided methods of treating nicotine addiction or facilitating smoking cessation, comprising administering to a mammalian subject in need thereof a therapeutically effective amount of a nicotine-degrading enzyme variant or composition as described herein. In some embodiments, the mammalian subject is a human subject. In some embodiments, the nicotine addiction

is associated with the ingestion of a nicotine product selected from tobacco products and electronic cigarettes.

[0023] In some embodiments, there are provided methods of treating nicotine poisoning, comprising administering to a mammalian subject in need thereof a therapeutically effective amount of a nicotine-degrading enzyme variant or composition as described herein. In some embodiments, the mammalian subject is a human subject, or, more specifically, a human child. In some embodiments, the nicotine poisoning is associated with the consumption of a nicotine product selected from tobacco products and electronic cigarettes.

[0024] Also provided are nicotine-degrading enzyme variants and compositions as described herein for use in treating nicotine addiction or facilitating smoking cessation.

[0025] Also provided are uses of nicotine-degrading enzyme variants and compositions as described herein in the manufacture of a medicament for the treatment of nicotine addiction or facilitation of smoking cessation.

[0026] The foregoing general description and following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] FIG. 1 shows SDS-PAGE analysis of His-tagged versions of wild-type NicA2 (SEQ ID NO:1) and NicA2Δ50 (SEQ ID NO:2). Lane 1: MW marker (Blue Plus2; Invitrogen); Lane 2: wild-type NicA2 (SEQ ID NO:1); and Lane 3: NicA2Δ50 (SEQ ID NO:2).

[0028] FIG. 2 shows results of a nicotine-degrading activity assay comparing wild-type NicA2 with variant nicotine-degrading enzymes as described herein. The assays used purified proteins at a final concentration of 80 nM.

[0029] FIG. 3 shows the residues around the active site in the NicA2 crystal structure (adapted from Tararina et al., *Biochem.* 55:6595-98 (2016)). Shell one is shown in dark grey and shell two is shown in light grey. The residues making up the first and second shell are shown in Table 2.

[0030] FIGS. 4A-E show the relative activities of specific NicA2 variants carrying mutations of Epitopes B (FIG. 4E), 1 (FIG. 4A), 2 (FIG. 4B), 3 (FIG. 4C) and 4 (FIG. 4D) (as listed in Table 3), predicted to reduce the immunogenic potential compared to wild-type NicA2.

[0031] FIG. 5 shows random PEGylation of NicA2 using SDS-PAGE analysis. These results indicate that PEGylation can be increased by an increase in molar excess of PEGylation reagent and PEG chain length.

[0032] FIG. 6 shows PEGylation enhances the pharmacokinetic (PK) properties of NicA2 in the serum of animals administered PEGylated NicA2.

[0033] FIG. 7 shows PEGylation can mask potentially immunogenic epitopes on NicA2.

[0034] FIG. 8 shows PEGylation can decrease titers of NicA2-specific antibodies in a transgenic HLA-DR4 mouse model of immunogenicity. PEGylation of NicA2 led to a significant (≥ 10 -fold) decrease in average NicA2-specific antibody titers in transgenic DR4 mice (4, 2, and 2, animals from groups NicA2-PEG1, -PEG2, and -PEG3, respectively, had titers below the limit of detection (LOD)), suggesting a lower immunogenic potential in a clinical setting.

[0035] FIG. 9 shows PEGylation attenuates the human T-cell proliferation response and cytokine TNF γ release mediated by exposure to NicA2. PEGylation of NicA2 led to a significant decrease in average T Cell proliferation Stimulation Index (left panel) as well as a decrease in IFN γ

secretion levels (right panel). An increase of $3\geq$ (dashed line) is considered a significant increase and a positive response.

[0036] FIG. 10 shows PEGylated NicA2 enzymes retain full nicotine degrading activity in serum. PEGylation did not appear to impede NicA2's ability to degrade nicotine in rat serum.

[0037] FIG. 11 shows that a NicA2 variant with a A107R substitution has increased activity in serum at low nicotine concentrations compared to wild-type NicA2.

DETAILED DESCRIPTION

[0038] Described herein are nicotine-degrading enzyme variants that exhibit increased nicotine-degrading activity and/or decreased immunogenicity relative to the wild-type NicA2 or NOX enzymes, compositions comprising them, and methods using them, including methods for treating nicotine addiction and methods for facilitating nicotine cessation (e.g., smoking cessation) in a subject in need thereof.

I. Definitions

[0039] As used herein, the singular forms “a” “an” and “the” are used interchangeably and intended to include the plural forms as well and fall within each meaning, unless the context clearly indicates otherwise. Also, as used herein, “and/or” refers to and encompasses any and all possible permutations and combinations of one or more of the listed items.

[0040] As used herein, the term “about” will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, “about” will mean up to plus or minus 10% of the particular term.

[0041] As used herein, the phrases “therapeutically effective amount” and “therapeutic level” mean a nicotine-degrading enzyme dosage or plasma concentration in a subject that provides the specific pharmacological effect for which the nicotine-degrading enzyme is administered to a subject in need of such treatment, i.e. to degrade nicotine in the subject, and/or treat nicotine addiction and/or facilitate smoking cessation and/or treat nicotine poisoning. It is emphasized that a therapeutically effective amount or therapeutic level of a nicotine-degrading enzyme will not always be effective in treating the nicotine addiction or facilitate smoking cessation of a given subject, even though such dosage is deemed to be a therapeutically effective amount by those of skill in the art. For convenience only, exemplary amounts are provided below.

[0042] Those skilled in the art can adjust such amounts in accordance with standard practices as needed to treat a specific subject. The therapeutically effective amount may vary based on the route of administration and dosage form, the age and weight of the subject, and/or the subject's condition, including the degree of nicotine addiction, amount of nicotine generally consumed/ingested by the subject, and/or the subject's plasma levels of nicotine at the time of treatment and/or the amount of nicotine localized in the brain at the time of treatment.

[0043] The terms “treatment” or “treating” as used herein with reference to nicotine addiction or smoking cessation refer to one or more of: reducing, ameliorating or eliminat-

ing one or more symptoms or effects of nicotine withdrawal; reducing the number of cigarettes or the amount of nicotine consumed by a subject; and/or reducing the subject's plasma levels of nicotine and/or reducing the amount of nicotine localized in specific tissues of the subject (e.g., brain/central nervous system, heart and vasculature, etc.).

[0044] The terms “treatment” or “treating” as used herein with reference to nicotine poisoning refer to reducing, ameliorating or eliminating one or more symptoms or effects of nicotine and/or reducing the subject's plasma levels of nicotine and/or reducing the amount of nicotine localized in specific tissues of the subject (e.g., brain/central nervous system, heart and vasculature, etc.).

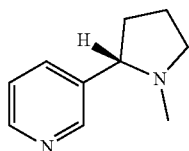
[0045] The terms “individual,” “subject,” and “patient” are used interchangeably herein, and refer to any individual mammal subject, e.g., bovine, canine, feline, equine, or human.

[0046] In accordance with FDA guidance, as used herein, “child” refers to a human subject from 0 through about 19 years of age. A child can be a subject that begins a course of treatment prior to turning about 19 years of age, even if the subject continues treatment beyond 19 years of age.

II. Nicotine, Nicotine Addiction, and Nicotine Toxicity

[0047] Nicotine is a nitrogen-containing chemical made by several types of plants including tobacco and other members of the nightshade family. When humans, mammals and most other types of animals are exposed to nicotine, it increases their heart rate, heart muscle oxygen consumption rate, and heart stroke volume. The consumption of nicotine is also linked to raised alertness, euphoria, and a sensation of being relaxed. However, nicotine is highly addictive.

[0048] By binding to nicotinic acetylcholine receptors in the brain, nicotine elicits its psychoactive effects and increases the levels of several neurotransmitters in various brain structures. Nicotine has a higher affinity for nicotinic receptors in the brain than those in skeletal muscle, though at toxic doses it can induce contractions and respiratory paralysis. Nicotine's selectivity is thought to be due to a particular amino acid difference on these receptor subtypes. The structure of nicotine is shown in Formula I below.



Formula I

[0049] People who regularly consume nicotine and then suddenly stop experience withdrawal symptoms, which may include cravings, a sense of emptiness, anxiety, depression, moodiness, irritability, and inattentiveness. The American Heart Association says that nicotine (from smoking tobacco) is one of the hardest substances to quit—at least as hard as heroin.

[0050] Nicotine poisoning can occur when an individual consumes loose tobacco, cigarettes, nicotine gum, patches, or the “e-liquid” of electronic cigarettes (e.g., the nicotine-containing liquid that is used in electronic cigarettes and other vaporizing devices) or other products containing

tobacco or tobacco extracts, or other products, supplies or intermediates containing nicotine. Indeed, a recent study showed that the incidence of nicotine poisoning from exposure to e-cigarettes increased 1492.9% between January 2012 and April 2015 (Kamboj et al. *PEDIATRICS* 137(6): e20160041 (2016)). Although exposure can occur through inhalation of tobacco smoke (either primary or second hand), nicotine poisoning or nicotine overdose more commonly results when a subject (typically a child) ingests nicotine, for example by chewing or ingesting nicotine gum, ingesting cigarettes or other tobacco leaf products, ingesting nicotine patches, or ingesting e-liquid. Additionally, nicotine can be dermally absorbed, and therefore nicotine poisoning can result from toxic levels of nicotine coming into direct contact with the skin.

[0051] Nicotine poisoning can produce neurological symptoms (convulsions, coma, depression, confusion, fainting, headache), cardiovascular symptoms (rapid heartbeat, high blood pressure), respiratory symptoms (difficulty breathing, rapid breathing), gastrointestinal symptoms (increased salivation, abdominal cramps, vomiting), and musculoskeletal symptoms (Muscular twitching, weakness), as well as death.

III. Nicotine-Degrading Enzyme Variants

[0052] Described herein are nicotine-degrading enzyme variants comprising an amino acid sequence that is a variant of the amino acid sequence of the wild-type NicA2 or NOX enzyme set forth in SEQ ID NO: 1 or SEQ ID NO: 57, respectively, wherein the variant sequence has at least one substitution, addition, or deletion relative to SEQ ID NO: 1 or SEQ ID NO: 57 that increases the nicotine-degrading activity and/or decreases the immunogenicity of the variant relative to the wild-type NicA2 or NOX enzyme, respectively.

[0053] NicA2 (nicotine oxidoreductase; PPS_4081; GenBank accession number: AEJ14620.1), was isolated from *Pseudomonas putida* strain S16. See, e.g., Tang et al., *PLoS GENETICS*, 9(10): e1003923 (2013). The activity of NicA2 is the first committed step of S16's degradation of nicotine, catalyzing the oxidation of nicotine to N-methylmyosmine. It is reported to be an essential enzyme in the *P. putida* S16 metabolic cascade responsible for breaking down nicotine. A structural analysis of the wild-type NicA2 enzyme has been reported in Tararina et al., *Biochem.* 55:6595-98 (2016).

[0054] NOX (nicotine amine oxidase; GenBank accession number: AGH68979.1) has been isolated from *Pseudomonas* sp. HZN6 (See, e.g., Qiu et al., *Appl. Environ. Microbiol.* 78, 2154-2160 (2012); Qiu et al., *Appl. Environ. Microbiol.* 79, 2164-2171 (2013)). NOX is closely related to NicA2, with an amino acid identity of 83%. NOX was reported to have a catalytic activity like NicA2, degrading nicotine to N-methylmyosmine.

[0055] The present disclosure provides variants of wild-type NicA2 and NOX with improved activity and/or decreased immunogenicity. In some embodiments, the disclosed variants may have an amino acid identity that is about 80, about 85, about 90, about 95, about 96, about 97, about 98, or about 99 percent of wild-type NicA2 or NOX. In some embodiments, the disclosed variants may share about 80, about 85, about 90, about 95, about 96, about 97, about 98, or about 99 percent homology with wild-type NicA2 or

NOX. For instance, in some embodiments, the disclosed variants may comprise the amino acids residues conserved between NicA2 and NOX.

[0056] The amino acid sequence of wild-type NicA2, wild-type NOX, and exemplary variants thereof are set forth in Table 1 below. The disclosed variants were produced with

a linker and His-tag (GGGGSGSGHHHHHH, SEQ ID NO: 139) at the C-terminal end, which was subsequently removed. The His-tag was used to assist in purification of the variants, but other means or methods of purification that do not require a His-tag may also be used.

TABLE 1

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
Wild-Type NicA2	1	M *SDKTKTNEGFSRRSF IGSA AAVVTA GVAGLG AIDAASATQKTNRAS TV KGGFDYDVVVVGGGFAGATAARECGLQGYRTLLLEARSRLGGRTFTSR FAGQEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYN DGSVESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSV LDRIKTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFA CGGWN YD AFMDTETHYRIQGGT IGLINAMLT DSGA EVRMSVPVTAVEQVNGGVKIK TDDDEIITAG VVVM TVPLNTY KHIGFTPALSKGKQRFIKEGQLSKGAKLY VHVKQNLGRVFAFADEQQPLNWWQTHDYSDEL GTILSIT IARKETIDVND RDAVTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ KDLQA AEGRI L FAGAETS NGWHANIDGAVESGLRAGREV KQLLS**†
NicA2Δ50 (N-terminal deletion of residues 1-50)	2	GFDYDVVVVGGGFAGATAARECGLQGYRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFA CGGWN YDAFM DTETHYRIQGGTIGLINAMLTDSGA EVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSIT IARKETIDVND RDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWHANIDGAVESGLRAGREV KQLLS
NicA2Δ25 (N-terminal deletion of residues 1-25)	3	GVAGLG AIDAASATQKTNRAS TVKGGFDYDVVVVGGGFAGATAARECG LQGYRTLLLEARSRLGGRTFTSRFAGQEIEFGGAWVHWLQPHVWAEMQ RYGLGVVEDPLTNLDKTLIMYNDGSVESISPDEFGKNIRIAFEKLCHDAW EVFP RPHEPMF TERARELDKSSVLDRIKTLGLSRLQQAQINSYMALYAGE TTDKFGLPGLVKLFA CGGWN YDAFM DTETHYRIQGGTIGLINAMLTDSG AEVRMSVPVTAVEQVNGGVKIKTDDDEIITAGVVMTVPLNTYKHIGFT PALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQ HDYSDELGTILSIT IARKETIDVND RDAVTREVQKMPGVEVLGTAAYDW TADPFSLGAWAAYGVGQLSRLKDLQA AEGRI L FAGAETS NGWHANIDG AVESGLRAGREV KQLLS
NicA2Δ38 (N-terminal deletion of residues 1-38)	4	TQKTNRAS TVKGGFDYDVVVVGGGFAGATAARECGLQGYRTLLLEARS RLGGRTFTSRFAGQEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTN LDKTLIMYNDGSVESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TER ARELDKSSVLDRIKTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLF ACGGWN YDAFM DTETHYRIQGGTIGLINAMLTDSGA EVRMSVPVTAVE QVNGGVKIKTDDDEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEG QLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSIT I ARKETIDVND RDAVTREVQKMPGVEVLGTAAYDWTADPFSLGAWAA YGVGQLSRLKDLQA AEGRI L FAGAETS NGWHANIDGAVESGLRAGREV KQLLS
NicA2Δ50 W427Q (W427Q substitution; N-terminal deletion of residues 1-50)	5	GFDYDVVVVGGGFAGATAARECGLQGYRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFA CGGWN YDAFM DTETHYRIQGGTIGLINAMLTDSGA EVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSIT IARKETIDVND RDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAQAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWHANIDGAVESGLRAGREV KQLLS
NicA2Δ50 W427E (W427E substitution; N-terminal deletion of residues 1-50)	6	GFDYDVVVVGGGFAGATAARECGLQGYRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFA CGGWN YDAFM DTETHYRIQGGTIGLINAMLTDSGA EVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSIT IARKETIDVND RDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAQAAYGVGQLSRLKDLQA AEGRILFAGAETSNGWHANIDGAVESGLRAGREV KQLLS

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
NicA2Δ50 W427S (W427S substitution; N-terminal deletion of residues 1-50)	7	GFDYDVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLV LKFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLKDLQ AAGRILFAGAETSNGWANIDGAVESGLRAGREV KQLLS
NicA2Δ50 W427M (W427M substitution; N-terminal deletion of residues 1-50)	8	GFDYDVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLV LKFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLKDLQ AAGRILFAGAETSNGWANIDGAVESGLRAGREV KQLLS
NicA2Δ50 R91A (R91A substitution; N-terminal deletion of residues 1-50)	9	GFDYDVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLV LKFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLKDLQ AAGRILFAGAETSNGWANIDGAVESGLRAGREV KQLLS
NicA2Δ50 R91Q (R91Q substitution; N-terminal deletion of residues 1-50)	10	GFDYDVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFA GQEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDG SVESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLD RIKTLGLSRLQQAQINSYMALYAGETTDKFGLPGLV LKFACGGWNYDAF MDTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTD DDEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV VKQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRD AVTREVQKMPFPGVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLKDL QAAEGRILFAGAETSNGWANIDGAVESGLRAGREV KQLLS
NicA2Δ50 R91F (R91F substitution; N-terminal deletion of residues 1-50)	11	GFDYDVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLV LKFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLKDLQ AAGRILFAGAETSNGWANIDGAVESGLRAGREV KQLLS
NicA2Δ50 R91G (R91G substitution; N-terminal deletion of residues 1-50)	12	GFDYDVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFA GQEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDG SVESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLD RIKTLGLSRLQQAQINSYMALYAGETTDKFGLPGLV LKFACGGWNYDAF MDTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTD DDEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV VKQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRD AVTREVQKMPFPGVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLKDL QAAEGRILFAGAETSNGWANIDGAVESGLRAGREV KQLLS
NicA2Δ50 R91T (R91T substitution; N-terminal deletion of residues 1-50)	13	GFDYDVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLV LKFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLKDLQ AAGRILFAGAETSNGWANIDGAVESGLRAGREV KQLLS

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
NicA2Δ50 R91L (R91L substitution; N-terminal deletion of residues 1-50)	14	GFDYDVVVGGGFAGATAARECGLQGYRTLLEARSRLGGLTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGKQRFI KEGQLS KGA KLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 R91S (R91S substitution; N-terminal deletion of residues 1-50)	15	GFDYDVVVGGGFAGATAARECGLQGYRTLLEARSRLGGSTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGKQRFI KEGQLS KGA KLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 R91N (R91N substitution; N-terminal deletion of residues 1-50)	16	GFDYDVVVGGGFAGATAARECGLQGYRTLLEARSRLGGNTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGKQRFI KEGQLS KGA KLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T250G (T250G substitution; N-terminal deletion of residues 1-50)	17	GFDYDVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGGLPGVLKLFACGGWNYDAFM DTEGHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGKQRFI KEGQLS KGA KLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T250L (T250L substitution; N-terminal deletion of residues 1-50)	18	GFDYDVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGGLPGVLKLFACGGWNYDAFM DTELHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGKQRFI KEGQLS KGA KLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T250R (T250R substitution; N-terminal deletion of residues 1-50)	19	GFDYDVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGGLPGVLKLFACGGWNYDAFM DTERHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGKQRFI KEGQLS KGA KLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T250V (T250V substitution; N-terminal deletion of residues 1-50)	20	GFDYDVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGGLPGVLKLFACGGWNYDAFM DTEVHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGKQRFI KEGQLS KGA KLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
NicA2Δ50 K340P (K340P substitution; N-terminal deletion of residues 1-50)	21	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAPLYVHV KQNLGRVFAFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 K340I (K340I substitution; N-terminal deletion of residues 1-50)	22	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAILYVHV KQNLGRVFAFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 K340V (K340V substitution; N-terminal deletion of residues 1-50)	23	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAVLYVHV KQNLGRVFAFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 K340D (K340D substitution; N-terminal deletion of residues 1-50)	24	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGADLYVHV KQNLGRVFAFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 K340E (K340E substitution; N-terminal deletion of residues 1-50)	25	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAELYVHV KQNLGRVFAFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 Q366K (Q366K substitution; N-terminal deletion of residues 1-50)	26	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 Q366E (Q366E substitution; N-terminal deletion of residues 1-50)	27	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 Q366V (Q366V	28	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
substitution; N-terminal deletion of residues 1-50)		KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWVTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 Q366L (Q366L substitution; N-terminal deletion of residues 1-50)	29	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWVTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 Q366I (Q366I substitution; N-terminal deletion of residues 1-50)	30	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWVTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 Q366Y (Q366Y substitution; N-terminal deletion of residues 1-50)	31	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWVTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T381P (T381P substitution; N-terminal deletion of residues 1-50)	32	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWVTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T381I (T381I substitution; N-terminal deletion of residues 1-50)	33	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWVTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T381V (T381V substitution; N-terminal deletion of residues 1-50)	34	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWVTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 T381Q (T381Q substitution;	35	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLKLFACGGWNYDAFM

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
N-terminal deletion of residues 1-50)		DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSIQIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRIILFAGAETSNGWANIDGAVESGLRAGREVKKQLLS
NicA2Δ50 T381N (T381N substitution; N-terminal deletion of residues 1-50)	36	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHVLQPHVWAEQMRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSIQIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRIILFAGAETSNGWANIDGAVESGLRAGREVKKQLLS
NicA2Δ50 T381L (T381L substitution; N-terminal deletion of residues 1-50)	37	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHVLQPHVWAEQMRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSIQIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRIILFAGAETSNGWANIDGAVESGLRAGREVKKQLLS
NicA2Δ50 T381M (T381M substitution; N-terminal deletion of residues 1-50)	38	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHVLQPHVWAEQMRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSIQIARKETIDVNRD AVTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDL QAAEGRIILFAGAETSNGWANIDGAVESGLRAGREVKKQLLS
NicA2Δ50 N462L (N462L substitution; N-terminal deletion of residues 1-50)	39	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHVLQPHVWAEQMRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSIQIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRIILFAGAETSNGWALIDGAVESGLRAGREVKKQLLS
NicA2Δ50 N462Y (N462Y substitution; N-terminal deletion of residues 1-50)	40	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHVLQPHVWAEQMRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSIQIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRIILFAGAETSNGWAYIDGAVESGLRAGREVKKQLLS
NicA2Δ50 N462S (N462S substitution; N-terminal deletion of residues 1-50)	41	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHVLQPHVWAEQMRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSIQIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRIILFAGAETSNGWASIDGAVESGLRAGREVKKQLLS
NicA2Δ50 N462F (N462F substitution; N-terminal deletion of residues 1-50)	42	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHVLQPHVWAEQMRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
deletion of residues 1-50) NicA2Δ50		DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHAFIDGAVESGLRAGREVKQLLS
N462G (N462G substitution; N-terminal deletion of residues 1-50)	43	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG L P G V L K L F A C G G W N Y D A F M DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHAGIDGAVESGLRAGREVKQLLS
NicA2Δ50 N462E (N462E substitution; N-terminal deletion of residues 1-50)	44	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG L P G V L K L F A C G G W N Y D A F M DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHAGIDGAVESGLRAGREVKQLLS
NicA2Δ50 N462A (N462A substitution; N-terminal deletion of residues 1-50)	45	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG L P G V L K L F A C G G W N Y D A F M DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHAGIDGAVESGLRAGREVKQLLS
NicA2Δ50 I463F (I463F substitution; N-terminal deletion of residues 1-50)	46	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG L P G V L K L F A C G G W N Y D A F M DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANFDGAVESGLRAGREVKQLLS
NicA2Δ50 I463Y (I463Y substitution; N-terminal deletion of residues 1-50)	47	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG L P G V L K L F A C G G W N Y D A F M DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANYDGAVESGLRAGREVKQLLS
NicA2Δ50 I463A (I463A substitution; N-terminal deletion of residues 1-50)	48	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG L P G V L K L F A C G G W N Y D A F M DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANADGAVESGLRAGREVKQLLS
NicA2Δ50 I463V (I463V substitution; N-terminal	49	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG L P G V L K L F A C G G W N Y D A F M DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
deletion of residues 1-50)		DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANVDGAVESGLRAGREVKQLLS
NicA2Δ50 I463L (I463L substitution; N-terminal deletion of residues 1-50)	50	GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANLDGAVESGLRAGREVKQLLS
NicA2Δ50 L217Q (L217Q substitution; N-terminal deletion of residues 1-50)	51	GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMAQYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 L217G (L217G substitution; N-terminal deletion of residues 1-50)	52	GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMAQYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 L217E (L217E substitution; N-terminal deletion of residues 1-50)	53	GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMAEYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 L217I (L217I substitution; N-terminal deletion of residues 1-50)	54	GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMAIYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 L217C (L217C substitution; N-terminal deletion of residues 1-50)	55	GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMACYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2Δ50 L217S (L217S substitution; N-terminal deletion of residues 1-50)	56	GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMASYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
		VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWANIDGAVESGLRAGREVKQLLS
Wild-Type NOX	57	MDEKRNNGLSRRSFIGGAAVVTAGAAGLGLIGSANATENGTSKRATGFD YDVIVVGGGFAGATAARECGHOGYKTLLEARSRLGGRTFTSHFAGQEI EFGGAWVHWLQPHVWSEMQR YGLGVVEDPLTNLDKTLVMYNDGSVED LPPEVFGTNIQVAFKMKCHDAWEAFPRPHEPMPF TERARKLDKMSVLDRI NQLELTRAQRAELNSYMALYGGETTDKYGLPGVLKLFACGGWYNNAF MDTETHYRIEGGTIGLINAMLADSGAEVRLNMPVISVEQLNGGVR VETD DGETITAGTIIIMTVPLNTYRHINFTPALSEGKQRFIQEGQLSKGAKLYVHV KENLGRVF AFADEQQPLNWVQTHDYGDELGTILSITIARAETIDVNRDA VTREIRKLFP GVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLTDLQQP EGRILFAGAETSNGWANIDGAVESGLRAGREAKEIL
NOXW423A (W423A Substitution)	58	MDEKRNNGLSRRSFIGGAAVVTAGAAGLGLIGSANATENGTSKRATGFD YDVIVVGGGFAGATAARECGHOGYKTLLEARSRLGGRTFTSHFAGQEI EFGGAWVHWLQPHVWSEMQR YGLGVVEDPLTNLDKTLVMYNDGSVED LPPEVFGTNIQVAFKMKCHDAWEAFPRPHEPMPF TERARKLDKMSVLDRI NQLELTRAQRAELNSYMALYGGETTDKYGLPGVLKLFACGGWYNNAF MDTETHYRIEGGTIGLINAMLADSGAEVRLNMPVISVEQLNGGVR VETD DGETITAGTIIIMTVPLNTYRHINFTPALSEGKQRFIQEGQLSKGAKLYVHV KENLGRVF AFADEQQPLNWVQTHDYGDELGTILSITIARAETIDVNRDA VTREIRKLFP GVEVLGTAAYDWTADPFSLGAAAYGVGQLSRLTDLQQP EGRILFAGAETSNGWANIDGAVESGLRAGREAKEIL
NOXW423S (W423S Substitution)	59	MDEKRNNGLSRRSFIGGAAVVTAGAAGLGLIGSANATENGTSKRATGFD YDVIVVGGGFAGATAARECGHOGYKTLLEARSRLGGRTFTSHFAGQEI EFGGAWVHWLQPHVWSEMQR YGLGVVEDPLTNLDKTLVMYNDGSVED LPPEVFGTNIQVAFKMKCHDAWEAFPRPHEPMPF TERARKLDKMSVLDRI NQLELTRAQRAELNSYMALYGGETTDKYGLPGVLKLFACGGWYNNAF MDTETHYRIEGGTIGLINAMLADSGAEVRLNMPVISVEQLNGGVR VETD DGETITAGTIIIMTVPLNTYRHINFTPALSEGKQRFIQEGQLSKGAKLYVHV KENLGRVF AFADEQQPLNWVQTHDYGDELGTILSITIARAETIDVNRDA VTREIRKLFP GVEVLGTAAYDWTADPFSLGASAAAYGVGQLSRLTDLQQPE GRILFAGAETSNGWANIDGAVESGLRAGREAKEIL
NOXW423E (W423E Substitution)	60	MDEKRNNGLSRRSFIGGAAVVTAGAAGLGLIGSANATENGTSKRATGFD YDVIVVGGGFAGATAARECGHOGYKTLLEARSRLGGRTFTSHFAGQEI EFGGAWVHWLQPHVWSEMQR YGLGVVEDPLTNLDKTLVMYNDGSVED LPPEVFGTNIQVAFKMKCHDAWEAFPRPHEPMPF TERARKLDKMSVLDRI NQLELTRAQRAELNSYMALYGGETTDKYGLPGVLKLFACGGWYNNAF MDTETHYRIEGGTIGLINAMLADSGAEVRLNMPVISVEQLNGGVR VETD DGETITAGTIIIMTVPLNTYRHINFTPALSEGKQRFIQEGQLSKGAKLYVHV KENLGRVF AFADEQQPLNWVQTHDYGDELGTILSITIARAETIDVNRDA VTREIRKLFP GVEVLGTAAYDWTADPFSLGAEAAAYGVGQLSRLTDLQQPE GRILFAGAETSNGWANIDGAVESGLRAGREAKEIL
NOXW423H (W423H Substitution)	61	MDEKRNNGLSRRSFIGGAAVVTAGAAGLGLIGSANATENGTSKRATGFD YDVIVVGGGFAGATAARECGHOGYKTLLEARSRLGGRTFTSHFAGQEI EFGGAWVHWLQPHVWSEMQR YGLGVVEDPLTNLDKTLVMYNDGSVED LPPEVFGTNIQVAFKMKCHDAWEAFPRPHEPMPF TERARKLDKMSVLDRI NQLELTRAQRAELNSYMALYGGETTDKYGLPGVLKLFACGGWYNNAF MDTETHYRIEGGTIGLINAMLADSGAEVRLNMPVISVEQLNGGVR VETD DGETITAGTIIIMTVPLNTYRHINFTPALSEGKQRFIQEGQLSKGAKLYVHV KENLGRVF AFADEQQPLNWVQTHDYGDELGTILSITIARAETIDVNRDA VTREIRKLFP GVEVLGTAAYDWTADPFSLGAHAAYGVGQLSRLTDLQQP EGRILFAGAETSNGWANIDGAVESGLRAGREAKEIL
NicA2Δ50 W427Q; I262A (W427Q & I262A substitution; N-terminal deletion of residues 1-50)	62	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAE MQR YGLGVVEDPLTNLDKTLIMYNDGS VESISPDEF GKNIRIAFEKLC HDAWEVFP RPHEPMPF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVLFACGGWNYDAFM DTETHYRIQGGTIGLANAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSGKQRFIKEGQLSKGAKLYVHV KQNLGRVF AFADEQQPLNWVQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAQAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNGWANIDGAVESGLRAGREVKQLLS
NicA2Δ50 W427Q; I262T;N263R (W427Q, I262T &	63	GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAE MQR YGLGVVEDPLTNLDKTLIMYNDGS VESISPDEF GKNIRIAFEKLC HDAWEVFP RPHEPMPF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGLVLFACGGWNYDAFM DTETHYRIQGGTIGLTRAMLTD SGAEVRMSVPVTAVEQVNGGVKIKTDD

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
N263R substitution; N-terminal deletion of residues 1-50)		DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2 A107R (A107R substitution)	124	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRAS TVKG GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGRWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFKGNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2A107K (A107K substitution)	125	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRAS TVKG GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFKGNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2A107T (A107T substitution)	126	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRAS TVKG GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGTWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFKGNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2F355C (F355C substitution)	127	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRAS TVKG GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFKGNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFACADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2F355V (F355V substitution)	128	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRAS TVKG GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFKGNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLDRI KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAPADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2R91Q (R91Q substitution)	129	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRAS TVKG GFDYDVVVVGGGFAGATAARECGLQGVRTLLLEARSRLGGRTFTSRFA GQEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDG SVESISPDEFKGNIRIAFEKLCHDAWEVFP RPHEPMF TERARELDKSSVLD RIKTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAF MDTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTD DDEIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVH VKQNLGRVFAPADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRD AVTREVQKMPFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDL QAAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
NicA2Q366K (Q336K substitution)	130	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGAI DAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTL LLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG LPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2T381V (T381V substitution)	131	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGAI DAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTL LLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG LPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSIVIARKETIDVNRDRA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2N462L (N462L substitution)	132	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGAI DAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTL LLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG LPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWHALIDGAVESGLRAGREVKQLLS
NicA2N462Y (N462Y substitution)	133	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGAI DAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTL LLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG LPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWHAYIDGAVESGLRAGREVKQLLS
NicA2Δ50 A107R (A107R substitution; N-terminal deletion of residues 1-50)	134	GFDYDVVVVGGGFAGATAARECGLQGYRTL LLEARSRLGGRTFTSRFAG QEIEFGGRVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG LPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRILFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2W427R (W427R substitution)	135	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGAI DAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTL LLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG LPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV KQNLGRVFAFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDRA VTREVQKMPGVEVLGTAAYDWTADPFSLGARAAAYGVGQLSRLKDLQA AEGRILFAGAETSNGWHANIDGAVESGLRAGREVKQLLS
NicA2T250P (T250P substitution)	136	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGAI DAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTL LLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFG LPGVLKLFACGGWNYDAFM DTEPHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDD DEIITAGVVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHV

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
		KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2W427H; N462F (W427H & N462F substitution)	137	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHAFIDGAVESGLRAGREVKQLLS
NicA2W427L; N462M (W427L & N462M substitution)	138	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGALAAAYGVGQLSRLKDLQA AEGRI LFAGAETSNNGWHAMIDGAVESGLRAGREVKQLLS
NicA2F104L (F104L substitution)	140	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIELGGAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2G106S (G106S substitution)	141	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGSAWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGSV ESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I TLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFMD TETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDDD EIIITAGVVMTVPLNTYKHIGFTPALS KKGQRFIKEGQLSKGAKLYVHV QNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDAV TREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQA AEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2A107H (A107H substitution)	142	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGHWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGS VESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I KTLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFM DTETHYRIQGGTIGLINAMLTDSGAEVMSVPVTAVEQVNGGVKIKTDD DEIITAGVVMTVPLNTYKHIGFTPALS KKGQRFIKEGQLSKGAKLYVHV KQNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNRDA VTREVQKMPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQ AAEGRI LFAGAETSNNGWHANIDGAVESGLRAGREVKQLLS
NicA2A107P (A107P substitution)	143	SDKTKTNEGFSRRSFIGSAAVVTAGVAGLGIDAASATQKTNRASTVKG GFDYDVVVVGGGFAGATAARECGLQGYRTLLEARSRLGGRTFTSRFAG QEIEFGGFWVHWLQPHVWAEMQRYGLGVVEDPLTNLDKTLIMYNDGSV ESISPDEFGKNIRIAFEKLCHDAWEVFP RPHEPMFTERARELDKSSVLDR I TLGLSRLQQAQINSYMALYAGETTDKFGLPGVLKLFACGGWNYDAFMD

TABLE 1-continued

Amino Acid Sequences of NicA2 & NOX and Exemplary Variants		
Enzyme	SEQ ID NO.	Sequence
		TETHYRIQGGTIGLINAMLTDSGAEVRMSVPVTAVEQVNGGVKIKTDDD EIITAGVVMTVPLNTYKHIGFTPALSKGKQRFIKEGQLSKGAKLYVHVK QNLGRVFAPFADEQQPLNWWQTHDYSDELGTILSITIARKETIDVNDRAV TREVQKMFPGVEVLGTAAYDWTADPFSLGAWAAYGVGQLSRLKDLQA AEGRILFAGAETSNGWANIDGAVESGLRAGREVKQLLS

*The N-terminal methionine residue (M) of SEQ ID NO: 1 is cleaved off in the purified product; however all amino acid position designations disclosed herein take the methionine residue into account for the purpose of maintaining amino acid numbering conventions used in the art for the wild-type NicA2 sequence.

**Underlined sequences in wild-type NicA2 identify the six highest ranked immunogenic regions identified by the online MHC-II Binding Predictions tool on the Immune Epitope Database and Analysis Resource website (iedb.org) using the specific human MHC allele HLA DRB1*0401.

†Residues highlighted in grey were identified as MHCII epitopes as disclosed in Example 2.

[0057] As noted above the nicotine-degrading enzyme variants may exhibit increased nicotine-degrading activity and/or decreased immunogenicity relative to the wild-type NicA2 enzyme. The variants may comprise one or more mutations to the amino acid sequence of wild-type NicA2, including one or more deletions, additions, or substitutions. A substitution mutation may be “conservative” or “non-conservative.” “Conservative” refers to a substitution within the same family of amino acids, while “non-conservative” refers to substitutions across families of amino acids. Families of amino acids and “conservative” and “non-conservative” substitutions relative thereto are known in the art. For example, the naturally occurring amino acids may be divided into the following four families and conservative substitutions will take place within those families, while non-conservative substitutions will take place across different families.

- [0058] 1) Amino acids with basic side chains: lysine, arginine, histidine.
- [0059] 2) Amino acids with acidic side chains: aspartic acid, glutamic acid
- [0060] 3) Amino acids with uncharged polar side chains: asparagine, glutamine, serine, threonine, tyrosine.
- [0061] 4) Amino acids with nonpolar side chains: glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, cysteine.

[0062] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations in an active site of the wild-type NicA2 enzyme relevant to its nicotine-degrading activity, such as a mutation at one or more positions selected from any one of amino acid residues 90-93, 95, 102-109, 113, 116, 130, 132, 138, 155, 159, 210, 213-215, 217-220, 234, 245, 246, 248-251, 253, 254, 258, 334, 336, 339-342, 353, 355, 363-367, 378-382, 415-418, 423-429, 459-463, 465, or 466 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions in positions listed in Table 2 and shown on the structure in FIG. 3. The Shell One residues identified in Table 2 make up the cavity surface, while the Shell Two residues contacting Shell One (see FIG. 3). For instance, in some embodiments, the disclosed nicotine-degrading enzyme variants can comprise at least one substitution at amino acid position 91, 104, 106, 107, 217, 250,

340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1. In some embodiments, the variants may comprise one, two, or three or more substitutions.

TABLE 2

NicA2 Active Site Residues	
Shell One	Shell Two
ARG91	GLY90
PHE104	THR92
GLY105	PHE93
GLY106	SER95
ALA107	ILE102
TRP108	GLU103
TYR214	VAL109
TYR218	GLN113
GLU249	VAL116
THR250	ASP130
LYS340	LEU132
PHE355	THR138
TRP364	PHE155
GLN366	ILE159
THR381	GLN210
TRP417	SER213
ALA426	MET215
TRP427	LEU217
ALA461	ALA219
ASN462	GLY220
ILE463	LEU234
	PHE245
	MET246
	THR248
	HIS251
	ARG253
	ILE254
	THR258
	GLN334
	SER336
	ALA339
	LEU341
	TYR342
	PHE353
	ASN363
	VAL365
	THR367
	LEU378
	SER379
	ILE380
	ILE382
	TYR415
	ASP416
	THR418
	SER423
	LEU424

TABLE 2-continued

NicA2 Active Site Residues	
Shell One	Shell Two
	GLY425
	ALA428
	ALA429
	TRP459
	HIS460
	GLY465
	ALA466

Active site based on published crystal structure from Tararina et al., 2016.
Amino acid residue numbers correspond to SEQ ID NO: 1.

[0063] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations to the wild-type NOX enzyme relevant to its nicotine-degrading activity, such as a mutation at amino acid residue 423 of SEQ ID NO: 57, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. For instance, in some embodiments, the variant may comprise the substitution W423A, W423S, W423E, or W423H.

[0064] In some embodiments, at least one mutation that increases the nicotine-degrading activity or increases the catalytic activity of the enzyme is introduced into the variant, allowing the variant to more rapidly and/or more efficiently break-down nicotine. In some embodiments, such a mutation may improve various measures of enzymatic performance, including but not limited to, increasing k_{cat} , lowering K_M , increasing k_{cat}/K_M and/or increasing V_{max} . Thus, in some embodiments, a variant may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more mutations in an active site of the wild-type NicA2 or wild-type NOX enzymes and/or in the aromatic cage, and exhibit increased nicotine-degrading activity as measured by increased k_{cat} , lowered K_M , increased k_{cat}/K_M , and/or increased V_{max} relative to the wild-type NicA2 enzyme.

[0065] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations in the aromatic cage of the wild-type NicA2 enzyme formed by the tryptophan at position 427 and the asparagine at position 462 of SEQ ID NO:1, such as a mutation at one or more of these positions, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 427 or 462 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 427 or 462 of SEQ ID NO:1.

[0066] In some embodiments, the mutation at position 427 is the substitution W427Q, where the tryptophan (W) at position 427 of SEQ ID NO:1 (in the aromatic cage) is substituted with glutamine (Q). The variant having the amino acid sequence of SEQ ID NO:5 is an example of this type of variant. This is a non-conservative substitution in which a non-polar, aromatic amino acid is replaced with a polar, uncharged amino acid. Generally, a non-conservative substitution to an active site of an enzyme would be expected to render the enzyme dysfunctional or inoperative, yet, surprisingly, this variant exhibits significantly increased enzyme-degrading activity as compared to the wild-type NicA2 enzyme.

[0067] In some embodiments, the mutation at position 427 is the substitution W427E, where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with glutamic acid (E). The variant having the amino acid sequence of SEQ ID NO:6 is an example of this type of variant. In some embodiments, the mutation is the substitution W427S, where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with serine (S). The variant having the amino acid sequence of SEQ ID NO:7 is an example of this type of variant. In some embodiments, the mutation is the substitution W427M, where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with methionine (M). The variant having the amino acid sequence of SEQ ID NO:8 is an example of this type of variant. In some embodiments, the mutation is the substitution W427R, where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with arginine (R). The variant having the amino acid sequence of SEQ ID NO:135 is an example of this type of variant. In some embodiments, the mutation is the substitution W427H, where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with histidine (H). The variant having the amino acid sequence of SEQ ID NO:137 is an example of this type of variant. In some embodiments, the mutation is the substitution W427L, where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with leucine (L). The variant having the amino acid sequence of SEQ ID NO:138 is an example of this type of variant.

[0068] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the arginine (R) at position 91 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0069] In some embodiments, the mutation at position 91 is the substitution R91A, where the arginine (R) at position 91 of SEQ ID NO:1 is substituted with alanine (A). The variant having the amino acid sequence of SEQ ID NO:9 is an example of this type of variant. In some embodiments, the mutation at position 91 is the substitution R91Q, where the arginine (R) at position 91 of SEQ ID NO:1 is substituted with glutamine (Q). The variants having the amino acid sequences of SEQ ID NOs:10 and 129 are examples of this type of variant. In some embodiments, the mutation at position 91 is the substitution R91F, where the arginine (R) at position 91 of SEQ ID NO:1 is substituted with phenylalanine (F). The variant having the amino acid sequence of SEQ ID NO:11 is an example of this type of variant. In some embodiments, the mutation at position 91 is the substitution R91G, where the arginine (R) at position 91 of SEQ ID NO:1 is substituted with glycine (G). The variant having the amino acid sequence of SEQ ID NO:12 is an example of this type of variant. In some embodiments, the mutation at position 91 is the substitution R91T, where the arginine (R) at position 91 of SEQ ID NO:1 is substituted with threonine (T). The variant having the amino acid sequence of SEQ ID NO:13 is an example of this type of variant. In some embodiments, the mutation at position 91 is the substitution R91L, where the arginine (R) at position 91 of SEQ ID NO:1

is substituted with leucine (L). The variant having the amino acid sequence of SEQ ID NO:14 is an example of this type of variant. In some embodiments, the mutation at position 91 is the substitution R91S, where the arginine (R) at position 91 of SEQ ID NO:1 is substituted with serine (S). The variant having the amino acid sequence of SEQ ID NO:15 is an example of this type of variant. In some embodiments, the mutation at position 91 is the substitution R91N, where the arginine (R) at position 91 of SEQ ID NO:1 is substituted with asparagine (N). The variant having the amino acid sequence of SEQ ID NO:16 is an example of this type of variant.

[0070] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the threonine (T) at position 250 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0071] In some embodiments, the mutation at position 250 is the substitution T250G, where the threonine (T) at position 250 of SEQ ID NO:1 is substituted with glycine (G). The variant having the amino acid sequence of SEQ ID NO:17 is an example of this type of variant. In some embodiments, the mutation at position 250 is the substitution T250L, where the threonine (T) at position 250 of SEQ ID NO:1 is substituted with leucine (L). The variant having the amino acid sequence of SEQ ID NO:18 is an example of this type of variant. In some embodiments, the mutation at position 250 is the substitution T250R, where the threonine (T) at position 250 of SEQ ID NO:1 is substituted with arginine (R). The variant having the amino acid sequence of SEQ ID NO:19 is an example of this type of variant. In some embodiments, the mutation at position 250 is the substitution T250V, where the threonine (T) at position 250 of SEQ ID NO:1 is substituted with valine (V). The variant having the amino acid sequence of SEQ ID NO:20 is an example of this type of variant. In some embodiments, the mutation at position 250 is the substitution T250P, where the threonine (T) at position 250 of SEQ ID NO:1 is substituted with proline (P). The variant having the amino acid sequence of SEQ ID NO:136 is an example of this type of variant.

[0072] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the lysine (K) at position 340 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0073] In some embodiments, the mutation at position 340 is the substitution K340P, where the lysine (K) at position 340 of SEQ ID NO:1 is substituted with proline (P). The variant having the amino acid sequence of SEQ ID NO:21 is an example of this type of variant. In some embodiments,

the mutation at position 340 is the substitution K340I, where the lysine (K) at position 340 of SEQ ID NO:1 is substituted with isoleucine (I). The variant having the amino acid sequence of SEQ ID NO:22 is an example of this type of variant. In some embodiments, the mutation at position 340 is the substitution K340V, where the lysine (K) at position 340 of SEQ ID NO:1 is substituted with valine (V). The variant having the amino acid sequence of SEQ ID NO:23 is an example of this type of variant. In some embodiments, the mutation at position 340 is the substitution K340D, where the lysine (K) at position 340 of SEQ ID NO:1 is substituted with aspartic acid (D). The variant having the amino acid sequence of SEQ ID NO:24 is an example of this type of variant. In some embodiments, the mutation at position 340 is the substitution K340E, where the lysine (K) at position 340 of SEQ ID NO:1 is substituted with glutamic acid (E). The variant having the amino acid sequence of SEQ ID NO:25 is an example of this type of variant.

[0074] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the glutamine (Q) at position 366 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0075] In some embodiments, the mutation at position 366 is the substitution Q366K, where the glutamine (Q) at position 366 of SEQ ID NO:1 is substituted with lysine (K). The variants having the amino acid sequences of SEQ ID NOs:26 and 130 are examples of this type of variant. In some embodiments, the mutation at position 366 is the substitution Q366E, where the glutamine (Q) at position 366 of SEQ ID NO:1 is substituted with glutamic acid (E). The variant having the amino acid sequence of SEQ ID NO:27 is an example of this type of variant. In some embodiments, the mutation at position 366 is the substitution Q366V, where the glutamine (Q) at position 366 of SEQ ID NO:1 is substituted with valine (V). The variant having the amino acid sequence of SEQ ID NO:28 is an example of this type of variant. In some embodiments, the mutation at position 366 is the substitution Q366L, where the glutamine (Q) at position 366 of SEQ ID NO:1 is substituted with leucine (L). The variant having the amino acid sequence of SEQ ID NO:29 is an example of this type of variant. In some embodiments, the mutation at position 366 is the substitution Q366I, where the glutamine (Q) at position 366 of SEQ ID NO:1 is substituted with isoleucine (I). The variant having the amino acid sequence of SEQ ID NO:30 is an example of this type of variant. In some embodiments, the mutation at position 366 is the substitution Q366Y, where the glutamine (Q) at position 366 of SEQ ID NO:1 is substituted with tyrosine (Y). The variant having the amino acid sequence of SEQ ID NO:31 is an example of this type of variant.

[0076] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the threonine (T) at position 381 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions.

Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0077] In some embodiments, the mutation at position 381 is the substitution T381P, where the threonine (T) at position 381 of SEQ ID NO:1 is substituted with proline (P). The variant having the amino acid sequence of SEQ ID NO:32 is an example of this type of variant. In some embodiments, the mutation at position 381 is the substitution T381I, where the threonine (T) at position 381 of SEQ ID NO:1 is substituted with isoleucine (I). The variant having the amino acid sequence of SEQ ID NO:33 is an example of this type of variant. In some embodiments, the mutation at position 381 is the substitution T381V, where the threonine (T) at position 381 of SEQ ID NO:1 is substituted with valine (V). The variants having the amino acid sequences of SEQ ID NOs:34 and 131 are examples of this type of variant. In some embodiments, the mutation at position 381 is the substitution T381Q, where the threonine (T) at position 381 of SEQ ID NO:1 is substituted with glutamine (Q). The variant having the amino acid sequence of SEQ ID NO:35 is an example of this type of variant. In some embodiments, the mutation at position 381 is the substitution T381N, where the threonine (T) at position 381 of SEQ ID NO:1 is substituted with asparagine (N). The variant having the amino acid sequence of SEQ ID NO:36 is an example of this type of variant. In some embodiments, the mutation at position 381 is the substitution T381L, where the threonine (T) at position 381 of SEQ ID NO:1 is substituted with leucine (L). The variant having the amino acid sequence of SEQ ID NO:37 is an example of this type of variant. In some embodiments, the mutation at position 381 is the substitution T381M, where the threonine (T) at position 381 of SEQ ID NO:1 is substituted with methionine (M). The variant having the amino acid sequence of SEQ ID NO:38 is an example of this type of variant.

[0078] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the asparagine (N) at position 462 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0079] In some embodiments, the mutation at position 462 is the substitution N462L, where the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with leucine (L). The variants having the amino acid sequences of SEQ ID NOs: 39 and 132 are examples of this type of variant. In some embodiments, the mutation at position 462 is the substitution N462Y, where the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with tyrosine (Y). The variants having the amino acid sequences of SEQ ID NOs: 40 and 133 are examples of this type of variant. In some embodiments, the mutation at position 462 is the substitution N462S, where the asparagine (N) at position 462 of

SEQ ID NO:1 is substituted with serine (S). The variant having the amino acid sequence of SEQ ID NO:41 is an example of this type of variant. In some embodiments, the mutation at position 462 is the substitution N462F, where the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with phenylalanine (F). The variants having the amino acid sequences of SEQ ID NOs:42 and 137 are examples of this type of variant. In some embodiments, the mutation at position 462 is the substitution N462G, where the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with glycine (G). The variant having the amino acid sequence of SEQ ID NO:43 is an example of this type of variant. In some embodiments, the mutation at position 462 is the substitution N462E, where the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with glutamic acid (E). The variant having the amino acid sequence of SEQ ID NO:44 is an example of this type of variant. In some embodiments, the mutation at position 462 is the substitution N462A, where the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with alanine (A). The variant having the amino acid sequence of SEQ ID NO:45 is an example of this type of variant. In some embodiments, the mutation at position 462 is the substitution N462M, where the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with methionine (M). The variant having the amino acid sequence of SEQ ID NO:138 is an example of this type of variant.

[0080] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the isoleucine (I) at position 463 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0081] In some embodiments, the mutation at position 463 is the substitution I463F, where the isoleucine (I) at position 463 of SEQ ID NO:1 is substituted with phenylalanine (F). The variant having the amino acid sequence of SEQ ID NO:46 is an example of this type of variant. In some embodiments, the mutation at position 463 is the substitution I463Y, where the isoleucine (I) at position 463 of SEQ ID NO:1 is substituted with tyrosine (Y). The variant having the amino acid sequence of SEQ ID NO:47 is an example of this type of variant. In some embodiments, the mutation at position 463 is the substitution I463A, where the isoleucine (I) at position 463 of SEQ ID NO:1 is substituted with alanine (A). The variant having the amino acid sequence of SEQ ID NO:48 is an example of this type of variant. In some embodiments, the mutation at position 463 is the substitution I463V, where the isoleucine (I) at position 463 of SEQ ID NO:1 is substituted with valine (V). The variant having the amino acid sequence of SEQ ID NO:49 is an example of this type of variant. In some embodiments, the mutation at position 463 is the substitution I463L, where the isoleucine (I) at position 463 of SEQ ID NO:1 is substituted with leucine (L). The variant having the amino acid sequence of SEQ ID NO:50 is an example of this type of variant.

[0082] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the leucine (L) at position 217 of SEQ ID

NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 217, 250, 340, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0083] In some embodiments, the mutation at position 217 is the substitution L217Q, where the leucine (L) at position 217 of SEQ ID NO:1 is substituted with glutamine (Q). The variant having the amino acid sequence of SEQ ID NO:51 is an example of this type of variant. In some embodiments, the mutation at position 217 is the substitution L217G, where the leucine (L) at position 217 of SEQ ID NO:1 is substituted with glycine (G). The variant having the amino acid sequence of SEQ ID NO:52 is an example of this type of variant. In some embodiments, the mutation at position 217 is the substitution L217E, where the leucine (L) at position 217 of SEQ ID NO:1 is substituted with glutamic acid (E). The variant having the amino acid sequence of SEQ ID NO:53 is an example of this type of variant. In some embodiments, the mutation at position 217 is the substitution L217I, where the leucine (L) at position 217 of SEQ ID NO:1 is substituted with isoleucine (I). The variant having the amino acid sequence of SEQ ID NO:54 is an example of this type of variant. In some embodiments, the mutation at position 217 is the substitution L217C, where the leucine (L) at position 217 of SEQ ID NO:1 is substituted with cysteine (C). The variant having the amino acid sequence of SEQ ID NO:55 is an example of this type of variant. In some embodiments, the mutation at position 217 is the substitution L217S, where the leucine (L) at position 217 of SEQ ID NO:1 is substituted with serine (S). The variant having the amino acid sequence of SEQ ID NO:56 is an example of this type of variant.

[0084] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the alanine (A) at position 107 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0085] In some embodiments, the mutation at position 107 is the substitution A107R, where the alanine (A) at position 107 of SEQ ID NO:1 is substituted with arginine (R). The variants having the amino acid sequences of SEQ ID NOs: 124 or 134 are examples of this type of variant. In some embodiments, the mutation at position 107 is the substitution A107K, where the alanine (A) at position 107 of SEQ ID NO:1 is substituted with lysine (K). The variant having the amino acid sequence of SEQ ID NO:125 is an example of this type of variant. In some embodiments, the mutation at position 107 is the substitution A107T, where the alanine (A) at position 107 of SEQ ID NO:1 is substituted with tyrosine (T). The variant having the amino acid sequence of SEQ ID NO:126 is an example of this type of variant. In some embodiments, the mutation at position 107 is the

substitution A107H, where the alanine (A) at position 107 of SEQ ID NO:1 is substituted with histidine (H). The variant having the amino acid sequence of SEQ ID NO:142 is an example of this type of variant. In some embodiments, the mutation at position 107 is the substitution A107P, where the alanine (A) at position 107 of SEQ ID NO:1 is substituted with proline (P). The variant having the amino acid sequence of SEQ ID NO:143 is an example of this type of variant.

[0086] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the phenylalanine (F) at position 355 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0087] In some embodiments, the mutation at position 355 is the substitution F355C, where the phenylalanine (F) at position 355 of SEQ ID NO:1 is substituted with cysteine (C). The variant having the amino acid sequence of SEQ ID NO:127 is an example of this type of variant. In some embodiments, the mutation at position 355 is the substitution F355V, where the phenylalanine (F) at position 355 of SEQ ID NO:1 is substituted with valine (V). The variant having the amino acid sequence of SEQ ID NO:128 is an example of this type of variant.

[0088] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the phenylalanine (F) at position 104 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0089] In some embodiments, the mutation at position 104 is the substitution F104L, where the phenylalanine (F) at position 104 of SEQ ID NO:1 is substituted with leucine (L). The variant having the amino acid sequence of SEQ ID NO:140 is an example of this type of variant.

[0090] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the glycine (G) at position 106 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, or 463 of SEQ ID NO:1.

[0091] In some embodiments, the mutation at position 106 is the substitution G106S where the glycine (G) at position 106 of SEQ ID NO:1 is substituted with serine (S). The variant having the amino acid sequence of SEQ ID NO:141 is an example of this type of variant.

[0092] In some embodiments, the nicotine-degrading enzyme variants comprise one, two, or three or more mutations to SEQ ID NO:1. For instance, in some embodiment, the nicotine-degrading enzyme may comprise a mutation in the tryptophan (W) at position 427 of SEQ ID NO:1, a mutation in the isoleucine (I) at position 262, and a mutation in the asparagine (N) at position 263, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, at least one mutation that increases the nicotine-degrading activity is at one, two, or three or more of positions 107, 355, 262, 263, 217, 91, 463, 381, 366, 340, 250, 427, or 462 of SEQ ID NO:1, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 107, 355, 262, 263, 217, 91, 463, 381, 366, 340, 250, 427, or 462 of SEQ ID NO:1.

[0093] In some embodiments, the mutation is a bi-substitution of W427Q and I262A, such as where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with glutamine (Q) and the isoleucine (I) at position 262 of SEQ ID NO:1 is substituted with alanine (A). The variant having the amino acid sequence of SEQ ID NO:62 is an example of this type of variant. In some embodiments, the mutation is a bi-substitution of W427H and N462F, such as where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with histidine (H) and the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with phenylalanine (F). The variant having the amino acid sequence of SEQ ID NO:137 is an example of this type of variant. In some embodiments, the mutation is a bi-substitution of W427L and N462M, such as where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with leucine (L) and the asparagine (N) at position 462 of SEQ ID NO:1 is substituted with methionine (M). The variant having the amino acid sequence of SEQ ID NO:138 is an example of this type of variant. In some embodiments, the mutation is a tri-substitution of W427Q, I262T, and N263R, such as where the tryptophan (W) at position 427 of SEQ ID NO:1 is substituted with glutamine (Q), the isoleucine (I) at position 262 of SEQ ID NO:1 is substituted with threonine (T), and the asparagine (N) at position 263 of SEQ ID NO:1 is substituted with arginine (R). The variant having the amino acid sequence of SEQ ID NO:63 is an example of this type of variant.

[0094] In some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations including a mutation in the tryptophan (W) at position 423 of SEQ ID NO:57, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions. Thus, in some embodiments, a mutation that increases the nicotine-degrading activity is at one or more of positions including 423 of SEQ ID NO:57, such as a conservative substitution, non-conservative substitution, addition, or deletion at one or more of positions 423 of SEQ ID NO:57.

[0095] In some embodiments, the mutation at position 423 is the substitution W423A, where the tryptophan (W) at position 423 of SEQ ID NO:57 is substituted with alanine (A). The variant having the amino acid sequence of SEQ ID NO:58 is an example of this type of variant. In some embodiments, the mutation at position 423 is the substitution W423S, where the tryptophan (W) at position 423 of SEQ ID NO:57 is substituted with serine (S). The variant having the amino acid sequence of SEQ ID NO:59 is an example of this type of variant. In some embodiments, the

mutation at position 423 is the substitution W423E, where the tryptophan (W) at position 423 of SEQ ID NO:57 is substituted with glutamic acid (E). The variant having the amino acid sequence of SEQ ID NO:60 is an example of this type of variant. In some embodiments, the mutation at position 423 is the substitution W423H, where the tryptophan (W) at position 423 of SEQ ID NO:57 is substituted with histidine (H). The variant having the amino acid sequence of SEQ ID NO:61 is an example of this type of variant.

[0096] Additionally or alternatively, in some embodiments, the nicotine-degrading enzyme variants comprise one or more mutations within an immunogenic T-cell epitope, such as one or more mutations within an immunogenic T-cell epitope within a region selected from positions 10-32, 68-94, 189-225, 248-285, 296-327, 336-391, or 435-459 of SEQ ID NO:1, such as one or more mutations within an immunogenic T-cell epitope selected from positions 16-24, 73-81, 258-266, 302-310, 373-381, or 447-455 of SEQ ID NO:1, such as one or more conservative substitutions, non-conservative substitutions, additions, or deletions in one or more of these regions. Thus, in some embodiments, a variant may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more mutations in 1, 2, 3, 4, 5, 6, or 7, immunogenic T-cells epitopes. In some embodiments, such variants exhibit reduced immunogenicity when administered to a mammalian subject.

[0097] In some embodiments, the nicotine-degrading enzymes variants comprise a mutation in an immunogenic T-cell epitope at one or more positions selected from 74, 77, 78, 80, 262-266, 303, 304, 306, 310, 374, 377, 378, 382, 383, 450-452, or 457 of SEQ ID NO:1, including all permutations and combinations thereof. For example, a variant may include any one or more of the mutations set forth below, including one or more of the exemplary mutations in Epitope B, one or more of the exemplary mutations in Epitope 1, one or more of the exemplary mutations in Epitope 2, one or more of the exemplary mutations in Epitope 3, and/or one or more of the exemplary mutations in Epitope 4. For instance, in some embodiments, the nicotine-degrading enzyme may have an amino acid substitution at position 262 and/or 263 of SEQ ID NO: 1, such as an I262A substitution or I262T/N263R substitutions.

TABLE 3

Exemplary Mutations in the NicA2 Epitopes (numbering based on SEQ ID NO: 1)				
Epitope B	Epitope 1	Epitope 2	Epitope 3	Epitope 4
L74N Y77R	I262A, A264Q	V303T, V304N, M306I	L374Q, I377S	I448Q, F450S
L74N, Y77K L74Q, Y77R L74Q, Y77N	I262K, L266D I262T	V304A, M306Q V304A, M306N V304A	L374A, I377A L374Q, I377A L374N, I377A	I448E, F450N I448A, F450N I448Q, F450Q
L74N, Y77Q L74N, Y77H L74N, L80H L80F	I262D, L266K I262A	V304A, M306H V304N, M306H V304Q, M306H I262T, N263R	I374N, I382Q I377A, I382T I377A, L378N V304N, I377T, I382T	I448T, F450Q I448E, F450L T455K L449H, F450A

TABLE 3-continued

Exemplary Mutations in the NicA2 Epitopes (numbering based on SEQ ID NO: 1)				
Epitope B	Epitope 1	Epitope 2	Epitope 3	Epitope 4
Y77R	M265H	V304T, M306I	I377T	F450A
R78Q	I262A, A264N	M306I, L310R	L374N, A383Q	I448A, F450Y

[0098] Additionally or alternatively, in some embodiments, the nicotine-degrading enzyme variants comprise an N-terminal deletion of from 1 to 52 amino acid residues of SEQ ID NO:1 or SEQ ID NO:57. For example, in some embodiments a variant comprises an N-terminal deletion of amino acid residues 1-16, 1-25, 1-38, 1-50, 1-51, or 1-52 of SEQ ID NO:1 or SEQ ID NO:57. Thus, the disclosed variants may comprise an N-terminal deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52 consecutive amino acids.

[0099] In some embodiments, the disclosed variants may additionally or alternatively comprise a deletion at the C-terminus of the peptide. For example, the disclosed variants may comprise a deletion of one or more amino acids at the C-terminus of the peptide. For example, in a NicA2 variant, the amino acid corresponding to S482 of the wild-type sequence may be deleted.

[0100] In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the wild-type NicA2 enzyme (SEQ ID NO:1), or to an N-terminal deletion variant thereof having a deletion of up to 52 N-terminal amino acid residues of SEQ ID NO:1.

[0101] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:5. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:5.

[0102] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:6. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:6.

[0103] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:7. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about

96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:7.

[0104] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:8. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:8.

[0105] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:9. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:9.

[0106] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:10. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:10.

[0107] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:11. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:11.

[0108] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:12. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:12.

[0109] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:13. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% sequence identity with the variant sequence of SEQ ID NO:13.

[0110] In some embodiments, a nicotine-degrading enzyme variant as described herein is or comprises SEQ ID NO:14. In some embodiments, a nicotine-degrading enzyme variant as described herein has at least about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about

about 2250%, about 2500%, about 2750%, about 3000%, about 3250%, about 3500%, about 3750%, about 4000%, about 4250%, about 4500%, about 4750%, or about 5000% or more than that of the wild-type NicA2 or NOX enzymes, as determined by an assay such as an AMPLEX® Red assay (Thermo Fisher Scientific).

[0180] In some embodiments, a variant as described herein exhibits increased nicotine-degrading activity relative to the wild-type NicA2 or NOX enzymes, such that its activity is at least about 110%, about 120%, about 130%, about 140%, about 150%, about 160%, about 170%, about 180%, about 190%, about 200%, about 210%, about 220%, about 230%, about 240%, about 250%, about 260%, about 270%, about 280%, about 290%, about 300%, about 310%, about 320%, about 330%, about 340%, about 350%, about 360%, about 370%, about 380%, about 390%, about 400%, about 410%, about 420%, about 430%, about 440%, about 450%, about 460%, about 470%, about 480%, about 490%, about 500%, about 550%, about 600%, about 650%, about 700%, about 750%, about 800%, about 850%, about 900%, about 950%, about 1000%, about 1100%, about 1200%, about 1300%, about 1400%, about 1500%, about 1600%, about 1700%, about 1800%, about 1900%, about 2000%, about 2250%, about 2500%, about 2750%, about 3000%, about 3250%, about 3500%, about 3750%, about 4000%, about 4250%, about 4500%, about 4750%, or about 5000% or more than that of the wild-type NicA2 or NOX enzymes as determined by an assay where residual nicotine concentrations are measured using Gas Chromatography (GC; Hieda et al.: Immunization of rats reduces nicotine distribution to brain. *Psychopharmacology*, 143, 150-157, 1999) after incubation with a fixed concentration of enzyme in either buffer or rat serum at 37° C. and quenching activity at fixed time points by mixing with MeOH.

[0181] In some embodiments, a variant as described herein exhibits decreased immunogenicity in a mammalian subject relative to wild-type NicA2 or NOX, such that it is at least about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100% less immunogenic than the wild-type NicA2 or NOX enzymes. Unless otherwise specified, “decreased immunogenicity” as compared to the wild-type NicA2 or NOX enzymes as used herein refers decreased immunogenicity as shown by one or more of *in silico* approaches, *in vitro* assays, *in vivo* studies (e.g., using transgenic animals), *ex vivo* studies using human T-cells, or clinical studies with human subjects.

IV. Pharmaceutical Compositions

[0182] The nicotine-degrading enzyme variants disclosed herein can be formulated into pharmaceutical compositions suitable for administration to the target subject (i.e., a human or other mammal) via a predetermined route of administration, as discussed in more detail below.

[0183] Pharmaceutical compositions may include one or more variants as described herein and a pharmaceutically acceptable carrier or diluent.

[0184] The compositions may be formulated for intravenous, subcutaneous, intraperitoneal, intramuscular, oral, nasal, pulmonary, ocular, vaginal, or rectal administration. In some embodiments, the compositions are formulated for intravenous, subcutaneous, intraperitoneal, or intramuscular

administration, such as in a solution, suspension, emulsion, liposome formulation, etc. The pharmaceutical compositions can be formulated to be an immediate-release composition, sustained-release composition, delayed-release composition, etc., using techniques known in the art

[0185] Pharmaceutically acceptable carriers for various dosage forms are known in the art. For example, excipients, lubricants, binders, and disintegrants for solid preparations are known; solvents, solubilizing agents, suspending agents, isotonicity agents, buffers, and soothing agents for liquid preparations are known. In some embodiments, the pharmaceutical compositions include one or more additional components, such as one or more preservatives, antioxidants, colorants, sweetening/flavoring agents, adsorbing agents, wetting agents and the like.

[0186] In some embodiments, the composition is formulated for administration by injection or infusion. In some embodiments, the composition is formulated for oral administration.

[0187] In some embodiments, the nicotine-degrading enzyme variant is a long-acting variant that has been modified in order to extend its half-life *in vivo* (after administration). Various techniques are known in the art for extending the circulating half-life of peptides. For example, in some embodiments the variant is conjugated to polyethylene glycol (PEG) or a similar polymer that prolongs half-life. As discussed in more detail in Example 3 below, conjugating PEG to the disclosed nicotine-degrading enzyme variants can improve the pharmacokinetic properties of the variant. In some embodiments PEGylation has one or more effects selected from masking one or more immunogenic epitopes of the variant, decreasing variant-specific antibody titers, and attenuating T-cell proliferation and/or cytokine responses. Additionally or alternatively, in some embodiments, conjugating the variants to PEG does not decrease the enzymatic activity of the nicotine-degrading enzyme variants, or does not significantly decrease the enzymatic activity, or does not eliminate the enzymatic activity.

[0188] The PEG chain length and architecture (i.e. linear vs. branched) may be selected and varied to impact, impart, or promote different properties, as illustrated in the examples below. PEG can be conjugated to the variants by methods known for conjugating PEG to proteins, including those illustrated in the examples below. Any of the variants described herein can be PEGylated, including variants defined by or comprising any of SEQ ID NOs: 2-56, 58-63, or 124-134. For the purposes of conjugating PEG to the disclosed enzyme variants, the size or length of the PEG polymers can vary. For example, linear PEG conjugated to the disclosed enzyme variants may be in the range of 1-50 kDa, 5-40 kDa, or 10-20 kDa, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, or 50 kDa. Additionally, the PEG polymers may be branched, with size in the range of 20-80 kDa, such as 20, 40, 60 or 80 kDa.

[0189] In some embodiments, the variant is fused to an albumin-binding peptide, an albumin-binding protein domain, human serum albumin, or an inert polypeptide. Exemplary inert polypeptides that have been used to increase the circulating half-life of peptides include, but are not limited to, XTEN® (also known as recombinant PEG or “rPEG”), a homo-amino acid polymer (HAP; HAPylation), a proline-alanine serine polymer (PAS; PASylation), or an elastin-like peptide (ELP; ELPylation). As used herein,

“fused to” includes genetic fusion, directly or through a linker, resulting in a single polypeptide containing multiple domains, unless otherwise specified.

V. Methods of Treating Nicotine Addiction or Facilitating Smoking Cessation

[0190] As noted above, the variants described herein are useful in methods of treating nicotine addiction and/or facilitating smoking cessation (or the cessation of use of other tobacco products) or preventing relapse of smoking (or consumption of other tobacco products) in a mammalian subject in need thereof. (For convenience, in the discussion that follows, these methods are referred to collectively as treating nicotine addiction or facilitating smoking cessation.) In some embodiments, the subject is a human subject addicted to nicotine or desiring to quit smoking or maintain abstinence from smoking or consumption of other nicotine products, or prevent relapse of smoking or consumption of other nicotine products.

[0191] The methods generally involve administering a therapeutically effective amount of a nicotine-degrading enzyme variant as described herein (or a pharmaceutical composition comprising the same) to the subject. However, in some embodiments, the methods comprise administering a nucleic acid encoding the nicotine-degrading enzyme variant in a construct that expresses the variant in vivo. For example, in such embodiments, the nucleic acid can be provided in a suitable vector, such as an adeno-associated virus (AAV) gene transfer vector. Other exemplary vectors that are suitable for use in such methods are known in the art. See, e.g., Lukashev and Zamyatnin, *Biochem.*, 81(7): 700-8 (2016)). Exemplary vectors may include one or more enhancers (e.g., a cytomegalovirus (CMV) enhancer), promoters (e.g., chicken β -actin promoter), and/or other elements enhancing the properties of the expression cassette. Methods of making suitable vectors and general methods of using expression vectors in vivo are known in the art. See, e.g., (see Hicks et al., *Sci. Transl. Med.*, 4(140): 140ra87 (2012)).

[0192] In some embodiments, a subject in need of treatment for nicotine addiction or facilitation of smoking cessation is a human subject who consumes nicotine products, such as smoking tobacco, chewing tobacco, electronic cigarettes, and/or other nicotine delivery devices. Such a subject may or may not be physically addicted to nicotine and/or psychologically addicted to consuming nicotine products. Typical subjects in need of smoking cessation treatment smoke or use tobacco or other nicotine products daily, such as smoking at least 1 cigarette a day, or more, such as at least about 5, at least about 10, at least about 15, at least about 20, or more, cigarettes per day, including fewer than 10, 10-20, 20-30, 30-40, or 40 or more (or the equivalent use of other tobacco or nicotine products).

[0193] In some embodiments, a therapeutically effective amount of a nicotine-degrading enzyme variant is an amount effective to reduce plasma levels of nicotine, to reduce levels of nicotine localized in the brain, or both.

[0194] Nicotine exerts many of its significant effects after it crosses the blood brain barrier. In some embodiments, the methods and uses described herein reduce or prevent nicotine from crossing the blood-brain-barrier. Thus, in some embodiments, administration of a nicotine-degrading enzyme variant as described herein degrades nicotine circulating in the bloodstream of the subject, thereby reducing

or preventing the nicotine from crossing the blood-brain-barrier. Thus, in some embodiments, the methods described herein reduce or prevent the physiological and psychological effects of nicotine that originate in the brain. Because the subject will experience a lessening or cessation of these effects, he/she will lose the desire to consume nicotine products. Additionally or alternatively, the disclosed nicotine-degrading enzyme variants may exert an effect by affecting the ability of nicotine to stimulate the peripheral nervous system.

[0195] The specific amount of a nicotine-degrading enzyme that is administered may depend on one or more of the age and/or weight of the subject, the amount of nicotine routinely consumed (e.g., smoked, chewed, or inhaled), and/or the level of nicotine in the subject's brain or plasma at the time of treatment. In some embodiments, a variant is administered at a dose of from about 0.01 to about 20 mg/kg, about 0.1 mg/kg to about 18 mg/kg, about 1 mg/kg to about 16 mg/kg, about 2 mg/kg to about 14 mg/kg, or about 5 mg/kg to about 10 mg/kg. In some embodiments, a variant is administered at a dose of about 0.01 mg/kg, about 0.02 mg/kg, about 0.03 mg/kg, about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, about 10 mg/kg, about 10.5 mg/kg, about 12 mg/kg, about 12.5 mg/kg, about 13 mg/kg, about 13.5 mg/kg, about 14 mg/kg, about 14.5 mg/kg, about 15 mg/kg, about 15.5 mg/kg, about 16 mg/kg, about 16.5 mg/kg, about 17 mg/kg, about 17.5 mg/kg, about 18 mg/kg, about 18.5 mg/kg, about 19 mg/kg, about 19.5 mg/kg, or about 20 mg/kg. In some embodiments, a variant is administered at a dose of about 0.5 mg, about 1 mg, about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, about 1500 mg, about 1550 mg, about 1600 mg, about 1650 mg, about 1700 mg, about 1750 mg, about 1800 mg, about 1850 mg, about 1900 mg, about 1950 mg, about 2000 mg, about 2050 mg, about 2100, about 2150 mg, about 2200 mg, about 2250 mg, about 2300 mg, about 2350 mg, about 2400 mg, about 2450 mg, or about 2500 mg. When more than one variant is administered, the total amount of variants administered may be in accordance with the foregoing guidance.

[0196] In some embodiments, the methods comprise administering a single dose of a nicotine-degrading enzyme variant(s) (or composition comprising the same). In some embodiments, the method comprises administering repeated doses, such as for a predetermined period of time of until the symptoms or effects of nicotine addiction are reduced, ameliorated, or eliminated or until the subject has ceased smoking or otherwise consuming nicotine. In some embodi-

ments, treatment is repeated with additional doses of the variant(s) if signs/symptoms/effects persist or if the subject continues to have nicotine cravings or experiences them anew.

[0197] In some embodiments, the methods comprise administering a nicotine-degrading enzyme variant(s) (or composition comprising the same) three or more times a day, twice a day, or once a day. In some embodiments, the methods comprise administering a nicotine-degrading enzyme variant(s) (or composition comprising the same) once every other day, three times a week, twice a week, once a week, once every other week, once every three weeks, once a month, or less frequently. In such embodiments, the nicotine-degrading enzyme variant may be a long-acting nicotine-degrading enzyme variant as described above.

[0198] In some embodiments, treatment may continue for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 or more days; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 or weeks months; or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 or more months; or 1, 2, or 3 or more years or until the subject no longer experiences nicotine cravings or other nicotine withdrawal symptoms, or has ceased smoking or using other tobacco products.

VI. Methods of Treating Nicotine Poisoning

[0199] The disclosed nicotine-degrading enzyme variants may be used to treat nicotine poisoning, nicotine overdose, or nicotine toxicity. For convenience, these methods are referred to herein collectively as treating nicotine poisoning. In some aspects, the methods of treating nicotine poisoning described herein comprise administering to a mammalian subject in need thereof a nicotine-degrading enzyme variant as disclosed herein, or a pharmaceutical composition comprising the same. In some embodiments, the methods comprise administering a nicotine-degrading enzyme variant to a subject that has ingested or consumed a toxic amount of nicotine. In some embodiments, the methods may comprise administering both a nicotine-degrading enzyme variant and another compound that is useful for treating nicotine poisoning, such as activated charcoal or another agent. In such embodiments, the enzyme variant and the second compound (e.g., activated charcoal) can be administered sequentially or simultaneously, from the same or different compositions. Thus, the treatment may include administering activated charcoal and/or other supportive treatments to address the symptoms and/or effects of nicotine poisoning.

[0200] In some embodiments, the therapeutically effective amount of the nicotine-degrading enzyme variant is effective to reduce, ameliorate, or eliminate one or more symptoms or effects of nicotine poisoning or overdose. The specific amount administered may depend on one or more of the age and/or weight of the subject, the amount of nicotine believed to have been ingested, and/or the subject's plasma level of nicotine at the time of treatment, and/or the subject's brain level of nicotine at the time of treatment. In some embodiments, the subject that is being treated for nicotine poisoning is an adult, while in some embodiments, the subject is a child (i.e., less than 19 years of age). In some embodiments, a therapeutically effective amount of a nicotine-degrading enzyme variant is an amount effective to reduce plasma levels of nicotine, and/or to reduce the amount of nicotine localized in specific tissues of the subject (e.g., brain/central nervous system, heart and vasculature, etc.). In specific embodiments, a therapeutically effective amount of a nico-

tine-degrading enzyme variant is an amount effective to reduce plasma levels of nicotine, to reduce levels of nicotine localized in the brain, or both.

[0201] The specific amount of a nicotine-degrading enzyme that is administered may depend on one or more of the age and/or weight of the subject, the amount of nicotine that was acutely consumed, and/or the level of nicotine in the subject's brain or plasma at the time of treatment. In some embodiments, a variant is administered at a dose of from about 0.01 to about 20 mg/kg, about 0.1 mg/kg to about 18 mg/kg, about 1 mg/kg to about 16 mg/kg, about 2 mg/kg to about 14 mg/kg, or about 5 mg/kg to about 10 mg/kg. In some embodiments, a variant is administered at a dose of about 0.01 mg/kg, about 0.02 mg/kg, about 0.03 mg/kg, about 0.04 mg/kg, about 0.05 mg/kg, about 0.06 mg/kg, about 0.07 mg/kg, about 0.08 mg/kg, about 0.09 mg/kg, about 0.1 mg/kg, about 0.2 mg/kg, about 0.3, about 0.4 mg/kg, about 0.5 mg/kg, about 0.6 mg/kg, about 0.7 mg/kg, about 0.8 mg/kg, about 0.9 mg/kg, about 1 mg/kg, about 1.5 mg/kg, about 2 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 3.5 mg/kg, about 4 mg/kg, about 4.5 mg/kg, about 5 mg/kg, about 5.5 mg/kg, about 6 mg/kg, about 6.5 mg/kg, about 7 mg/kg, about 7.5 mg/kg, about 8 mg/kg, about 8.5 mg/kg, about 9 mg/kg, about 9.5 mg/kg, about 10 mg/kg, about 10.5 mg/kg, about 12 mg/kg, about 12.5 mg/kg, about 13 mg/kg, about 13.5 mg/kg, about 14 mg/kg, about 14.5 mg/kg, about 15 mg/kg, about 15.5 mg/kg, about 16 mg/kg, about 16.5 mg/kg, about 17 mg/kg, about 17.5 mg/kg, about 18 mg/kg, about 18.5 mg/kg, about 19 mg/kg, about 19.5 mg/kg, or about 20 mg/kg. In some embodiments, a variant is administered at a dose of about 0.5 mg, about 1 mg, about 2.5 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 50 mg, about 75 mg, about 100 mg, about 150 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, about 1500 mg, about 1550 mg, about 1600 mg, about 1650 mg, about 1700 mg, about 1750 mg, about 1800 mg, about 1850 mg, about 1900 mg, about 1950 mg, about 2000 mg, about 2050 mg, about 2100, about 2150 mg, about 2200 mg, about 2250 mg, about 2300 mg, about 2350 mg, about 2400 mg, about 2450 mg, or about 2500 mg. When more than one variant is administered, the total amount of variants administered may be in accordance with the foregoing guidance.

[0202] Because nicotine poisoning is associated with vomiting, a non-oral route of administration may be used. Moreover, intravenous administration may be more effective than intraperitoneal administration. Thus, in some embodiments of methods of treating nicotine poisoning, a nicotine-degrading enzyme variant(s) (or composition comprising the same) is administered intravenously.

[0203] In some embodiments, the methods comprise administering a single dose of a nicotine-degrading enzyme variant(s) (or composition comprising the same). In some embodiments, the method comprises administering repeated doses, such as for a predetermined period of time or until the symptoms or effects of nicotine poisoning or toxicity are reduced, ameliorated, or eliminated or until the subject has ceased smoking or otherwise consuming nicotine. In some

embodiments, treatment is repeated with additional doses of the variant(s) if signs/symptoms/effects persist.

[0204] In some embodiments, treatment may continue for one or more days following overdose, such as for 1-3 days, or 1-5 days, or for 1, 2, 3, 4, or 5 days following overdose. In some embodiments, treatment may continue until the subject no longer experiences any symptoms of nicotine poisoning or toxicity or until the levels of nicotine in the subject's plasma and/or brain have decreased to a sufficiently safe level. In some embodiments, the nicotine-degrading enzyme variant may be a long-acting nicotine-degrading enzyme variant as described above.

[0205] One skilled in the art will readily appreciate that the present disclosure is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. Modifications therein and other uses will occur to those skilled in the art. These modifications are encompassed within the spirit of the disclosure. The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not limited to the specific conditions or details of these examples.

EXAMPLES

Example 1—Development and Testing of Nicotine-Degrading Enzyme Variants

[0206] A synthetic gene (custom DNA synthesis by GeneArt/Invitrogen), codon optimized for *E. coli* expression of wild-type (wt) NicA2 amino acid sequence (GenBank accession number: AEJ14620.1) with a C-terminal His6-tag was cloned into the NdeI-XhoI sites of pET-22b(+) (Novagen), and the expression plasmid transformed into *E. coli* BL21(DE3). The predicted wild-type NicA2 amino acid sequence as expressed from this construct is shown in Table 1 (SEQ ID NO:1).

[0207] A heterogeneity in size was identified by SDS-PAGE of protein expressed from the construct encoding SEQ ID NO: 1 and purified by Immobilized Metal Affinity Chromatography (IMAC) using Cobalt TALON™ His-Tag Purification Resin (Clontech) according to the manufacturers protocol (FIG. 1; major and minor band around 49 kDa marker). As the protein was purified via the His-tag, the heterogeneity was inferred to be at the N-terminus. Utilizing the online search tool PRED-TAT (compgen.org/tools/PRED-TAT; Pantelis et al., Combined prediction of Tat and-Sec signalpeptides with Hidden Markov Models, 2010 BIO-INFORMATICS), a putative TAT-leader sequence with an associated cleavage site following the alanine (A) residue at position 37 of SEQ ID NO:1 was identified.

[0208] In an effort to eliminate any non-essential bacterial sequence (including a specific in silico predicted T-cell epitope sequence at positions 16-24 of SEQ ID NO:1), to reduce immunogenic risk, as well as to eliminate the putative N-terminal cleavage site and the associated product heterogeneity, a deletion construct was made removing the first 50 N-terminal residues (NicA2Δ50; SEQ ID NO:2).

[0209] It was believed that this region could potentially be deleted without compromising catalytically activity; consequently NicA2Δ50 (SEQ ID NO: 2) was expressed in *E. coli* and purified as described above. As seen in FIG. 1, the purified NicA2Δ50 (SEQ ID NO: 2) appears homogeneous by SDS-PAGE analysis.

[0210] Analysis of enzymatic activity on purified protein was conducted using an Amplex Red assay (Reszka, et al., *Effects of peroxidase substrates on the Amplex red/oxidase assay: Antioxidant properties of anthracyclines*, ANALYTICAL BIOCHEMISTRY 342: 327-337 (2005)). Briefly, the oxidation of nicotine by NicA2 results in generation of H₂O₂ which is coupled to the conversion of the colorless Amplex Red reagent into its red-fluorescent product, resorufin by HRP (horseradish peroxidase). The assay was performed essentially as recommended by the supplier of the assay kit (Thermo; Cat #A22188) with the exception of addition of S-Nicotine (Sigma) to a final assay concentration of 10 μM. Assays were run in a total volume of 100 μL/well in a black half-area flat bottom 96-well assay plate (Corning Cat #3993). Development of fluorescence was detected in a SpectraMax M2 multimode microplate plate reader (Molecular Devices) using the settings Ex at 555 nm; Em at 590 nm, employing a “Plate Blank” well to subtract values derived from a no enzyme control for each time point in the SoftMax Pro data evolution package (Molecular Devices). Activities were expressed as the relative slopes of increase in fluorescence plotted as a function of time compared to the wild-type NicA2 enzyme, which was run in parallel.

[0211] The Amplex Red assay revealed that the purified NicA2Δ50 protein showed a 230 reduction in activity relative to wild-type NicA2 (FIG. 2 and Table 4). Two shorter deletion constructs (NicA2Δd25 (SEQ ID NO:3) and NicA2Δ38 SEQ ID NOA) were generated and tested, but similarly showed decreases in activity compared to the wild-type enzyme. In summary, all three deletion mutants showed reduced activity relative to wild-type (Table 5).

TABLE 4

Relative Activities of NicA2 Variants (purified proteins)	
Variant	Activity in Amplex Red assay (rel. to wt NicA2); slopes of curves in FIG. 2
Wt NicA2 (SEQ ID NO: 1)	100%
NicA2Δ50 (SEQ ID NO: 2)	77%
NicA2Δ50W427Q (SEQ ID NO: 5)	276%
NicA2Δ50W427Q; I262A (SEQ ID NO: 62)	111%
NicA2Δ50W427Q; I262T; N263R (SEQ ID NO: 63)	248%

TABLE 5

Relative Activities of NicA2 and NOX Variants (Screening Assay Format)	
Variant	Activity in Amplex Red assay (rel. to wt NicA2)
Wt NicA2 (SEQ ID NO: 1)	100%
NicA2Δ25 (SEQ ID NO: 3)	74%
NicA2Δ38 (SEQ ID NO: 4)	71%
NicA2Δ50 (SEQ ID NO: 2)	68%
NicA2Δ50W427Q (SEQ ID NO: 5)	296%
NicA2Δ50W427E (SEQ ID NO: 6)	305%
NicA2Δ50W427S (SEQ ID NO: 7)	330%
NicA2Δ50W427M (SEQ ID NO: 8)	275%
NicA2Δ50R91A (SEQ ID NO: 9)	376%
NicA2Δ50R91Q (SEQ ID NO: 10)	306%
NicA2Δ50R91F (SEQ ID NO: 11)	270%
NicA2Δ50R91G (SEQ ID NO: 12)	248%
NicA2Δ50R91T (SEQ ID NO: 13)	237%
NicA2Δ50R91L (SEQ ID NO: 14)	236%
NicA2Δ50R91S (SEQ ID NO: 15)	205%

TABLE 5-continued

Relative Activities of NicA2 and NOX Variants (Screening Assay Format)

Variant	Activity in Amplex Red assay (rel. to wt NicA2)
NicA2A50R91N (SEQ ID NO: 16)	142%
NicA2A50T250G (SEQ ID NO: 17)	469%
NicA2A50T250L (SEQ ID NO: 18)	351%
NicA2A50T250R (SEQ ID NO: 19)	193%
NicA2A50T250V (SEQ ID NO: 20)	123%
NicA2A50K340P (SEQ ID NO: 21)	207%
NicA2A50K340I (SEQ ID NO: 22)	148%
NicA2A50K340V (SEQ ID NO: 23)	130%
NicA2A50K340D (SEQ ID NO: 24)	119%
NicA2A50K340E (SEQ ID NO: 25)	96%
NicA2A50Q366K (SEQ ID NO: 26)	493%
NicA2A50Q366E (SEQ ID NO: 27)	322%
NicA2A50Q366V (SEQ ID NO: 28)	236%
NicA2A50Q366L (SEQ ID NO: 29)	219%
NicA2A50Q366I (SEQ ID NO: 30)	191%
NicA2A50Q366Y (SEQ ID NO: 31)	140%
NicA2A50T381P (SEQ ID NO: 32)	548%
NicA2A50T381I (SEQ ID NO: 33)	537%
NicA2A50T381V (SEQ ID NO: 34)	488%
NicA2A50T381Q (SEQ ID NO: 35)	425%
NicA2A50T381N (SEQ ID NO: 36)	265%
NicA2A50T381L (SEQ ID NO: 37)	238%
NicA2A50T381M (SEQ ID NO: 38)	213%
NicA2A50N462L (SEQ ID NO: 39)	647%
NicA2A50N462Y (SEQ ID NO: 40)	566%
NicA2A50N462S (SEQ ID NO: 41)	421%
NicA2A50N462F (SEQ ID NO: 42)	312%
NicA2A50N462G (SEQ ID NO: 43)	302%
NicA2A50N462E (SEQ ID NO: 44)	264%
NicA2A50N462A (SEQ ID NO: 45)	221%
NicA2A50I463F (SEQ ID NO: 46)	366%
NicA2A50I463Y (SEQ ID NO: 47)	351%
NicA2A50I463A (SEQ ID NO: 48)	129%
NicA2A50I463V (SEQ ID NO: 49)	125%
NicA2A50I463L (SEQ ID NO: 50)	114%
NicA2A50L217Q (SEQ ID NO: 51)	278%
NicA2A50L217G (SEQ ID NO: 52)	233%
NicA2A50L217E (SEQ ID NO: 53)	203%
NicA2A50L217I (SEQ ID NO: 54)	178%
NicA2A50L217C (SEQ ID NO: 55)	179%
NicA2A50L217S (SEQ ID NO: 56)	168%
Wt NOX (SEQ ID NO: 57)	80%
NOXW423A (SEQ ID NO: 58)	456%
NOXW423S (SEQ ID NO: 59)	248%
NOXW423E (SEQ ID NO: 60)	229%
NOXW423H (SEQ ID NO: 61)	181%
NicA2A107R (SEQ ID NO: 124)	1900%
NicA2A107K (SEQ ID NO: 125)	670%
NicA2A107T (SEQ ID NO: 126)	590%
NicA2F355C (SEQ ID NO: 127)	260%
NicA2R91Q (SEQ ID NO: 129)	330%
NicA2Q366K (SEQ ID NO: 130)	310%
NicA2T381V (SEQ ID NO: 131)	190%
NicA2N462Y (SEQ ID NO: 133)	315%
NicA2A50A107R (SEQ ID NO: 134)	1730%
NicA2F104L	320%
NicA2G106S	385%
NicA2A107H	480%
NicA2A107P	350%

[0212] Thus, it was decided to design and identify NicA2A50 variants that would exhibit nicotine-degrading activity at least equivalent to the wild-type. NOX variants were identified for further testing as well.

[0213] The NicA2 protein was visualized using Discovery Studio 4.5 (Dassault Systemes, BIOVIA Corp., San Diego CA) to determine active site residues. Based on inspection of the structure, location of FAD and reporting of putative critical active site residues (Tararina et al, 2016), a presumed active site cavity was defined. All residues making up the

exposed surface of this cavity, including both side chains and backbone atoms, were classified as shell one residues (Table 2). Based on the defined shell one residues, residues similarly in direct contact with shell one were defined as shell two (Table 2). Residue locations were confirmed by manual inspection of the structure.

[0214] A synthetic double-stranded DNA fragment:

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                                (SEQ ID NO: 64)
AAAGCAATATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTTCAG

AAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGATTGGAC

CGCAGATCCGTTTAGCTTAGGTGCCNNKGCCTGATGGTGTGGTCAGC

TGTCACGCTCTGAAAGATCTGCAGGCAGCAGAAAGTCGTATTCTGTTTGGC

GGTGCAGAAACCAGCAATGGTTGGCATGCAATATTGATGGTGCAGTTGA

AAGCGGTCTGCGTGCAGGTCGTGAAGTTAAACAGCTGCTGAGCGGTGGTG

GTGGATCCATAGG

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was digested with restriction enzymes ClaI and BamHI and the fragment cloned into the corresponding unique sites in the expression plasmid used for expression of NicA2A50.

[0215] The resulting library had an NNK randomization of shell one codon W427 (Table 2) resulting in a 32-member library (at the DNA/codon level) encoding variants with all possible 20 amino acids in said position. The library was transformed into BL21 Gold(DE3), and single colonies were picked and grown overnight in a 96 well plate in LB media containing Carbenicillin (100 µg/ml). Overnight LB cultures were diluted into a new 96-deep well plate containing 475 µl of auto-inducing Magic Media (Invitrogen)+Carbenicillin (100 µg/ml), and grown for 18 h at room temperature with vigorous shaking. Bacteria were harvested in the plate by centrifugation at 4000 rpm for 15 minutes, and pellets frozen at -80° C. Pellets were lysed by dissolving in 100 µl of room temperature Bug Buster HT Reagent (Novagen) containing 1KU of r-Lysozyme (Novagen) per 1 ml, and incubating on a shaking platform for 20 min at room temperature. Cleared lysates were prepared by diluting 1:1 (vol.:vol.) with Bug Buster HT Reagent and removing insoluble cell debris by centrifugation at 4400 rpm for 20 min at 4° C. 25 µl of cleared lysates were transferred to a new 96 well plate and diluted 1:1 (vol.:vol.) in 2% milk in PBS. Diluted lysates were transferred to the assay plate (black 96-well half area high binding plate (Corning) coated o/n at 4° C. with 5 µg/ml of anti-His Tag antibody (R&D Systems) in PBS; 50 µl per well; then blocked with 4% milk (in PBS) for 2 h at RT) and incubated at room temperature gently shaking for 3 h to ensure saturation of the immobilized anti-His mAb with the molar excess of expressed His-tagged enzyme. This step essentially results in normalizing any differences in concentration afforded by differences in growth, induction conditions, intrinsic expression levels, etc., and ensures a consistent amount of enzyme is assayed for activity in each well in the subsequent steps. This also ablates the need for quantification of enzyme in individual wells to precisely measure and compare activity of variants. Plate was washed 6x with PBST and 1x with Amplex Red Reagent Buffer (Thermo) to remove unbound material. Enzyme assay was conducted by adding 50 µl of Amplex Red Solution including 10 µM S-Nicotine (as described above) to each well and monitoring development of fluorescence over time.

[0216] From one 96 well assay plate, the ten variants with the highest assay activities (all enhanced compared to values from included colonies expressing wild-type NicA2) were isolated from the master plate with overnight LB culture, and plasmid DNA was prepared and sequenced. Sequencing revealed sequence changes relative to NicA2Δ50 (SEQ ID NO:2) affording single mutations of W427 to Q, E, S, and M, resulting in SEQ ID NOs: 5, 6, 7, and 8, respectively. Additionally, further variants with mutations at positions 91, 217, 463, 381, 366, 340, 250, 427, or 462 of SEQ ID NO:1 were also designed and screened, as well as variants of NOX with mutations at position 423 of SEQ ID NO:57. A list of the activities of the identified variants in the Amplex Red screening assay (average for 8 individual colonies of each variant re-assayed as described above) is shown in Table 5.

[0217] The variant NicA2Δ50W427Q (SEQ ID NO:5) was expressed and purified as described above. An activity assay was conducted in parallel with purified wild-type NicA2 and NicA2Δ50 using the Amplex Red assay as described above. As shown in FIG. 2 and Table 4, the W427Q mutation surprisingly not only “restored” but actually significantly increased activity, with the NicA2Δ50W427Q variant exhibiting about 250% activity (a 2.5-fold increase) relative to the wild-type NicA2 enzyme. This data obtained using purified proteins is in agreement with the data generated in the screening assay format (Table 5).

[0218] A Protein BLAST homology search using NicA2 as query sequence yielded NOX, Nicotine amine oxidase from *Pseudomonas* sp. HZN6 (Qiu et al., Appl. Environ. Microbiol. 78, 2154-2160 (2012); Qiu et al., Appl. Environ. Microbiol. 79, 2164-2171 (2013)) as the closest relative, with an identity of 83%. A synthetic gene (custom synthesis by GeneArt/Invitrogen) codon optimized for *E. coli* expression of wild-type (wt) NOX amino acid sequence (GenBank accession number: AGH68979.1) with a C-terminal His6-tag was cloned into the NdeI-XhoI sites of pET-22b(+), and the expression plasmid transformed into *E. coli* BL21(DE3). The predicted wild-type NOX amino acid sequence as expressed from this construct is shown in Table 1 (SEQ ID NO:57). NOX was purified and assayed in the Amplex Red activity assay. The enzyme did indeed display activity consistent with nicotine degradation and H₂O₂ formation, albeit at a 20% decreased activity relative to wt NicA2 (Table 5). Identifying improved NicA2 variants carrying mutations in W427 prompted the generation and screening of an NNK randomization library of the homologous W423 position of NOX. The library was generated using the QuikChange Site-Directed Mutagenesis Kit (Agilent), the DNA template encoding NOX in the pET22 expression vector described above, and the NNK primer NOX-W423NNK (Table 6, SEQ ID NO:65) as per the kit instructions. After plating transformations and incubation overnight at 37° C., 8 random colonies were sequenced for library QC, and remainder colonies scraped off the agar plates and pooled for DNA miniprep. This DNA was then transformed into the BL21 (DE3) expression strain, and individual clones screened as described for the NicA2Δ50W427NNK library above. Screening this library identified 4 variants with between 2- and 4.5-fold improved activity over wt NicA2: NOXW423A, S, E, and H (Table 5). Interestingly, given all these variants have an increased activity compared to wt NicA2, these could potentially be equally good starting points for biotherapeutic development, and beneficial muta-

tions identified in NicA2 (e.g. all mutations disclosed in Table 5) may also be advantageous in the homologous positions in the NOX backbone.

[0219] Given the successful identification of improved variants in NicA2 position W427, similar “NNK libraries” in the NicA2Δ50 backbone were generated for other active site (shell one) positions: R91, T250, K340, Q366, T381, N462, I463F, as well as second shell residue L217. These later libraries were generated using the QUIKCHANGE™ Site-Directed Mutagenesis Kit (Agilent), a DNA template encoding NicA2Δ50 in the pET22 expression vector, and the NNK primers listed in Table 6, SEQ ID NO’s 66-73, respectively, as per the kit instructions. As indicated in Table 5, screening of these libraries as described above led to identification of variants that had a range of 1- to 6.5-fold improvement in activity over wild-type NicA2 (SEQ ID NO’s:5-56), in spite of carrying the Δ50 N-terminal deletion. Interestingly, even though L217 is a second shell residue (Table 3), 6 substitutions conferring 1.7- to 2.8-fold increased activity over wild-type were identified in this position (Table 5).

TABLE 6

Exemplary synthetic DNA oligonucleotides used for generation of NNK libraries			
Name:	SEQ ID NO:	Sequence	
NOX- W423NNK	65	TCC GTT TAG CCT GGG TGC ANN KGC AGC GTA TGG TGT TGG	
NicA2- R91NNK	66	GAA GCA CGT AGC CGT TTA GGT GGT NNK ACC TTT ACC AGC CGT TTT	
NicA2- T250NNK	67	ATG ATG CAT TCA TGG ATA CCG AAN NNK ATT ATC GTA TTC AGG GTG GCA C	
NicA2- K340NNK	68	GGT CAG CTG AGT AAA GGT GCC NNK CTG TAT GTT CAT GTG AAA CAG	
NicA2- Q366NNK	69	GAT GAA CAG CAG CCG CTG AAT TGG GTT NNK ACC CAT GAT TAT AGT GAT G	
NicA2- T381NNK	70	GAA CTG GGC ACC ATT CTG AGC ATT NNK ATT GCA CGT AAA GAA ACC ATT G	
NicA2- N462NNK	71	ACC AGC AAT GGT TGG CAT GCA NNK ATT GAT GGT GCA GTT GAA AGC	
NicA2- I463NNK	72	GAA ACC AGC AAT GGT TGG CAT GCA AAT NNK GAT GGT GCA GTT GAA AGC	
NicA2- L217NNK	73	GCA CAG ATT AAT AGC TAT ATG GCA NNK TAT GCC GGT GAA ACC ACC GAT AAA	

[0220] To cover additional active site positions listed in Table 2, a custom Comprehensive Saturation Mutagenesis (CSM) library was supplied by Revolve Biotechnologies, Inc. (Firnberg et al., *PLoS One*, 7:e52031 (2012)), probing all single amino acid (aa) substitutions (one mutation per variant) in the following positions of full-length NicA2: PHE104, GLY105, GLY106, ALA107, TRP108, TYR214, TYR218, GLU249, P1TE355, TRP364, TRP417, ALA426, and ALA461.

[0221] Screening of this library in the Amplex Red screening assay as described earlier lead to identification of improved variants NicA2A107R, NicA2A107K, NicA2A107T and NicA2F355C (Table 5; SEQ ID NO’s 124-127).

[0222] In order to more efficiently screen through larger library sizes, a Fluorescence-Activated Cell Sorting (FACS) sorting protocol was implemented. Libraries containing NicA2 variants were cloned into the vector pET22b and transformed into the *E. coli* BL21(DE3) strain. Cells were inoculated in LB+100 µg/mL Ampicillin and grown at 37° C. until the OD600 reached 0.6-0.8. Expression was induced with 1 mM IPTG and the cultures were moved to 18° C. and expressed overnight. The next day, cells were washed in 5×PBS+1 mM EDTA to permeabilize the cells to nicotine before resuspending in the same buffer containing 5 µM Dihydrorhodamine 123 (DHR123), a redox sensitive dye that becomes fluorescent upon reaction with the hydrogen peroxide released by NicA2, and 5 mM nicotine. The cell suspension was transferred to a clean, sterile flask and incubated at room temperature while shaking. After 2 hours, cells were washed with 5×PBS+1 mM EDTA and sorted by FACS using the FITC channel to quantify DHR123 fluorescence. Recovered cells were re-grown in LB+100 µg/mL Ampicillin, and the protocol was repeated as needed.

[0223] Sequencing of clones from outputs resulting from one round of sorting of the CSM library enriched for putative NicA2 activity yielded the variants NicA2W427R, NicA2T250P, NicA2W427H; N462F, and NicA2W427L; N462M (SEQ ID NOs: 135-138, respectively).

[0224] Based on the results of improved variants NicA2Δ50R91Q, NicA2Δ50Q366K, NicA2Δ50T381V and NicA2Δ50N462Y, the same mutations were introduced into full-length NicA2, (resulting in NicA2R91Q, NicA2Q366K, NicA2T381V and NicA2N462Y with SEQ ID NOs: 129, 130, 131, 133, respectively), and activity assessed in the Amplex Red assay as previously described. As shown in Table 5, all these exemplary mutations provided activity enhancement in the full-length enzyme. Correspondingly, the high activity of the NicA2A107R variant (SEQ ID NO: 124) was retained in the NicA2Δ50A107R variant (SEQ ID NO: 134). Consequently, it is expected that all mutations listed in Table 5 will improve activity in the context of both full-length NicA2 and a deletion variant such as NicA2Δ50, NicA2Δ25, NicA2Δ38, or any similar deletion up to and including at least the first 50 N-terminal residues of NicA2, or any N- or C-terminal deletion variant provided this has an enzymatic activity of at least 20% of full-length wt NicA2.

[0225] Specific variants carrying mutations in multiple residues chosen from Table 5 can be generated by site-specific mutagenesis, and libraries consisting of multiple mutations in multiple positions chosen from Table 5 can be generated and screened as described above. These efforts could allow for the identification of variants with mutations in several positions in the same molecule with enzymatic activities higher than any of the individual single mutations listed in Table 5.

[0226] Having shown with the variants having SEQ ID NOs 2-4 that it is possible to delete the first epitope with only moderate effect on activity, and that this decrease in activity could be mitigated or overcome by mutations listed in Table 5, multiple other epitopes were investigated further.

[0227] Structure coordinates for the NicA2 protein were examined on Discovery Studio 4.5 (Dassault Systemes, BIOVIA Corp., San Diego CA) to determine immunogenic epitopes. Regions of the NicA2 sequence flagged as potential epitopes via T cell epitope scanning against the MHC allele DRB1*0401 (Immune Epitope Database and Analysis Resource, IEDB, iedb.org) were evaluated using a predictive site saturation mutagenesis protocol implemented in Discovery Studio to evaluate mutational energies. Favorably-scoring mutations in each previously defined region were cross-referenced with new rounds of IEDB T cell epitope predictions against MHC allele DRB1*0401 to find alterations that reduced predicted immunogenicity. The final selection of possible mutations was confirmed by visualization and manual evaluation of the probable structural changes in Discovery Studio. Table 3 sets forth exemplary mutations in the immunogenic T-cell epitopes that were predicted to decrease immunogenicity (based on in silico scoring as described above) while substantially retaining nicotine-degrading activity and stability.

[0228] Ten variants based on the mutations for Epitope 1 were generated by cloning the synthetic double-stranded DNA fragments (SEQ ID NOs: 74-83, Table 7) digested with restriction enzymes EcoRI and SacII into the corresponding unique sites in the expression plasmid used for expression of wild-type NicA2 (SEQ ID NO:1). The nucleic acid sequences of other exemplary epitope variants are shown in SEQ ID NOs: 84-113, as discussed in further detail below.

TABLE 7

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
SEQ ID NO:	Sequence
SEQ ID NO: 74	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAAGTGGATAAATCAAGCGTTCTGGATC GTATTAACACTGGGTCTGAGCCGCTGACGACGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACACCGATAAATTG GTCTGCCTGGTGTCTGAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCCTTTATGGATACCGAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGGCGAATCAGATGCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCCGGTTACCGCGGATAGG
SEQ ID NO: 75	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAAGTGGATAAATCAAGCGTTCTGGATC GTATTAACACTGGGTCTGAGCCGCTGACGACGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACACCGATAAATTG GTCTGCCTGGTGTCTGAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCCTTTATGGATACCGAACGCACTATCGTATTCAAGGTG

TABLE 7-continued

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
SEQ ID NO:	Sequence
	GCACCATTTGGTCTGAAAAATGCAATGGATACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG
SEQ ID NO: 76	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTTCCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCACCGATAAATTG GTCTGCCTGGTGTCTGAAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCTTTATGGATACCGAAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGACCAATGCAATGCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG
SEQ ID NO: 77	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTTCCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCACCGATAAATTG GTCTGCCTGGTGTCTGAAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCTTTATGGATACCGAAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGAGCAATGCAATGCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG
SEQ ID NO: 78	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTTCCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCACCGATAAATTG GTCTGCCTGGTGTCTGAAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCTTTATGGATACCGAAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGGATAATGCAATGAAACCAGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG
SEQ ID NO: 79	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTTCCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCACCGATAAATTG GTCTGCCTGGTGTCTGAAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCTTTATGGATACCGAAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGGCGAATGCAATGCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG
SEQ ID NO: 80	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTTCCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCACCGATAAATTG GTCTGCCTGGTGTCTGAAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCTTTATGGATACCGAAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGACCAATCTGATGCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG
SEQ ID NO: 81	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTTCCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCACCGATAAATTG GTCTGCCTGGTGTCTGAAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCTTTATGGATACCGAAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGACCCGTGCAATGCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG
SEQ ID NO: 82	GCAATGAATTCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTCATGATGCATGGGAAGTTTTTCCGCGTCCGCATGAACCGATGT TTACCGAACGTGCCCCGTAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCACCGATAAATTG GTCTGCCTGGTGTCTGAAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCTTTATGGATACCGAAACGCACTATCGTATTCAAGGTG GCACCATTTGGTCTGATTAAATGCACATCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCGGTTACCGCGGATAGG

TABLE 7-continued

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
SEQ ID NO:	Sequence
SEQ ID NO: 83	GCAATGAATTCCGGTAAAAACATTGCGATTGCCTTTGAAAACTGT GTATGATGTCATGGGAAGTTTTCGCGCTCCGATGAACCGATGT TTACCGAACGTGCCCGTGAACCTGGATAAATCAAGCGTTCTGGATC GTATTAACCACTGGGTCTGAGCCGTCTGCAGCAGGCACAGATTA ATAGCTATATGGCACTGTATGCCGGTGAAACCAACCGATAAATTG GTCTGCCTGGTGTCTGAACTGTTTGCATGTGGTGGTTGGAATT ATGATGCCCTTATGGATACCGAAACGCACTATCGTATTCAGGTG GCACCATTTGGTCTGGCGAATAACATGCTGACCGATAGCGGTGCCG AAGTTCGTATGAGCGTTCCGGTTACCGCGATAGG
SEQ ID NO: 84	GCAATCATATGAGCGACAAACCAAAACCAATGAAGGTTTTAGCC GTGCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTTGCCGGTGCAACCGCAGCACGTGAATGTG GTAACCAAGGTCGTGCTACCTGCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCTGACCTTTACCGCCGTTTTCAGGTCAAGAAATTG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 85	GCAATCATATGAGCGACAAACCAAAACCAATGAAGGTTTTAGCC GTGCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTTGCCGGTGCAACCGCAGCACGTGAATGTG GTAACCAAGGTAACGTAACCTGCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCTGACCTTTACCGCCGTTTTCAGGTCAAGAAATTG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 86	GCAATCATATGAGCGACAAACCAAAACCAATGAAGGTTTTAGCC GTGCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTTGCCGGTGCAACCGCAGCACGTGAATGTG GTCAGCAGGTCGTGCTACCTGCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCTGACCTTTACCGCCGTTTTCAGGTCAAGAAATTG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 87	GCAATCATATGAGCGACAAACCAAAACCAATGAAGGTTTTAGCC GTGCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTTGCCGGTGCAACCGCAGCACGTGAATGTG GTCAGCAGGTAACCGTACCTGCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCTGACCTTTACCGCCGTTTTCAGGTCAAGAAATTG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 88	GCAATCATATGAGCGACAAACCAAAACCAATGAAGGTTTTAGCC GTGCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTTGCCGGTGCAACCGCAGCACGTGAATGTG GTAACCAAGGTCAGCGTACCTGCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCTGACCTTTACCGCCGTTTTCAGGTCAAGAAATTG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG

TABLE 7-continued

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
SEQ ID NO:	Sequence
SEQ ID NO: 89	GCAATCATATGAGCGACAAAACCAAAACCAATGAAGGTTTATAGCC GTCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTGCCCGGTGCAACCGCAGCACGTGAATGTG GTAACCAAGGGTCATCGTACCTGCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCGTACCTTTACCAGCCGTTTTCAGGTCAGAAATG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 90	GCAATCATATGAGCGACAAAACCAAAACCAATGAAGGTTTATAGCC GTCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTGCCCGGTGCAACCGCAGCACGTGAATGTG GTAACCAAGGGTTATCGTACCTCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCGTACCTTTACCAGCCGTTTTCAGGTCAGAAATG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 91	GCAATCATATGAGCGACAAAACCAAAACCAATGAAGGTTTATAGCC GTCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTGCCCGGTGCAACCGCAGCACGTGAATGTG GTCTGCAGGGTTATCGTACCTTCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCGTACCTTTACCAGCCGTTTTCAGGTCAGAAATG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 92	GCAATCATATGAGCGACAAAACCAAAACCAATGAAGGTTTATAGCC GTCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTGCCCGGTGCAACCGCAGCACGTGAATGTG GTCTGCAGGGTTATCGTACCTTCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCGTACCTTTACCAGCCGTTTTCAGGTCAGAAATG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 93	GCAATCATATGAGCGACAAAACCAAAACCAATGAAGGTTTATAGCC GTCGAGCTTTATTGGTAGCGCAGCAGTTGTTACCGCAGGCGTTG CAGGTCCTGGGTGCAATTGATGCAGCAAGCGCAACCCAGAAAACCA ATCGTGCAAGCACCGTTAAAGGTGGCTTCGATTATGATGTTGTTG TGGTTGGTGGTGGTTTGCCCGGTGCAACCGCAGCACGTGAATGTG GTCTGCAGGGTTATCAGACCTGCTGCTGGAAGCACGTAGCCGTT TAGGTGGTCGTACCTTTACCAGCCGTTTTCAGGTCAGAAATG AATTTGGTGGTGCATGGGTTTCATTGGTTACAGCCGCATGTTGGG CAGAAATGCAGCGTTATGGTCTGGGTGTTGTTGAAGATCCGCTGA CCAATCTGGATAAAACCTGATTATGTATAATGACGGTAGCGTGG AAAGCATTAGTCCGGATGAATTCATAGG
SEQ ID NO: 94	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCACCAACGTTATTACCGT TCCGCTGAATACCTATAAACAATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGTCAAGTGAAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAACTCTGGGTCGTGTTTT TGCAATTCAGATGAACAGCAGCCGCTGAATTGGGTTACAGCCCA TGATTATAGTGATGAACGGGACCATTCGAGCATTACCATTCG ACGTAAGAAACCATCGATATAGG
SEQ ID NO: 95	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGCGGTTACAGCCGT

TABLE 7-continued

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
SEQ ID NO:	Sequence
	TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 96	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGCGGTTAACACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 97	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGCGGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 98	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGCGGTTTCATACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 99	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTAACGTTTCATACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 100	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTACGGTTTCATACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 101	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTAACGTTATTACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 102	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTACCGTTATTACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACCTGGGCACCATTTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 103	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATTACCGT

TABLE 7-continued

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
SEQ ID NO:	Sequence
	TCCGCGTAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACAGGACCCATTCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 104	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACAGGACCCAGCCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 105	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACAGGACCCGCGCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 106	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACAGGACCCGCGCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 107	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAAAACGGCACCCTGAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 108	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAAAACGGCACCATTCTGAGCATTACCAGGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 109	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACAGGACCCGCGCTGAGCATTACCACCGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 110	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTTCAGACCCA TGATTATAGTGATGAACAGGACCCGCGCAGCATTACCATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 111	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTTATGACCGT

TABLE 7-continued

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
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SEQ ID NO: 112	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTAGACCCA TGATTATAGTGATGAACTGGGCACCACTGAGCATTACCAATTGC ACGTAAAGAAACCATCGATATAGG
SEQ ID NO: 113	GCAATCCGCGGTTGAACAGGTTAATGGTGGTGTAAAAATCAAAAC CGATGACGATGAAATCATTACCGCAGGCGTTGTTGTATGACCGT TCCGCTGAATACCTATAAACATATTGGTTTTACACCGGCACTGAG CAAAGGTAACAGCGTTTTATCAAAGAAGGTCAGCTGAGTAAAGG TGCCAAACTGTATGTTTCATGTGAAACAGAATCTGGGTCGTGTTTT TGCAATTTGCAGATGAACAGCAGCCGCTGAATTGGGTTAGACCCA TGATTATAGTGATGAAACAGGCACTTCTGAGCATTACCAATTCA CGTAAAGAAACCATCGATATAGG
SEQ ID NO: 114	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTGAGCTGAGCGCGGTGCAGAAACAGCAATGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
SEQ ID NO: 115	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTGAACTGAACGCGGGTGCAGAAACAGCAATGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
SEQ ID NO: 116	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTGAGCTGAACGCGGGTGCAGAAACAGCAATGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
SEQ ID NO: 117	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTGAGCTGCAGGCGGGTGCAGAAACAGCAATGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
SEQ ID NO: 118	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTACCTGCAGGCGGGTGCAGAAACAGCAATGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
SEQ ID NO: 119	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA

TABLE 7-continued

Exemplary synthetic DNA fragments for generation of variants listed in Table 3	
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	TTGGACCGCAGATCCGTTTAGCTTAGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTGAACTGCTGGCGGGTGCAGAAACCAGCAATGGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
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SEQ ID NO: 121	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTATTCTGCGCGGGTGCAGAAACCAGCAATGGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
SEQ ID NO: 122	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTATTCTGGCGCGGGTGCAGAAACCAGCAATGGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG
SEQ ID NO: 123	AACCATCGATGTGAATGATCGTGATGCAGTTACCCGTGAAGTTCA GAAAATGTTTCCGGGTGTTGAAGTTCTGGGCACCGCAGCCTATGA TTGGACCGCAGATCCGTTTAGCTTAGTGCCTGGGCAGCGTATGG TGTTGGTCAGCTGTACGCTCTGAAAGATCTGCAGGCAGCAGAAGG TCGTGCGCTGTATGCGGGTGCAGAAACCAGCAATGGTTGGCATGC AAATATTGATGGTGCAGTTGAAAGCGGTCTGCGTGCAGGTCGTGA AGTTAAACAGCTGCTGAGCGGTGGTGGTGGATCCGGTAGCGGTCA TCATCACCATCATCATTAACTCGAGAATCG

[0229] The ligations for the individual constructs were transformed in parallel into BL21 Gold(DE3), and 8 single colonies from each transformation were picked and grown overnight in a 96 well plate in LB media containing Carbenicillin (100 µg/ml). Screening of the 8 individual clones of each variant was performed as described above for screening of the W427NNK library, and the assay values reported as an average after eliminating potential rare outliers (never more than 1 of 8 clones total; typically resulting from clones without an insert or mutations within the cloned synthetic DNA) as seen in FIG. 4A-E. Five variants were identified with assay values $\geq 90\%$ of the activity of the wild-type NicA2 enzyme, indicating that these five variants (I262T; I262S; I262A; I262T & A264L; and I262T & N263R; see FIG. 4) have enzymatic activity similar to wild-type, and, based on in silico predictions, are predicted to have lower immunogenic potential.

[0230] Variants based on mutations in four other epitopes set forth in Table 3 were prepared and assessed as described above. For Epitope B, variants were generated by cloning the synthetic double-stranded DNA fragments (SEQ ID NOs 84-93, Table 7) digested with restriction enzymes NdeI and

EcoRI; for Epitope 2 DNA fragments (SEQ ID NOs 94-103, Table 7) digested with SacII-ClaI; for Epitope 3 DNA fragments (SEQ ID Nos 102-113, Table 7) cut with SacII-ClaI; and for Epitope 4 DNA fragments (SEQ ID Nos 114-123, Table 7) cut with ClaI-XhoI, into the corresponding unique sites in the expression plasmid used for expression of wild-type NicA2 (SEQ ID NO:1). 8 random colonies from each ligation and transformation were tested as described above. As seen in FIGS. 4A-E, 2, 4, and 7 variants were identified with assay values $\geq 90\%$ of the activity of the wild-type NicA2 enzyme from Epitope B, 2, and 3, respectively, indicating that these variants (L74N & Y77R, R78Q, V304A & M306Q, V394A, V304T & M306I, M306I & L310R, L374Q & I377S, L374A & I377A; L374N & I382Q, I377A & I382T, I377T & I382T, I377T, and L374N & A383Q; see FIG. 4) have enzymatic activity similar to wild-type, and, based on in silico predictions, are predicted to have lower immunogenic potential. Interestingly, none of the variants proposed for Epitope 4 showed more than 40% activity compared to wt NicA2 (see FIG. 4D). The mutations identified in this Example (one mutation from each epitope) can be introduced into the d50W427Q backbone, or com-

bined with any of the other identified single mutation variants described in Table 5, or any number of mutations resulting from combination of mutations listed in Table 5, to produce a single de-immunized enzyme variant with enzymatic activity equal to or better than wt NicA2.

[0231] Two variants were generated combining deletion of the first epitope within the TAT leader sequence, mutating Epitope 1 using variants identified in FIG. 4, and containing the activity enhancing mutation W427Q: NicA2Δ50W427Q;I262A (SEQ ID: 62) and NicA2Δ50W427Q;I262T;N263R (SEQ ID: 63). These variants were purified, and enzymatic activity assessed in the Amplex Red assay as described above. As seen in Table 4, both variants have increased activity over wt despite having alteration that are likely to reduce immunogenicity.

Example 2—Identifying NicA2 MHCII Epitopes

[0232] An in silico search of NicA2 MHCH epitopes was performed based on the 8 most common HLA-DR alleles (Cantoret et al., *PNAS* 108: 1272-1277 (2011); DRB1*0101, DRB1*1501, DRB1*1301, DRB1*1101, DRB1*0801, DRB1*0701, DRB1*0401, and DRB1*0301). The search was done using the immune epitope database (Vita et al., *Nucleic acids research*, 43: D405-D412 (2015)) and percentile consensus rank method to assess the predicted immunogenic potential of NicA2. The percentile consensus score for each overlapping 15-mer NicA2 peptide was averaged across all the 8 HLA-DR alleles. The immunogenic potential of NicA2 was then determined by selecting all NicA2 sequences that were >1 standard deviation in tighter predicted binding to MHCH as compared to the overall averaged binding score. This method revealed eight contiguous sequences across 45% of the NicA2 sequence reflective of residues 10-32, 68-94, 189-225, 248-285, 296-327, 336-391 and 435-459 of SEQ ID NO:1 (highlighted in grey in Table 1) that are predicted to be broadly immunogenic.

[0233] A more narrow search using only the DRB1*0401 allele was performed in silico. This search yielded the six highest ranked immunogenic T-cell epitopes underlined in SEQ ID NO:1 of Table 1.

Example 3—PEGylation of Nicotine-Degrading Enzymes

[0234] Experiments were undertaken to develop protocols for conjugating polyethylene glycol (PEG) to NicA2 and other nicotine-degrading enzymes, such as NOX and the disclosed NOX variants, and to determine the effect of PEGylation on activity and half-life.

[0235] SDS-PAGE analysis indicated that the degree of PEGylation increases as the molar excess of PEGylation reagent and PEG chain length increases. Random PEGylation of wild-type (wt) NicA2 was performed at a protein concentration of 5 mg/mL and using 10 or 20 kDa NHS-PEG reagent (Sunbright® ME-100TS or ME-200TS; NOF America Corporation) at 7- or 20-fold molar excess in 100 mM Na₃PO₄, pH 7.6 on ice for ≥2 hours. Elimination of unconjugated PEG reagent was performed using Amicon Ultra-15 centrifugal filter units with a 50 kDa cutoff. Samples corresponding to 2 μg protein were loaded on an SDS-PAGE gel run in MOPS running buffer, and stained using SimplyBlue SafeStain (Invitrogen). As seen in FIG. 5, the degree of PEGylation could be controlled by controlling the excess molar ratios of PEGylation reagent. Preparations

NicA2-PEG1, -PEG2, and -PEG3 where no residual unconjugated protein was detected by SDS-PAGE were chosen for further analysis.

[0236] In order to determine whether PEGylation could enhance the pharmacokinetic (PK) properties of NicA2, serum concentrations were determined as a function of time after intravenous (i.v.) dosing in rats (5 mg/mL; N=4; 2M+2F). The data from these experiments are shown in FIG. 6. Briefly, MaxiSorp ELISA plates (Nunc) were coated overnight with anti-His tag antibody (R&D Systems), which could bind to the C-terminal His-tag on the NicA2 and PEGylated NicA2 proteins. Plates were blocked with 1% non-fat dry milk (NFDM) in phosphate buffered saline (PBS) for approximately 1 hour. Dilutions of NicA2 and NicA2-PEG1-3 standards and serum samples in 1% NFDM in PBS+0.1% Tween-20 were added to the plates and incubated for 2 hours at room temperature. After washing away unbound substances (all wash steps performed in PBS+0.1% Tween-20), rabbit anti-NicA2 polyclonal primary detection antibody was added to the wells for a 1 hour incubation. A wash step was followed by addition of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Fc) (KPL International). Plates were washed, and the remaining binding complex was detected with TMB substrate (3,3',5,5'-tetramethylbenzidine; KPL International). Once stopped with acid, plates were read on a spectrophotometer at 450 nm and data analyzed in SoftMax® Pro, version 5.4 (Molecular Devices).

[0237] Additional experiments were performed to determine whether PEGylation masks epitopes on NicA2. Serial dilutions in PBS of unPEGylated or PEGylated preparations of NicA2 were tested in the same sandwich ELISA assay used for measurement of serum concentrations in the PK experiment described above (FIG. 6), and signal (A450) plotted as a function of concentration. Assay sensitivity is dramatically reduced with increasing degree of PEGylation (approx. 1000-fold higher concentration of NicA2-PEG2 and -PEG3 required to obtain an A450 of 1.0 relative to unPEGylated molecule), indicating that epitopes recognized by the detection antibody reagents are less accessible in the PEGylated molecules. These results are shown in FIG. 7.

[0238] PEGylation was shown to decrease titers of NicA2-specific antibodies in a transgenic HLA-DR4 mouse model of immunogenicity. In particular, the reduction of NicA2-specific antibody titers 10 days after subcutaneous (s.c.) injection in Freund's Incomplete Adjuvant in human DR4 transgenic mice (N=6; 3M+3F; Taconic Biosciences) was studied. This mouse model carries a hybrid MHC class II molecule with the antigen binding domains of human HLA-DRA and HLA-DRB*0401 (representative of the DR4 supertype) and does not express endogenous mouse MHC class II molecules. Titer was defined as serum dilution to achieve OD450=0.5 in ELISA using NicA2 coated plates, and detection by goat α-mouse IgG-γ-HRP. The lowest serum dilution tested was 50-fold (Limit Of Detection (LOD); indicated by a dashed line in FIG. 8). PEGylation of NicA2 led to a significant ≥10-fold decrease in average NicA2-specific antibody titers in transgenic DR4 mice (FIGS. 8; 4, 2, and 2, animals from groups NicA2-PEG1, -PEG2, and -PEG3, respectively, had titers below LOD), indicating that PEGylated variants may exhibit lower immunogenicity in a clinical setting.

[0239] It was also observed that PEGylation attenuates the human T-cell proliferation response and cytokine TNFγ

release mediated by exposure to NicA2, as shown in FIG. 9. PEGylation of NicA2 led to a significant decrease in average T Cell proliferation Stimulation Index (FIG. 9, left panel) as well as decrease in IFN γ secretion levels (FIG. 9, right panel) (Independent experiments from five healthy volunteers). Positive control: phytohaemagglutinin (PHA). Test results for five independent experiments are shown for T cell proliferation response stimulation indexes (SI; measured by 3H-Thymidine uptake) (left) and fold increase in IFN γ secretion levels (measured by flow cytometry and a Cytometric Bead Array kit (BD Biosciences)) (right) for NicA2 and NicA2-PEG2 at three test concentrations in comparison to baseline responses (PBMC). An increase of ≥ 3 (dashed line) is considered a significant increase and a positive response.

[0240] It also was shown that PEGylated enzymes retained full nicotine degrading activity in serum, as shown in FIG. 10. Wt NicA2 or NicA2-PEG2 was added to a final concentration of 0.075 mg/ml into rat serum containing 40 ng/mL S-nicotine (250 nM, which is equivalent to plasma levels observed in a typical smoker) pre-incubated at 37° C. Samples were withdrawn at various time points, and enzymatic activity immediately quenched by addition of methanol and rapid mixing. Residual nicotine concentrations were determined by gas chromatography (GC). LOD of the GC assay was 2 ng/mL indicated by the dashed line. PEGylation did not appear to impede NicA2's ability to degrade nicotine.

Example 4—Nicotine Degrading Activity in Serum

[0241] To confirm that activity was improved not only in assay buffer and at the high (10 μ M) nicotine concentration of the Amplex Red assay (discussed in Example 1; see, e.g., Tables 4 and 5), but also in serum concentrations more relevant to those encountered in a typical smoker, an exemplary representative variant from the Amplex Red assay (NicA2A107R) was assessed in an assay utilizing serum rather than a buffer solution (the same assay used to produce the data shown in FIG. 10).

[0242] Briefly, NicA2A107R was added to a final concentration of 0.075 mg/ml into rat serum containing 40/ng/ml of S-(–)-nicotine (250 nM). As seen in FIG. 11, variant NicA2A107R has increased nicotine degrading activity under these conditions compared to wt NicA2, at serum concentrations that would be found in smokers.

Example 5—Treating Nicotine Poisoning

[0243] The maximum sub-lethal dose of nicotine in balb/c mice was determined to be 2 mg/kg intraperitoneally. The effects of nicotine were dose dependent, and included sedation, straub tail, tremors, tachypnea, back arching, rapid movements of the legs, wild running, loss of righting response, and clonic/tonic seizures. In this experiment, wild-type NicA2 was used as a proof of concept to show that the disclosed enzyme variants are capable of treating nicotine poisoning or toxicity.

[0244] Seven to eight week old balb/c mice (N=5 for each group) were pretreated with 775 mg/kg of wtNicA2 or a negative control (both i.v.) 15 minutes prior to administration of nicotine at the sub-lethal dose of 2 mg/kg. Over the course of 5 minutes, the mice were monitored for seizures and phenotypic indications of nicotine poisoning or toxicity and scored according to the rubric in Table 8, in which a

score of less than 3 indicates that there was no seizure. Sensitivity to seizure was computed by determining the percentage of animals that had a score of 4 or 5.

TABLE 8

Nicotine Poisoning Phenotypic Scoring	
Score	Signs & Symptoms
0	no visible effects
1	locomotor effects including increased exploring activity and/or sedation
2	tachypnea, tremors, back arching
3	any combination of the symptoms in 1 and 2 plus rapid movements of the legs, wild running, or partial loss of righting reflex
4	any combination of the previous symptoms plus complete loss of righting reflex, clonic seizures, and tonic seizures
5	any combination of the preceding symptoms plus death, with or without hyperextension of the limbs along the axis of the body (soldier position)

[0245] As shown in Table 9 below, all untreated mice had severe seizures, while none of the mice pretreated with NicA2 had seizures. Accordingly, these results indicate that the disclosed nicotine-degrading enzyme variants can be used to treat nicotine poisoning.

TABLE 9

Score	Dose	Sensitivity to Seizure	<4	≥ 4	0	1	2	3	4	5
Control	0	100%		N = 5						5
wt NicA2	775 mg/kg	0%	N = 5			2	2		1*	

*Administration was i.p. rather than i.v.

[0246] Similar experiments in a rat seizure model (using a challenge dose of nicotine of 4 mg/kg i.p. since rats are more tolerant of nicotine than mice) using lower doses of wtNicA2 (70 mg/kg and 140 mg/kg) were not effective. Thus, the dose of enzyme may be important to providing effective treatment.

[0247] The 775 mg/kg dose used successfully in the mouse experiment is not a practical dose for humans. However, as shown above, nicotine-degrading enzyme variants described herein have greater nicotine-degrading activity than the wild-type enzyme. Thus, it is believed that the enzyme variants disclosed herein will be effective against nicotine poisoning at doses suitable for use in humans.

Example 6—Treating Nicotine Addiction and/or Facilitating Smoking Cessation

[0248] This example illustrates methods of using a variant as described herein to treat nicotine addiction and/or facilitate smoking cessation in a human adult.

[0249] An adult human subject who regularly smokes cigarettes but wishes to quit is administered a therapeutically effective amount of a pharmaceutical compositions comprising a nicotine-degrading enzyme variant (e.g., NicA2 Δ 50W427Q; SEQ ID NO: 5, or a long-acting version thereof) orally or by intravenous or subcutaneous injection. The subject is evaluated for levels of nicotine circulating in plasma, as well as for the presence and/or severity of signs and symptoms associated with nicotine withdrawal, such as headache, irritability, anxiety, and sleeplessness, as well as the number of cigarettes smoked in a given day. The subject is treated with repeated administrations until levels of nico-

tine circulating in plasma reach a target (reduced) level, and/or until one or more signs/symptoms of nicotine withdrawal are reduced, ameliorated, or eliminated, and/or until the subject has reduced the level of consumption of nicotine products (e.g., is smoking fewer cigarettes per day), and/or until the subject has ceased consumption of nicotine products (e.g., has quit smoking).

Example 7—Treatment of a Pediatric Patient with a Nicotine-Degrading Enzyme Variant

[0250] This example illustrates methods using nicotine-degrading enzyme variants in the treatment of nicotine poisoning in a pediatric patient.

[0251] A child known to have or suspected of having ingested nicotine is administered a therapeutically effective amount of a pharmaceutical composition comprising a nicotine-degrading enzyme variant, by intravenous, intramuscular, or subcutaneous injection. The child is evaluated for the presence and/or severity of signs and symptoms associated with nicotine poisoning, including, but not limited to, seizures, coma, shortness of breath, and increased heart rate, and the child is treated until one or more signs/symptoms is reduced, ameliorated, or eliminated. Optionally, another dose of the pharmaceutical composition is administered if signs/symptoms persist and/or if nicotine plasma/brain levels remain elevated.

SEQUENCE LISTING

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SEQUENCE: 3
GVAGLGAI DAASATQKTNRA STVKGGFDYD VVVGGGFAG ATAARECGLQ GYRTLLLEAR 60
SRLGGRTFTS RFAGQIEIFG GAWVHWLQPH VWAEMQRYGL GVVEDPLTNL DKTLMYNDG 120
SVESISPDEF GKNIRIAFEK LCHDAWEVFP RPHEPMTFER ARELDKSSVL DRIKTLGLSR 180
LQQAQINSYM ALYAGETTDK FGLPGVLKLF ACGGWNYDAF MDTETHYRIQ GGTIGLINAM 240
LTDSGAEVRM SVPVTAVEQV NGGVKIKTDD DEIITAGVVV MTVPLNTYKH IGFTPALSKG 300
KQRFIKEGQL SKGAKLYVHV KQNLGRVFAF ADEQQPLNWV QTHDYSDELG TILSITIARK 360
ETIDVNDRDA VTREVGKMF GVEVLGTAA YDWTADPFSLG AWAAYGVGQL SRLKDLQAAE 420
GRILFAGAET SNGWHANIDG AVESGLRAGR EVKQLLS 457

SEQ ID NO: 4          moltype = AA  length = 444
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FEATURE Location/Qualifiers
 REGION 1..444
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..444
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 4
 TQKTNRASV KGGFDYDVVV VGGGFAGATA ARECGLQGYR TLLLEARSRL GGRTFTSRFA 60
 GQEIFEGGAW VHWLQPHVWA EMQRYGLGVV EDPLTNLDKT LIMYNDGSVE SISPDEFGKN 120
 IRIAFEKLCH DAEVFPFRPH EPMFTERARE LDKSSVLDRI KTLGLSRLQQ AQINSYMALY 180
 AGETTDKFGL PGVLKLFACG GWNDAFMDT ETHYRIQGGT IGLINAMLTG SGAEVMSVP 240
 VTAVEQVNGG VKIKTDDDEI ITAGVVMTV PLNTYKHIGF TPALSKGKQR FIKEGQLSKG 300
 AKLYVHVQKN LGRVFAFADE QQPLNWWQTH DYSDELGTIL SITIARKETI DVNDRDAVTR 360
 EVQKMFPQVE VLGTAAYDWT ADPFSLGAWA AYGVGQLSRL KDLQAAEGRI LFAGAETSNG 420
 WHANIDGAVE SGLRAGREVK QLLS 444

SEQ ID NO: 5 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 5
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
 WEVFPFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNLG 300
 RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPQVEVL 360
 GTAAYDWTAD PFSLGAQAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
 LRAGREVKQL LS 432

SEQ ID NO: 6 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 6
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
 WEVFPFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNLG 300
 RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPQVEVL 360
 GTAAYDWTAD PFSLGAAEAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
 LRAGREVKQL LS 432

SEQ ID NO: 7 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 7
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
 WEVFPFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNLG 300
 RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPQVEVL 360
 GTAAYDWTAD PFSLGASAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
 LRAGREVKQL LS 432

SEQ ID NO: 8 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide

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source                1..432
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 8
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAMAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 9          moltype = AA length = 432
FEATURE              Location/Qualifiers
REGION              1..432
                    note = Description of Artificial Sequence: Synthetic
                    polypeptide
source              1..432
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 9
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG ATFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 10         moltype = AA length = 432
FEATURE              Location/Qualifiers
REGION              1..432
                    note = Description of Artificial Sequence: Synthetic
                    polypeptide
source              1..432
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 10
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG QTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 11         moltype = AA length = 432
FEATURE              Location/Qualifiers
REGION              1..432
                    note = Description of Artificial Sequence: Synthetic
                    polypeptide
source              1..432
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 11
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG FTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 12         moltype = AA length = 432
FEATURE              Location/Qualifiers
REGION              1..432
                    note = Description of Artificial Sequence: Synthetic
                    polypeptide
source              1..432
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 12

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GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG GTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 13      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 13
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG TTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 14      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 14
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG LTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 15      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 15
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG STFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 16      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 16
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG NTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVMSVPVT AVEQVNGGVK 240

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IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 17      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 17
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTEG HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 18      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 18
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTEL HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 19      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 19
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTER HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 20      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 20
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTEV HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

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SEQ ID NO: 21	moltype = AA length = 432	
FEATURE	Location/Qualifiers	
REGION	1..432	
	note = Description of Artificial Sequence: Synthetic polypeptide	
source	1..432	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 21		
GFDYDVVVVG	GGFAGATAAR	ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM	QRYGLGVVED	PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP	MFTERARELD	KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW	NYDAFMDTET	HYRIQGGTIG LINAMLTDSG AEV RMSVPVT AVEQVNGGVK 240
IKTDDDEIIT	AGVVVMTVPL	NTYKHIGFTP ALSKGKQRFI KEGQLSKGAP LYVHV KQNLG 300
RVFAFADEQQ	PLNWVQTHDY	SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD	PFSLGAWAAY	GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL	LS	432
SEQ ID NO: 22		
moltype = AA length = 432		
Location/Qualifiers		
1..432		
note = Description of Artificial Sequence: Synthetic polypeptide		
1..432		
mol_type = protein		
organism = synthetic construct		
SEQUENCE: 22		
GFDYDVVVVG	GGFAGATAAR	ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM	QRYGLGVVED	PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP	MFTERARELD	KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW	NYDAFMDTET	HYRIQGGTIG LINAMLTDSG AEV RMSVPVT AVEQVNGGVK 240
IKTDDDEIIT	AGVVVMTVPL	NTYKHIGFTP ALSKGKQRFI KEGQLSKGAI LYVHV KQNLG 300
RVFAFADEQQ	PLNWVQTHDY	SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD	PFSLGAWAAY	GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL	LS	432
SEQ ID NO: 23		
moltype = AA length = 432		
Location/Qualifiers		
1..432		
note = Description of Artificial Sequence: Synthetic polypeptide		
1..432		
mol_type = protein		
organism = synthetic construct		
SEQUENCE: 23		
GFDYDVVVVG	GGFAGATAAR	ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM	QRYGLGVVED	PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP	MFTERARELD	KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW	NYDAFMDTET	HYRIQGGTIG LINAMLTDSG AEV RMSVPVT AVEQVNGGVK 240
IKTDDDEIIT	AGVVVMTVPL	NTYKHIGFTP ALSKGKQRFI KEGQLSKGAV LYVHV KQNLG 300
RVFAFADEQQ	PLNWVQTHDY	SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD	PFSLGAWAAY	GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL	LS	432
SEQ ID NO: 24		
moltype = AA length = 432		
Location/Qualifiers		
1..432		
note = Description of Artificial Sequence: Synthetic polypeptide		
1..432		
mol_type = protein		
organism = synthetic construct		
SEQUENCE: 24		
GFDYDVVVVG	GGFAGATAAR	ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM	QRYGLGVVED	PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP	MFTERARELD	KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW	NYDAFMDTET	HYRIQGGTIG LINAMLTDSG AEV RMSVPVT AVEQVNGGVK 240
IKTDDDEIIT	AGVVVMTVPL	NTYKHIGFTP ALSKGKQRFI KEGQLSKGAD LYVHV KQNLG 300
RVFAFADEQQ	PLNWVQTHDY	SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD	PFSLGAWAAY	GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL	LS	432
SEQ ID NO: 25		
moltype = AA length = 432		
Location/Qualifiers		
1..432		

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note = Description of Artificial Sequence: Synthetic
      polypeptide
source      1..432
            mol_type = protein
            organism = synthetic construct

SEQUENCE: 25
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAE LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 26      moltype = AA length = 432
FEATURE            Location/Qualifiers
REGION             1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source             1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 26
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVKTTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 27      moltype = AA length = 432
FEATURE            Location/Qualifiers
REGION             1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source             1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 27
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVETHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 28      moltype = AA length = 432
FEATURE            Location/Qualifiers
REGION             1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source             1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 28
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVETHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 29      moltype = AA length = 432
FEATURE            Location/Qualifiers
REGION             1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source             1..432
                  mol_type = protein

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                                organism = synthetic construct
SEQUENCE: 29
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGGAK LYVHVQNQLG 300
RVFAFADEQQ PLNVWLTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 30      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 30
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGGAK LYVHVQNQLG 300
RVFAFADEQQ PLNVVITHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 31      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 31
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGGAK LYVHVQNQLG 300
RVFAFADEQQ PLNVVYTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 32      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 32
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGGAK LYVHVQNQLG 300
RVFAFADEQQ PLNVVQTHDY SDELGITLSI PIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 33      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 33
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120

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WEVFPRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGKAK LYVHVQNGLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI IARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 34      moltype = AA  length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 34
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFPRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGKAK LYVHVQNGLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI VIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 35      moltype = AA  length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 35
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFPRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGKAK LYVHVQNGLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI QIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 36      moltype = AA  length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 36
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFPRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGKAK LYVHVQNGLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI NIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 37      moltype = AA  length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 37
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFPRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSGKAK LYVHVQNGLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI LIARKETIDV NDRDAVTREV QKMFPGVEVL 360

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GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAGEGRILF AGAETSNGWH ANIDGAVESG 420
 LLAGREVKKQL LS 432

SEQ ID NO: 38 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic
 polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 38
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMVTPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGTILSI MIARKETIDV NDRDAVTREV QKMFPGEVEVL 360
 GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAGEGRILF AGAETSNGWH ANIDGAVESG 420
 LLAGREVKKQL LS 432

SEQ ID NO: 39 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic
 polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 39
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMVTPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGEVEVL 360
 GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAGEGRILF AGAETSNGWH ALIDGAVESG 420
 LLAGREVKKQL LS 432

SEQ ID NO: 40 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic
 polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 40
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMVTPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGEVEVL 360
 GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAGEGRILF AGAETSNGWH AYIDGAVESG 420
 LLAGREVKKQL LS 432

SEQ ID NO: 41 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic
 polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 41
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMVTPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGEVEVL 360
 GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAAGEGRILF AGAETSNGWH ASIDGAVESG 420
 LLAGREVKKQL LS 432

SEQ ID NO: 42 moltype = AA length = 432

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FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct
 SEQUENCE: 42
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
 GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH AFIDGAVESG 420
 LRAGREV KQL LS 432

SEQ ID NO: 43 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct
 SEQUENCE: 43
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
 GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH AGIDGAVESG 420
 LRAGREV KQL LS 432

SEQ ID NO: 44 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct
 SEQUENCE: 44
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
 GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH AEIDGAVESG 420
 LRAGREV KQL LS 432

SEQ ID NO: 45 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct
 SEQUENCE: 45
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
 RVFAFADEQQ PLNVVQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFP PGVEVL 360
 GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQA AEGRILF AGAETSNGWH AADGAVESG 420
 LRAGREV KQL LS 432

SEQ ID NO: 46 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide

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source                1..432
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 46
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANFDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 47          moltype = AA length = 432
FEATURE               Location/Qualifiers
REGION                1..432
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                1..432
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 47
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANYDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 48          moltype = AA length = 432
FEATURE               Location/Qualifiers
REGION                1..432
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                1..432
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 48
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANADGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 49          moltype = AA length = 432
FEATURE               Location/Qualifiers
REGION                1..432
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                1..432
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 49
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWWQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFP GVEVL 360
GTAAYDWTAD PPSLGAWAAY GVGQLSRLKD LQAABGRILF AGAETSNGWH ANVDGAVESG 420
LRAGREV KQL LS 432

SEQ ID NO: 50          moltype = AA length = 432
FEATURE               Location/Qualifiers
REGION                1..432
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                1..432
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 50

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GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANLDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 51      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 51
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMAQYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 52      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 52
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMAGYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 53      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 53
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMAEYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNQWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 54      moltype = AA length = 432
FEATURE           Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 54
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFRPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMAIYAG ETTDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240

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IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNGLG 300
RVFAFADEQQ PLNVVQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVQQL LS 432

SEQ ID NO: 55 moltype = AA length = 432
FEATURE Location/Qualifiers
REGION 1..432
 note = Description of Artificial Sequence: Synthetic
 polypeptide
source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 55
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMACYAG ETDDKFGPLG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNGLG 300
RVFAFADEQQ PLNVVQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVQQL LS 432

SEQ ID NO: 56 moltype = AA length = 432
FEATURE Location/Qualifiers
REGION 1..432
 note = Description of Artificial Sequence: Synthetic
 polypeptide
source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 56
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMASYAG ETDDKFGPLG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNGLG 300
RVFAFADEQQ PLNVVQTHDY SDELGITLSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFSLGAWAAY GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVQQL LS 432

SEQ ID NO: 57 moltype = AA length = 477
FEATURE Location/Qualifiers
source 1..477
 mol_type = protein
 organism = Pseudomonas sp.
 note = Pseudomonas sp. HZN6

SEQUENCE: 57
MDEKRNNGLS RRSFIGGAHV VTAGAAGLGL IGSANATENG TSKRATGFDY DVIVVGGGFA 60
GATAARECGH QGYKTLLEA RSRLLGRTFT SHFAGQEIEF GGAWVHWLQP HVWSEMQRYS 120
LGVVEDPLTN LDKTLVMYND GSVEDLPPEV FGTNIQVAF KMCHDAWEAF PRPHEPMFTE 180
RARKLDKMSV LDRINQLELT RAQRAELNSY MALYGGETTD KYGLPGVLKL FACGGWNYNA 240
FMDTETHYRI EGGTIGLINA MLADSGAEVR LNMPVISVEQ LNGGVRVETD DGETITAGTI 300
IMTVPLNTYR HINFTPALSE GKQRFIQEGQ LSKGAKLYVH VKENLGRVFA FADEQQPLNW 360
VQTHDYGDEL GTILSITIAE AETIDVNRD AVTREIRKLF PGVEVLGIAA YDWTADPFSL 420
GAWAAYGVGQ LSRLTDLQOP EGRILFAGAE TSNGWHANID GAVESGLRAG REAKEIL 477

SEQ ID NO: 58 moltype = AA length = 477
FEATURE Location/Qualifiers
REGION 1..477
 note = Description of Artificial Sequence: Synthetic
 polypeptide
source 1..477
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 58
MDEKRNNGLS RRSFIGGAHV VTAGAAGLGL IGSANATENG TSKRATGFDY DVIVVGGGFA 60
GATAARECGH QGYKTLLEA RSRLLGRTFT SHFAGQEIEF GGAWVHWLQP HVWSEMQRYS 120
LGVVEDPLTN LDKTLVMYND GSVEDLPPEV FGTNIQVAF KMCHDAWEAF PRPHEPMFTE 180
RARKLDKMSV LDRINQLELT RAQRAELNSY MALYGGETTD KYGLPGVLKL FACGGWNYNA 240
FMDTETHYRI EGGTIGLINA MLADSGAEVR LNMPVISVEQ LNGGVRVETD DGETITAGTI 300
IMTVPLNTYR HINFTPALSE GKQRFIQEGQ LSKGAKLYVH VKENLGRVFA FADEQQPLNW 360
VQTHDYGDEL GTILSITIAE AETIDVNRD AVTREIRKLF PGVEVLGIAA YDWTADPFSL 420
GAAAYGVGQ LSRLTDLQOP EGRILFAGAE TSNGWHANID GAVESGLRAG REAKEIL 477

SEQ ID NO: 59 moltype = AA length = 477

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FEATURE Location/Qualifiers
 REGION 1..477
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..477
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 59
 MDEKRNNGLS RRSFIGGAHV VTAGAAGLGL IGSANATENG TSKRATGFYD DVIVVGGGFA 60
 GATAARECGH QGYKTLLEA RSRLGGRTFT SHFAGQEIEF GGAWVHWLQP HVWSEMQRYG 120
 LGVVEDPLTN LDKTLVMYND GSVEDLPPEV FGTNIQVAPE KMCHDAWEAF PRPHEPMFTE 180
 RARKLDKMSV LDRINQLELT RAQRAELNSY MALYGGETTD KYGLPGVLKL FACGGWNYNA 240
 FMDTETHYRI EGGTIGLINA MLADSGAEVR LNMPVISVEQ LNGGVRVETD DGETITAGTI 300
 IMTVPLNTYR HINFTPALSE GKQRFIQEGQ LSKGAKLYVH VKENLGRVFA FADEQQPLNW 360
 VQTHDYGDEL GTILSITIAI AETIDVNDRD AVTREIRKLF PGVEVLGIAA YDWTADPFSL 420
 GASAAYGVGQ LSRLTDLQQP EGRILFAGAE TSNGWHANID GAVESGLRAG REAKEIL 477

SEQ ID NO: 60 moltype = AA length = 477
 FEATURE Location/Qualifiers
 REGION 1..477
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..477
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 60
 MDEKRNNGLS RRSFIGGAHV VTAGAAGLGL IGSANATENG TSKRATGFYD DVIVVGGGFA 60
 GATAARECGH QGYKTLLEA RSRLGGRTFT SHFAGQEIEF GGAWVHWLQP HVWSEMQRYG 120
 LGVVEDPLTN LDKTLVMYND GSVEDLPPEV FGTNIQVAPE KMCHDAWEAF PRPHEPMFTE 180
 RARKLDKMSV LDRINQLELT RAQRAELNSY MALYGGETTD KYGLPGVLKL FACGGWNYNA 240
 FMDTETHYRI EGGTIGLINA MLADSGAEVR LNMPVISVEQ LNGGVRVETD DGETITAGTI 300
 IMTVPLNTYR HINFTPALSE GKQRFIQEGQ LSKGAKLYVH VKENLGRVFA FADEQQPLNW 360
 VQTHDYGDEL GTILSITIAI AETIDVNDRD AVTREIRKLF PGVEVLGIAA YDWTADPFSL 420
 GAEEAYGVGQ LSRLTDLQQP EGRILFAGAE TSNGWHANID GAVESGLRAG REAKEIL 477

SEQ ID NO: 61 moltype = AA length = 477
 FEATURE Location/Qualifiers
 REGION 1..477
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..477
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 61
 MDEKRNNGLS RRSFIGGAHV VTAGAAGLGL IGSANATENG TSKRATGFYD DVIVVGGGFA 60
 GATAARECGH QGYKTLLEA RSRLGGRTFT SHFAGQEIEF GGAWVHWLQP HVWSEMQRYG 120
 LGVVEDPLTN LDKTLVMYND GSVEDLPPEV FGTNIQVAPE KMCHDAWEAF PRPHEPMFTE 180
 RARKLDKMSV LDRINQLELT RAQRAELNSY MALYGGETTD KYGLPGVLKL FACGGWNYNA 240
 FMDTETHYRI EGGTIGLINA MLADSGAEVR LNMPVISVEQ LNGGVRVETD DGETITAGTI 300
 IMTVPLNTYR HINFTPALSE GKQRFIQEGQ LSKGAKLYVH VKENLGRVFA FADEQQPLNW 360
 VQTHDYGDEL GTILSITIAI AETIDVNDRD AVTREIRKLF PGVEVLGIAA YDWTADPFSL 420
 GAHAAYGVGQ LSRLTDLQQP EGRILFAGAE TSNGWHANID GAVESGLRAG REAKEIL 477

SEQ ID NO: 62 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide
 source 1..432
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 62
 GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
 WLQPHVWAEH QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
 WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGPLPG 180
 VLKLFACGGW NYDAFMDTET HYRIQGGTIG LANAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
 IKTDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVKNLGL 300
 RVFAFADEQQ PLNVVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPVGEVL 360
 GTAAYDWTAD PFSLGAQAAY GVGQLSRLKD LQAAGRILF AGAETSNGWH ANIDGAVESG 420
 LRAREVQQL LS 432

SEQ ID NO: 63 moltype = AA length = 432
 FEATURE Location/Qualifiers
 REGION 1..432
 note = Description of Artificial Sequence: Synthetic polypeptide

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source                1..432
                      mol_type = protein
                      organism = synthetic construct

SEQUENCE: 63
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGAWVH 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFKGNIR IAFEKLCHDA 120
WEVFP RPHEP MFERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETDDKFGPLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LTRAMLTDSG AEVRMSVPVT AVEQVNGGVK 240
IKTDDDEIIT AGVVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQNQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGEVVL 360
GTAAYDWTAD PPSLGAQAAY GVGQLSRLKD LQAABEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 64         moltype = DNA length = 313
FEATURE              Location/Qualifiers
misc_difference       126..127
                      note = modified_base - a, c, t, g, unknown or other
misc_feature          1..313
                      note = Description of Artificial Sequence: Synthetic
                      polynucleotide
source                1..313
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 64
aaagcaatat cgatgtgaat gatcgatgat cagttaccgc tgaagttcag aaaatgtttc 60
cgggtgttga agttctgggc accgcagcct atgattggac cgcagatccg ttagcttag 120
gtgcnnkgc cgcgatgggt gttggtcagc tgtcacgtct gaaagatctg caggcagcag 180
aaggtcgtat tctgtttgcg ggtgcagaaa ccagcaatgg ttggcatgca aatattgatg 240
gtgcagttga aagcggctcg cgtgcaggtc gtgaagttaa acagctgctg agcgggtggtg 300
gtggatccat agg 313

SEQ ID NO: 65         moltype = DNA length = 39
FEATURE              Location/Qualifiers
misc_difference       20..21
                      note = modified_base - a, c, t, g, unknown or other
misc_feature          1..39
                      note = Description of Artificial Sequence: Synthetic
                      oligonucleotide
source                1..39
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 65
tccgttttagc ctgggtgcan nkgcagcgta tgggtgttgg 39

SEQ ID NO: 66         moltype = DNA length = 45
FEATURE              Location/Qualifiers
misc_difference       25..26
                      note = modified_base - a, c, t, g, unknown or other
misc_feature          1..45
                      note = Description of Artificial Sequence: Synthetic
                      oligonucleotide
source                1..45
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 66
gaagcacgta gccgttttagg ttgtnnkacc tttaccagcc gtttt 45

SEQ ID NO: 67         moltype = DNA length = 49
FEATURE              Location/Qualifiers
misc_difference       24..25
                      note = modified_base - a, c, t, g, unknown or other
misc_feature          1..49
                      note = Description of Artificial Sequence: Synthetic
                      oligonucleotide
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct

SEQUENCE: 67
atgatgcatt catggatacc gaannkcatt atcgatttca gggtggcac 49

SEQ ID NO: 68         moltype = DNA length = 45
FEATURE              Location/Qualifiers
misc_difference       22..23
                      note = modified_base - a, c, t, g, unknown or other
misc_feature          1..45
                      note = Description of Artificial Sequence: Synthetic

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source	oligonucleotide 1..45 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 68		
ggtcagctga gtaaagggtgc cnnkctgtat gttcatgtga aacag		45
SEQ ID NO: 69	moltype = DNA length = 49	
FEATURE	Location/Qualifiers	
misc_difference	28..29 note = modified_base - a, c, t, g, unknown or other	
misc_feature	1..49 note = Description of Artificial Sequence: Synthetic oligonucleotide	
source	1..49 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 69		
gatgaacagc agccgctgaa ttgggttnnk acccatgatt atagtgatg		49
SEQ ID NO: 70	moltype = DNA length = 49	
FEATURE	Location/Qualifiers	
misc_difference	25..26 note = modified_base - a, c, t, g, unknown or other	
misc_feature	1..49 note = Description of Artificial Sequence: Synthetic oligonucleotide	
source	1..49 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 70		
gaactgggca ccattctgag cattnnkatt gcacgtaaag aaaccattg		49
SEQ ID NO: 71	moltype = DNA length = 45	
FEATURE	Location/Qualifiers	
misc_difference	22..23 note = modified_base - a, c, t, g, unknown or other	
misc_feature	1..45 note = Description of Artificial Sequence: Synthetic oligonucleotide	
source	1..45 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 71		
accagcaatg gttggcatgc annkattgat ggtgcagttg aaagc		45
SEQ ID NO: 72	moltype = DNA length = 48	
FEATURE	Location/Qualifiers	
misc_difference	28..29 note = modified_base - a, c, t, g, unknown or other	
misc_feature	1..48 note = Description of Artificial Sequence: Synthetic oligonucleotide	
source	1..48 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 72		
gaaaccagca atggttgga tgcaaatnnk gatggtgcag ttgaaagc		48
SEQ ID NO: 73	moltype = DNA length = 51	
FEATURE	Location/Qualifiers	
misc_difference	25..26 note = modified_base - a, c, t, g, unknown or other	
misc_feature	1..51 note = Description of Artificial Sequence: Synthetic oligonucleotide	
source	1..51 mol_type = other DNA organism = synthetic construct	
SEQUENCE: 73		
gcacagatta atagctatat gccannktat gccggtgaaa ccaccgataa a		51
SEQ ID NO: 74	moltype = DNA length = 395	
FEATURE	Location/Qualifiers	
misc_feature	1..395 note = Description of Artificial Sequence: Synthetic	

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source                polynucleotide
                        1..395
                        mol_type = other DNA
                        organism = synthetic construct

SEQUENCE: 74
gcaatgaatt cggtaaaaac attcgcatgt cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctcg cctgggtgttc 240
tgaaactggt tgcattgtgt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctgg cgaatcagat gctgaccgat agcggtgccg 360
aagttcgtat gagcgttcgg gttaccgcgg atagg 395

SEQ ID NO: 75          moltype = DNA length = 395
FEATURE                Location/Qualifiers
misc_feature            1..395
                        note = Description of Artificial Sequence: Synthetic
                        polynucleotide
source                  1..395
                        mol_type = other DNA
                        organism = synthetic construct

SEQUENCE: 75
gcaatgaatt cggtaaaaac attcgcatgt cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctcg cctgggtgttc 240
tgaaactggt tgcattgtgt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctga aaaatgcaat ggataccgat agcggtgccg 360
aagttcgtat gagcgttcgg gttaccgcgg atagg 395

SEQ ID NO: 76          moltype = DNA length = 395
FEATURE                Location/Qualifiers
misc_feature            1..395
                        note = Description of Artificial Sequence: Synthetic
                        polynucleotide
source                  1..395
                        mol_type = other DNA
                        organism = synthetic construct

SEQUENCE: 76
gcaatgaatt cggtaaaaac attcgcatgt cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctcg cctgggtgttc 240
tgaaactggt tgcattgtgt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctga ccaatgcaat gctgaccgat agcggtgccg 360
aagttcgtat gagcgttcgg gttaccgcgg atagg 395

SEQ ID NO: 77          moltype = DNA length = 395
FEATURE                Location/Qualifiers
misc_feature            1..395
                        note = Description of Artificial Sequence: Synthetic
                        polynucleotide
source                  1..395
                        mol_type = other DNA
                        organism = synthetic construct

SEQUENCE: 77
gcaatgaatt cggtaaaaac attcgcatgt cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctcg cctgggtgttc 240
tgaaactggt tgcattgtgt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctga gcaatgcaat gctgaccgat agcggtgccg 360
aagttcgtat gagcgttcgg gttaccgcgg atagg 395

SEQ ID NO: 78          moltype = DNA length = 395
FEATURE                Location/Qualifiers
misc_feature            1..395
                        note = Description of Artificial Sequence: Synthetic
                        polynucleotide
source                  1..395
                        mol_type = other DNA
                        organism = synthetic construct

SEQUENCE: 78
gcaatgaatt cggtaaaaac attcgcatgt cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180

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atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctctg cctgggtgttc 240
tgaaactggt tgcattgtgtt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctgg ataatgcaat gaaaaccgat agcggtgccg 360
aagttcgtat gagcgttcoc gttaccgcgg atagg 395

SEQ ID NO: 79      moltype = DNA length = 395
FEATURE          Location/Qualifiers
misc_feature      1..395
                  note = Description of Artificial Sequence: Synthetic
                  polynucleotide
source            1..395
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 79
gcaatgaatt cggtaaaaac attcgcattg cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtcccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctctg cctgggtgttc 240
tgaaactggt tgcattgtgtt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctgg cgaatgcaat gctgaccgat agcggtgccg 360
aagttcgtat gagcgttcoc gttaccgcgg atagg 395

SEQ ID NO: 80      moltype = DNA length = 395
FEATURE          Location/Qualifiers
misc_feature      1..395
                  note = Description of Artificial Sequence: Synthetic
                  polynucleotide
source            1..395
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 80
gcaatgaatt cggtaaaaac attcgcattg cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtcccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctctg cctgggtgttc 240
tgaaactggt tgcattgtgtt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctga ccaatctgat gctgaccgat agcggtgccg 360
aagttcgtat gagcgttcoc gttaccgcgg atagg 395

SEQ ID NO: 81      moltype = DNA length = 395
FEATURE          Location/Qualifiers
misc_feature      1..395
                  note = Description of Artificial Sequence: Synthetic
                  polynucleotide
source            1..395
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 81
gcaatgaatt cggtaaaaac attcgcattg cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtcccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctctg cctgggtgttc 240
tgaaactggt tgcattgtgtt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctga cccgtgcaat gctgaccgat agcggtgccg 360
aagttcgtat gagcgttcoc gttaccgcgg atagg 395

SEQ ID NO: 82      moltype = DNA length = 395
FEATURE          Location/Qualifiers
misc_feature      1..395
                  note = Description of Artificial Sequence: Synthetic
                  polynucleotide
source            1..395
                  mol_type = other DNA
                  organism = synthetic construct

SEQUENCE: 82
gcaatgaatt cggtaaaaac attcgcattg cctttgaaaa actgtgtcat gatgcatggg 60
aagtttttcc gcgtcccgcat gaaccgatgt ttaccgaacg tgcccgtgaa ctggataaat 120
caagcgttct ggatcgtatt aaaacactgg gtctgagccg tctgcagcag gcacagatta 180
atagctatat ggcactgtat gccggtgaaa ccaccgataa atttggctctg cctgggtgttc 240
tgaaactggt tgcattgtgtt ggttgggaatt atgatgcctt tatggatacc gaaacgcact 300
atcgtattca aggtggcacc attggtctga ttaatgcaca tctgaccgat agcggtgccg 360
aagttcgtat gagcgttcoc gttaccgcgg atagg 395

SEQ ID NO: 83      moltype = DNA length = 395
FEATURE          Location/Qualifiers
misc_feature      1..395

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note = Description of Artificial Sequence: Synthetic
polynucleotide

source 1..395
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 83

gcaatgaatt	cggtaaaaac	attcgcattg	cctttgaaaa	actgtgtcat	gatgcatggg	60
aagtttttcc	gcgtccgcat	gaaccgatgt	ttaccgaacg	tgcccgtgaa	ctggataaat	120
caagcggtct	ggatcgattt	aaaacactgg	gtctgagccg	tctgcagcag	gcacagatta	180
atagctatat	ggcactgtat	gccggtgaaa	ccaccgataa	atttggctcg	cctgggtgtc	240
tgaaactggt	tgcattggtt	ggttggaatt	atgatgcctt	tatggatacc	gaaacgcact	300
atcgatttca	aggtggcacc	attggtctgg	cgaataacat	gctgaccgat	agcggtgccg	360
aagttcgtat	gagcgttccg	gttaccgcgg	atagg			395

SEQ ID NO: 84 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
note = Description of Artificial Sequence: Synthetic
polynucleotide

source 1..478
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 84

gcaatcatat	gagcgacaaa	acaaaaacca	atgaaggttt	tagccgtcgc	agctttattg	60
gtagcgagc	agttgttacc	gcaggcggtg	caggtctggg	tgcaattgat	gcagcaagcg	120
caaccagaaa	aaccaatcgt	gcaagcaccg	ttaaagggtg	cttcgattat	gatgtgtgtg	180
tggttggtgg	tggttttgcc	ggtgcaaccg	cagcacgtga	atgtggtaac	cagggtcgctc	240
gtaccctgct	gctggaagca	cgtagccgtt	taggtggtcg	tacctttacc	agccgttttg	300
caggtcaaga	aattgaattt	ggtggtgcat	gggttcattg	gttacagccg	catgtttggg	360
cagaaatgca	gcgttatggt	ctgggtgttg	ttgaagatcc	gctgaccaat	ctggataaaa	420
ccttgattat	gtataatgac	ggtagcgtgg	aaagcattag	tccggatgaa	ttcatagg	478

SEQ ID NO: 85 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
note = Description of Artificial Sequence: Synthetic
polynucleotide

source 1..478
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 85

gcaatcatat	gagcgacaaa	acaaaaacca	atgaaggttt	tagccgtcgc	agctttattg	60
gtagcgagc	agttgttacc	gcaggcggtg	caggtctggg	tgcaattgat	gcagcaagcg	120
caaccagaaa	aaccaatcgt	gcaagcaccg	ttaaagggtg	cttcgattat	gatgtgtgtg	180
tggttggtgg	tggttttgcc	ggtgcaaccg	cagcacgtga	atgtggtaac	cagggttaaac	240
gtaccctgct	gctggaagca	cgtagccgtt	taggtggtcg	tacctttacc	agccgttttg	300
caggtcaaga	aattgaattt	ggtggtgcat	gggttcattg	gttacagccg	catgtttggg	360
cagaaatgca	gcgttatggt	ctgggtgttg	ttgaagatcc	gctgaccaat	ctggataaaa	420
ccttgattat	gtataatgac	ggtagcgtgg	aaagcattag	tccggatgaa	ttcatagg	478

SEQ ID NO: 86 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
note = Description of Artificial Sequence: Synthetic
polynucleotide

source 1..478
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 86

gcaatcatat	gagcgacaaa	acaaaaacca	atgaaggttt	tagccgtcgc	agctttattg	60
gtagcgagc	agttgttacc	gcaggcggtg	caggtctggg	tgcaattgat	gcagcaagcg	120
caaccagaaa	aaccaatcgt	gcaagcaccg	ttaaagggtg	cttcgattat	gatgtgtgtg	180
tggttggtgg	tggttttgcc	ggtgcaaccg	cagcacgtga	atgtggtcag	cagggtcgctc	240
gtaccctgct	gctggaagca	cgtagccgtt	taggtggtcg	tacctttacc	agccgttttg	300
caggtcaaga	aattgaattt	ggtggtgcat	gggttcattg	gttacagccg	catgtttggg	360
cagaaatgca	gcgttatggt	ctgggtgttg	ttgaagatcc	gctgaccaat	ctggataaaa	420
ccttgattat	gtataatgac	ggtagcgtgg	aaagcattag	tccggatgaa	ttcatagg	478

SEQ ID NO: 87 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
note = Description of Artificial Sequence: Synthetic
polynucleotide

source 1..478
mol_type = other DNA
organism = synthetic construct

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SEQUENCE: 87
gcaatcatat gagcgacaaa accaaaacca atgaagggtt tagccgtcgc agctttattg 60
gtagcgagc agttgttacc gcaggcggtg caggtctggg tgcaattgat gcagcaagcg 120
caaccagaaa aaccaatcgt gcaagcaccg ttaaagggtg cttcgattat gatgttggtg 180
tggttggtgg tggttttgcc ggtgcaaccg cagcacgtga atgtggtcag cagggttaacc 240
gtaccctgct gctggaagca cgtagccgtt taggtgggtc tacctttacc agccgttttg 300
cagggtcaaga aattgaattt ggtggtgcat ggggttcattg gttacagccg catgtttggg 360
cagaaatgca gcgttatggt ctgggtgttg ttgaagatcc gctgaccaat ctggataaaa 420
ccctgattat gtataatgac ggtagcgtgg aaagcattag tccggatgaa ttcattagg 478

SEQ ID NO: 88 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
source 1..478
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 88
gcaatcatat gagcgacaaa accaaaacca atgaagggtt tagccgtcgc agctttattg 60
gtagcgagc agttgttacc gcaggcggtg caggtctggg tgcaattgat gcagcaagcg 120
caaccagaaa aaccaatcgt gcaagcaccg ttaaagggtg cttcgattat gatgttggtg 180
tggttggtgg tggttttgcc ggtgcaaccg cagcacgtga atgtggtaac cagggtcagc 240
gtaccctgct gctggaagca cgtagccgtt taggtgggtc tacctttacc agccgttttg 300
cagggtcaaga aattgaattt ggtggtgcat ggggttcattg gttacagccg catgtttggg 360
cagaaatgca gcgttatggt ctgggtgttg ttgaagatcc gctgaccaat ctggataaaa 420
ccctgattat gtataatgac ggtagcgtgg aaagcattag tccggatgaa ttcattagg 478

SEQ ID NO: 89 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
source 1..478
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 89
gcaatcatat gagcgacaaa accaaaacca atgaagggtt tagccgtcgc agctttattg 60
gtagcgagc agttgttacc gcaggcggtg caggtctggg tgcaattgat gcagcaagcg 120
caaccagaaa aaccaatcgt gcaagcaccg ttaaagggtg cttcgattat gatgttggtg 180
tggttggtgg tggttttgcc ggtgcaaccg cagcacgtga atgtggtaac cagggtcatc 240
gtaccctgct gctggaagca cgtagccgtt taggtgggtc tacctttacc agccgttttg 300
cagggtcaaga aattgaattt ggtggtgcat ggggttcattg gttacagccg catgtttggg 360
cagaaatgca gcgttatggt ctgggtgttg ttgaagatcc gctgaccaat ctggataaaa 420
ccctgattat gtataatgac ggtagcgtgg aaagcattag tccggatgaa ttcattagg 478

SEQ ID NO: 90 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
source 1..478
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 90
gcaatcatat gagcgacaaa accaaaacca atgaagggtt tagccgtcgc agctttattg 60
gtagcgagc agttgttacc gcaggcggtg caggtctggg tgcaattgat gcagcaagcg 120
caaccagaaa aaccaatcgt gcaagcaccg ttaaagggtg cttcgattat gatgttggtg 180
tggttggtgg tggttttgcc ggtgcaaccg cagcacgtga atgtggtaac cagggttatc 240
gtaccctctc gctggaagca cgtagccgtt taggtgggtc tacctttacc agccgttttg 300
cagggtcaaga aattgaattt ggtggtgcat ggggttcattg gttacagccg catgtttggg 360
cagaaatgca gcgttatggt ctgggtgttg ttgaagatcc gctgaccaat ctggataaaa 420
ccctgattat gtataatgac ggtagcgtgg aaagcattag tccggatgaa ttcattagg 478

SEQ ID NO: 91 moltype = DNA length = 478
FEATURE Location/Qualifiers
misc_feature 1..478
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
source 1..478
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 91
gcaatcatat gagcgacaaa accaaaacca atgaagggtt tagccgtcgc agctttattg 60
gtagcgagc agttgttacc gcaggcggtg caggtctggg tgcaattgat gcagcaagcg 120
caaccagaaa aaccaatcgt gcaagcaccg ttaaagggtg cttcgattat gatgttggtg 180

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tgggttggtgg tggttttgccc ggtgcaaccg cagcacgtga atgtggtctg cagggttatc 240
gtacctttct gctggaagca cgtagccgtt taggtggctg tacctttacc agccgttttg 300
caggccaaga aattgaattt ggtggtgcat ggggttcattg gttacagccg catgtttggg 360
cagaaatgca gcgttatggt ctgggtggtg ttgaagatcc gctgaccaat ctggataaaa 420
ccctgattat gtataatgac ggtagcgtgg aaagcattag tccggatgaa ttcattagg 478

SEQ ID NO: 92      moltype = DNA length = 478
FEATURE           Location/Qualifiers
misc_feature       1..478
                   note = Description of Artificial Sequence: Synthetic
                   polynucleotide
source             1..478
                   mol_type = other DNA
                   organism = synthetic construct

SEQUENCE: 92
gcaatcatat gagcgacaaa accaaaacca atgaagggtt tagccgtcgc agctttattg 60
gtagcgcagc agttgttacc gcaggcgttg caggctctgg tgcaattgat gcagcaagcg 120
caaccagaaa aaccaatcgt gcaagcaccg ttaaagggtg ctcgattat gatgttggtg 180
tggttggtgg tggttttgccc ggtgcaaccg cagcacgtga atgtggtctg cagggtcgtc 240
gtaccctgct gctggaagca cgtagccgtt taggtggctg tacctttacc agccgttttg 300
caggccaaga aattgaattt ggtggtgcat ggggttcattg gttacagccg catgtttggg 360
cagaaatgca gcgttatggt ctgggtggtg ttgaagatcc gctgaccaat ctggataaaa 420
ccctgattat gtataatgac ggtagcgtgg aaagcattag tccggatgaa ttcattagg 478

SEQ ID NO: 93      moltype = DNA length = 478
FEATURE           Location/Qualifiers
misc_feature       1..478
                   note = Description of Artificial Sequence: Synthetic
                   polynucleotide
source             1..478
                   mol_type = other DNA
                   organism = synthetic construct

SEQUENCE: 93
gcaatcatat gagcgacaaa accaaaacca atgaagggtt tagccgtcgc agctttattg 60
gtagcgcagc agttgttacc gcaggcgttg caggctctgg tgcaattgat gcagcaagcg 120
caaccagaaa aaccaatcgt gcaagcaccg ttaaagggtg ctcgattat gatgttggtg 180
tggttggtgg tggttttgccc ggtgcaaccg cagcacgtga atgtggtctg cagggttatc 240
agaccctgct gctggaagca cgtagccgtt taggtggctg tacctttacc agccgttttg 300
caggccaaga aattgaattt ggtggtgcat ggggttcattg gttacagccg catgtttggg 360
cagaaatgca gcgttatggt ctgggtggtg ttgaagatcc gctgaccaat ctggataaaa 420
ccctgattat gtataatgac ggtagcgtgg aaagcattag tccggatgaa ttcattagg 478

SEQ ID NO: 94      moltype = DNA length = 339
FEATURE           Location/Qualifiers
misc_feature       1..339
                   note = Description of Artificial Sequence: Synthetic
                   polynucleotide
source             1..339
                   mol_type = other DNA
                   organism = synthetic construct

SEQUENCE: 94
gcaatccgcg gttgaacagg ttaatggtgg tgttaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcaccaacg ttattaccgt tccgtgtaat acctataaac atattggtt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcagt tgaaacagaa tctgggtcgt gtttttgcatt tgcagatga 240
acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaactgg gcaccattct 300
gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 95      moltype = DNA length = 339
FEATURE           Location/Qualifiers
misc_feature       1..339
                   note = Description of Artificial Sequence: Synthetic
                   polynucleotide
source             1..339
                   mol_type = other DNA
                   organism = synthetic construct

SEQUENCE: 95
gcaatccgcg gttgaacagg ttaatggtgg tgttaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgccg ttcagaccgt tccgtgtaat acctataaac atattggtt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcagt tgaaacagaa tctgggtcgt gtttttgcatt tgcagatga 240
acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaactgg gcaccattct 300
gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 96      moltype = DNA length = 339
FEATURE           Location/Qualifiers

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misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 96
gcaatccgcg gttgaacagg ttaatggtgg tggtaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgagg ttaacaccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccattct 300
gagcattacc attgcacgta aagaaacat cgatatagg 339

SEQ ID NO: 97 moltype = DNA length = 339
FEATURE Location/Qualifiers

misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 97
gcaatccgcg gttgaacagg ttaatggtgg tggtaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgagg ttaacaccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccattct 300
gagcattacc attgcacgta aagaaacat cgatatagg 339

SEQ ID NO: 98 moltype = DNA length = 339
FEATURE Location/Qualifiers

misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 98
gcaatccgcg gttgaacagg ttaatggtgg tggtaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgagg ttcataccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccattct 300
gagcattacc attgcacgta aagaaacat cgatatagg 339

SEQ ID NO: 99 moltype = DNA length = 339
FEATURE Location/Qualifiers

misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 99
gcaatccgcg gttgaacagg ttaatggtgg tggtaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgagg ttcataccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccattct 300
gagcattacc attgcacgta aagaaacat cgatatagg 339

SEQ ID NO: 100 moltype = DNA length = 339
FEATURE Location/Qualifiers

misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 100
gcaatccgcg gttgaacagg ttaatggtgg tggtaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgagg ttcataccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccattct 300

-continued

gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 101 moltype = DNA length = 339
 FEATURE Location/Qualifiers
 misc_feature 1..339
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
 source 1..339
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 101
 gcaatccgcg gttgaacagg ttaatggtgg tgtaaatac aaaaccgatg acgatgaaat 60
 cattaccgca ggcgttaacg ttattaccgt tccgctgaat acctataaac atattgggtt 120
 tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
 tgccaaactg tatgttcacg tgaacacaga tctgggtcgt gtttttgcac ttgcagatga 240
 acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaactgg gcaccattct 300
 gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 102 moltype = DNA length = 339
 FEATURE Location/Qualifiers
 misc_feature 1..339
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
 source 1..339
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 102
 gcaatccgcg gttgaacagg ttaatggtgg tgtaaatac aaaaccgatg acgatgaaat 60
 cattaccgca ggcgttaacg ttattaccgt tccgctgaat acctataaac atattgggtt 120
 tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
 tgccaaactg tatgttcacg tgaacacaga tctgggtcgt gtttttgcac ttgcagatga 240
 acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaactgg gcaccattct 300
 gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 103 moltype = DNA length = 339
 FEATURE Location/Qualifiers
 misc_feature 1..339
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
 source 1..339
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 103
 gcaatccgcg gttgaacagg ttaatggtgg tgtaaatac aaaaccgatg acgatgaaat 60
 cattaccgca ggcgttggtt ttattaccgt tccgctgaat acctataaac atattgggtt 120
 tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
 tgccaaactg tatgttcacg tgaacacaga tctgggtcgt gtttttgcac ttgcagatga 240
 acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaactgg gcaccattct 300
 gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 104 moltype = DNA length = 339
 FEATURE Location/Qualifiers
 misc_feature 1..339
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
 source 1..339
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 104
 gcaatccgcg gttgaacagg ttaatggtgg tgtaaatac aaaaccgatg acgatgaaat 60
 cattaccgca ggcgttggtt ttatgaccgt tccgctgaat acctataaac atattgggtt 120
 tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
 tgccaaactg tatgttcacg tgaacacaga tctgggtcgt gtttttgcac ttgcagatga 240
 acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaacagg gcaccagcct 300
 gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 105 moltype = DNA length = 339
 FEATURE Location/Qualifiers
 misc_feature 1..339
 note = Description of Artificial Sequence: Synthetic
 polynucleotide
 source 1..339
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 105
 gcaatccgcg gttgaacagg ttaatggtgg tgtaaatac aaaaccgatg acgatgaaat 60

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cattaccgca ggcgttgttg ttatgaccgt tccgtgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaacggg gcaccgcgct 300
gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 106      moltype = DNA length = 339
FEATURE            Location/Qualifiers
misc_feature        1..339
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..339
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 106
gcaatccgcg gttgaacagg ttaatggttg tgtaaatac aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgtgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaacggg gcaccgcgct 300
gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 107      moltype = DNA length = 339
FEATURE            Location/Qualifiers
misc_feature        1..339
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..339
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 107
gcaatccgcg gttgaacagg ttaatggttg tgtaaatac aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgtgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaaaacg gcaccgcgct 300
gagcattacc attgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 108      moltype = DNA length = 339
FEATURE            Location/Qualifiers
misc_feature        1..339
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..339
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 108
gcaatccgcg gttgaacagg ttaatggttg tgtaaatac aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgtgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaaaacg gcaccattct 300
gagcattacc caggcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 109      moltype = DNA length = 339
FEATURE            Location/Qualifiers
misc_feature        1..339
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..339
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 109
gcaatccgcg gttgaacagg ttaatggttg tgtaaatac aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgtgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcacg tgaacacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagaccca tgattatagt gatgaactgg gcaccgcgct 300
gagcattacc accgcacgta aagaaacccat cgatatagg 339

SEQ ID NO: 110      moltype = DNA length = 339
FEATURE            Location/Qualifiers
misc_feature        1..339
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..339

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mol_type = other DNA
organism = synthetic construct

SEQUENCE: 110
gcaatccgcg gttgaacagg ttaatggtgg tgttaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcatg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccgcgaa 300
cagcattacc attgcacgta aagaaacat cgatatagg 339

SEQ ID NO: 111 moltype = DNA length = 339
FEATURE Location/Qualifiers
misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide
source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 111
gcaatccgcg gttgaacagg ttaatggtgg tgttaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcatg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccaccct 300
gagcattacc accgcacgta aagaaacat cgatatagg 339

SEQ ID NO: 112 moltype = DNA length = 339
FEATURE Location/Qualifiers
misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide
source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 112
gcaatccgcg gttgaacagg ttaatggtgg tgttaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcatg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaactgg gcaccaccct 300
gagcattacc attgcacgta aagaaacat cgatatagg 339

SEQ ID NO: 113 moltype = DNA length = 339
FEATURE Location/Qualifiers
misc_feature 1..339
note = Description of Artificial Sequence: Synthetic polynucleotide
source 1..339
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 113
gcaatccgcg gttgaacagg ttaatggtgg tgttaaaatc aaaaccgatg acgatgaaat 60
cattaccgca ggcgttgttg ttatgaccgt tccgctgaat acctataaac atattggttt 120
tacaccggca ctgagcaaag gtaaacagcg ttttatcaaa gaaggtcagc tgagtaaagg 180
tgccaaactg tatgttcatg tgaacagaa tctgggtcgt gtttttgcac ttgcagatga 240
acagcagccg ctgaattggg ttcagacca tgattatagt gatgaaaacg gcaccattct 300
gagcattacc attcagcgta aagaaacat cgatatagg 339

SEQ ID NO: 114 moltype = DNA length = 345
FEATURE Location/Qualifiers
misc_feature 1..345
note = Description of Artificial Sequence: Synthetic polynucleotide
source 1..345
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 114
aaccatcgat gtgaatgacg gtgatgcagt taccctgaa gttcagaaaa tgtttccggg 60
tgttgaagtt ctgggcaccg cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtcagctg agcgcggggtg cagaaaccag caatgggttg catgcaata ttgatgggtc 240
agttgaaagc ggtctgcgtg caggctcgtg agttaaacag ctgctgagcg gtgggtgggtg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcgc 345

SEQ ID NO: 115 moltype = DNA length = 345
FEATURE Location/Qualifiers

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misc_feature 1..345
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..345
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 115
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgtttcggg 60
tgttgaagtt ctgggcaccc cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtgaactg aacgcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtgc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtggtgg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg 345

SEQ ID NO: 116 moltype = DNA length = 345
FEATURE Location/Qualifiers

misc_feature 1..345
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..345
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 116
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgtttcggg 60
tgttgaagtt ctgggcaccc cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtgagctg aacgcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtgc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtggtgg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg 345

SEQ ID NO: 117 moltype = DNA length = 345
FEATURE Location/Qualifiers

misc_feature 1..345
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..345
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 117
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgtttcggg 60
tgttgaagtt ctgggcaccc cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtcagctg caggcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtgc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtggtgg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg 345

SEQ ID NO: 118 moltype = DNA length = 345
FEATURE Location/Qualifiers

misc_feature 1..345
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..345
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 118
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgtttcggg 60
tgttgaagtt ctgggcaccc cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtaccctg caggcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtgc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtggtgg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg 345

SEQ ID NO: 119 moltype = DNA length = 345
FEATURE Location/Qualifiers

misc_feature 1..345
note = Description of Artificial Sequence: Synthetic polynucleotide

source 1..345
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 119
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgtttcggg 60
tgttgaagtt ctgggcaccc cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtgaactg ctggcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtgc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtggtgg 300

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atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg          345

SEQ ID NO: 120      moltype = DNA length = 345
FEATURE            Location/Qualifiers
misc_feature        1..345
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..345
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 120
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgttccggg 60
tggtgaagtt ctgggcaccg cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtattctg tttgcgggtg cagaaaaaag caatgggttg catgcaaata ttgatgggtc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtgggtg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg          345

SEQ ID NO: 121      moltype = DNA length = 345
FEATURE            Location/Qualifiers
misc_feature        1..345
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..345
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 121
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgttccggg 60
tggtgaagtt ctgggcaccg cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtattctg gcggcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtgggtg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg          345

SEQ ID NO: 122      moltype = DNA length = 345
FEATURE            Location/Qualifiers
misc_feature        1..345
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..345
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 122
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgttccggg 60
tggtgaagtt ctgggcaccg cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtattctg gcggcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtgggtg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg          345

SEQ ID NO: 123      moltype = DNA length = 345
FEATURE            Location/Qualifiers
misc_feature        1..345
                    note = Description of Artificial Sequence: Synthetic
                    polynucleotide
source              1..345
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 123
aaccatcgat gtgaatgac gtgatgcagt taccctgaa gttcagaaaa tgttccggg 60
tggtgaagtt ctgggcaccg cagcctatga ttggaccgca gatccgttta gcttaggtgc 120
ctgggcagcg tatggtgttg gtcagctgtc acgtctgaaa gatctgcagg cagcagaagg 180
tcgtgcgctg tatgcgggtg cagaaaccag caatgggttg catgcaaata ttgatgggtc 240
agttgaaagc ggtctgcgtg caggtcgtga agttaaacag ctgctgagcg gtggtgggtg 300
atccggtagc ggtcatcatc accatcatca ttaactcgag aatcg          345

SEQ ID NO: 124      moltype = AA length = 481
FEATURE            Location/Qualifiers
REGION              1..481
                    note = Description of Artificial Sequence: Synthetic
                    polypeptide
source              1..481
                    mol_type = protein
                    organism = synthetic construct

SEQUENCE: 124
SDKTKTNEGFSRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60

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GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEFGGRVWHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFACADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 125          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 125
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEFGGKVVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFACADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 126          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 126
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEFGGTWWHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFACADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 127          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 127
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEFGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFACADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 128          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 128
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60

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GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEPGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFADADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 129          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 129
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEPGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFADADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 130          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 130
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEPGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFADADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 131          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 131
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IEPGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNQLGR VFADADEQQP 360
LNWVQTHDYS DELGTILSIV IARKETIDVN DRDAVTREVQ KMPFGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S                                                                 481

SEQ ID NO: 132          moltype = AA length = 481
FEATURE                Location/Qualifiers
REGION                 1..481
                        note = Description of Artificial Sequence: Synthetic
                        polypeptide
source                 1..481
                        mol_type = protein
                        organism = synthetic construct

SEQUENCE: 132
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60

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GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IFFGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGPLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQKQLGR VFADFADQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMFPGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA LIDGAVESGL RAGREVKQLL 480
S 481

SEQ ID NO: 133      moltype = AA length = 481
FEATURE            Location/Qualifiers
REGION            1..481
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..481
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 133
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IFFGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGPLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQKQLGR VFADFADQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMFPGVEVLG TAAWDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA YIDGAVESGL RAGREVKQLL 480
S 481

SEQ ID NO: 134      moltype = AA length = 432
FEATURE            Location/Qualifiers
REGION            1..432
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..432
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 134
GFDYDVVVVG GGFAGATAAR ECGLQGYRTL LLEARSRLGG RTFTSRFAGQ EIEFGGRVHW 60
WLQPHVWAEM QRYGLGVVED PLTNLDKTLI MYNDGSVESI SPDEFGKNIR IAFEKLCHDA 120
WEVFP RPHEP MFTERARELD KSSVLDRIKT LGLSRLQQAQ INSYMALYAG ETTDKFGLPG 180
VLKLFACGGW NYDAFMDTET HYRIQGGTIG LINAMLTDSG AEVMSVPVPT AVEQVNGGVK 240
IKTDDDEIIT AGVVMTVPL NTYKHIGFTP ALSKGKQRFI KEGQLSKGAK LYVHVQKQLG 300
RVFAFADEQQ PLNWVQTHDY SDELGTILSI TIARKETIDV NDRDAVTREV QKMFPGVEVL 360
GTAAYDWTAD PFLGAWAAYG GVGQLSRLKD LQAAEGRILF AGAETSNGWH ANIDGAVESG 420
LRAGREVKQL LS 432

SEQ ID NO: 135      moltype = AA length = 481
FEATURE            Location/Qualifiers
REGION            1..481
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..481
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 135
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IFFGGAWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGPLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQKQLGR VFADFADQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMFPGVEVLG TAAWDWTADP 420
FSLGARAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S 481

SEQ ID NO: 136      moltype = AA length = 481
FEATURE            Location/Qualifiers
REGION            1..481
                  note = Description of Artificial Sequence: Synthetic
                  polypeptide
source            1..481
                  mol_type = protein
                  organism = synthetic construct

SEQUENCE: 136
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTLL LEARSRLGGR TFTSRFAGQE IFFGGAWVHW LQPHVWAEMQ 120

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RYGLGVVEDP	LTNLDKTLIM	YNDGSVESIS	PDEFGKNIRI	APEKLCHDAW	EVFPRPHEPM	180
FTERARELDK	SSVLDRIKTL	GLSRLQQAQI	NSYMALYAGE	TTDKFGLPGV	LKLFACGGWN	240
YDAFMDTEPH	YRIQGGTIGL	INAMLTDSGA	EVMSVVPVTA	VEQVNGGVKI	KTDDDEIITA	300
GVVMTVPLN	TYKHIGFTPA	LSKGKQRFIK	EGQLSKGAKL	YVHVQNQLGR	VFAFADEQQP	360
LNWVQTHDYS	DELGTILSIT	IARKETIDVN	DRDAVTREVQ	KMFPGEVLG	TAAAYDWTADP	420
FSLGAWAAYG	VGQLSRLKDL	QAAEGRILFA	GAETSNQWHA	NIDGAVESGL	RAGREVKQLL	480
S						481

SEQ ID NO: 137 moltype = AA length = 481
FEATURE Location/Qualifiers
REGION 1..481
 note = Description of Artificial Sequence: Synthetic
 polypeptide
source 1..481
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 137

SDKTKTNEGF	SRRSFIGSAA	VVTAGVAGLG	AIDAASATQK	TNRASTVKGG	FDYDVVVVGG	60
GFAGATAARE	CGLQGYRTLL	LEARSRLGGR	TFTSRFAGQE	IEFGGAWVHW	LQPHVWAEMQ	120
RYGLGVVEDP	LTNLDKTLIM	YNDGSVESIS	PDEFGKNIRI	APEKLCHDAW	EVFPRPHEPM	180
FTERARELDK	SSVLDRIKTL	GLSRLQQAQI	NSYMALYAGE	TTDKFGLPGV	LKLFACGGWN	240
YDAFMDTETH	YRIQGGTIGL	INAMLTDSGA	EVMSVVPVTA	VEQVNGGVKI	KTDDDEIITA	300
GVVMTVPLN	TYKHIGFTPA	LSKGKQRFIK	EGQLSKGAKL	YVHVQNQLGR	VFAFADEQQP	360
LNWVQTHDYS	DELGTILSIT	IARKETIDVN	DRDAVTREVQ	KMFPGEVLG	TAAAYDWTADP	420
FSLGAHAAYG	VGQLSRLKDL	QAAEGRILFA	GAETSNQWHA	FIDGAVESGL	RAGREVKQLL	480
S						481

SEQ ID NO: 138 moltype = AA length = 481
FEATURE Location/Qualifiers
REGION 1..481
 note = Description of Artificial Sequence: Synthetic
 polypeptide
source 1..481
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 138

SDKTKTNEGF	SRRSFIGSAA	VVTAGVAGLG	AIDAASATQK	TNRASTVKGG	FDYDVVVVGG	60
GFAGATAARE	CGLQGYRTLL	LEARSRLGGR	TFTSRFAGQE	IEFGGAWVHW	LQPHVWAEMQ	120
RYGLGVVEDP	LTNLDKTLIM	YNDGSVESIS	PDEFGKNIRI	APEKLCHDAW	EVFPRPHEPM	180
FTERARELDK	SSVLDRIKTL	GLSRLQQAQI	NSYMALYAGE	TTDKFGLPGV	LKLFACGGWN	240
YDAFMDTETH	YRIQGGTIGL	INAMLTDSGA	EVMSVVPVTA	VEQVNGGVKI	KTDDDEIITA	300
GVVMTVPLN	TYKHIGFTPA	LSKGKQRFIK	EGQLSKGAKL	YVHVQNQLGR	VFAFADEQQP	360
LNWVQTHDYS	DELGTILSIT	IARKETIDVN	DRDAVTREVQ	KMFPGEVLG	TAAAYDWTADP	420
FSLGALAAAYG	VGQLSRLKDL	QAAEGRILFA	GAETSNQWHA	MIDGAVESGL	RAGREVKQLL	480
S						481

SEQ ID NO: 139 moltype = AA length = 14
FEATURE Location/Qualifiers
REGION 1..14
 note = Description of Artificial Sequence: Synthetic peptide
source 1..14
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 139

GGGGSGSGHH	HHHH					14
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SEQ ID NO: 140 moltype = AA length = 481
FEATURE Location/Qualifiers
REGION 1..481
 note = Description of Artificial Sequence: Synthetic
 polypeptide
source 1..481
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 140

SDKTKTNEGF	SRRSFIGSAA	VVTAGVAGLG	AIDAASATQK	TNRASTVKGG	FDYDVVVVGG	60
GFAGATAARE	CGLQGYRTLL	LEARSRLGGR	TFTSRFAGQE	IELGGAWVHW	LQPHVWAEMQ	120
RYGLGVVEDP	LTNLDKTLIM	YNDGSVESIS	PDEFGKNIRI	APEKLCHDAW	EVFPRPHEPM	180
FTERARELDK	SSVLDRIKTL	GLSRLQQAQI	NSYMALYAGE	TTDKFGLPGV	LKLFACGGWN	240
YDAFMDTETH	YRIQGGTIGL	INAMLTDSGA	EVMSVVPVTA	VEQVNGGVKI	KTDDDEIITA	300
GVVMTVPLN	TYKHIGFTPA	LSKGKQRFIK	EGQLSKGAKL	YVHVQNQLGR	VFAFADEQQP	360
LNWVQTHDYS	DELGTILSIT	IARKETIDVN	DRDAVTREVQ	KMFPGEVLG	TAAAYDWTADP	420
FSLGAWAAYG	VGQLSRLKDL	QAAEGRILFA	GAETSNQWHA	NIDGAVESGL	RAGREVKQLL	480
S						481

SEQ ID NO: 141 moltype = AA length = 481

-continued

FEATURE Location/Qualifiers
REGION 1..481
note = Description of Artificial Sequence: Synthetic polypeptide
source 1..481
mol_type = protein
organism = synthetic construct

SEQUENCE: 141
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTL LLEARSRLGGR TFTSRFAGQE IEFGSAAVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNLGR VFAPADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMFPGVEVLG TAAYDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S 481

SEQ ID NO: 142 moltype = AA length = 481
FEATURE Location/Qualifiers
REGION 1..481
note = Description of Artificial Sequence: Synthetic polypeptide
source 1..481
mol_type = protein
organism = synthetic construct

SEQUENCE: 142
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTL LLEARSRLGGR TFTSRFAGQE IEFGGHWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNLGR VFAPADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMFPGVEVLG TAAYDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S 481

SEQ ID NO: 143 moltype = AA length = 481
FEATURE Location/Qualifiers
REGION 1..481
note = Description of Artificial Sequence: Synthetic polypeptide
source 1..481
mol_type = protein
organism = synthetic construct

SEQUENCE: 143
SDKTKTNEGF SRRSFIGSAA VVTAGVAGLG AIDAASATQK TNRASTVKGG FDYDVVVVGG 60
GFAGATAARE CGLQGYRTL LLEARSRLGGR TFTSRFAGQE IEFGGPWVHW LQPHVWAEMQ 120
RYGLGVVEDP LTNLDKTLIM YNDGSVESIS PDEFGKNIRI AFEKLCHDAW EVFPRPHEPM 180
FTERARELDK SSVLDRIKTL GLSRLQQAQI NSYMALYAGE TTDKFGLPGV LKLFACGGWN 240
YDAFMDTETH YRIQGGTIGL INAMLTDSGA EVRMSVPVTA VEQVNGGVKI KTDDDEIITA 300
GVVMTVPLN TYKHIGFTPA LSKGKQRFIK EGQLSKGAKL YVHVQNLGR VFAPADEQQP 360
LNWVQTHDYS DELGTILSIT IARKETIDVN DRDAVTREVQ KMFPGVEVLG TAAYDWTADP 420
FSLGAWAAYG VGQLSRLKDL QAAEGRILFA GAETSNGWHA NIDGAVESGL RAGREVKQLL 480
S 481

SEQ ID NO: 144 moltype = AA length = 6
FEATURE Location/Qualifiers
REGION 1..6
note = Description of Artificial Sequence: Synthetic 6xHis tag
source 1..6
mol_type = protein
organism = synthetic construct

SEQUENCE: 144
HHHHHH 6

1-80. (canceled)

81. A method of treating nicotine addiction, facilitating smoking cessation, treating nicotine poisoning or treating nicotine toxicity comprising administering to a mammalian subject in need thereof a therapeutically effective amount of a nicotine-degrading enzyme variant, wherein the variant comprises an amino acid sequence having at least 90% sequence identity to a sequence selected from (i) SEQ ID NO: 1 and (ii) SEQ ID NO: 1 having an N-terminal deletion of up to 52 amino acids, and wherein the variant comprises at least one substitution at an amino acid position selected from positions 91, 104, 106, 107, 217, 250, 340, 355, 366, 381, 427, 462, and 463 of SEQ ID NO:1, and has nicotine-degrading activity, provided that if the variant is F335V then the variant has an N-terminal deletion of up to 52 amino acids.

82. The method of claim **81**, wherein the mammalian subject is a human subject.

83. The method of claim **82**, wherein the variant sequence has at least 95% sequence identity to a sequence selected from (i) SEQ ID NO: 1 and (ii) SEQ ID NO: 1 having an N-terminal deletion of up to 52 amino acids, and wherein the variant comprises at least one substitution at an amino acid position selected from positions 91, 104, 106, 107, 217, 250, 340, 366, 381, 427, 462, and 463 of SEQ ID NO:1

84. The method of claim **83**, wherein the variant comprises at least one substitution selected from:

- a. R91A, R91Q, R91F, R91G, R91T, R91L, R91S, and R91N;
- b. F104L;
- c. G106S;
- d. A107H, A107P, A107R, A107K, and A107T;
- e. L217Q, L217G, L217E, L217I, L217C, and L217S;
- f. T250P, T250G, T250L, T250R, and T250V;
- g. K340P, K340I, K340V, K340D and K340E;
- h. Q366K, Q366E, Q366V, Q366L, Q366I, and Q366Y;
- i. T381P, T381I, T381V, T381Q, T381N, T381L, and T381M;
- j. W427R, W427H, W427L, W427Q, W427E, W427S, or W427M
- k. N462M, N462L, N462Y, N462S, N462F, N462G, N462E, and N462A; and
- l. I463F, I463Y, I463A, I463V, and I463L.

85. The method of claim **82**, wherein the variant comprises an F355C substitution.

86. The method of claim **83**, wherein the variant comprises at least one substitution selected from A107R and A107T.

87. The method of claim **82**, wherein the variant further comprises at least one substitution, addition, or deletion in an immunogenic T-cell epitope at an amino acid position selected from positions 74, 77, 78, 80, 262-266, 303, 304, 306, 310, 374, 377, 378, 382, 383, 450-452, and 457 of SEQ ID NO:1.

88. The method of claim **82**, wherein the variant further comprises at least one substitution, addition, or deletion in an immunogenic T-cell epitope at an amino acid position selected from positions 16-24, 73-81, 258-266, 302-310, 373-381, and 447-455 of SEQ ID NO: 1.

89. The method of claim **82**, wherein the variant further comprises at least one substitution, addition, or deletion at a position selected from:

- (a) amino acid positions 74, 77, 78, or 80 of SEQ ID NO: 1;

- (b) amino acid positions 262, 263, 264, or 266 of SEQ ID NO: 1;

- (c) amino acid positions 303, 304, 306, or 310 of SEQ ID NO: 1;

- (d) amino acid positions 374, 377, 378, 382, or 383 of SEQ ID NO: 1; and/or

- (e) amino acid positions 450, 451, 452, or 457 of SEQ ID NO: 1.

90. The method of claim **83**, wherein the variant further comprises at least one substitution or substitution combination selected from:

- (a) L74N and Y77R;
- (b) L74N and Y77K;
- (c) L74Q and Y77R;
- (d) L74Q and Y77N;
- (e) L74N and Y77Q;
- (f) L74N and Y77H;
- (g) L74N and L80H;
- (h) L80F;
- (i) Y77R;
- (j) R78Q;
- (k) I262A and A264Q;
- (l) I262K and L266D;
- (m) I262T;
- (n) I262S;
- (o) I262D and L266K;
- (p) I262A
- (q) I262T and A264L;
- (r) I262T and N263R;
- (s) M265H;
- (t) I262A and A264N;
- (u) V303T, V304N, and M306I;
- (v) V304A and M306Q;
- (w) V304A, M306N;
- (x) V304A;
- (y) V304A and M306H;
- (z) V304N and M306H;
- (aa) V304Q and M306H;
- (bb) V304N, M306I;
- (cc) V304T and M306I;
- (dd) L374Q and I377S;
- (ee) L374A and I377A;
- (ff) L374Q and I377A;
- (gg) L374N and I377A;
- (hh) L374N and I382Q;
- (ii) I377A and I382T;
- (jj) I377A and L378N;
- (kk) I377T and I382T;
- (ll) I377T; and
- (mm) L374N and A383Q.
- (nn) I448Q and F450S;
- (oo) I448E and F450N;
- (pp) I448A and F450N;
- (qq) I448Q and F450Q;
- (rr) I448T and F450Q;
- (ss) I448E and F450L;
- (tt) T455K;
- (uu) L449H and F450A;
- (vv) F450A;
- (ww) I448A and F450Y.
- (xx) I262T;
- (yy) I262S;

(zz) I262A;
(aaa) I262T and A264L;
(bbb) I262T and N263R; and
(ccc) M306I and L310R.

91. The method of claim **82**, comprising a deletion of at least amino acids 1-38 of SEQ ID NO:1 or a deletion of amino acids 1-50 of SEQ ID NO:1.

92. The method of claim **86**, comprising a deletion of at least amino acids 1-38 of SEQ ID NO:1 or a deletion of amino acids 1-50 of SEQ ID NO:1.

93. The method of claim **86**, wherein the variant is fused to a compound selected from the group consisting of an albumin-binding peptide, an albumin-binding protein domain, human serum albumin, an inert polypeptide, recombinant PEG (XTEN), a homo-amino acid polymer (HAP), a proline-alanine serine polymer (PAS), and an elastin-like peptide (ELP), and polyethylene glycol (PEG).

94. The method of claim **86**, wherein the variant is PEGylated.

95. The method of claim **82**, wherein the variant comprises an amino acid sequence selected from SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 135, 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: 129, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 136, 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: 130, 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: 131, SEQ ID NO:

39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 132, SEQ ID NO: 133, 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: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 134, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 140, and SEQ ID NO: 141.

96. The method of claim **83**, wherein the variant comprises the amino sequence of any of SEQ ID NOs: 124, 126, or 134.

97. The method of claim **81**, wherein the variant consists of the amino sequence of any of SEQ ID NOs: 124, 126, or 134 optionally fused to a compound selected from the group consisting of an albumin-binding peptide, an albumin-binding protein domain, human serum albumin, an inert polypeptide, recombinant PEG (XTEN), a homo-amino acid polymer (HAP), a proline-alanine serine polymer (PAS), and an elastin-like peptide (ELP), and polyethylene glycol (PEG).

98. The method of claim **86**, wherein the method treats nicotine addiction or facilitates smoking cessation in a patient in need thereof.

99. The method of claim **86**, wherein the method treats nicotine poisoning or nicotine toxicity in a patient in need thereof.

100. The method of claim **97**, wherein the method treats nicotine addiction or facilitates smoking cessation in a patient in need thereof.

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