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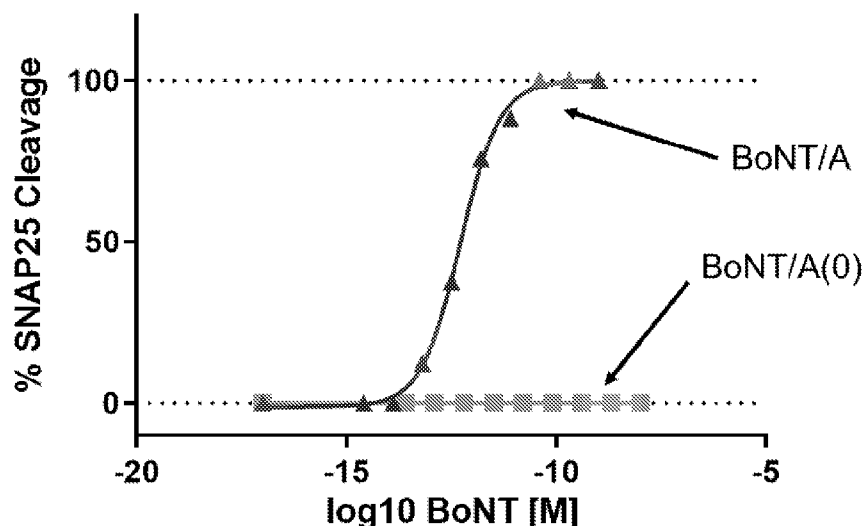
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FIGURE 1



(57) Abstract: The present invention is directed to a polypeptide for use in treating pain or an inflammatory disorder, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (HN domain) and/or a clostridial neurotoxin receptor binding domain (Hc domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. Also provided are corresponding methods of treatment and uses.

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CATALYTICALLY INACTIVE CLOSTRIDIAL NEUROTOXINS FOR THE TREATMENT OF PAIN & INFLAMMATORY DISORDERS

FIELD OF THE INVENTION

- 5 The present invention relates to polypeptides for use in therapy, for example, the use of polypeptides for the treatment of pain or an inflammatory disorder.

BACKGROUND

- Bacteria in the genus *Clostridia* produce highly potent and specific protein toxins, which can
10 poison neurons and other cells to which they are delivered. Examples of such clostridial toxins include the neurotoxins produced by *C. tetani* (TeNT) and by *C. botulinum* (BoNT) serotypes A-G, and X (see WO 2018/009903 A2), as well as those produced by *C. baratii* and *C. butyricum*. Both tetanus and botulinum toxins act by inhibiting the function of affected neurons, specifically the release of neurotransmitters. While botulinum toxin acts at the neuromuscular
15 junction and inhibits cholinergic transmission in the peripheral nervous system, tetanus toxin acts in the central nervous system.

- In nature, clostridial neurotoxins are synthesised as a single-chain polypeptide that is modified post-translationally by a proteolytic cleavage event to form two polypeptide chains joined
20 together by a disulphide bond. Cleavage occurs at a specific cleavage site, often referred to as the activation site that is located between the cysteine residues that provide the inter-chain disulphide bond. It is this di-chain form that is the active form of the toxin. The two chains are termed the heavy chain (H-chain), which has a molecular mass of approximately 100 kDa, and the light chain (L-chain), which has a molecular mass of approximately 50 kDa. The H-chain
25 comprises an N-terminal translocation component (H_N domain) and a C-terminal targeting component (H_C domain). The cleavage site is located between the L-chain and the translocation domain components. Following binding of the H_C domain to its target neuron and internalisation of the bound toxin into the cell via an endosome, the H_N domain translocates the L-chain across the endosomal membrane and into the cytosol, and the L-chain provides a
30 protease function (also known as a non-cytotoxic protease).

- Non-cytotoxic proteases act by proteolytically cleaving intracellular transport proteins known as SNARE proteins (e.g. SNAP-25, VAMP, or Syntaxin). The acronym SNARE derives from the term Soluble NSF Attachment Receptor, where NSF means N-ethylmaleimide-Sensitive
35 Factor. SNARE proteins are integral to intracellular vesicle fusion, and thus to secretion of molecules via vesicle transport from a cell. The protease function is a zinc-dependent endopeptidase activity and exhibits a high substrate specificity for SNARE proteins.

Accordingly, once delivered to a desired target cell, the non-cytotoxic protease is capable of inhibiting cellular secretion from the target cell. The L-chain proteases of clostridial neurotoxins are non-cytotoxic proteases that cleave SNARE proteins.

- 5 In view of the ubiquitous nature of SNARE proteins, clostridial neurotoxins such as botulinum toxin have been successfully employed in a wide range of therapies.

The clostridial neurotoxins are some of the most potent toxins known. By way of example, botulinum neurotoxins have median lethal dose (LD₅₀) values for mice ranging from 0.5 to 5
10 ng/kg, depending on the serotype. Thus, use of said toxins is not without risk. Spread of toxin away from an administration site and into surrounding tissue or systemic circulation is believed to be responsible for undesirable side effects of clostridial neurotoxin treatment that in extreme cases may be life threatening. This can be a particular concern when using clostridial neurotoxins at high doses, concentrations, and/or injection volumes. Adverse effects that have
15 been reported for commercial BoNT/A therapeutics include asthenia, generalised muscle weakness, diplopia, ptosis, dysphagia, dysphonia, dysarthria, urinary incontinence, and breathing difficulties. Swallowing and breathing difficulties can be life threatening and there have been reported deaths related to the spread of toxin effects.

- 20 The present invention overcomes one or more of the above-mentioned problems.

SUMMARY OF THE INVENTION

The present inventors have found that catalytically inactive clostridial neurotoxins are effective at treating pain. This finding is particularly surprising, as catalytic activity resulting in SNARE
25 protein cleavage was believed to be an essential mechanism of action underlying clostridial neurotoxin therapy. The polypeptides of the invention thus avoid the toxic side-effects associated with conventional catalytically active clostridial neurotoxin therapy and constitute a safer (substantially non-toxic) therapeutic. Advantageously, the polypeptides of the present invention may be dosed in greater amounts when compared to a conventional catalytically
30 active clostridial neurotoxin therapeutic. Furthermore, the reduced toxicity of the polypeptides of the invention allows for ease of manufacture and handling throughout the product lifecycle and removes the need for physicians to perform complex (e.g. personalised) dosing regimen calculations aimed at avoiding toxicity in a subject.

- 35 Equally surprisingly, the present inventors have found that catalytically inactive clostridial neurotoxins are effective at treating inflammatory disorders.

DETAILED DESCRIPTION

In one aspect, the invention provides a polypeptide (e.g. an analgesic polypeptide) for use in treating pain, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

In a related aspect, there is provided a method for treating pain, the method comprising administering a polypeptide (e.g. an analgesic polypeptide) to a subject, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

In another related aspect, the invention provides the use of a polypeptide (e.g. an analgesic polypeptide) in the manufacture of a medicament for treating pain, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

The polypeptide of the invention preferably has analgesic properties. In other words, a polypeptide of the invention is preferably an analgesic polypeptide.

Preferably, a polypeptide of the invention neither promotes neuronal growth nor neuronal repair to treat pain. In other words, preferably, the polypeptide does not treat pain by any of the following means: by promoting neuronal growth, by promoting neuronal repair, or by promoting neuronal growth and repair.

In one aspect, the invention provides a polypeptide for use in treating an inflammatory disorder, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

In a related aspect, there is provided a method for treating an inflammatory disorder, the method comprising administering a polypeptide to a subject, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain),
5 wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

In another related aspect, the invention provides the use of a polypeptide in the manufacture of a medicament for treating an inflammatory disorder, wherein the polypeptide comprises a
10 clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

The polypeptide of the invention may have anti-inflammatory properties. In other words, a
15 polypeptide of the invention may be an anti-inflammatory polypeptide.

Where a polypeptide is used in treating an inflammatory disorder as described herein, the polypeptide may comprise a botulinum neurotoxin serotype X (BoNT/X) L-chain, a BoNT/X H_N domain, and/or a BoNT/X H_C domain, wherein when the polypeptide comprises a clostridial
20 neurotoxin L-chain, the L-chain is catalytically inactive. For example, the polypeptide may be a chimeric botulinum neurotoxin (BoNT) comprising a catalytically inactive BoNT/X light-chain and translocation domain, and a receptor binding domain (H_C domain) from a different (i.e. non-BoNT/X) clostridial neurotoxin. Thus, in one aspect, the invention provides a polypeptide for use in treating an inflammatory disorder, wherein the polypeptide comprises a catalytically
25 inactive BoNT/X light-chain and translocation domain, and a receptor binding domain (H_C domain) from a different (i.e. non-BoNT/X) clostridial neurotoxin (preferably a BoNT/B H_C domain). Corresponding methods of treatment and uses are also provided.

Preferably, a polypeptide of the invention neither promotes neuronal growth nor neuronal
30 repair to treat an inflammatory condition. In other words, preferably, the polypeptide does not treat an inflammatory condition by any of the following means: by promoting neuronal growth, by promoting neuronal repair, or by promoting neuronal growth and repair

The term “promotes neuronal growth and/or neuronal repair” encompasses an increase in the
35 rate of neuronal growth and/or neuronal repair. The term “neuronal growth and/or neuronal repair” encompasses the rebuilding of damaged neuronal circuits, thereby restoring activity

and/or neuronal communication in a network or population of neurons. Thus, the term “neuronal repair” as used herein encompasses repair of a specific neuron as well as repair of a neuronal circuit. The term also encompasses neuronal plasticity. The term “neuronal plasticity” as used herein encompasses axonal sprouting, dendritic sprouting, neurogenesis (e.g. the production of new neurons), maturation, differentiation, and/or synaptic plasticity (e.g. including changes to synaptic strength, activity, anatomy, and/or connectivity). The term “promotes neuronal growth and/or neuronal repair” also encompasses promoting the establishment of functional synapses (e.g. at or near to a site of injury). The term “neuronal growth” as used herein encompasses growth of any part of a neuron, including growth of axons and/or dendrites. Said term encompasses an increase neurite length, neurite number (e.g. number of neurites per cell), and/or an increase the length and/or numbers of projections from a cell body or cell membrane of a neuron, e.g. axonal growth of a neuron and/or axonal sprouting, e.g. a neuron in a subject. Said axonal growth may promote connections and/or chemical communication between neurons.

Preferably, a polypeptide of the invention does not promote a neuroimmune response to treat pain or an inflammatory disorder. A neuroimmune response in this context encompasses a microglial response. Thus, in one embodiment a polypeptide of the invention does not promote a microglial response to treat pain or an inflammatory condition.

In a preferred embodiment, the pain is not pain associated with, or caused by, a brain disorder. In another preferred embodiment, the inflammatory disorder is not an inflammatory brain disorder. The term “brain disorder” used in this context is interchangeable with “brain disease”. A “brain disorder” as used in this context encompasses a disorder that originates from within or outside the brain, and includes disorders associated with bodily insults that cause brain tissue damage. Examples of brain disorders encompassed in this context include any one (or more) of traumatic brain injury, cancer (e.g. a brain tumour), infectious disease (e.g. encephalitis, meningitis, a brain abscess, and encephalitis), stroke, a neurodegenerative disorder (e.g. Alzheimer’s disease, Parkinson’s disease, Parkinson’s disease related disorders, motor neuron disease (e.g. amyotrophic lateral sclerosis), prion disease, Huntington’s disease, spinocerebellar ataxia, ataxia, Hallervorden-Spatz disease, and frontotemporal lobar degeneration), brain aneurysm, multiple sclerosis, anoxic injury, toxic injury and metabolic injury. A brain disorder may be caused by traumatic brain injury, cancer, infectious disease (e.g. encephalitis, meningitis, a brain abscess, and encephalitis), stroke, a neurodegenerative disorder (e.g. Alzheimer’s disease, Parkinson’s disease, Parkinson’s disease related disorders, motor neuron disease (e.g. amyotrophic lateral sclerosis), prion

disease, Huntington's disease, spinocerebellar ataxia, ataxia, Hallervorden-Spatz disease, and frontotemporal lobar degeneration), brain aneurysm, multiple sclerosis, anoxic injury, toxic injury and/or metabolic injury.

- 5 Active clostridial neurotoxin L-chain has non-cytotoxic protease activity. Specifically, active clostridial neurotoxin L-chain has endopeptidase activity and is capable of cleaving a protein of the exocytic fusion apparatus in a target cell. A protein of the exocytic fusion apparatus is preferably a SNARE protein, such as SNAP25, synaptobrevin/VAMP, or syntaxin.
- 10 The term "catalytically inactive" as used herein in respect of a clostridial neurotoxin L-chain means that said L-chain exhibits substantially no non-cytotoxic protease activity, preferably the term "catalytically inactive" as used herein in respect of a clostridial neurotoxin L-chain means that said L-chain exhibits no non-cytotoxic protease activity. In one embodiment, a catalytically inactive clostridial neurotoxin L-chain is one that does not cleave a protein of the exocytic
- 15 fusion apparatus in a target cell. The term "substantially no non-cytotoxic protease activity" means that the clostridial neurotoxin L-chain has less than 5% of the non-cytotoxic protease activity of a catalytically active clostridial neurotoxin L-chain, for example less than 2%, 1% or preferably less than 0.1% of the non-cytotoxic protease activity of a catalytically active clostridial neurotoxin L-chain. Non-cytotoxic protease activity can be determined *in vitro* by
- 20 incubating a test clostridial neurotoxin L-chain with a SNARE protein and comparing the amount of SNARE protein cleaved by the test clostridial neurotoxin L-chain when compared to the amount of SNARE protein cleaved by a catalytically active clostridial neurotoxin L-chain under the same conditions. Routine techniques, such as SDS-PAGE and Western blotting can be used to quantify the amount of SNARE protein cleaved. Suitable *in vitro* assays are
- 25 described in WO 2019/145577 A1, which is incorporated herein by reference.

Cell-based and *in vivo* assays may also be used to determine if a clostridial neurotoxin comprising an L-chain and a functional cell binding and translocation domain has non-cytotoxic protease activity. Assays such as the Digit Abduction Score (DAS) assay, the dorsal root ganglia (DRG) assay, spinal cord neuron (SCN) assay, and mouse phrenic nerve hemidiaphragm (PNHD) assay are routine in the art. A suitable assay for determining non-cytotoxic protease activity may be one described in Aoki KR, Toxicon 39: 1815-1820; 2001 or Donald *et al* (2018), Pharmacol Res Perspect, e00446, 1-14, which are incorporated herein by reference.

A catalytically inactive L-chain may have one or more mutations that inactivate said catalytic activity. Thus, a catalytically active L-chain (e.g. as described herein) may be modified to introduce one or more mutations that inactivate the catalytic activity of the L-chain. For example, a catalytically inactive L-chain may comprise a mutation of an active site residue. A mutation may be a substitution or a deletion, however a substitution is preferred, in particular substitution with a chemically-similar amino acid. Glutamic acid may be substituted with glutamine, histidine may be substituted with tyrosine, arginine may be substituted with glutamine, and/or tyrosine may be substituted with phenylalanine. Alternatively, any residue may be substituted with alanine.

A catalytically inactive BoNT/A L-chain may comprise a mutation at H223, E224, H227, E262, R363, and/or Y366, preferably at at least E224 and H227. Preferably, a catalytically inactive BoNT/A L-chain may comprise substitution at E224 with glutamine (E224Q) and substitution at H227 with tyrosine (H227Y). The position numbering corresponds to the amino acid positions of SEQ ID NO: 60 and can be determined by aligning a polypeptide with SEQ ID NO: 60. As the presence of a methionine residue at position 1 of SEQ ID NO: 60 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering. For example, where SEQ ID NO: 60 includes a methionine, the position numbering will be as defined above (e.g. His223 will be His223 of SEQ ID NO: 60). Alternatively, where the methionine is absent from SEQ ID NO: 60 the amino acid residue numbering should be modified by -1 (e.g. His223 will be His222 of SEQ ID NO: 60). Similar considerations apply when the methionine at position 1 of the other polypeptide sequences described herein is present/absent, and the skilled person will readily determine the correct amino acid residue numbering using techniques routine in the art.

A catalytically inactive BoNT/B L-chain may comprise a mutation at E231 and/or H234, preferably E231 and H234. Preferably, a catalytically inactive BoNT/B L-chain comprises substitution at E231 with glutamine (E231Q) and substitution at H234 with tyrosine (H234Y). The position numbering corresponds to the amino acid positions of SEQ ID NO: 52 and can be determined by aligning a polypeptide with SEQ ID NO: 52. As the presence of a methionine residue at position 1 of SEQ ID NO: 52 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering.

A catalytically inactive BoNT/C L-chain may comprise a mutation at H229, E230 and/or H233, preferably H229, E230 and H233. Preferably, a catalytically inactive BoNT/C L-chain

comprises substitution at H229 with glycine (H229G), substitution at E230 with threonine (E230T), and substitution at H233 with asparagine (H233N). The position numbering corresponds to the amino acid positions of SEQ ID NO: 53 and can be determined by aligning a polypeptide with SEQ ID NO: 53. As the presence of a methionine residue at position 1 of SEQ ID NO: 53 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering.

A catalytically inactive BoNT/D L-chain may comprise a mutation at H229, E230, H233 and/or H236, preferably at at least E230 and H236. Preferably, a catalytically inactive BoNT/D L-chain comprises at least substitution at E230 with glutamine (E230Q) and substitution at H236 with tyrosine (H236Y). The position numbering corresponds to the amino acid positions of SEQ ID NO: 54 and can be determined by aligning a polypeptide with SEQ ID NO: 54. As the presence of a methionine residue at position 1 of SEQ ID NO: 54 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering.

A catalytically inactive BoNT/E L-chain may comprise a mutation at E213 and/or H216, preferably at E213 and H216. Preferably, a catalytically inactive BoNT/E L-chain comprises substitution at E213 with glutamine (E213Q) and H216 with tyrosine (H216Y). The position numbering corresponds to the amino acid positions of SEQ ID NO: 55 and can be determined by aligning a polypeptide with SEQ ID NO: 55. As the presence of a methionine residue at position 1 of SEQ ID NO: 55 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering.

A catalytically inactive BoNT/F L-chain may comprise a mutation at E228 and/or H231, preferably at E228 and H231. Preferably, a catalytically inactive BoNT/F L-chain comprises substitution at E228 with glutamine (E228Q) and H231 with tyrosine (H231Y). The position numbering corresponds to the amino acid positions of SEQ ID NO: 56 and can be determined by aligning a polypeptide with SEQ ID NO: 56. As the presence of a methionine residue at position 1 of SEQ ID NO: 56 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering.

A catalytically inactive BoNT/G L-chain may comprise a mutation at E231 and/or H234, preferably at E231 and H234. Preferably, a catalytically inactive BoNT/G L-chain comprises substitution at E231 with glutamine (E231Q) and H234 with tyrosine (H234Y). The position numbering corresponds to the amino acid positions of SEQ ID NO: 57 and can be determined

by aligning a polypeptide with SEQ ID NO: 57. As the presence of a methionine residue at position 1 of SEQ ID NO: 57 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering.

- 5 A catalytically inactive BoNT/X L-chain may comprise a mutation at E228 and/or H231, preferably at E228 and H231. Preferably, a catalytically inactive BoNT/X L-chain comprises substitution at E228 with glutamine (E228Q) and H231 with tyrosine (H231Y). The position numbering corresponds to the amino acid positions of SEQ ID NO: 59 and can be determined by aligning a polypeptide with SEQ ID NO: 59. As the presence of a methionine residue at
10 position 1 of SEQ ID NO: 59 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering.

- A catalytically inactive TeNT L-chain may comprise a mutation at E234, R372, and/or Y375, preferably at at least R372 and Y375 (e.g. at E234, R372, and Y375). Preferably, a catalytically
15 inactive TeNT L-chain comprises substitution at R372 with glutamine or alanine (R372Q or R372A), more preferably with alanine, and substitution at Y375 with phenylalanine (Y375F). The position numbering corresponds to the amino acid positions of SEQ ID NO: 58 and can be determined by aligning a polypeptide with SEQ ID NO: 58. As the presence of a methionine residue at position 1 of SEQ ID NO: 58 is optional, the skilled person will take the
20 presence/absence of the methionine residue into account when determining amino acid residue numbering.

- The polypeptide of the invention may comprise a full-length clostridial neurotoxin (with the proviso that the L-chain is catalytically inactive) or fragments of clostridial neurotoxins that do
25 not have non-cytotoxic protease activity (e.g. the H_N domain and/or H_C domain). In other words, the polypeptides of the invention do not have non-cytotoxic protease activity.

- The term "clostridial neurotoxin" embraces toxins produced by *C. botulinum* (botulinum neurotoxin serotypes A, B, C₁, D, E, F, G, and X), *C. tetani* (tetanus neurotoxin), *C. butyricum*
30 (botulinum neurotoxin serotype E), and *C. baratii* (botulinum neurotoxin serotype F). A reference BoNT/A sequence is shown as SEQ ID NO: 51. A reference BoNT/B sequence is shown as SEQ ID NO: 52. A reference BoNT/C sequence is shown as SEQ ID NO: 53. A reference BoNT/D sequence is shown as SEQ ID NO: 54. A reference BoNT/E sequence is shown as SEQ ID NO: 55. A reference BoNT/F sequence is shown as SEQ ID NO: 56. A
35 reference BoNT/G sequence is shown as SEQ ID NO: 57. A reference TeNT sequence is shown as SEQ ID NO: 58. A reference BoNT/X sequence is shown as SEQ ID NO: 59. The

term “clostridial neurotoxin” may also embrace newly discovered botulinum neurotoxin protein family members expressed by non-clostridial microorganisms, such as the *Enterococcus* encoded toxin which has closest sequence identity to BoNT/X, the *Weissella oryzae* encoded toxin called BoNT/Wo (NCBI Ref Seq: WP_027699549.1), which cleaves VAMP2 at W89-W90, the *Enterococcus faecium* encoded toxin (GenBank: OTO22244.1), which cleaves VAMP2 and SNAP25, and the *Chryseobacterium piperi* encoded toxin (NCBI Ref.Seq: WP_034687872.1).

Thus, a clostridial neurotoxin may be selected from BoNT/A, BoNT/B, BoNT/C, BoNT/D, BoNT/E, BoNT/F, BoNT/G, BoNT/X, and TeNT (tetanus neurotoxin). Preferably, a clostridial neurotoxin is a botulinum neurotoxin, such as a botulinum neurotoxin selected from BoNT/A, BoNT/B, BoNT/C, BoNT/D, BoNT/E, BoNT/F, BoNT/G, and BoNT/X. For example, a clostridial neurotoxin H_N domain may be a H_N domain from BoNT A B, C₁, D, E, F, G, X or TeNT. Similarly, an L-chain may be an L-chain from BoNT A B, C₁, D, E, F, G, X or TeNT with the proviso that said L-chain is catalytically inactive (e.g. has been modified to render it catalytically inactive). More preferably, the clostridial neurotoxin is BoNT/A.

As discussed above, (full-length) clostridial neurotoxins are formed from two polypeptide chains, the heavy chain (H-chain), which has a molecular mass of approximately 100 kDa, and the light chain (L-chain), which has a molecular mass of approximately 50 kDa. The H-chain comprises a C-terminal targeting component (receptor binding domain or H_C domain) and an N-terminal translocation component (H_N domain). Botulinum neurotoxin (BoNT) is produced by *C. botulinum* in the form of a large protein complex, consisting of BoNT itself complexed to a number of accessory proteins. There are at present eight different classes of botulinum neurotoxin, namely: botulinum neurotoxin serotypes A, B, C₁, D, E, F, G, and X all of which share similar structures and modes of action. Different BoNT serotypes can be distinguished based on inactivation by specific neutralising anti-sera, with such classification by serotype correlating with percentage sequence identity at the amino acid level. BoNT proteins of a given serotype are further divided into different subtypes on the basis of amino acid percentage sequence identity.

Conventional (catalytically active) BoNTs are absorbed in the gastrointestinal tract, and, after entering the general circulation, bind to the presynaptic membrane of cholinergic nerve terminals and prevent the release of their neurotransmitter acetylcholine. BoNT/B, BoNT/D, BoNT/F and BoNT/G cleave synaptobrevin/vesicle-associated membrane protein (VAMP); BoNT/C₁, BoNT/A and BoNT/E cleave the synaptosomal-associated protein of 25 kDa (SNAP-25); and BoNT/C₁ cleaves syntaxin. BoNT/X has been found to cleave SNAP-25, VAMP1,

VAMP2, VAMP3, VAMP4, VAMP5, Ykt6, and syntaxin 1. Tetanus toxin is produced in a single serotype by *C. tetani*. *C. butyricum* produces BoNT/E, while *C. baratii* produces BoNT/F.

In one embodiment, a polypeptide of the invention may be encoded by a nucleotide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, or 49 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. In one embodiment, a polypeptide of the invention may be encoded by a nucleotide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, or 49 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. Preferably, a polypeptide of the invention may be encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 1, 7, 9, 11, 13, 15, 17, 21, 25, 29, 33, 37, 41, 43, 45, 47, or 49.

In one embodiment a polypeptide of the invention may comprise a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 74, 75 or 76 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. In one embodiment a polypeptide of the invention may comprise a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 74, 75 or 76 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. Preferably, a polypeptide of the invention may comprise a polypeptide sequence of any one of SEQ ID NOs: 2, 8, 10, 12, 14, 16, 18, 22, 26, 30, 34, 38, 42, 44, 46, 48, 50, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 74, 75 or 76.

In one embodiment a polypeptide of the invention may comprise a fragment of a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 2, 10, 12, 14, 16, 18, 26, 34, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. In one embodiment a polypeptide of the invention may comprise a fragment of a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 2, 10, 12, 14, 16, 18, 26, 34, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60,

61, 62, 63, 64, 65, 66, 67, 68, 69 or 70 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. Preferably, a polypeptide of the invention may comprise a fragment of a polypeptide sequence comprising any one of SEQ ID NOs: 2, 10, 12, 14, 16, 18, 26, 34, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63,
5 64, 65, 66, 67, 68, 69 or 70 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive. The fragment may be a catalytically inactive L-chain, H_N domain or H_C domain of any of said SEQ ID NOs.

Preferably, a polypeptide of the invention comprises (or consists of) a catalytically inactive
10 clostridial neurotoxin L-chain. Reference to a catalytically inactive clostridial neurotoxin in this context also encompasses a fragment of a clostridial neurotoxin L-chain. A fragment of a clostridial neurotoxin L-chain may have ≤ 400 , ≤ 350 , ≤ 300 , ≤ 250 , ≤ 200 , ≤ 150 , ≤ 100 or ≤ 50 amino acid residues of a clostridial neurotoxin L-chain. In one embodiment, a fragment of a clostridial neurotoxin L-chain has at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 or 200
15 amino acid residues of a clostridial neurotoxin L-chain. For example, a fragment of a clostridial neurotoxin L-chain may have 20-400, 50-300 or 100-200 amino acid residues of a clostridial neurotoxin L-chain. It is preferred, however, that reference to a catalytically inactive clostridial neurotoxin is reference to a full-length catalytically inactive clostridial neurotoxin L-chain.

20 Examples of L-chain reference sequences include:

Botulinum type A neurotoxin: amino acid residues 1-448
Botulinum type B neurotoxin: amino acid residues 1-440
Botulinum type C1 neurotoxin: amino acid residues 1-441
25 Botulinum type D neurotoxin: amino acid residues 1-445
Botulinum type E neurotoxin: amino acid residues 1-422
Botulinum type F neurotoxin: amino acid residues 1-439
Botulinum type G neurotoxin: amino acid residues 1-441
Tetanus neurotoxin: amino acid residues 1-457

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For recently-identified BoNT/X, the L-chain has been reported as corresponding to amino acids 1-439 thereof, with the L-chain boundary potentially varying by approximately 25 amino acids (e.g. 1-414 or 1-464).

The above-identified reference sequences should be considered a guide, as slight variations may occur according to sub-serotypes. By way of example, US 2007/0166332 (hereby incorporated by reference in its entirety) cites slightly different clostridial sequences:

- 5 Botulinum type A neurotoxin: amino acid residues M1-K448
- Botulinum type B neurotoxin: amino acid residues M1-K441
- Botulinum type C1 neurotoxin: amino acid residues M1-K449
- Botulinum type D neurotoxin: amino acid residues M1-R445
- Botulinum type E neurotoxin: amino acid residues M1-R422
- 10 Botulinum type F neurotoxin: amino acid residues M1-K439
- Botulinum type G neurotoxin: amino acid residues M1-K446
- Tetanus neurotoxin: amino acid residues M1-A457

Suitable clostridial neurotoxin L-chains are described herein.

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A clostridial neurotoxin L-chain may comprise a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 6, 24, 32, 40, 74 or 76 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). In one embodiment a clostridial neurotoxin L-chain comprises a polypeptide sequence having at least 20 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 6, 24, 32, 40, 74 or 76 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). Preferably, a clostridial neurotoxin L-chain comprises (more preferably consists of) a polypeptide sequence comprising any one of SEQ ID NOs: 6, 24, 32 or 40 that has been modified to catalytically inactivate the L-chain, for example SEQ ID NO: 74 or 76

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A clostridial neurotoxin L-chain may be one encoded by a nucleotide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 5, 23, 31 or 39 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). In one embodiment a clostridial neurotoxin L-chain is one encoded by a nucleotide sequence having 30 at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 5, 23, 31 or 39 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). Preferably, a clostridial neurotoxin L-chain is one encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 5, 23, 31 or 39 that has been modified to catalytically inactivate the encoded L-chain.

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Given that catalytic activity of the light-chain was not required for efficacy in treating pain, it is credible that a polypeptide that does not comprise an L-chain (or only comprises a fragment of an L-chain) can treat pain. For similar reasons, it is credible that a polypeptide that does not comprise an L-chain (or only comprises a fragment of an L-chain) can treat an inflammatory condition. Thus, in one embodiment, the polypeptide may comprise a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain). In one embodiment, a polypeptide of the invention does not comprise both a clostridial neurotoxin translocation domain (H_N domain) and a clostridial neurotoxin receptor binding domain (H_C domain).

In one embodiment, a polypeptide of the invention comprises (or consists of) a clostridial neurotoxin heavy chain (H-chain). Said H-chain comprises a clostridial neurotoxin translocation domain (H_N domain) and a receptor binding domain (H_C domain). Reference to a clostridial neurotoxin H-chain in this context also encompasses a fragment of a clostridial neurotoxin H-chain. A fragment of a clostridial neurotoxin H-chain may have ≤ 800 , ≤ 700 , ≤ 600 , ≤ 500 , ≤ 400 , ≤ 350 , ≤ 300 , ≤ 250 , ≤ 200 , ≤ 150 , ≤ 100 or ≤ 50 amino acid residues of a clostridial neurotoxin H-chain. In one embodiment, a fragment of a clostridial neurotoxin H-chain has at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 or 200 amino acid residues of a clostridial neurotoxin H-chain. For example, a fragment of a clostridial neurotoxin H-chain may have 20-800, 30-600, 40-400, 50-300 or 100-200 amino acid residues of a clostridial neurotoxin H-chain. It is preferred, however, that reference to an H-chain is reference to a full-length H-chain.

In one embodiment, a polypeptide of the invention comprises (or consists of) a clostridial neurotoxin translocation domain (H_N domain). Reference to a clostridial neurotoxin translocation domain in this context also encompasses a fragment of a translocation domain. A fragment of a clostridial neurotoxin translocation domain may have ≤ 400 , ≤ 350 , ≤ 300 , ≤ 250 , ≤ 200 , ≤ 150 , ≤ 100 or ≤ 50 amino acid residues of a clostridial neurotoxin translocation domain. In one embodiment, a fragment of a clostridial neurotoxin translocation domain has at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 or 200 amino acid residues of a clostridial neurotoxin translocation domain. For example, a fragment of a clostridial neurotoxin translocation domain may have 20-400, 50-300 or 100-200 amino acid residues of a clostridial neurotoxin translocation domain. It is preferred, however, that reference to a translocation domain is reference to a full-length translocation domain.

The translocation domain is a fragment of the H-chain of a clostridial neurotoxin approximately equivalent to the amino-terminal half of the H-chain, or the domain corresponding to that fragment in the intact H-chain. In one embodiment the H_C function of the H-chain may be removed by deletion of the H_C amino acid sequence (either at the DNA synthesis level, or at the post-synthesis level by nuclease or protease treatment). Alternatively, the H_C function may be inactivated by chemical or biological treatment. Thus, in some embodiments the H-chain may be incapable of binding to the Binding Site on a target cell to which native clostridial neurotoxin (i.e. holotoxin) binds.

Examples of suitable (reference) Translocation Domains include:

- Botulinum type A neurotoxin - amino acid residues (449-871)
- Botulinum type B neurotoxin - amino acid residues (441-858)
- Botulinum type C neurotoxin - amino acid residues (442-866)
- Botulinum type D neurotoxin - amino acid residues (446-862)
- Botulinum type E neurotoxin - amino acid residues (423-845)
- Botulinum type F neurotoxin - amino acid residues (440-864)
- Botulinum type G neurotoxin - amino acid residues (442-863)
- Tetanus neurotoxin - amino acid residues (458-879)

The above-identified reference sequence should be considered a guide as slight variations may occur according to sub-serotypes. By way of example, US 2007/0166332 (hereby incorporated by reference thereto) cites slightly different clostridial sequences:

- Botulinum type A neurotoxin - amino acid residues (A449-K871)
- Botulinum type B neurotoxin - amino acid residues (A442-S858)
- Botulinum type C neurotoxin - amino acid residues (T450-N866)
- Botulinum type D neurotoxin - amino acid residues (D446-N862)
- Botulinum type E neurotoxin - amino acid residues (K423-K845)
- Botulinum type F neurotoxin - amino acid residues (A440-K864)
- Botulinum type G neurotoxin - amino acid residues (S447-S863)
- Tetanus neurotoxin - amino acid residues (S458-V879)

In the context of the present invention, a variety of clostridial neurotoxin H_N regions comprising a translocation domain can be useful in aspects of the present invention. The H_N regions from the heavy chains of clostridial neurotoxins are approximately 410-430 amino acids in length

and comprise a translocation domain. Research has shown that the entire length of a H_N region from a clostridial neurotoxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment can include clostridial neurotoxin H_N regions comprising a translocation domain having a length of, for example, at least 350 amino acids, at least 375 amino acids, at least 400 amino acids and at least 425 amino acids. Other aspects of this embodiment can include clostridial neurotoxin H_N regions comprising a translocation domain having a length of, for example, at most 350 amino acids, at most 375 amino acids, at most 400 amino acids and at most 425 amino acids.

For further details on the genetic basis of toxin production in *Clostridium botulinum* and *C. tetani*, see Henderson *et al* (1997) in *The Clostridia: Molecular Biology and Pathogenesis*, Academic press.

The term H_N embraces naturally-occurring neurotoxin H_N portions, and modified H_N portions having amino acid sequences that do not occur in nature and/ or synthetic amino acid residues. In one embodiment said modified H_N portions still demonstrate the above-mentioned translocation function.

In one embodiment, a polypeptide of the invention comprises (or consists of) a clostridial neurotoxin receptor binding domain (H_C domain). Reference to a clostridial neurotoxin receptor binding domain (H_C) in this context also encompasses a fragment of a clostridial neurotoxin receptor binding domain (H_C). A fragment of a clostridial neurotoxin receptor binding domain (H_C) may have ≤350, ≤300, ≤250, ≤200, ≤150, ≤100 or ≤50 amino acid residues of a clostridial neurotoxin receptor binding domain (H_C). In one embodiment, a fragment of a clostridial neurotoxin receptor binding domain (H_C) has at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 or 200 amino acid residues of a clostridial neurotoxin receptor binding domain (H_C). For example, a fragment of a clostridial neurotoxin receptor binding domain (H_C) may have 20-350, 50-300 or 100-200 amino acid residues of a clostridial neurotoxin receptor binding domain (H_C). It is preferred, however, that reference to a clostridial neurotoxin receptor binding domain (H_C) is reference to a full-length clostridial neurotoxin receptor binding domain (H_C).

Examples of clostridial neurotoxin receptor binding domain (H_C) reference sequences include:

BoNT/A - N872-L1296

BoNT/B - E859-E1291

BoNT/C1 - N867-E1291

BoNT/D - S863-E1276

BoNT/E - R846-K1252

BoNT/F - K865-E1274

BoNT/G - N864-E1297

5 TeNT - I880-D1315

For recently-identified BoNT/X, the H_C domain has been reported as corresponding to amino acids 893-1306 thereof, with the domain boundary potentially varying by approximately 25 amino acids (e.g. 868-1306 or 918-1306).

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A clostridial neurotoxin H-chain (e.g. the H_C domain portion) may further comprise a translocation facilitating domain (or a fragment thereof may be translocation facilitating domain fragment). Said domain facilitates delivery of the L-chain into the cytosol of the target cell and are described, for example, in WO 08/008803 and WO 08/008805, each of which is herein
15 incorporated by reference thereto.

By way of example, a translocation facilitating domain may comprise a clostridial neurotoxin H_{CN} domain or a fragment or variant thereof. In more detail, a clostridial neurotoxin H_{CN} translocation facilitating domain may have a length of at least 200 amino acids, at least 225
20 amino acids, at least 250 amino acids, at least 275 amino acids. In this regard, a clostridial neurotoxin H_{CN} translocation facilitating domain preferably has a length of at most 200 amino acids, at most 225 amino acids, at most 250 amino acids, or at most 275 amino acids. Specific (reference) examples include:

25 Botulinum type A neurotoxin - amino acid residues (872-1110)
Botulinum type B neurotoxin - amino acid residues (859-1097)
Botulinum type C neurotoxin - amino acid residues (867-1111)
Botulinum type D neurotoxin - amino acid residues (863-1098)
Botulinum type E neurotoxin - amino acid residues (846-1085)
30 Botulinum type F neurotoxin - amino acid residues (865-1105)
Botulinum type G neurotoxin - amino acid residues (864-1105)
Tetanus neurotoxin - amino acid residues (880-1127)

The above sequence positions may vary a little according to serotype/ sub-type, and further
35 examples of suitable (reference) clostridial neurotoxin H_{CN} domains include:

Botulinum type A neurotoxin - amino acid residues (874-1110)

Botulinum type B neurotoxin - amino acid residues (861-1097)

Botulinum type C neurotoxin - amino acid residues (869-1111)

Botulinum type D neurotoxin - amino acid residues (865-1098)

5 Botulinum type E neurotoxin - amino acid residues (848-1085)

Botulinum type F neurotoxin - amino acid residues (867-1105)

Botulinum type G neurotoxin - amino acid residues (866-1105)

Tetanus neurotoxin - amino acid residues (882-1127)

10 Suitable clostridial neurotoxin H_C domains are described herein.

A clostridial neurotoxin H_C domain may comprise a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 8, 22, 30, 38, 42, 44, 46, 48 or 50. In one embodiment a clostridial neurotoxin H_C domain comprises a polypeptide sequence having at
15 least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 8, 22, 30, 38, 42, 44, 46, 48 or 50. Preferably, a clostridial neurotoxin H_C domain comprises (more preferably consists of) a polypeptide sequence comprising any one of SEQ ID NOs: 8, 22, 30, 38, 42, 44, 46, 48 or 50.

20 A clostridial neurotoxin H_C domain may be one encoded by a nucleotide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 7, 21, 29, 37, 41, 43, 45, 47 or 49. In one embodiment a clostridial neurotoxin H_C domain is one encoded by a nucleotide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 7, 21, 29, 37, 41, 43, 45, 47 or 49. Preferably, a clostridial neurotoxin H_C domain is one encoded by
25 a nucleotide sequence comprising any one of SEQ ID NOs: 7, 21, 29, 37, 41, 43, 45, 47 or 49.

Any of the above-described facilitating domains may be combined with any of the previously described translocation domain peptides that are suitable for use in the present invention. Thus, by way of example, a non-clostridial facilitating domain may be combined with a non-
30 clostridial translocation domain peptide or with clostridial translocation domain peptide. Alternatively, a clostridial neurotoxin H_{CN} translocation facilitating domain may be combined with a non-clostridial translocation domain peptide. Alternatively, a clostridial neurotoxin H_{CN} facilitating domain may be combined with a clostridial translocation domain peptide, examples of which include:

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Botulinum type A neurotoxin - amino acid residues (449-1110)

Botulinum type B neurotoxin - amino acid residues (442-1097)
 Botulinum type C neurotoxin - amino acid residues (450-1111)
 Botulinum type D neurotoxin - amino acid residues (446-1098)
 Botulinum type E neurotoxin - amino acid residues (423-1085)
 5 Botulinum type F neurotoxin - amino acid residues (440-1105)
 Botulinum type G neurotoxin - amino acid residues (447-1105)
 Tetanus neurotoxin - amino acid residues (458-1127)

In some embodiments the polypeptides of the present invention may lack a functional H_C domain of a clostridial neurotoxin. In one embodiment, the polypeptides preferably lack the last 10 50 C-terminal amino acids of a clostridial neurotoxin holotoxin. In another embodiment, the polypeptides preferably lack the last 100, preferably the last 150, more preferably the last 200, particularly preferably the last 250, and most preferably the last 300 C-terminal amino acid residues of a clostridial neurotoxin holotoxin. Alternatively, the H_C binding activity may be 15 negated/ reduced by mutagenesis – by way of example, referring to BoNT/A for convenience, modification of one or two amino acid residue mutations (W1266 to L and Y1267 to F) in the ganglioside binding pocket causes the H_C region to lose its receptor binding function. Analogous mutations may be made to non-serotype A clostridial peptide components, e.g. a construct based on botulinum B with mutations (W1262 to L and Y1263 to F) or botulinum E 20 (W1224 to L and Y1225 to F). Other mutations to the active site achieve the same ablation of H_C receptor binding activity, e.g. Y1267S in botulinum type A toxin and the corresponding highly conserved residue in the other clostridial neurotoxins. Details of this and other mutations are described in Rummel et al (2004) (Molecular Microbiol. 51:631-634), which is hereby incorporated by reference thereto.

25 The H_C peptide of a native clostridial neurotoxin comprises approximately 400-440 amino acid residues, and consists of two functionally distinct domains of approximately 25kDa each, namely the N-terminal region (commonly referred to as the H_{CN} peptide or domain) and the C-terminal region (commonly referred to as the H_{CC} peptide or domain). This fact is confirmed by 30 the following publications, each of which is herein incorporated in its entirety by reference thereto: Umland TC (1997) Nat. Struct. Biol. 4: 788-792; Herreros J (2000) Biochem. J. 347: 199-204; Halpern J (1993) J. Biol. Chem. 268: 15, pp. 11188-11192; Rummel A (2007) PNAS 104: 359-364; Lacey DB (1998) Nat. Struct. Biol. 5: 898-902; Knapp (1998) Am. Cryst. Assoc. Abstract Papers 25: 90; Swaminathan and Eswaramoorthy (2000) Nat. Struct. Biol. 7: 1751- 35 1759; and Rummel A (2004) Mol. Microbiol. 51(3), 631-643. Moreover, it has been well documented that the C-terminal region (H_{CC}), which constitutes the C-terminal 160-200 amino

acid residues, is responsible for binding of a clostridial neurotoxin to its natural cell receptors, namely to nerve terminals at the neuromuscular junction - this fact is also confirmed by the above publications. Thus, reference throughout this specification to a clostridial heavy-chain lacking a functional heavy chain H_C peptide (or domain) such that the heavy-chain is incapable of binding to cell surface receptors to which a native clostridial neurotoxin binds means that the clostridial heavy-chain simply lacks a functional H_{CC} peptide. In other words, the H_{CC} peptide region may be either partially or wholly deleted, or otherwise modified (e.g. through conventional chemical or proteolytic treatment) to reduce its native binding ability for nerve terminals at the neuromuscular junction.

Thus, in one embodiment, a clostridial neurotoxin H_N peptide of the present invention lacks part of a C-terminal peptide portion (H_{CC}) of a clostridial neurotoxin and thus lacks the H_C binding function of native clostridial neurotoxin. By way of example, in one embodiment, the C-terminally extended clostridial H_N peptide lacks the C-terminal 40 amino acid residues, or the C-terminal 60 amino acid residues, or the C-terminal 80 amino acid residues, or the C-terminal 100 amino acid residues, or the C-terminal 120 amino acid residues, or the C-terminal 140 amino acid residues, or the C-terminal 150 amino acid residues, or the C-terminal 160 amino acid residues of a clostridial neurotoxin heavy-chain. In another embodiment, the clostridial H_N peptide of the present invention lacks the entire C-terminal peptide portion (H_{CC}) of a clostridial neurotoxin and thus lacks the H_C binding function of native clostridial neurotoxin. By way of example, in one embodiment, the clostridial H_N peptide lacks the C-terminal 165 amino acid residues, or the C-terminal 170 amino acid residues, or the C-terminal 175 amino acid residues, or the C-terminal 180 amino acid residues, or the C-terminal 185 amino acid residues, or the C-terminal 190 amino acid residues, or the C-terminal 195 amino acid residues of a clostridial neurotoxin heavy-chain. By way of further example, the clostridial H_N peptide of the present invention lacks a clostridial H_{CC} reference sequence selected from the group consisting of:

- Botulinum type A neurotoxin - amino acid residues (Y1111-L1296)
- Botulinum type B neurotoxin - amino acid residues (Y1098-E1291)
- Botulinum type C neurotoxin - amino acid residues (Y1112-E1291)
- Botulinum type D neurotoxin - amino acid residues (Y1099-E1276)
- Botulinum type E neurotoxin - amino acid residues (Y1086-K1252)
- Botulinum type F neurotoxin - amino acid residues (Y1106-E1274)
- Botulinum type G neurotoxin - amino acid residues (Y1106-E1297)
- Tetanus neurotoxin - amino acid residues (Y1128-D1315).

The above-identified reference sequences should be considered a guide as slight variations may occur according to sub-serotypes.

- 5 In other embodiments a fragment of an H_C domain may comprise an H_{CC} peptide as described herein.

A polypeptide of the invention may comprise a catalytically inactive clostridial neurotoxin L-chain and a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial
10 neurotoxin receptor binding domain (H_C domain). For example, a polypeptide may comprise a catalytically inactive clostridial neurotoxin L-chain and a clostridial neurotoxin translocation domain (H_N).

Suitable polypeptides comprising a catalytically inactive clostridial neurotoxin L-chain and
15 translocation domain are described herein.

A polypeptide comprising a clostridial neurotoxin L-chain and translocation domain may comprise a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 4, 20, 28, 36 or 75 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). In one a polypeptide comprising a clostridial
20 neurotoxin L-chain and translocation domain comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 4, 20, 28, 36 or 75 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). Preferably, a polypeptide comprising a clostridial neurotoxin L-chain and
25 translocation domain comprises (more preferably consists of) a polypeptide sequence comprising any one of SEQ ID NOs: 4, 20, 28, 36 or 75 that has been modified to catalytically inactivate the L-chain, such as SEQ ID NO: 75.

A polypeptide comprising (or consisting of) a clostridial neurotoxin L-chain and translocation
30 domain may be one encoded by a nucleotide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 3, 19, 27 or 35 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). In one embodiment a polypeptide comprising (or consisting of) a clostridial neurotoxin L-chain and translocation domain is one encoded by a nucleotide sequence having at least 80%, 90%, 95% or 98%
35 sequence identity to any one of SEQ ID NOs: 3, 19, 27 or 35 with the proviso that the L-chain is catalytically inactive (e.g. the L-chain has been inactivated by modification). Preferably, a

polypeptide comprising (or consisting of) a clostridial neurotoxin L-chain and translocation domain is one encoded by a nucleotide sequence comprising any one of SEQ ID NOs: 3, 19, 27 or 35 that has been modified to catalytically inactivate the encoded L-chain.

- 5 Preferably, the polypeptide comprises a catalytically inactive clostridial neurotoxin L-chain, a clostridial neurotoxin translocation domain (H_N domain), and a clostridial neurotoxin receptor binding domain (H_C domain).

10 In one embodiment, a polypeptide of the invention does not comprise a clostridial neurotoxin receptor binding domain (H_C) or at least the C-terminal portion of a clostridial neurotoxin receptor binding domain (H_{CC}). Thus, in one embodiment a polypeptide of the present invention lacks a C-terminal portion of a clostridial neurotoxin receptor binding domain (H_{CC}). Advantageously, such polypeptides lack the endogenous clostridial neurotoxin receptor binding capabilities and may thus exhibit fewer off-target effects in a subject administered said
15 polypeptide.

A polypeptide of the invention may consist essentially of a catalytically inactive clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain). For example, a
20 polypeptide may consist essentially of a catalytically inactive clostridial neurotoxin L-chain and a clostridial neurotoxin translocation domain (H_N).

Preferably, the polypeptide consists essentially of a catalytically inactive clostridial neurotoxin L-chain, a clostridial neurotoxin translocation domain (H_N domain), and a clostridial neurotoxin
25 receptor binding domain (H_C domain).

The term “consists essentially of” as used in this context means that the polypeptide does not further comprise one or more amino acid residues that confer additional functionality to the polypeptide, e.g. when administered to a subject. In other words, a polypeptide that “consists
30 essentially of” a catalytically inactive clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain) may further comprise one or more amino acid residues (to those of the catalytically inactive clostridial neurotoxin light-chain (L-chain), clostridial neurotoxin translocation domain (H_N domain) and/or clostridial neurotoxin receptor binding domain (H_C domain)) but said one or more further amino acid residues do not confer additional functionality
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to the polypeptide, e.g. when administered to a subject. Additional functionality may include enzymatic activity, binding activity and/or any physiological activity whatsoever.

A polypeptide of the invention may consist of a catalytically inactive clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain). For example, a polypeptide may consist of a catalytically inactive clostridial neurotoxin L-chain and a clostridial neurotoxin translocation domain (H_N).

Preferably, the polypeptide consists of a catalytically inactive clostridial neurotoxin L-chain, a clostridial neurotoxin translocation domain (H_N domain), and a clostridial neurotoxin receptor binding domain (H_C domain).

In one embodiment a polypeptide may comprise non-clostridial neurotoxin sequences in addition to any clostridial neurotoxin sequences so long as the non-clostridial neurotoxin sequences do not disrupt the ability of a polypeptide to achieve its therapeutic effect (e.g. to treat pain). Preferably, the non-clostridial neurotoxin sequence is not one having catalytic activity, e.g. enzymatic activity. In one embodiment the polypeptide of the invention does not comprise a catalytically active domain (e.g. a non-clostridial catalytically active domain). In one embodiment, the non-clostridial sequence is not one that binds to a cellular receptor. In other words, in one embodiment, the non-clostridial sequence is not a ligand for a cellular receptor. A cellular receptor may be a proteinaceous cellular receptor, such as an integral membrane protein. Examples of cellular receptors can be found in the IUPHAR Guide to Pharmacology Database, version 2019.4, available at https://www.guidetopharmacology.org/download.jsp#db_reports. Non-clostridial neurotoxin sequences may include tags to aid in purification, such as His-tags. In one embodiment, a polypeptide of the invention does not comprise a label or a site for adding a label, such as a sortase acceptor or donor site.

In a preferred embodiment, a polypeptide of the invention does not comprise a therapeutic or diagnostic agent (e.g. a nucleic acid, protein, peptide or small molecule therapeutic or diagnostic agent) additional to the catalytically inactive clostridial neurotoxin L-chain, H_N domain and/or H_C domain. For example, in one embodiment, the polypeptide may not comprise a covalently or non-covalently associated therapeutic or diagnostic agent. Thus, a polypeptide of the invention preferably does not function as a delivery vehicle for a further therapeutic or diagnostic agent.

The polypeptide of the invention may comprise (or consist of) a modified clostridial neurotoxin or derivative thereof or modified clostridial neurotoxin fragment or derivative fragment, including but not limited to those described below with the proviso that any L-chain present is catalytically inactive. A modified clostridial neurotoxin or derivative (or modified clostridial neurotoxin fragment or derivative fragment) may contain one or more amino acids that has been modified as compared to the native (unmodified) form of the clostridial neurotoxin (or clostridial neurotoxin fragment), or may contain one or more inserted amino acids that are not present in the native (unmodified) form of the clostridial neurotoxin (or clostridial neurotoxin fragment). By way of example, a modified clostridial neurotoxin (or clostridial neurotoxin fragment) may have modified amino acid sequences in one or more domains relative to the native (unmodified) clostridial neurotoxin sequence (or clostridial neurotoxin fragment). Such modifications may modify functional aspects of the toxin (or toxin fragment). Thus, in one embodiment, the polypeptide of the invention is or comprises a modified clostridial neurotoxin, or a modified clostridial neurotoxin derivative, or a clostridial neurotoxin derivative or a modified clostridial neurotoxin fragment or derivative fragment (e.g. a catalytically inactive L-chain, H_N domain and/or H_C domain) with the proviso that any L-chain present is catalytically inactive.

A polypeptide of the invention may comprise (or consist of) a modified clostridial neurotoxin or clostridial neurotoxin fragment (e.g. H_C domain) having one or more modifications in the amino acid sequence of the heavy chain (such as a modified H_C domain), wherein said modified heavy chain binds to target nerve cells with a higher or lower affinity than the native (unmodified) clostridial neurotoxin or clostridial neurotoxin fragment, with the proviso that any L-chain present is catalytically inactive. Such modifications in the H_C domain can include modifying residues in the ganglioside binding site of the H_C domain or in the protein (SV2 or synaptotagmin) binding site that alter binding to the ganglioside receptor and/or the protein receptor of the target nerve cell. Examples of such modified clostridial neurotoxins are described in WO 2006/027207 and WO 2006/114308, both of which are hereby incorporated by reference in their entirety.

A modified clostridial neurotoxin (or clostridial neurotoxin fragment) may be one that comprises one or more modifications that increases the isoelectric point of the clostridial neurotoxin when compared to an equivalent unmodified clostridial neurotoxin (or clostridial neurotoxin fragment) lacking said one or more modifications, with the proviso that any L-chain present is catalytically inactive. Suitable modified clostridial neurotoxins (with the proviso that any L-chain present has been modified to be catalytically inactive) are described below and in WO 2015/004461

A1 and WO 2016/110662 A1, which are incorporated herein by reference. Exemplary sequences include SEQ ID NOs: 42 and 62 described herein.

In one embodiment, a polypeptide of the invention may comprise a modified BoNT/A or
5 fragment thereof (e.g. a BoNT/A H_C domain or fragment thereof). The modified BoNT/A or
fragment thereof may be one that comprises a modification at one or more amino acid
residue(s) selected from: ASN 886, ASN 905, GLN 915, ASN 918, GLU 920, ASN 930, ASN
954, SER 955, GLN 991, GLU 992, GLN 995, ASN 1006, ASN 1025, ASN 1026, ASN 1032,
ASN 1043, ASN 1046, ASN 1052, ASP 1058, HIS 1064, ASN 1080, GLU 1081, GLU 1083,
10 ASP 1086, ASN 1188, ASP 1213, GLY 1215, ASN 1216, GLN 1229, ASN 1242, ASN 1243,
SER 1274, and THR 1277.

The modification may be a modification when compared to catalytically inactive BoNT/A shown
as SEQ ID NO: 2, wherein the amino acid residue numbering is determined by alignment with
15 SEQ ID NO: 2. As the presence of a methionine residue at position 1 of SEQ ID NO: 2 (as
well as the SEQ ID NOs corresponding to modified BoNT/A polypeptides or fragments thereof
described herein) is optional, the skilled person will take the presence/absence of the
methionine residue into account when determining amino acid residue numbering. For
example, where SEQ ID NO: 2 includes a methionine, the position numbering will be as defined
20 above (e.g. ASN 886 will be ASN 886 of SEQ ID NO: 2). Alternatively, where the methionine
is absent from SEQ ID NO: 2 the amino acid residue numbering should be modified by -1 (e.g.
ASN 886 will be ASN 885 of SEQ ID NO: 2). Similar considerations apply when the methionine
at position 1 of the other polypeptide sequences described herein is present/absent, and the
skilled person will readily determine the correct amino acid residue numbering using
25 techniques routine in the art.

An alignment described herein for determining amino acid residue numbering may be carried
out using any of the methods described herein for determining sequence homology and/or %
sequence identity.

30 The amino acid residue(s) indicated for modification above are surface exposed amino acid
residue(s).

A modified BoNT/A or fragment thereof may comprise a modification at one or more amino
35 acid residue(s) selected from: ASN 886, ASN 930, ASN 954, SER 955, GLN 991, ASN 1025,

ASN 1026, ASN 1052, ASN 1188, ASP 1213, GLY 1215, ASN 1216, GLN 1229, ASN 1242, ASN 1243, SER 1274 and THR 1277.

The term “one or more amino acid residue(s)” when used in the context of a modified BoNT/A or fragment thereof preferably means at least 2, 3, 4, 5, 6 or 7 of the indicated amino acid residue(s). Thus, a modified BoNT/A or fragment thereof may comprise at least 2, 3, 4, 5, 6 or 7 (preferably 7) modifications at the indicated amino acid residue(s). A modified BoNT/A or fragment thereof may comprise 1-30, 3-20, or 5-10 amino acid modifications. More preferably, the term “one or more amino acid residue(s)” when used in the context of modified BoNT/A or fragment thereof means all of the indicated amino acid residue(s).

Preferably, beyond the one or more amino acid modification(s) at the indicated amino acid residue(s), the modified BoNT/A or fragment thereof does not contain any further amino acid modifications when compared to SEQ ID NO: 2.

The modification may be selected from:

- i. substitution of an acidic surface exposed amino acid residue with a basic amino acid residue;
- ii. substitution of an acidic surface exposed amino acid residue with an uncharged amino acid residue;
- iii. substitution of an uncharged surface exposed amino acid residue with a basic amino acid residue;
- iv. insertion of a basic amino acid residue; and
- v. deletion of an acidic surface exposed amino acid residue.

A modification as indicated above results in a modified BoNT/A or fragment thereof that has an increased positive surface charge and increased isoelectric point when compared to the corresponding unmodified BoNT/A or fragment thereof.

The isoelectric point (pI) is a specific property of a given protein. As is well known in the art, proteins are made from a specific sequence of amino acids (also referred to when in a protein as amino acid residues). Each amino acid of the standard set of twenty has a different side chain (or R group), meaning that each amino acid residue in a protein displays different chemical properties such as charge and hydrophobicity. These properties may be influenced by the surrounding chemical environment, such as the temperature and pH. The overall chemical characteristics of a protein will depend on the sum of these various factors.

Certain amino acid residues (detailed below) possess ionisable side chains that may display an electric charge depending on the surrounding pH. Whether such a side chain is charged or not at a given pH depends on the pKa of the relevant ionisable moiety, wherein pKa is the negative logarithm of the acid dissociation constant (Ka) for a specified proton from a conjugate base.

For example, acidic residues such as aspartic acid and glutamic acid have side chain carboxylic acid groups with pKa values of approximately 4.1 (precise pKa values may depend on temperature, ionic strength and the microenvironment of the ionisable group). Thus, these side chains exhibit a negative charge at a pH of 7.4 (often referred to as “physiological pH”). At low pH values, these side chains will become protonated and lose their charge.

Conversely, basic residues such as lysine and arginine have nitrogen-containing side chain groups with pKa values of approximately 10-12. These side chains therefore exhibit a positive charge at a pH of 7.4. These side chains will become de-protonated and lose their charge at high pH values.

The overall (net) charge of a protein molecule therefore depends on the number of acidic and basic residues present in the protein (and their degree of surface exposure) and on the surrounding pH. Changing the surrounding pH changes the overall charge on the protein. Accordingly, for every protein there is a given pH at which the number of positive and negative charges is equal and the protein displays no overall net charge. This point is known as the isoelectric point (pI). The isoelectric point is a standard concept in protein biochemistry with which the skilled person would be familiar.

The isoelectric point (pI) is therefore defined as the pH value at which a protein displays a net charge of zero. An increase in pI means that a higher pH value is required for the protein to display a net charge of zero. Thus, an increase in pI represents an increase in the net positive charge of a protein at a given pH. Conversely, a decrease in pI means that a lower pH value is required for the protein to display a net charge of zero. Thus, a decrease in pI represents a decrease in the net positive charge of a protein at a given pH.

Methods of determining the pI of a protein are known in the art and would be familiar to a skilled person. By way of example, the pI of a protein can be calculated from the average pKa values of each amino acid present in the protein (“calculated pI”). Such calculations can be

performed using computer programs known in the art, such as the Compute pI/MW Tool from ExPASy (https://web.expasy.org/compute_pi/), which is the preferred method for calculating pI in accordance with the present invention. Comparisons of pI values between different molecules should be made using the same calculation technique/program.

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Where appropriate, the calculated pI of a protein can be confirmed experimentally using the technique of isoelectric focusing (“observed pI”). This technique uses electrophoresis to separate proteins according to their pI. Isoelectric focusing is typically performed using a gel that has an immobilised pH gradient. When an electric field is applied, the protein migrates through the pH gradient until it reaches the pH at which it has zero net charge, this point being the pI of the protein. Results provided by isoelectric focusing are typically relatively low-resolution in nature, and thus the present inventors believe that results provided by calculated pI (as described above) are more appropriate to use.

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15 Throughout the present specification, “pI” means “calculated pI” unless otherwise stated.

The pI of a protein may be increased or decreased by altering the number of basic and/or acidic groups displayed on its surface. This can be achieved by modifying one or more amino acids of the protein. For example, an increase in pI may be provided by reducing the number of acidic residues, or by increasing the number of basic residues.

20

A modified BoNT/A or fragment thereof of the invention may have a pI value that is at least 0.2, 0.4, 0.5 or 1 pI units higher than that of a catalytically inactive BoNT/A (e.g. SEQ ID NO: 2) or fragment thereof. Preferably, a modified BoNT/A or fragment thereof may have a pI of at least 6.6, e.g. at least 6.8.

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The properties of the 20 standard amino acids are indicated in the table below:

Amino Acid			Side Chain
Aspartic acid	Asp	D	Charged (acidic)
Glutamic acid	Glu	E	Charged (acidic)
Arginine	Arg	R	Charged (basic)
Lysine	Lys	K	Charged (basic)
Histidine	His	H	Uncharged (polar)
Asparagine	Asn	N	Uncharged (polar)
Glutamine	Gln	Q	Uncharged (polar)
Serine	Ser	S	Uncharged (polar)

Threonine	Thr	T	Uncharged (polar)
Tyrosine	Tyr	Y	Uncharged (polar)
Methionine	Met	M	Uncharged (polar)
Tryptophan	Trp	W	Uncharged (polar)
Cysteine	Cys	C	Uncharged (polar)
Alanine	Ala	A	Uncharged (hydrophobic)
Glycine	Gly	G	Uncharged (hydrophobic)
Valine	Val	V	Uncharged (hydrophobic)
Leucine	Leu	L	Uncharged (hydrophobic)
Isoleucine	Ile	I	Uncharged (hydrophobic)
Proline	Pro	P	Uncharged (hydrophobic)
Phenylalanine	Phe	F	Uncharged (hydrophobic)

The following amino acids are considered charged amino acids: aspartic acid (negative), glutamic acid (negative), arginine (positive), and lysine (positive).

- 5 At a pH of 7.4, the side chains of aspartic acid (pKa 3.1) and glutamic acid (pKa 4.1) have a negative charge, while the side chains of arginine (pKa 12.5) and lysine (pKa 10.8) have a positive charge. Aspartic acid and glutamic acid are referred to as acidic amino acid residues. Arginine and lysine are referred to as basic amino acid residues.
- 10 The following amino acids are considered uncharged, polar (meaning they can participate in hydrogen bonding) amino acids: asparagine, glutamine, histidine, serine, threonine, tyrosine, cysteine, methionine, and tryptophan.

- The following amino acids are considered uncharged, hydrophobic amino acids: alanine, valine, leucine, isoleucine, phenylalanine, proline, and glycine.
- 15

- In an amino acid insertion, an additional amino acid residue (one that is not normally present) is incorporated into the BoNT/A polypeptide sequence or fragment thereof, thus increasing the total number of amino acid residues in said sequence. In an amino acid deletion, an amino acid residue is removed from the clostridial toxin amino acid sequence, thus reducing the total number of amino acid residues in said sequence.
- 20

- Preferably, the modification is a substitution, which advantageously maintains the same number of amino acid residues in the modified BoNT/A or fragment thereof. In an amino acid substitution, an amino acid residue that forms part of the BoNT/A polypeptide sequence or
- 25

fragment thereof is replaced with a different amino acid residue. The replacement amino acid residue may be one of the 20 standard amino acids, as described above. Alternatively, the replacement amino acid in an amino acid substitution may be a non-standard amino acid (an amino acid that is not part of the standard set of 20 described above). By way of example, the replacement amino acid may be a basic non-standard amino acid, e.g. L-Ornithine, L-2-amino-3-guanidinopropionic acid, or D-isomers of Lysine, Arginine and Ornithine). Methods for introducing non-standard amino acids into proteins are known in the art and include recombinant protein synthesis using *E. coli* auxotrophic expression hosts.

In one embodiment, the substitution is selected from: substitution of an acidic amino acid residue with a basic amino acid residue, substitution of an acidic amino acid residue with an uncharged amino acid residue, and substitution of an uncharged amino acid residue with a basic amino acid residue. In one embodiment, wherein the substitution is a substitution of an acidic amino acid residue with an uncharged amino acid residue, the acidic amino acid residue is replaced with its corresponding uncharged amide amino acid residue (i.e. aspartic acid is replaced with asparagine, and glutamic acid is replaced with glutamine).

Preferably, the basic amino acid residue is a lysine residue or an arginine residue. In other words, the substitution is substitution with lysine or arginine. Most preferably, the modification is substitution with lysine.

Preferably, a modified BoNT/A or fragment thereof for use in the invention comprises between 4 and 40 amino acid modifications located in the clostridial toxin H_{CN} domain. Said modified BoNT/A or fragment thereof preferably also has pI of at least 6.6. Said modified BoNT/A preferably comprises modifications of at least 4 amino acids selected from: ASN 886, ASN 930, ASN 954, SER 955, GLN 991, ASN 1025, ASN 1026, and ASN 1052, wherein said modification comprises substitution of the amino acids with a lysine residue or an arginine residue. For example, said modified BoNT/A or fragment thereof may comprise modifications of at least 5 amino acids selected from: ASN 886, ASN 930, ASN 954, SER 955, GLN 991, ASN 1025, ASN 1026, ASN 1052, and GLN 1229, wherein said modification comprises substitution of the amino acids with a lysine residue or an arginine residue.

Methods for modifying proteins by substitution, insertion or deletion of amino acid residues are known in the art. By way of example, amino acid modifications may be introduced by modification of a DNA sequence encoding a polypeptide (e.g. encoding unmodified BoNT/A or a fragment thereof). This can be achieved using standard molecular cloning techniques, for

example by site-directed mutagenesis where short strands of DNA (oligonucleotides) coding for the desired amino acid(s) are used to replace the original coding sequence using a polymerase enzyme, or by inserting/deleting parts of the gene with various enzymes (e.g., ligases and restriction endonucleases). Alternatively, a modified gene sequence can be
5 chemically synthesised.

In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 42 and/or a polypeptide sequence that is encoded by a nucleotide sequence having at least 70% sequence identity to
10 SEQ ID NO: 41. In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to SEQ ID NO: 42. Preferably, a polypeptide for use according to the invention comprises a polypeptide sequence shown as SEQ ID NO: 42. In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence that is encoded by a nucleotide sequence
15 having at least 80%, 90%, 95% or 98% sequence identity to SEQ ID NO: 41. Preferably, a polypeptide for use according to the invention comprises a polypeptide sequence that is encoded by a nucleotide sequence shown as SEQ ID NO: 41.

In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 62. In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to SEQ ID NO: 62. Preferably, a polypeptide for use according to the invention comprises (more preferably consists of) a polypeptide sequence shown as SEQ ID NO: 62.

25 SEQ ID NO: 42 is an example of a modified BoNT/A fragment and SEQ ID NO: 62 is an example of a modified BoNT/A polypeptide that is catalytically inactive. Such modified BoNT/A polypeptides and fragments are particularly preferred for use in the present invention. The polypeptides shown as SEQ ID NO: 42 and 62 have a number of amino acid modifications
30 (e.g. substitutions) when compared to wild-type BoNT/A, which increase the isoelectric point of the polypeptide. Without wishing to be bound by theory, it is believed that the increased net positive charge promotes electrostatic interactions between the polypeptide and anionic extracellular components, thereby promoting binding between the polypeptide and cell surface thus increasing retention at a site of administration and/or duration of action. Thus, it is
35 envisaged that treatment using SEQ ID NO: 42 and 62 will be improved compared to equivalent polypeptides lacking said modifications.

In one embodiment a polypeptide comprising a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 42 or 62 and/or comprising a polypeptide sequence that is encoded by a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 41 comprises a substitution at one or more (preferably two or more, three or more, four or more, five or more or six or more, more preferably at all) of positions 930, 955, 991, 1026, 1052, 1229, and 886.

Preferably, the polypeptide comprising a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 42 or 62 and/or comprising a polypeptide sequence that is encoded by a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 41 comprises lysine or arginine (more preferably lysine) at one or more of positions 930, 955, 991, 1026, 1052, 1229, and 886. In one embodiment, the polypeptide comprises lysine or arginine (more preferably lysine) at least two, three, four, five, six or all of positions 930, 955, 991, 1026, 1052, 1229, and 886. Most preferably, the polypeptide comprises lysine or arginine (more preferably lysine) at all of positions 930, 955, 991, 1026, 1052, 1229, and 886.

In one embodiment a clostridial neurotoxin H_C domain for use in the invention is a modified BoNT/A H_C domain comprising a modification of one or more amino acids residues selected from Y1117, F1252, H1253, and L1278. For example, a modified BoNT/A H_C domain may comprise one or more (preferably two or more) of the following modifications Y1117V, F1252Y, H1253K, and L1278F or L1278H.

In one embodiment a modified BoNT/A H_C domain comprises the following modifications: Y1117V and H1253K; or Y1117V, F1252Y, H1253K, and L1278F; or Y1117V, F1252Y, H1253K, and L1278H.

Preferably, a modified BoNT/A H_C domain comprises the following modifications: Y1117V and H1253K; or Y1117V, F1252Y, H1253K, and L1278H.

The modification may be a modification when compared to catalytically inactive BoNT/A shown as SEQ ID NO: 2, wherein the amino acid residue numbering is determined by alignment with SEQ ID NO: 2. As the presence of a methionine residue at position 1 of SEQ ID NO: 2 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering. For example, where SEQ ID NO: 2 includes a methionine, the position numbering will be as defined above (e.g. Y1117 will align

against Y1117 of SEQ ID NO: 2). Alternatively, where the methionine is absent from SEQ ID NO: 2 the amino acid residue numbering should be modified by -1 (e.g. Y1117 will align against Y1116 of SEQ ID NO: 2). Similar considerations apply when the methionine at position 1 of the other polypeptide sequences described herein is present/absent, and the skilled person will readily determine the correct amino acid residue numbering using techniques routine in the art.

A modified BoNT/A H_C domain may comprise a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 46, 48 or 50 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 46, 48 or 50 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain comprises a polypeptide sequence having at least 99% or 99.9% sequence identity to any one of SEQ ID NOs: 46, 48 or 50 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. Preferably, a modified BoNT/A H_C domain comprises (more preferably consists of) a polypeptide sequence comprising any one of SEQ ID NOs: 46, 48 or 50.

A modified BoNT/A H_C domain may comprise a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 46 or 50 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 46 or 50 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain comprises a polypeptide sequence having at least 99% or 99.9% sequence identity to any one of SEQ ID NOs: 46 or 50 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. Preferably, a modified BoNT/A H_C domain comprises (more preferably consists of) a polypeptide sequence comprising any one of SEQ ID NOs: 46 or 50.

A modified BoNT/A H_C domain may be one encoded by a nucleotide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 45, 47 or 49 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain be one encoded by a nucleotide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 45, 47 or 49

with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain be one encoded by a nucleotide sequence having at least 99% or 99.9% sequence identity to any one of SEQ ID NOs: 45, 47 or 49 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. Preferably, a modified BoNT/A H_C domain be one encoded by any one of SEQ ID NOs: 45, 47 or 49.

A modified BoNT/A H_C domain may be one encoded by a nucleotide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 45 or 49 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain be one encoded by a nucleotide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 45 or 49 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. In one embodiment a modified BoNT/A H_C domain be one encoded by a nucleotide sequence having at least 99% or 99.9% sequence identity to any one of SEQ ID NOs: 45 or 49 with the proviso that the modified BoNT/A H_C domain comprises a modification as described above. Preferably, a modified BoNT/A H_C domain be one encoded by any one of SEQ ID NOs: 45 or 49.

A polypeptide of the present invention may comprise (or consist of) a hybrid or chimeric clostridial neurotoxin (or a fragment of a hybrid or chimeric clostridial neurotoxin), with the proviso that the L-chain is catalytically inactive (when present). A hybrid clostridial neurotoxin comprises at least a portion of a light chain from one clostridial neurotoxin or subtype thereof, and at least a portion of a heavy chain from another clostridial neurotoxin or clostridial neurotoxin subtype. In one embodiment the hybrid clostridial neurotoxin may contain the entire light chain of a light chain from one clostridial neurotoxin subtype and the heavy chain from another clostridial neurotoxin subtype, with the proviso that the L-chain is catalytically inactive (when present). In another embodiment, a chimeric clostridial neurotoxin may contain a portion (e.g. the binding domain) of the heavy chain of one clostridial neurotoxin subtype, with another portion of the heavy chain being from another clostridial neurotoxin subtype. Similarly or alternatively, the therapeutic element may comprise light chain portions from different clostridial neurotoxins, with the proviso that the L-chain is catalytically inactive (when present). Such hybrid or chimeric clostridial neurotoxins are useful, for example, as a means of delivering the therapeutic benefits of such clostridial neurotoxins to subjects who are immunologically resistant to a given clostridial neurotoxin subtype, to subjects who may have a lower than average concentration of receptors to a given clostridial neurotoxin heavy chain binding domain, or to subjects who may have a protease-resistant variant of the membrane or vesicle

toxin substrate (e.g., SNAP-25, VAMP and syntaxin). Hybrid and chimeric clostridial neurotoxins are described in US 8,071,110, which publication is hereby incorporated by reference in its entirety. Thus, in one embodiment, the polypeptide of the invention is or comprises a hybrid clostridial neurotoxin, or a chimeric clostridial neurotoxin with the proviso
5 that the L-chain is catalytically inactive.

In a particularly preferred embodiment, a polypeptide of the invention may be a chimeric clostridial neurotoxin comprising (preferably consisting of) a catalytically inactive BoNT/A light-chain and translocation domain (LH_N domain), and a BoNT/B receptor binding domain (H_C
10 domain) or a portion thereof. A suitable chimeric and/or hybrid clostridial neurotoxin may be one taught in WO 2017/191315 A1, which is incorporated herein by reference, with the proviso that the L-chain is catalytically inactive (e.g. has been inactivated by a modification). Such preferred sequences include SEQ ID NOs: 44 and 61.

15 The catalytically inactive BoNT/A LH_N domain may be covalently linked to the BoNT/B H_C domain. Said chimeric BoNT/A is also referred to herein as “BoNT/AB” or a “BoNT/AB chimera”.

The C-terminal amino acid residue of the LH_N domain may correspond to the first amino acid
20 residue of the 3₁₀ helix separating the LH_N and H_C domains of BoNT/A, and the N-terminal amino acid residue of the H_C domain may correspond to the second amino acid residue of the 3₁₀ helix separating the LH_N and H_C domains in BoNT/B.

Reference herein to the “first amino acid residue of the 3₁₀ helix separating the LH_N and H_C
25 domains of BoNT/A” means the N-terminal residue of the 3₁₀ helix separating the LH_N and H_C domains.

Reference herein to the “second amino acid residue of the 3₁₀ helix separating the LH_N and H_C
domains of BoNT/B” means the amino acid residue following the N-terminal residue of the 3₁₀
30 helix separating the LH_N and H_C domains.

A “3₁₀ helix” is a type of secondary structure found in proteins and polypeptides, along with α-helices, β-sheets and reverse turns. The amino acids in a 3₁₀ helix are arranged in a right-handed helical structure where each full turn is completed by three residues and ten atoms
35 that separate the intramolecular hydrogen bond between them. Each amino acid corresponds to a 120° turn in the helix (i.e., the helix has three residues per turn), and a translation of 2.0 Å

(= 0.2 nm) along the helical axis, and has 10 atoms in the ring formed by making the hydrogen bond. Most importantly, the N-H group of an amino acid forms a hydrogen bond with the C = O group of the amino acid three residues earlier; this repeated $i + 3 \rightarrow i$ hydrogen bonding defines a 3_{10} helix. A 3_{10} helix is a standard concept in structural biology with which the skilled person is familiar.

This 3_{10} helix corresponds to four residues which form the actual helix and two cap (or transitional) residues, one at each end of these four residues. The term “ 3_{10} helix separating the LH_N and H_C domains” as used herein consists of those 6 residues.

Through carrying out structural analyses and sequence alignments, a 3_{10} helix separating the LH_N and H_C domains was identified. This 3_{10} helix is surrounded by an α -helix at its N-terminus (i.e. at the C-terminal part of the LH_N domain) and by a β -strand at its C-terminus (i.e. at the N-terminal part of the H_C domain). The first (N-terminal) residue (cap or transitional residue) of the 3_{10} helix also corresponds to the C-terminal residue of this α -helix.

The 3_{10} helix separating the LH_N and H_C domains can be for example determined from publicly available crystal structures of botulinum neurotoxins, for example 3BTA (<http://www.rcsb.org/pdb/explore/explore.do?structureId=3BTA>) and 1EPW (<http://www.rcsb.org/pdb/explore/explore.do?structureId=1EPW>) for botulinum neurotoxins A1 and B1 respectively.

In silico modelling and alignment tools which are publicly available can also be used to determine the location of the 3_{10} helix separating the LH_N and H_C domains in other neurotoxins, for example the homology modelling servers LOOPP (Learning, Observing and Outputting Protein Patterns, <http://loopp.org>), PHYRE (Protein Homology/analogY Recognition Engine, <http://www.sbg.bio.ic.ac.uk/phyre2/>) and Rosetta (<https://www.rosettacommons.org/>), the protein superposition server SuperPose (<http://wishart.biology.ualberta.ca/superpose/>), the alignment program Clustal Omega (<http://www.clustal.org/omega/>), and a number of other tools/services listed at the Internet Resources for Molecular and Cell Biologists (<http://molbiol-tools.ca/>). In particular that the region around the “ H_N/H_{CN} ” junction is structurally highly conserved which renders it an ideal region to superimpose different serotypes.

For example, the following methodology may be used to determine the sequence of this 3_{10} helix in other neurotoxins:

1. The structural homology modelling tool LOOP (<http://loopp.org>) was used to obtain a predicted structure of other BoNT serotypes based on the BoNT/A1 crystal structure (3BTA.pdb);
2. The structural (pdb) files thus obtained were edited to include only the N-terminal end of the H_{CN} domain and about 80 residues before it (which are part of the H_N domain), thereby retaining the “H_N/H_{CN}” region which is structurally highly conserved;
3. The protein superposition server SuperPose (<http://wishart.biology.ualberta.ca/superpose/>) was used to superpose each serotype onto the 3BTA.pdb structure;
4. The superposed pdb files were inspected to locate the 3₁₀ helix at the start of the H_C domain of BoNT/A1, and corresponding residues in the other serotype were then identified;
5. The other BoNT serotype sequences were aligned with Clustal Omega in order to check that corresponding residues were correct.

Examples of LH_N, H_C and 3₁₀ helix domains determined by this method are presented below:

Neurotoxin	Accession Number (Plus Sequence after Version Decimal)	LH _N	H _C	3 ₁₀ helix
Catalytically inactive BoNT/A1 (SEQ ID NO: 2)	N/A	1-872	873-1296	⁸⁷² NIINTS ⁸⁷⁷
BoNT/A2	X73423.3	1-872	873-1296	⁸⁷² NIVNTS ⁸⁷⁷
BoNT/A3	DQ185900.1 (aka Q3LRX9.1)	1-872	873-1292	⁸⁷² NIVNTS ⁸⁷⁷
BoNT/A4	EU341307.1 (aka Q3LRX8.1)	1-872	873-1296	⁸⁷² NITNAS ⁸⁷⁷
BoNT/A5	EU679004.1 (aka C1IPK2.1)	1-872	873-1296	⁸⁷² NIINTS ⁸⁷⁷
BoNT/A6	FJ981696.1	1-872	873-1296	⁸⁷² NIINTS ⁸⁷⁷
BoNT/A7	JQ954969.1 (aka K4LN57.1)	1-872	873-1296	⁸⁷² NIINTS ⁸⁷⁷
BoNT/A8	KM233166.1	1-872	873-1297	⁸⁷² NITNTS ⁸⁷⁷

Neurotoxin	Accession Number (Plus Sequence after Version Decimal)	LH _N	H _C	3 ₁₀ helix
BoNT/B1 (a.k.a. SEQ ID NO: 52)	B1INP5.1	1-859	860-1291	⁸⁵⁹ EILNNI ⁸⁶⁴
BoNT/B2	AB084152.1 (aka Q8GR96.1)	1-859	860-1291	⁸⁵⁹ EILNNI ⁸⁶⁴
BoNT/B3	EF028400.1 (aka A2I2S2.1)	1-859	860-1291	⁸⁵⁹ EILNNI ⁸⁶⁴
BoNT/B4	EF051570.1 (aka A2I2W0.1)	1-859	860-1291	⁸⁵⁹ EILNNI ⁸⁶⁴
BoNT/B5	EF033130.1 (aka A2I2U6.1)	1-859	860-1291	⁸⁵⁹ DILNNI ⁸⁶⁴
BoNT/B6	AB302852.1 (aka A8R089.1)	1-859	860-1291	⁸⁵⁹ EILNNI ⁸⁶⁴
BoNT/B7	JQ354985.1 (aka H9CNK9.1)	1-859	860-1291	⁸⁵⁹ EILNNI ⁸⁶⁴
BoNT/B8	JQ964806.1 (aka I6Z8G9.1)	1-859	860-1292	⁸⁵⁹ EILNNI ⁸⁶⁴

Using structural analysis and sequence alignments, it was found that the β -strand following the 3₁₀ helix separating the LH_N and H_C domains is a conserved structure in all botulinum and tetanus neurotoxins and starts at the 8th residue when starting from the first residue of the 3₁₀ helix separating the LH_N and H_C domains (e.g., at residue 879 for BoNT/A1).

A BoNT/AB chimera may comprise an LH_N domain from BoNT/A (having a catalytically inactive L-chain) covalently linked to a H_C domain from BoNT/B,

- wherein the C-terminal amino acid residue of the LH_N domain corresponds to the eighth amino acid residue N-terminally to the β -strand located at the beginning (N-term) of the H_C domain of BoNT/A, and
- wherein the N-terminal amino acid residue of the H_C domain corresponds to the seventh amino acid residue N-terminally to the β -strand located at the beginning (N-term) of the H_C domain of BoNT/B.

A BoNT/AB chimera may comprise an LH_N domain from BoNT/A (having a catalytically inactive L-chain) covalently linked to a H_C domain from BoNT/B,

- wherein the C-terminal amino acid residue of the LH_N domain corresponds to the C-terminal amino acid residue of the α -helix located at the end (C-term) of LH_N domain of BoNT/A, and
- wherein the N-terminal amino acid residue of the H_C domain corresponds to the amino acid residue immediately C-terminal to the C-terminal amino acid residue of the α -helix located at the end (C-term) of LH_N domain of BoNT/B.

The rationale of the design process of the BoNT/AB chimera was to try to ensure that the secondary structure was not compromised and thereby minimise any changes to the tertiary structure. Without wishing to be bound by theory, it is hypothesized that by not disrupting the four central amino acid residues of the 3₁₀ helix in the BoNT/AB chimera ensures an optimal conformation for the chimeric neurotoxin.

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The catalytically inactive LH_N domain from BoNT/A may correspond to amino acid residues 1 to 872 of SEQ ID NO: 2 or 61, or a polypeptide sequence having at least 70% sequence identity thereto. The catalytically inactive LH_N domain from BoNT/A may correspond to amino acid residues 1 to 872 of SEQ ID NO: 2 or 61, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the catalytically inactive LH_N domain from BoNT/A corresponds to amino acid residues 1 to 872 of SEQ ID NO: 2 or 61.

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The H_C domain from BoNT/B may correspond to amino acid residues 860 to 1291 of SEQ ID NO: 52, or a polypeptide sequence having at least 70% sequence identity thereto. The H_C domain from BoNT/B may correspond to amino acid residues 860 to 1291 of SEQ ID NO: 52, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the H_C domain from BoNT/B corresponds to amino acid residues 860 to 1291 of SEQ ID NO: 52.

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Preferably, the catalytically inactive LH_N domain corresponds to amino acid residues 1 to 872 of BoNT/A (SEQ ID NO: 2 or 61) and the H_C domain corresponds to amino acid residues 860 to 1291 of BoNT/B (SEQ ID NO: 52).

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Preferably, a BoNT/B H_C domain further comprises at least one amino acid residue substitution, addition or deletion in the H_{CC} subdomain which has the effect of increasing the binding affinity of BoNT/B neurotoxin for human Syt II as compared to the natural BoNT/B

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sequence. Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain have been disclosed in WO 2013/180799 and in WO 2016/154534 (both herein incorporated by reference).

5 Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain include substitution mutations selected from the group consisting of: V1118M; Y1183M; E1191M; E1191I; E1191Q; E1191T; S1199Y; S1199F; S1199L; S1201V; E1191C, E1191V, E1191L, E1191Y, S1199W, S1199E, S1199H, W1178Y, W1178Q, W1178A, W1178S, Y1183C, Y1183P and combinations thereof.

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Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain further include combinations of two substitution mutations selected from the group consisting of: E1191M and S1199L, E1191M and S1199Y, E1191M and S1199F, E1191Q and S1199L, E1191Q and S1199Y, E1191Q and S1199F, E1191M and S1199W, E1191M and W1178Q, E1191C and S1199W, E1191C and S1199Y, E1191C and W1178Q, E1191Q and S1199W, E1191V and S1199W, E1191V and S1199Y, or E1191V and W1178Q.

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Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain also include a combination of three substitution mutations which are E1191M, S1199W and W1178Q.

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Preferably, the suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain includes a combination of two substitution mutations which are E1191M and S1199Y.

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The modification may be a modification when compared to unmodified BoNT/B shown as SEQ ID NO: 52, wherein the amino acid residue numbering is determined by alignment with SEQ ID NO: 52. As the presence of a methionine residue at position 1 of SEQ ID NO: 52 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering. For example, where SEQ ID NO: 52 includes a methionine, the position numbering will be as defined above (e.g. E1191 will be E1191 of SEQ ID NO: 52). Alternatively, where the methionine is absent from SEQ ID NO: 52 the amino acid residue numbering should be modified by -1 (e.g. E1191 will be E1190 of SEQ ID NO: 52). Similar considerations apply when the methionine at position 1 of the other polypeptide sequences described herein is present/absent, and the skilled person will readily determine the correct amino acid residue numbering using techniques routine in the art.

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In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 61. In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to SEQ ID NO: 61. Preferably, a polypeptide for use according to the invention comprises (more preferably consists of) a polypeptide sequence shown as SEQ ID NO: 61.

A chimeric and/or hybrid clostridial neurotoxin for use in the present invention may comprise a portion of a BoNT/A polypeptide and a portion of a BoNT/B polypeptide, an example of which includes the polypeptide described herein as SEQ ID NO: 44.

In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 44 and/or a polypeptide sequence that is encoded by a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 43. In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to SEQ ID NO: 44. Preferably, a polypeptide for use according to the invention comprises a polypeptide sequence shown as SEQ ID NO: 44. In one embodiment a polypeptide for use according to the invention comprises a polypeptide sequence that is encoded by a nucleotide sequence having at least 80%, 90%, 95% or 98% sequence identity to SEQ ID NO: 43. Preferably, a polypeptide for use according to the invention comprises a polypeptide sequence that is encoded by a nucleotide sequence shown as SEQ ID NO: 43.

Suitable chimeric clostridial neurotoxins may include BoNT/FA, with the proviso that any L-chain present is catalytically inactive. Thus, a polypeptide of the invention may comprise BoNT/FA or a fragment thereof, with the proviso that any L-chain present is catalytically inactive. Catalytically inactive forms of BoNT/FA are described herein as SEQ ID NO: 26 and 34. Suitable fragments of BoNT/FA are also described herein as SEQ ID NOs: 28, 30, and 32.

In another preferred embodiment, a polypeptide of the invention may be a chimeric clostridial neurotoxin comprising a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and a receptor binding domain (H_C domain) or a portion thereof from a different (i.e. non-BoNT/X) clostridial neurotoxin. A suitable chimeric and/or hybrid clostridial neurotoxin may be one taught in WO 2020/065336 A1, which is incorporated herein by reference, with the

proviso that the L-chain is catalytically inactive (e.g. has been inactivated by a modification). Such preferred sequences include SEQ ID NOs: 63-70 described herein.

A chimeric clostridial neurotoxin may comprise a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and:

- (i) a BoNT/A receptor binding domain (H_C domain) or a portion thereof; or
- (ii) a BoNT/B receptor binding domain (H_C domain) or a portion thereof; or
- (iii) a BoNT/C receptor binding domain (H_C domain) or a portion thereof; or
- (iv) a BoNT/D receptor binding domain (H_C domain) or a portion thereof; or
- (v) a BoNT/E receptor binding domain (H_C domain) or a portion thereof; or
- (vi) a BoNT/F receptor binding domain (H_C domain) or a portion thereof; or
- (vii) a BoNT/G receptor binding domain (H_C domain) or a portion thereof; or
- (viii) a TeNT receptor binding domain (H_C domain) or a portion thereof.

In one embodiment, the receptor binding domain (H_C domain) or portion thereof from a different (i.e. non-BoNT/X) clostridial neurotoxin domain may be one that binds to synaptotagmin I and/or II (Syt I/II).

Preferably, a chimeric clostridial neurotoxin may comprise a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and a BoNT/B receptor binding domain (H_C domain) or a portion thereof.

A polypeptide comprising a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and a receptor binding domain (H_C domain) or a portion thereof from a different (i.e. non-BoNT/X) clostridial neurotoxin may comprise a polypeptide sequence having at least 70% sequence identity to any one of SEQ ID NOs: 63-70. In one embodiment a polypeptide comprising a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and a receptor binding domain (H_C domain) or a portion thereof from a different (i.e. non-BoNT/X) clostridial neurotoxin comprises a polypeptide sequence having at least 80%, 90%, 95% or 98% sequence identity to any one of SEQ ID NOs: 63-70. Preferably, a polypeptide comprising a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and a receptor binding domain (H_C domain) or a portion thereof from a different (i.e. non-BoNT/X) clostridial neurotoxin (more preferably consists of) any one of SEQ ID NOs: 63-70. Of those polypeptides, SEQ ID NOs: 63-66 are most preferred.

The polypeptide comprising a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and a receptor binding domain (H_C domain) or a portion thereof from a different (i.e. non-BoNT/X) clostridial neurotoxin may comprise the following N-terminal amino acid sequence MGS. Where SEQ ID NOs: 63-66 contain said N-terminal amino acid sequence, said sequence is optional. In one embodiment SEQ ID NOs: 63-66 lack an N-terminal amino acid sequence shown as MGS. In one embodiment SEQ ID NOs: 63-66 comprise an N-terminal amino acid sequence shown as MGS.

The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 1 to 899 of SEQ ID NO: 63, or a polypeptide sequence having at least 70% sequence identity thereto. The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 1 to 899 of SEQ ID NO: 63, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 1 to 899 of SEQ ID NO: 63.

The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 4 to 899 of SEQ ID NO: 63, or a polypeptide sequence having at least 70% sequence identity thereto. The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 4 to 899 of SEQ ID NO: 63, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 4 to 899 of SEQ ID NO: 63.

The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 1 to 866 of SEQ ID NO: 65, or a polypeptide sequence having at least 70% sequence identity thereto. The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 1 to 866 of SEQ ID NO: 65, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 1 to 866 of SEQ ID NO: 65.

The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 4 to 866 of SEQ ID NO: 65, or a polypeptide sequence having at least 70% sequence identity thereto. The catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 4 to 866 of SEQ ID NO: 65, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the catalytically inactive LH_N domain from BoNT/X may correspond to amino acid residues 4 to 866 of SEQ ID NO: 65.

The H_C domain from BoNT/B may correspond to amino acid residues 860 to 1291 of SEQ ID NO: 52, or a polypeptide sequence having at least 70% sequence identity thereto. The H_C domain from BoNT/B may correspond to amino acid residues 860 to 1291 of SEQ ID NO: 52, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto.

5 Preferably, the H_C domain from BoNT/B corresponds to amino acid residues 860 to 1291 of SEQ ID NO: 52.

10 Preferably, a BoNT/B H_C domain further comprises at least one amino acid residue substitution, addition or deletion in the H_{CC} subdomain which has the effect of increasing the binding affinity of BoNT/B neurotoxin for human Syt II as compared to the natural BoNT/B sequence. Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain have been disclosed in WO 2013/180799 and in WO 2016/154534 (both herein incorporated by reference).

15 Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain include substitution mutations selected from the group consisting of: V1118M; Y1183M; E1191M; E1191I; E1191Q; E1191T; S1199Y; S1199F; S1199L; S1201V; E1191C, E1191V, E1191L, E1191Y, S1199W, S1199E, S1199H, W1178Y, W1178Q, W1178A, W1178S, Y1183C, Y1183P and combinations thereof.

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Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain further include combinations of two substitution mutations selected from the group consisting of: E1191M and S1199L, E1191M and S1199Y, E1191M and S1199F, E1191Q and S1199L, E1191Q and S1199Y, E1191Q and S1199F, E1191M and S1199W, E1191M and W1178Q, 25 E1191C and S1199W, E1191C and S1199Y, E1191C and W1178Q, E1191Q and S1199W, E1191V and S1199W, E1191V and S1199Y, or E1191V and W1178Q.

Suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain also include a combination of three substitution mutations which are E1191M, S1199W and 30 W1178Q.

Preferably, the suitable amino acid residue substitution, addition or deletion in the BoNT/B H_{CC} subdomain includes a combination of two substitution mutations which are E1191M and S1199Y.

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The modification may be a modification when compared to unmodified BoNT/B shown as SEQ ID NO: 52, wherein the amino acid residue numbering is determined by alignment with SEQ ID NO: 52. As the presence of a methionine residue at position 1 of SEQ ID NO: 52 is optional, the skilled person will take the presence/absence of the methionine residue into account when determining amino acid residue numbering. For example, where SEQ ID NO: 52 includes a methionine, the position numbering will be as defined above (e.g. E1191 will be E1191 of SEQ ID NO: 52). Alternatively, where the methionine is absent from SEQ ID NO: 52 the amino acid residue numbering should be modified by -1 (e.g. E1191 will be E1190 of SEQ ID NO: 52). Similar considerations apply when the methionine at position 1 of the other polypeptide sequences described herein is present/absent, and the skilled person will readily determine the correct amino acid residue numbering using techniques routine in the art.

The H_C domain from BoNT/A may correspond to amino acid residues 873 to 1296 of SEQ ID NO: 60, or a polypeptide sequence having at least 70% sequence identity thereto. The H_C domain from BoNT/A may correspond to amino acid residues 873 to 1296 of SEQ ID NO: 60, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the H_C domain from BoNT/B corresponds to amino acid residues 873 to 1296 of SEQ ID NO: 60.

In one embodiment, where the polypeptide is for use in treating an inflammatory disorder, the polypeptide used does not comprise a catalytically inactive BoNT/X L-chain, a BoNT/X translocation domain (H_N domain), and a BoNT/A receptor binding domain (H_C domain). Thus, in one embodiment, where a polypeptide is for use in treating an inflammatory disorder, the polypeptide may comprise a catalytically inactive BoNT/X L-chain, a BoNT/X translocation domain (H_N domain) and: (i) a BoNT/B receptor binding domain (H_C domain); (ii) a BoNT/D receptor binding domain (H_C domain); or (iii) a BoNT/F receptor binding (H_C domain).

Similarly, in one embodiment, where the polypeptide is for use in treating pain, the polypeptide used does not comprise a catalytically inactive BoNT/X L-chain, a BoNT/X translocation domain (H_N domain), and a BoNT/A receptor binding domain (H_C domain). Thus, in one embodiment, where a polypeptide is for use in treating pain, the polypeptide may comprise a catalytically inactive BoNT/X L-chain, a BoNT/X translocation domain (H_N domain) and: (i) a BoNT/B receptor binding domain (H_C domain); (ii) a BoNT/D receptor binding domain (H_C domain); or (iii) a BoNT/F receptor binding (H_C domain).

The H_C domain from BoNT/D may correspond to amino acid residues 865 to 1276 of SEQ ID NO: 54, or a polypeptide sequence having at least 70% sequence identity thereto. The H_C domain from BoNT/D may correspond to amino acid residues 865 to 1276 of SEQ ID NO: 54, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto.

- 5 Preferably, the H_C domain from BoNT/D corresponds to amino acid residues 865 to 1276 of SEQ ID NO: 54.

The H_C domain from BoNT/F may correspond to amino acid residues 866 to 1278 of SEQ ID NO: 56, or a polypeptide sequence having at least 70% sequence identity thereto. The H_C domain from BoNT/F may correspond to amino acid residues 866 to 1278 of SEQ ID NO: 56, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the H_C domain from BoNT/F corresponds to amino acid residues 866 to 1278 of SEQ ID NO: 56 having a histidine to lysine substitution at position 1241 (H1241K).

- 10 domain from BoNT/F may correspond to amino acid residues 866 to 1278 of SEQ ID NO: 56, or a polypeptide sequence having at least 80%, 90% or 95% sequence identity thereto. Preferably, the H_C domain from BoNT/F corresponds to amino acid residues 866 to 1278 of SEQ ID NO: 56 having a histidine to lysine substitution at position 1241 (H1241K).
- 15 Preferably, the chimeric clostridial neurotoxin may comprise (preferably consist of) a catalytically inactive BoNT/X light-chain and translocation domain (LH_N domain), and a receptor binding domain (H_C domain) or a portion thereof from a different (i.e. non-BoNT/X) clostridial neurotoxin and Cys-(Xaa)_a-Ile-Asp/Glu-Gly-Arg-(Yaa)_b-Cys (SEQ ID NO: 71), wherein a = 1-10 and b = 4-15. SEQ ID NO: 71 is an activation loop consensus sequence
- 20 based on the BoNT/C1 activation loop.

Moreover, any polypeptide of the invention may comprise Cys-(Xaa)_a-Ile-Asp/Glu-Gly-Arg-(Yaa)_b-Cys (SEQ ID NO: 71), wherein a = 1-10 and b = 4-15. Said activation loop may suitably replace any activation loop sequences present in either a clostridial neurotoxin L-chain and/or translocation domain (H_N domain) present in a polypeptide described herein.

- 25
- 30 Xaa or Yaa when used in the context of SEQ ID NO: 71 can be any amino acid. The number of amino acids at position Xaa and Yaa are indicated by the letters 'a' and 'b', respectively. In one embodiment 'a' and 'b' can be any integer that allows for proteolytic cleavage of the activation loop and yields an active di-chain clostridial neurotoxin. In one embodiment 'a' is at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In one embodiment 'b' is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15. In one embodiment 'a' is ≤12, ≤11, ≤10, ≤9, ≤8, ≤7, ≤6, ≤5 or ≤4. In one embodiment 'b' is ≤20, ≤19, ≤18, ≤17, ≤16, ≤15, ≤14, ≤13, ≤12, ≤11, ≤10 or ≤9.

In one embodiment 'a' is 1-12, for example 1-10. Preferably 'a' is 1-7, such as 2-4. More preferably 'a' is 3. In one embodiment 'b' is 1-20, for example 4-15. Preferably 'b' is 6-10. More preferably 'b' is 8.

5 It is not intended that Xaa or Yaa be limited to only one type of amino acid. Thus, one or more residues present at position Xaa may be independently selected from the standard amino acids: aspartic acid, glutamic acid, arginine, lysine, histidine, asparagine, glutamine, serine, threonine, tyrosine, methionine, tryptophan, cysteine, alanine, glycine, valine, leucine, isoleucine, proline, and phenylalanine. One or more residues present at position Yaa may be
10 independently selected from the standard amino acids: aspartic acid, glutamic acid, arginine, lysine, histidine, asparagine, glutamine, serine, threonine, tyrosine, methionine, tryptophan, cysteine, alanine, glycine, valine, leucine, isoleucine, proline, and phenylalanine. Preferably an amino acid at position Yaa (more preferably immediately C-terminal to the Arg residue of SEQ ID NO: 71) is not proline.

15

Alternatively/additionally, one or more residues present at position Xaa or Yaa may be independently selected from a non-standard amino acid (an amino acid that is not part of the standard set of 20 described above). By way of example, non-standard amino acids may include 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline, α -methyl
20 serine, trans-3-methylproline, 2,4-methano-proline, cis-4-hydroxyproline, trans-4-hydroxyproline, N-methylglycine, allo-threonine, methyl-threonine, hydroxy-ethylcysteine, hydroxyethylhomo-cysteine, nitro-glutamine, homoglutamine, pipecolic acid, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenyl-alanine, 4-azaphenyl-alanine, L-Ornithine, L-2-amino-3-guanidinopropionic acid, or D-isomers of Lysine, Arginine and/or Ornithine, and 4-
25 fluorophenylalanine. Methods for introducing non-standard amino acids into proteins are known in the art, and include recombinant protein synthesis using *E. coli* auxotrophic expression hosts.

The sequence Ile-Asp/Glu-Gly-Arg comprised in SEQ ID NO: 71 refers to a site surprisingly
30 found in WO 2020/065336 A1 to be recognised by enterokinase (as well as factor Xa). Said document describes suitable methods for cleaving at Ile-Asp/Glu-Gly-Arg, thereby producing a di-chain polypeptide. Preferably the sequence is Ile-Asp-Gly-Arg, e.g. Cys-(Xaa)_a-Ile-Asp-Gly-Arg-(Yaa)_b-Cys. It is believed that enterokinase and factor Xa hydrolyse a peptide bond immediately C-terminal to Arg of SEQ ID NO: 71 (i.e. the peptide bond between Arg and Yaa).

35

In one embodiment an amino acid residue at Xaa immediately N-terminal to Ile of SEQ ID NO: 71 is an uncharged hydrophobic amino acid, preferably alanine. In some embodiments 'a' is at least 2, and Xaa comprises at least a C-terminal uncharged polar amino acid and a charged basic amino acid immediately N-terminal thereto. The charged basic amino acid is preferably lysine. Thus in embodiments where 'a' is at least 2, Xaa may comprise at least Lys-Ala, wherein Ala is immediately N-terminal to Ile of SEQ ID NO: 71.

In one embodiment Xaa comprises or consists of the sequence HKA.

In one embodiment an amino acid residue at Yaa immediately C-terminal to Arg of SEQ ID NO: 71 is an uncharged polar amino acid, preferably serine. In some embodiments 'b' is at least 2, and Yaa comprises at least an N-terminal uncharged polar amino acid and an uncharged hydrophobic amino acid immediately C-terminal thereto. The uncharged hydrophobic amino acid is preferably leucine. Thus in embodiments where 'b' is at least 2, Yaa may comprise at least Ser-Leu, wherein Ser is immediately C-terminal to Arg of SEQ ID NO: 71.

In one embodiment Yaa comprises or consists of the sequence SLYNKTLDC.

In some embodiments a polypeptide herein comprises an activation loop having at least 70% sequence identity to SEQ ID NO: 72. In one embodiment a polypeptide herein comprises an activation loop having at least 80%, 85% or 90% sequence identity to SEQ ID NO: 72. Preferably a polypeptide herein comprises an activation loop having at least 95% sequence identity to SEQ ID NO: 72. More preferably, a polypeptide herein comprises an activation loop having at least 99% sequence identity to SEQ ID NO: 72.

In a particularly preferred embodiment a polypeptide herein comprises an activation loop comprising SEQ ID NO: 72, more preferably consisting of SEQ ID NO: 72.

The activation loop may also be a variant of SEQ ID NO: 72, such as SEQ ID NO: 73 or a sequence having at least 70% sequence identity thereto. SEQ ID NO: 73 is a variant of SEQ ID NO: 72 in which the enterokinase recognition site IDGR has been mutated to IEGR. In one embodiment a polypeptide herein comprises an activation loop having at least 70% sequence identity to SEQ ID NO: 73. In one embodiment a polypeptide herein comprises an activation loop having at least 80%, 85% or 90% sequence identity to SEQ ID NO: 73. Preferably a polypeptide herein comprises an activation loop having at least 95% sequence identity to SEQ

ID NO: 73. More preferably, a polypeptide herein comprises an activation loop having at least 99% sequence identity to SEQ ID NO: 73.

5 In a particularly preferred embodiment a polypeptide herein comprises an activation loop comprising SEQ ID NO: 73, more preferably consisting of SEQ ID NO: 73.

10 In one embodiment an activation loop described herein (e.g. SEQ ID NO: 71, 72 or 73) may be modified to include an additional or alternative protease site. For example, a protease site shown as SEQ ID NO: 77. An example of such a modified activation loop is shown as SEQ ID NO: 78. Thus, in one embodiment a polypeptide herein comprises an activation loop having at least 70% sequence identity to SEQ ID NO: 78. In one embodiment a polypeptide herein comprises an activation loop having at least 80%, 85% or 90% sequence identity to SEQ ID NO: 78. Preferably a polypeptide herein comprises an activation loop having at least 95% sequence identity to SEQ ID NO: 78. More preferably, a polypeptide herein comprises an activation loop having at least 99% sequence identity to SEQ ID NO: 78. In a particularly preferred embodiment a polypeptide herein comprises an activation loop comprising SEQ ID NO: 78, more preferably consisting of SEQ ID NO: 78.

20 In one embodiment, a polypeptide of the invention may comprise (or consist of) a re-targeted clostridial neurotoxin, with the proviso that any L-chain present is catalytically inactive. In a re-targeted clostridial neurotoxin, the clostridial neurotoxin is modified to include an exogenous ligand known as a Targeting Moiety (TM). The TM is selected to provide binding specificity for a desired target cell, and as part of the re-targeting process the native binding portion of the clostridial neurotoxin (e.g. the H_C domain, or the H_{CC} domain) may be removed. Re-targeting technology is described, for example, in: EP-B-0689459; WO 1994/021300; EP-B-0939818; US 6,461,617; US 7,192,596; WO 1998/007864; EP-B-0826051; US 5,989,545; US 6,395,513; US 6,962,703; WO 1996/033273; EP-B-0996468; US 7,052,702; WO 1999/017806; EP-B-1107794; US 6,632,440; WO 2000/010598; WO 2001/21213; WO 2006/059093; WO 2000/62814; WO 2000/04926; WO 1993/15766; WO 2000/61192; and WO 1999/58571; all of which are hereby incorporated by reference in their entirety. Thus, in one embodiment, the polypeptide of the invention is a re-targeted clostridial neurotoxin, with the proviso that any L-chain present is catalytically inactive. The polypeptide of the present invention may lack a functional H_C domain of a clostridial neurotoxin and also lack any functionally equivalent TM.

35 In embodiments where a polypeptide described herein has a tag for purification (e.g. a His-tag) and/or a linker, said tag and/or linker are optional.

The polypeptides of the present invention may be free from the complexing proteins that are present in a naturally occurring clostridial neurotoxin complex.

- 5 The polypeptides of the present invention can be produced using recombinant nucleic acid technologies. Thus, in one embodiment, a polypeptide (as described above) is a recombinant polypeptide.

10 In one embodiment a nucleic acid (for example, a DNA) comprising a nucleic acid sequence encoding a polypeptide is provided. In one embodiment, the nucleic acid sequence is prepared as part of a DNA vector comprising a promoter and a terminator. The nucleic acid sequence may be selected from any of the nucleic acid sequences described herein.

In a preferred embodiment, the vector has a promoter selected from:

15

Promoter	Induction Agent	Typical Induction Condition
Tac (hybrid)	IPTG	0.2 mM (0.05-2.0mM)
AraBAD	L-arabinose	0.2% (0.002-0.4%)
T7-lac operator	IPTG	0.2 mM (0.05-2.0mM)

20

In another preferred embodiment, the vector has a promoter selected from:

Promoter	Induction Agent	Typical Induction Condition
Tac (hybrid)	IPTG	0.2 mM (0.05-2.0mM)
25 AraBAD	L-arabinose	0.2% (0.002-0.4%)
T7-lac operator	IPTG	0.2 mM (0.05-2.0mM)
T5-lac operator	IPTG	0.2 mM (0.05-2.0mM)

30 The nucleic acid molecules may be made using any suitable process known in the art. Thus, the nucleic acid molecules may be made using chemical synthesis techniques. Alternatively, the nucleic acid molecules of the invention may be made using molecular biology techniques.

The DNA construct of the present invention is preferably designed *in silico*, and then synthesised by conventional DNA synthesis techniques.

35

The above-mentioned nucleic acid sequence information is optionally modified for codon-biasing according to the ultimate host cell (e.g. *E. coli*) expression system that is to be employed.

- 5 The terms “nucleotide sequence” and “nucleic acid” are used synonymously herein. Preferably the nucleotide sequence is a DNA sequence.

A polypeptide of the invention (and especially any clostridial neurotoxin portion thereof) may be present as a single-chain or as a di-chain. However, it is preferred that the polypeptide is
10 present as a di-chain in which the catalytically inactive L-chain is linked to the H-chain (or component thereof, e.g. the H_N domain) via a di-sulphide bond.

The invention provides a method of producing a single-chain polypeptide having a catalytically inactive light chain and a heavy chain, the method comprising expressing a nucleic acid
15 described herein in an expression host, lysing the host cell to provide a host cell homogenate containing the single-chain polypeptide, and isolating the single-chain polypeptide. In one aspect, the present invention provides a method of proteolytically processing a polypeptide described herein, the method comprising contacting the polypeptide with a protease that hydrolyses a peptide bond in the activation loop of the polypeptide, thereby converting the
20 (single-chain) polypeptide into a corresponding di-chain polypeptide (e.g. wherein the catalytically inactive light chain and heavy chain are joined together by a disulphide bond).

The present invention therefore provides a di-chain polypeptide obtainable by a method of the invention.

25

A “subject” as used herein may be a mammal, such as a human or other mammal. Preferably “subject” means a human subject.

The term “disorder” as used herein also encompasses a “disease”. In one embodiment the
30 disorder is a disease.

The term “treat” or “treating” as used herein encompasses prophylactic treatment (e.g. to prevent onset of a disorder [e.g. pain]) as well as corrective treatment (treatment of a subject already suffering from a disorder [e.g. pain]). Preferably “treat” or “treating” as used herein
35 means corrective treatment.

The term “treat” or “treating” as used herein refers to the disorder (e.g. pain) and/or a symptom thereof .

Therefore a polypeptide of the invention may be administered to a subject in a therapeutically effective amount or a prophylactically effective amount. Preferably a polypeptide of the invention is administered to a subject in a therapeutically effective amount.

A “therapeutically effective amount” is any amount of the polypeptide, which when administered alone or in combination with another agent to a subject for treating said disorder (e.g. pain) (or a symptom thereof) is sufficient to effect such treatment of the disorder, or symptom thereof.

A “prophylactically effective amount” is any amount of the polypeptide that, when administered alone or in combination with another agent to a subject inhibits or delays the onset or reoccurrence of a disorder (e.g. pain) (or a symptom thereof). In some embodiments, the prophylactically effective amount prevents the onset or reoccurrence of a disorder (e.g. pain) entirely. “Inhibiting” the onset means either lessening the likelihood of a disorder’s onset (e.g. where the disorder is pain) (or symptom thereof), or preventing the onset entirely.

The polypeptides of the invention may be formulated in any suitable manner for administration to a subject, for example as part of a pharmaceutical composition. Thus, in one aspect, the invention provides a pharmaceutical composition comprising a polypeptide of the invention and a pharmaceutically acceptable carrier, excipient, adjuvant, propellant and/or salt.

The polypeptides of the present invention may be formulated for oral, parenteral, continuous infusion, inhalation or topical application. Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

In the case of a polypeptide that is to be delivered locally, the polypeptide may be formulated as a cream (e.g. for topical application), or for sub-dermal injection.

Local delivery means may include an aerosol, or other spray (e.g. a nebuliser). In this regard, an aerosol formulation of a polypeptide enables delivery to the lungs and/or other nasal and/or bronchial or airway passages.

Polypeptides of the invention may be administered to a subject by intrathecal or epidural injection in the spinal column at the level of the spinal segment involved in the innervation of an affected organ.

5 A route of administration may be via laproscopic and/ or localised injection. In one embodiment a polypeptide of the invention is administered at or near to a site to be treated, preferably at a site to be treated. For example, the polypeptide may be administered intrathecally or intraspinally. In one embodiment the route of administration of a polypeptide of the invention may be perineural, intraneural, intraspinal, and/or intrathecal.

10

In one embodiment a polypeptide of the invention may be administered peripherally. In one embodiment, the polypeptide may be administered intradermally subcutaneously or intramuscularly. Preferably, a polypeptide of the invention is administered intradermally.

15 The dosage ranges for administration of the polypeptides of the present invention are those to produce the desired therapeutic and/or prophylactic effect. It will be appreciated that the dosage range required depends on the precise nature of the clostridial neurotoxin or composition, the route of administration, the nature of the formulation, the age of the subject, the nature, extent or severity of the subject's condition, contraindications, if any, and the
20 judgement of the attending physician. Variations in these dosage levels can be adjusted using standard empirical routines for optimisation.

In one embodiment a dosage of the polypeptide is a flat dose. A flat dose may be in the range of 50 pg to 250 µg, preferably 100 pg to 100 µg. In one embodiment a flat dose may be at
25 least 50 pg, 100 pg, 500 pg, 1 ng, 50 ng, 100 ng, 500 ng, 1 µg or 50 µg. Said dose may be a single flat dose.

In a preferred embodiment, a polypeptide may be dosed in an amount of greater than 250 µg. In one embodiment, a polypeptide of the invention may be dosed in an amount of greater than
30 500 µg, 1 mg, 10 mg, 100 mg, 500 mg, 1 g or 5 g. In one embodiment, a polypeptide of the invention may be dosed in an amount equal to or less than 10 g, 5 g, 1 g, 500 mg, 100 mg, 10 mg or 1 mg. Preferably, a polypeptide of the invention is dosed at an amount of 251 µg to 10 g, 251 µg to 5 g, 251 µg to 1 g, 251 µg to 500 mg, 251 µg to 100 mg, 251 µg to 10 mg, or 251 µg to 1000 µg, e.g. 251 µg to 500 µg. In one embodiment, a polypeptide of the invention is
35 dosed in an amount of 500 µg to 5 g, e.g. 1 mg to 1 g or 1 g to 3 g. This is made possible by the non-toxic (e.g. substantially non-toxic) nature of the polypeptides of the invention.

Fluid dosage forms are typically prepared utilising the polypeptide and a pyrogen-free sterile vehicle. The clostridial neurotoxin, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. In preparing solutions the polypeptide can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and or local anaesthetic agents may be dissolved in the vehicle.

Dry powders, which are dissolved or suspended in a suitable vehicle prior to use, may be prepared by filling pre-sterilised ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the ingredients may be dissolved into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for an administration route described herein, are prepared in substantially the same manner, except that the sterile components are suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The components may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation.

Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition(s) to facilitate uniform distribution of the components.

Administration in accordance with the present invention may take advantage of a variety of delivery technologies including microparticle encapsulation, or high-pressure aerosol impingement.

The polypeptides of the invention are preferably administered iteratively (e.g. up to 5, 10, 15 or 20 times) as part of treatment regimen. Iterative administration means administration at least two times, e.g. at least 5, 10, 15 or 20 times. Thus, in one embodiment, polypeptides of the invention may be administered two or more times to treat a subject (e.g. to treat pain in a subject). This is particularly pertinent for the treatment of chronic conditions, such as chronic pain, where ongoing treatment is typically necessary. In one embodiment a polypeptide of the

invention may be administered weekly, twice monthly, monthly, every two months, every six months or annually, preferably at least twice annually or annually. In one embodiment, a polypeptide of the invention is administered two or more times in a period of 10 years, 5 years, 2 years or 1 year. Preferably, a polypeptide of the invention is administered two or more times
5 in a period of 1 year. Treatment may continue for at least 6 months, 1 year, 2 years, 3 years, 5 years, 10 years, 15 years, 20 years, 25 years or 30 years.

It is preferred that the polypeptide is not administered together with a further therapeutic or diagnostic agent (e.g. a nucleic acid, protein, peptide or small molecule therapeutic or
10 diagnostic agent) additional to the catalytically inactive clostridial neurotoxin L-chain, H_N domain and/or H_C domain. For example, in one embodiment the polypeptide is not administered with a further analgesic and/or anti-inflammatory. In one embodiment a polypeptide of the invention is not administered together with a covalently associated therapeutic agent. In one embodiment a polypeptide of the invention is not administered
15 together with a non-covalently associated therapeutic agent.

The polypeptides described herein may be used to treat a subject suffering from one or more types of pain. The pain may be chronic or acute pain. The pain may be one or more selected from the following four categories of pain: nociceptive pain; neuropathic pain; mixed pain; and
20 pain of an unknown origin. Nociceptive pain may be caused by a known noxious stimulus to a nociceptor (pain receptor) and may be somatic or visceral. Neuropathic pain may be pain initiated or caused by a primary lesion or dysfunction in the nervous system. Mixed pain may be a combination of nociceptive pain and neuropathic pain.

Examples of pain (e.g. of chronic pain) treated by the present invention include neuropathic pain, inflammatory pain, headache pain, somatic pain, visceral pain, referred pain, allodynia, mixed pain, and post-operative pain.

The term "pain" as used here, means any unpleasant sensory experience, usually associated
30 with a physical disorder. The physical disorder may or may not be apparent to a clinician. Pain is of two types: chronic and acute. An "acute pain" is a pain of short duration having a sudden onset. One type of acute pain, for example, is cutaneous pain felt on injury to the skin or other superficial tissues, such as caused by a cut or a burn. Cutaneous nociceptors terminate just below the skin, and due to the high concentration of nerve endings, produce a well-defined,
35 localized pain of short duration. "Chronic pain" is a pain other than an acute pain.

The polypeptides of the invention may be used to treat pain caused by or otherwise associated with any of the following neuropathic pain conditions. "Neuropathic pain" means abnormal sensory input, resulting in discomfort, from the peripheral nervous system, central nervous systems, or both. Symptoms of neuropathic pain can involve persistent, spontaneous pain, as well as allodynia (a painful response to a stimulus that normally is not painful), hyperalgesia (an accentuated response to a painful stimulus that usually causes only a mild discomfort, such as a pin prick), or hyperpathia (where a short discomfort becomes a prolonged severe pain). Neuropathic pain may be caused by any of the following:

1. A traumatic insult, such as, for example, a nerve compression injury (e.g., a nerve crush, a nerve stretch, a nerve entrapment or an incomplete nerve transection); a spinal cord injury (e.g., a hemisection of the spinal cord); a limb amputation; a contusion; an inflammation (e.g., an inflammation of the spinal cord); or a surgical procedure.
2. An ischemic event, including, for example, a stroke and heart attack.
3. An infectious agent.
4. Exposure to a toxic agent, including, for example, a drug, an alcohol, a heavy metal (e.g., lead, arsenic, mercury), an industrial agent (e.g., a solvent, fumes from a glue) or nitrous oxide.
5. A disease, including, for example, an inflammatory disorder, a neoplastic tumour, an acquired immune deficiency syndrome (AIDS), Lyme disease, a leprosy, a metabolic disease, a peripheral nerve disorder, like neuroma, a mononeuropathy or a polyneuropathy.

Types of neuropathic pain include the following:

1. Neuralgia.

A neuralgia is a pain that radiates along the course of one or more specific nerves usually without any demonstrable pathological change in the nerve structure. The causes of neuralgia are varied. Chemical irritation, inflammation, trauma (including surgery), compression by nearby structures (for instance, tumours), and infections may all lead to neuralgia. In many cases, however, the cause is unknown or unidentifiable. Neuralgia is most common in elderly persons, but it may occur at any age. A neuralgia, includes, without limitation, a trigeminal neuralgia, a post-herpetic neuralgia, a postherpetic neuralgia, a glossopharyngeal neuralgia, a sciatica and an atypical facial pain.

Neuralgia is pain in the distribution of a nerve or nerves. Examples are trigeminal neuralgia, atypical facial pain, and postherpetic neuralgia (caused by shingles or herpes). The affected nerves are responsible for sensing touch, temperature, and pressure in the facial area from the jaw to the forehead. The disorder generally causes short episodes of excruciating pain,

usually for less than two minutes and on only one side of the face. The pain can be described in a variety of ways such as "stabbing," "sharp," "like lightning," "burning," and even "itchy". In the atypical form of TN, the pain can also present as severe or merely aching and last for extended periods. The pain associated with TN is recognized as one the most excruciating
5 pains that can be experienced.

Simple stimuli such as eating, talking, washing the face, or any light touch or sensation can trigger an attack (even the sensation of a gentle breeze). The attacks can occur in clusters or as an isolated attack.

10 Symptoms include sharp, stabbing pain or constant, burning pain located anywhere, usually on or near the surface of the body, in the same location for each episode; pain along the path of a specific nerve; impaired function of an affected body part due to pain, or muscle weakness due to concomitant motor nerve damage; increased sensitivity of the skin or numbness of the
15 affected skin area (feeling similar to a local anaesthetic such as a Novocaine shot); and any touch or pressure is interpreted as pain. Movement may also be painful.

Trigeminal neuralgia is the most common form of neuralgia. It affects the main sensory nerve of the face, the trigeminal nerve ("trigeminal" literally means "three origins", referring to the
20 division of the nerve into 3 branches). This condition involves sudden and short attacks of severe pain on the side of the face, along the area supplied by the trigeminal nerve on that side. The pain attacks may be severe enough to cause a facial grimace, which is classically referred to as a painful tic (tic douloureux). Sometimes, the cause of trigeminal neuralgia is a blood vessel or small tumour pressing on the nerve. Disorders such as multiple sclerosis (an
25 inflammatory disease affecting the brain and spinal cord), certain forms of arthritis, and diabetes (high blood sugar) may also cause trigeminal neuralgia, but a cause is not always identified. In this condition, certain movements such as chewing, talking, swallowing, or touching an area of the face may trigger a spasm of excruciating pain.

30 A related but rather uncommon neuralgia affects the glosso-pharyngeal nerve, which provides sensation to the throat. Symptoms of this neuralgia are short, shock-like episodes of pain located in the throat.

Neuralgia may occur after infections such as shingles, which is caused by the varicella-zoster
35 virus, a type of herpesvirus. This neuralgia produces a constant burning pain after the shingles rash has healed. The pain is worsened by movement of or contact with the affected area. Not

all of those diagnosed with shingles go on to experience postherpetic neuralgia, which can be more painful than shingles. The pain and sensitivity can last for months or even years. The pain is usually in the form of an intolerable sensitivity to any touch but especially light touch. Postherpetic neuralgia is not restricted to the face; it can occur anywhere on the body but usually occurs at the location of the shingles rash. Depression is not uncommon due to the pain and social isolation during the illness.

Postherpetic neuralgia may be debilitating long after signs of the original herpes infection have disappeared. Other infectious diseases that may cause neuralgia are syphilis and Lyme disease.

Diabetes is another common cause of neuralgia. This very common medical problem affects almost 1 out of every 20 Americans during adulthood. Diabetes damages the tiny arteries that supply circulation to the nerves, resulting in nerve fibre malfunction and sometimes nerve loss. Diabetes can produce almost any neuralgia, including trigeminal neuralgia, carpal tunnel syndrome (pain and numbness of the hand and wrist), and meralgia paresthetica (numbness and pain in the thigh due to damage to the lateral femoral cutaneous nerve). Strict control of blood sugar may prevent diabetic nerve damage and may accelerate recovery in subjects who do develop neuralgia.

Other medical conditions that may be associated with neuralgias are chronic renal insufficiency and porphyria -- a hereditary disease in which the body cannot rid itself of certain substances produced after the normal breakdown of blood in the body. Certain drugs may also cause this problem.

2. Deafferentation.

Deafferentation indicates a loss of the sensory input from a portion of the body, and can be caused by interruption of either peripheral sensory fibres or nerves from the central nervous system. A deafferentation pain syndrome, includes, without limitation, an injury to the brain or spinal cord, a post-stroke pain, a phantom pain, a paraplegia, a brachial plexus avulsion injuries, lumbar radiculopathies.

3. Complex regional pain syndromes (CRPSs)

CRPS is a chronic pain syndrome resulting from sympathetically-maintained pain, and presents in two forms. CRPS 1 currently replaces the term "reflex sympathetic dystrophy syndrome". It is a chronic nerve disorder that occurs most often in the arms or legs after a

minor or major injury. CRPS 1 is associated with severe pain; changes in the nails, bone, and skin; and an increased sensitivity to touch in the affected limb. CRPS 2 replaces the term causalgia, and results from an identified injury to the nerve. A CRPS, includes, without limitation, a CRPS Type I (reflex sympathetic dystrophy) and a CRPS Type II (causalgia).

5

4. Neuropathy.

A neuropathy is a functional or pathological change in a nerve and is characterized clinically by sensory or motor neuron abnormalities.

10 Central neuropathy is a functional or pathological change in the central nervous system.

Peripheral neuropathy is a functional or pathological change in one or more peripheral nerves. The peripheral nerves relay information from your central nervous system (brain and spinal cord) to muscles and other organs and from your skin, joints, and other organs back to your brain. Peripheral neuropathy occurs when these nerves fail to carry information to and from
15 the brain and spinal cord, resulting in pain, loss of sensation, or inability to control muscles. In some cases, the failure of nerves that control blood vessels, intestines, and other organs results in abnormal blood pressure, digestion problems, and loss of other basic body processes. Risk factors for neuropathy include diabetes, heavy alcohol use, and exposure to
20 certain chemicals and drugs. Some people have a hereditary predisposition for neuropathy. Prolonged pressure on a nerve is another risk for developing a nerve injury. Pressure injury may be caused by prolonged immobility (such as a long surgical procedure or lengthy illness) or compression of a nerve by casts, splints, braces, crutches, or other devices. Polyneuropathy implies a widespread process that usually affects both sides of the body
25 equally. The symptoms depend on which type of nerve is affected. The three main types of nerves are sensory, motor, and autonomic. Neuropathy can affect any one or a combination of all three types of nerves. Symptoms also depend on whether the condition affects the whole body or just one nerve (as from an injury). The cause of chronic inflammatory polyneuropathy is an abnormal immune response. The specific antigens, immune processes, and triggering
30 factors are variable and in many cases are unknown. It may occur in association with other conditions such as HIV, inflammatory bowel disease, lupus erythematosus, chronic active hepatitis, and blood cell abnormalities.

Peripheral neuropathy may involve a function or pathological change to a single nerve or nerve
35 group (mononeuropathy) or a function or pathological change affecting multiple nerves (polyneuropathy).

Peripheral neuropathies may include the following:

Hereditary disorders

Charcot-Marie-Tooth disease

5 Friedreich's ataxia

Systemic or metabolic disorders

Diabetes (diabetic neuropathy)

Dietary deficiencies (especially vitamin B-12)

Excessive alcohol use (alcoholic neuropathy)

10 Uremia (from kidney failure)

Cancer

Infectious or inflammatory conditions

AIDS

Hepatitis

15 Colorado tick fever

diphtheria

Guillain-Barre syndrome

HIV infection without development of AIDS

leprosy

20 Lyme

polyarteritis nodosa

rheumatoid arthritis

sarcoidosis

Sjogren syndrome

25 syphilis

systemic lupus erythematosus

amyloid

Exposure to toxic compounds

sniffing glue or other toxic compounds

30 nitrous oxide

industrial agents -- especially solvents

heavy metals (lead, arsenic, mercury, etc.)

Neuropathy secondary to drugs like analgesic nephropathy

Miscellaneous causes

35 ischemia (decreased oxygen/decreased blood flow)

prolonged exposure to cold temperature

a. Polyneuropathy

Polyneuropathy is a peripheral neuropathy involving the loss of movement or sensation to an area caused by damage or destruction to multiple peripheral nerves. Polyneuropathic pain, includes, without limitation, post-polio syndrome, postmastectomy syndrome, diabetic neuropathy, alcohol neuropathy, amyloid, toxins, AIDS, hypothyroidism, uremia, vitamin
5 deficiencies, chemotherapy-induced pain, 2',3'-dideoxycytidine (ddC) treatment, Guillain-Barré syndrome or Fabry's disease.

b. Mononeuropathy

Mononeuropathy is a peripheral neuropathy involving loss of movement or sensation to an
10 area caused by damage or destruction to a single peripheral nerve or nerve group. Mononeuropathy is most often caused by damage to a local area resulting from injury or trauma, although occasionally systemic disorders may cause isolated nerve damage (as with mononeuritis multiplex). The usual causes are direct trauma, prolonged pressure on the nerve, and compression of the nerve by swelling or injury to nearby body structures. The damage
15 includes destruction of the myelin sheath (covering) of the nerve or of part of the nerve cell (the axon). This damage slows or prevents conduction of impulses through the nerve. Mononeuropathy may involve any part of the body. Mononeuropathic pain, includes, without limitation, a sciatic nerve dysfunction, a common peroneal nerve dysfunction, a radial nerve dysfunction, an ulnar nerve dysfunction, a cranial mononeuropathy VI, a cranial
20 mononeuropathy VII, a cranial mononeuropathy III (compression type), a cranial mononeuropathy III (diabetic type), an axillary nerve dysfunction, a carpal tunnel syndrome, a femoral nerve dysfunction, a tibial nerve dysfunction, a Bell's palsy, a thoracic outlet syndrome, a carpal tunnel syndrome and a sixth (abducent) nerve palsy

c. Generalized peripheral neuropathies

25 Generalized peripheral neuropathies are symmetrical, and usually due to various systematic illnesses and disease processes that affect the peripheral nervous system in its entirety. They are further subdivided into several categories:

i. Distal axonopathies are the result of some metabolic or toxic derangement of neurons. They may be caused by metabolic diseases such as diabetes, renal failure, deficiency
30 syndromes such as malnutrition and alcoholism, or the effects of toxins or drugs. Distal axonopathy (aka dying back neuropathy) is a type of peripheral neuropathy that results from some metabolic or toxic derangement of peripheral nervous system (PNS) neurons. It is the most common response of nerves to metabolic or toxic disturbances, and as such may be caused by metabolic diseases such as diabetes, renal failure, deficiency syndromes such as
35 malnutrition and alcoholism, or the effects of toxins or drugs. The most common cause of distal axonopathy is diabetes, and the most common distal axonopathy is diabetic neuropathy.

ii. Myelinopathies are due to a primary attack on myelin causing an acute failure of impulse conduction. The most common cause is acute inflammatory demyelinating polyneuropathy (AIDP; aka Guillain-Barré syndrome), though other causes include chronic inflammatory demyelinating syndrome (CIDP), genetic metabolic disorders (e.g., leukodystrophy), or toxins. Myelinopathy is due to primary destruction of myelin or the myelinating Schwann cells, which leaves the axon intact, but causes an acute failure of impulse conduction. This demyelination slows down or completely blocks the conduction of electrical impulses through the nerve. The most common cause is acute inflammatory demyelinating polyneuropathy (AIDP, better known as Guillain-Barré syndrome), though other causes include chronic inflammatory demyelinating polyneuropathy (CIDP), genetic metabolic disorders (e.g., leukodystrophy or Charcot-Marie-Tooth disease), or toxins.

iii. Neuronopathies are the result of destruction of peripheral nervous system (PNS) neurons. They may be caused by motor neurone diseases, sensory neuronopathies (e.g., Herpes zoster), toxins or autonomic dysfunction. Neurotoxins may cause neuronopathies, such as the chemotherapy agent vincristine. Neuronopathy is dysfunction due to damage to neurons of the peripheral nervous system (PNS), resulting in a peripheral neuropathy. It may be caused by motor neurone diseases, sensory neuronopathies (e.g., Herpes zoster), toxic substances or autonomic dysfunction. A person with neuronopathy may present in different ways, depending on the cause, the way it affects the nerve cells, and the type of nerve cell that is most affected.

iv. Focal entrapment neuropathies (e.g., carpal tunnel syndrome).

Where the pain is neuropathic pain, in one embodiment, the polypeptide used does not comprise a catalytically inactive BoNT/X L-chain, a BoNT/X translocation domain (H_N domain) and/or a BoNT/X receptor binding domain (H_C domain). In one embodiment, where the pain is neuropathic pain the polypeptide used does not comprise a catalytically inactive BoNT/X L-chain and translocation domain (H_N domain), optionally in combination with an H_C domain from a different (i.e. non-BoNT/X) clostridial neurotoxin (e.g. an H_C domain from BoNT/B). Preferably, where the pain is neuropathic pain the polypeptide used does not comprise a catalytically inactive BoNT/X L-chain and translocation domain (H_N domain), and a BoNT/B H_C domain.

The polypeptides of the invention may be used to treat pain caused by or otherwise associated with any of the following inflammatory conditions. Similarly, a polypeptide of the invention may be used to treat one or more of the following inflammatory conditions.

A. Arthritic disorder

Arthritic disorders include, for example, a rheumatoid arthritis; a juvenile rheumatoid arthritis; a systemic lupus erythematosus (SLE); a gouty arthritis; a scleroderma; an osteoarthritis; a psoriatic arthritis; an ankylosing spondylitis; a Reiter's syndrome (reactive arthritis); an adult
5 Still's disease; an arthritis from a viral infection; an arthritis from a bacterial infection, such as, e.g., a gonococcal arthritis and a non-gonococcal bacterial arthritis (septic arthritis); a Tertiary Lyme disease; a tuberculous arthritis; and an arthritis from a fungal infection, such as, e.g. a blastomycosis

10 B. Autoimmune diseases

Autoimmune diseases include, for example, a Guillain-Barré syndrome, a Hashimoto's thyroiditis, a pernicious anemia, an Addison's disease, a type I diabetes, a systemic lupus erythematosus, a dermatomyositis, a Sjogren's syndrome, a lupus erythematosus, a multiple sclerosis, a myasthenia gravis, a Reiter's syndrome and a Grave's disease.

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C. Connective tissue disorder

Connective tissue disorders include, for example, a spondyloarthritis a dermatomyositis, and a fibromyalgia.

20 D. Injury

Inflammation caused by injury, including, for example, a crush, puncture, stretch of a tissue or joint, may cause chronic inflammatory pain.

E. Infection

25 Inflammation caused by infection, including, for example, a tuberculosis or an interstitial keratitis may cause chronic inflammatory pain.

F. Neuritis

Neuritis is an inflammatory process affecting a nerve or group of nerves. Symptoms depend
30 on the nerves involved, but may include pain, paresthesias, paresis, or hypesthesia (numbness).

Examples include:

a. Brachial neuritis

b. Retrobulbar neuropathy, an inflammatory process affecting the part of the optic nerve
35 lying immediately behind the eyeball.

c. Optic neuropathy, an inflammatory process affecting the optic nerve causing sudden, reduced vision in the affected eye. The cause of optic neuritis is unknown. The sudden inflammation of the optic nerve (the nerve connecting the eye and the brain) leads to swelling and destruction of the myelin sheath. The inflammation may occasionally be the result of a viral infection, or it may be caused by autoimmune diseases such as multiple sclerosis. Risk factors are related to the possible causes.

d. Vestibular neuritis, a viral infection causing an inflammatory process affecting the vestibular nerve.

10 G. Joint inflammation

Inflammation of the joint, such as that caused by bursitis or tendonitis, for example, may cause chronic inflammatory pain.

H. Sunburn and/or UV-induced damage

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The polypeptides of the invention may be used to treat pain caused by or otherwise associated with any of the following headache conditions. A headache (medically known as cephalgia) is a condition of mild to severe pain in the head; sometimes neck or upper back pain may also be interpreted as a headache. It may indicate an underlying local or systemic disease or be a disorder in itself.

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A. Muscular/myogenic headache

Muscular/myogenic headaches appear to involve the tightening or tensing of facial and neck muscles; they may radiate to the forehead. Tension headache is the most common form of myogenic headache.

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A tension headache is a condition involving pain or discomfort in the head, scalp, or neck, usually associated with muscle tightness in these areas. Tension headaches result from the contraction of neck and scalp muscles. One cause of this muscle contraction is a response to stress, depression or anxiety. Any activity that causes the head to be held in one position for a long time without moving can cause a headache. Such activities include typing or use of computers, fine work with the hands, and use of a microscope. Sleeping in a cold room or sleeping with the neck in an abnormal position may also trigger this type of headache. A tension-type headache, includes, without limitation, an episodic tension headache and a chronic tension headache.

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B. Vascular headache

The most common type of vascular headache is migraine. Other kinds of vascular headaches include cluster headaches, which cause repeated episodes of intense pain, and headaches resulting from high blood pressure

5 1. Migraine

A migraine is a heterogeneous disorder that generally involves recurring headaches. Migraines are different from other headaches because they occur with other symptoms, such as, e.g., nausea, vomiting, or sensitivity to light. In most people, a throbbing pain is felt only on one side of the head. Clinical features such as type of aura symptoms, presence of prodromes,
10 or associated symptoms such as vertigo, may be seen in subgroups of subjects with different underlying pathophysiological and genetic mechanisms. A migraine headache, includes, without limitation, a migraine without aura (common migraine), a migraine with aura (classic migraine), a menstrual migraine, a migraine equivalent (acephalic headache), a complicated migraine, an abdominal migraine and a mixed tension migraine.

15 2. Cluster headache

Cluster headaches affect one side of the head (unilateral) and may be associated with tearing of the eyes and nasal congestion. They occurs in clusters, happening repeatedly every day at the same time for several weeks and then remitting.

20 D. High blood pressure headache

E. Traction and inflammatory headache

Traction and inflammatory headaches are usually symptoms of other disorders, ranging from stroke to sinus infection.

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F. Hormone headache

G. Rebound headache

Rebound headaches, also known as medication overuse headaches, occur when medication
30 is taken too frequently to relieve headache. Rebound headaches frequently occur daily and can be very painful.

H. Chronic sinusitis headache

Sinusitis is inflammation, either bacterial, fungal, viral, allergic or autoimmune, of the paranasal
35 sinuses. Chronic sinusitis is one of the most common complications of the common cold. Symptoms include: Nasal congestion; facial pain; headache; fever; general malaise; thick

green or yellow discharge; feeling of facial 'fullness' worsening on bending over. In a small number of cases, chronic maxillary sinusitis can also be brought on by the spreading of bacteria from a dental infection. Chronic hyperplastic eosinophilic sinusitis is a noninfective form of chronic sinusitis.

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I. An organic headache

J. Ictal headaches

Ictal headaches are headaches associated with seizure activity.

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The polypeptides of the invention may be used to treat pain caused by or otherwise associated with any of the following somatic pain conditions. Somatic pain originates from ligaments, tendons, bones, blood vessels, and even nerves themselves. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, poorly-localized pain of longer duration than cutaneous pain; examples include sprains and broken bones. Additional examples include the following.

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A. Excessive muscle tension

Excessive muscle tension can be caused, for example, by a sprain or a strain.

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B. Repetitive motion disorders

Repetitive motion disorders can result from overuse of the hands, wrists, elbows, shoulders, neck, back, hips, knees, feet, legs, or ankles.

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C. Muscle disorders

Muscle disorders causing somatic pain include, for example, a polymyositis, a dermatomyositis, a lupus, a fibromyalgia, a polymyalgia rheumatica, and a rhabdomyolysis.

D. Myalgia

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Myalgia is muscle pain and is a symptom of many diseases and disorders. The most common cause for myalgia is either overuse or over-stretching of a muscle or group of muscles. Myalgia without a traumatic history is often due to viral infections. Longer-term myalgias may be indicative of a metabolic myopathy, some nutritional deficiencies or chronic fatigue syndrome.

E. Infection

Infection can cause somatic pain. Examples of such infection include, for example, an abscess in the muscle, a trichinosis, an influenza, a Lyme disease, a malaria, a Rocky Mountain spotted fever, Avian influenza, the common cold, community-acquired pneumonia, meningitis, monkeypox, Severe Acute Respiratory Syndrome, toxic shock syndrome, trichinosis, typhoid fever, and upper respiratory tract infection.

F. Drugs

Drugs can cause somatic pain. Such drugs include, for example, cocaine, a statin for lowering cholesterol (such as atorvastatin, simvastatin, and lovastatin), and an ACE inhibitor for lowering blood pressure (such as enalapril and captopril)

The polypeptides of the invention may be used to treat pain caused by or otherwise associated with any of the following visceral pain conditions. Visceral pain originates from body's viscera, or organs. Visceral nociceptors are located within body organs and internal cavities. The even greater scarcity of nociceptors in these areas produces pain that is usually more aching and of a longer duration than somatic pain. Visceral pain is extremely difficult to localise, and several injuries to visceral tissue exhibit "referred" pain, where the sensation is localised to an area completely unrelated to the site of injury. Examples of visceral pain include the following.

A. Functional visceral pain

Functional visceral pain includes, for example, an irritable bowel syndrome and a chronic functional abdominal pain (CFAP), a functional constipation and a functional dyspepsia, a non-cardiac chest pain (NCCP) and a chronic abdominal pain.

B. Chronic gastrointestinal inflammation

Chronic gastrointestinal inflammation includes, for example, a gastritis, an inflammatory bowel disease, like, e.g., a Crohn's disease, an ulcerative colitis, a microscopic colitis, a diverticulitis and a gastroenteritis; an interstitial cystitis; an intestinal ischemia; a cholecystitis; an appendicitis; a gastroesophageal reflux; an ulcer, a nephrolithiasis, an urinary tract infection, a pancreatitis and a hernia.

C. Autoimmune pain

Autoimmune pain includes, for example, a sarcoidosis and a vasculitis.

D. Organic visceral pain

Organic visceral pain includes, for example, pain resulting from a traumatic, inflammatory or degenerative lesion of the gut or produced by a tumour impinging on sensory innervation.

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E. Treatment-induced visceral pain

Treatment-induced visceral pain includes, for example, a pain attendant to chemotherapy therapy or a pain attendant to radiation therapy.

10 The polypeptides of the invention may be used to treat pain caused by or otherwise associated with any of the following referred pain conditions.

Referred pain arises from pain localized to an area separate from the site of pain stimulation. Often, referred pain arises when a nerve is compressed or damaged at or near its origin. In this circumstance, the sensation of pain will generally be felt in the territory that the nerve serves, even though the damage originates elsewhere. A common example occurs in

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intervertebral disc herniation, in which a nerve root arising from the spinal cord is compressed by adjacent disc material. Although pain may arise from the damaged disc itself, pain will also be felt in the region served by the compressed nerve (for example, the thigh, knee, or foot). Relieving the pressure on the nerve root may ameliorate the referred pain, provided that permanent nerve damage has not occurred. Myocardial ischaemia (the loss of blood flow to a part of the heart muscle tissue) is possibly the best known example of referred pain; the sensation can occur in the upper chest as a restricted feeling, or as an ache in the left shoulder, arm or even hand.

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The polypeptides of the invention may be used to treat post-operative pain.

Post-operative (e.g. post-surgical) pain is an unpleasant sensation that results from a surgical procedure. Post-operative pain may be caused by damage to tissue by an incision, the procedure itself, the closing of the wound, and any force that is applied during the procedure. Pain after surgery (e.g. post-operative pain) can also stem from factors that accompany surgery. For example, a subject may suffer back pain due to the way the subject was positioned on the surgical table, or chest pain may be due to an incision in the chest area. Throat pain may also occur after general anesthesia because the insertion of the breathing tube can cause irritation. However, most common is post-operative pain caused by cutting into the skin and muscle from a surgical incision.

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For example, the surgical procedure (or more particularly, surgical incision) may represent a 'noxious stimulus' causing pain. Noxious stimuli, stimuli which can elicit tissue damage, can activate the release of neurotransmitters from nociceptive afferent terminals and the release of neuropeptides such as Substance P and Calcitonin gene related peptide (CGRP) from sensory terminals. The noxious information is then transduced from the peripheral nervous system to the central nervous system, where pain is perceived by the individual.

Post-operative pain can be caused by the combination of inflammation and neural tissue damage. For example, degranulation of activated mast cells in response to tissue injury can result in the release of various substances including proteases, cytokines, serotonin and extracellular space. These substances can sensitize (activate at a lower threshold) primary afferent neurons to produce pain hypersensitivity. As tissue is extensively innervated, any region of the body is susceptible to nerve damage from surgery.

Reference to surgery means a medical procedure involving the treatment of an injury or disease in a subject comprising subjecting a part of the body to an incision (optionally removing or repairing a damaged part of the body). Although the level of invasiveness (e.g. level of surgical incision required) may vary amongst surgery types, surgery having a level of invasiveness that causes pain in the subject once surgery is complete is intended to be encompassed.

The surgery may comprise an incision to skin and/or fascia and/or muscle. Preferably, the surgery comprises an incision to the skin.

The surgery is not limited to that which may be carried out by a physician, but also includes for example dental surgery. Non-limiting examples of surgery include appendectomy, breast biopsy, breast augmentation or reduction, facelift, cholecystectomy, coronary artery bypass, debridement (e.g. of a wound, a burn, or infection), skin graft, organ transplant and tonsillectomy.

Preferably, "post-operative" may refer to a time period beginning at most one day subsequent to surgery (e.g. post-surgery). In other words, the term "post-operative" may refer to a time period beginning not greater than one day post-surgery. For example, the term "post-operative" may refer to a time point beginning 1-20 hours post-surgery; optionally 2-15 hours post-surgery; optionally 5-10 hours post-surgery. Such time may represent a time period

beginning at the chronological interface at which the analgesic effects from a surgical anaesthetic administered to a subject diminish (e.g. taper) and thus the subject begins to perceive pain.

- 5 Furthermore, the term “post-operative” may be used interchangeably with the term “post-surgical”, as ‘operative’ is used in the sense of ‘surgery’ herein.

Similarly, the term “post-operative pain” may refer to pain that is perceived (or more particularly, begins to be perceived) for a time period beginning at most one day subsequent
10 to surgery (e.g. post-surgery). In other words, the term “post-operative pain” may refer to pain that is perceived by a subject for a time period beginning not greater than one day post-surgery. For example, the term “post-operative pain” may refer to pain that is perceived for a time period beginning 1-20 hours post-surgery; optionally 2-15 hours post-surgery; optionally 5-10 hours post-surgery.

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Said time period may be 1-50 weeks; for example 5-45 weeks, 10-40 weeks or 10-35 weeks post-surgery.

This contrasts with the term “peri-operative”, which may refer, for example, to a time period at
20 or around the time that a subject is undergoing surgery (e.g. the time when the subject is in the operating theatre), suitably a period beginning at least 1 hour pre-surgery and/or ending less than 1 hour post-surgery.

The present invention addresses a wide range of pain conditions, e.g. chronic pain conditions.
25 In some embodiments, the polypeptides of the invention are for treating cancerous and non-cancerous pain.

Preferably, polypeptides of the invention are used to treat neuropathic pain. The neuropathic pain may be acute or chronic. In one embodiment the neuropathic pain is injury-induced
30 neuropathic pain (neuropathic pain associated with an injury). In one embodiment the neuropathic pain is chemotherapy-induced neuropathic pain (neuropathic pain associated with chemotherapy).

Preferably, polypeptides of the invention are used to treat inflammatory pain. The inflammatory
35 pain may be acute or chronic. In one embodiment the inflammatory pain may be a burn. For example, the inflammatory pain may be caused by UV damage (e.g. UV-B damage).

Most preferably, the polypeptides of the invention are used to treat bladder pain syndrome, phantom limb pain, or migraine pain. The bladder pain syndrome may be caused by or associated with interstitial cystitis.

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Treating pain preferably means reducing pain. In other words, in one embodiment, administration of a polypeptide of the invention reduces pain in a subject.

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In more detail, reference to “reduced” or “reducing” (in terms of pain) preferably means a lower level of pain is perceived by the subject after administration with a polypeptide of the invention (post-administration) when compared with a level of pain perceived by the subject prior to administration (pre-administration). For example, the level of pain perceived may be reduced by at least 15%, 25%, 35%, 45%, 55%, 65%, 75%, 85% or 95% post-administration relative to pre-administration. For example, the level of pain perceived may be reduced by at least 75%;

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preferably at least 85%; more preferably at least 95% post-administration.

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A variety of means for assessing pain perception are known to those skilled in the art. For example, evaluation of mechanical allodynia (either static or dynamic) is routinely used in human pain studies as described in Pogatzki-Zahn *et. al.* (Pain Rep. 2017 Mar; 2(2): e588), incorporated herein by reference.

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A suitable (albeit non-limiting) method for assessing pain perception in a subject includes the following: Numerical Rating Scale (NRS) score; although the skilled person is aware of other methods which may be used additionally or alternatively such as sensory threshold, pain perception threshold, static mechanical allodynia, dynamic mechanical allodynia, temporal summation, pressure pain threshold, conditioned pain modulation, and temperature threshold.

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Other non-limiting examples of pain perception measures include: change from baseline in SF-36 scores at each scheduled time point; amount of rescue medication taken during the study and time to first intake of rescue medication. These may be considered “exploratory” endpoints or pain perception assessment measures.

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Thus, in a preferred embodiment, following the administration of a polypeptide of the invention, pain perception may assessed by one or more of: (a) a Numerical Rating Scale (NRS); (b) stimulus-evoked NRS; (c) temperature of the painful area; (d) size of the painful area; (e) time

to onset of analgesic effect; (f) peak analgesic effect; (g) time to peak analgesic effect; (h) duration of analgesic effect; and (i) SF-36 quality of life.

The skilled person is aware of such methods for assessing pain perception. For convenience,
5 further description of the Numerical Rating Score and Quality of Life questionnaire Short Form-36 are provided below.

Numerical Rating Scale (NRS): Typically pain perception according to the present invention
10 uses the Numerical Rating Scale (NRS). The NRS is an 11-point scale to assess subject pain perception. Subjects are asked to give a number between 0 and 10 that fits best to their pain intensity. Zero represents 'no pain at all' whereas the upper limit, 10, represents 'the worst pain possible'.

The NRS can be used to assess numerous facets of pain, including spontaneous average
15 pain, spontaneous worst pain and spontaneous current pain. Spontaneous average pain is assessed by asking a subject to select a number that best describes the subject's average pain (e.g. perceived pain) over a period of time, for example at least 6 hours, 12 hours, 24 hours, or at least 48 hours. Spontaneous worst pain is assessed by asking a subject to select a number that best describes the subject's pain at its worst during a specified period, e.g. at
20 least the previous 6 hours, 12 hours, 24 hours or previous 48 hours. Spontaneous current pain is assessed by asking a subject to select a number that best describes how much pain the subject is in at the time of assessment.

The NRS can also be used to assess a subject's pain perception in response to a variety of
25 different stimuli. To assess pain perception in response to a stimulus, the subject will be submitted to stimuli of various nature applied to the painful area. Subjects will be asked what are their current NRS scores pre-dose and post-stimulus.

Examples of stimuli used include: (i) light touch (which can be assessed by measuring pain on
30 the surface of the painful area on radial spokes following application of a von Frey filament as described herein); (ii) pressure (pressure pain threshold), which can be assessed by asking the subject to give a NRS score as increasing pressure is applied using a pressure algometer; and (iii) temperature (which can be assessed by asking the subject for an NRS score for warm, cold and hot stimulation using a thermode applied to the painful area).

Preferably, administration of a polypeptide of the invention reduces the subject's NRS score post-administration (e.g. from a rating of ≥ 7 to a rating of ≤ 6) when compared with the subject's NRS score pre-administration.

- 5 Quality of Life questionnaire Short Form-36 (SF-36): The SF-36 quality of life questionnaire may be used to assess a subject's pain perception. The SF-36 is a 36-item, subject-reported survey of subject health. The SF-36 consists of eight scaled scores (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning and mental health). Each scale is directly transformed into
10 a 0-100 scale on the assumption that each question carries equal weight. The higher the score recorded in the SF-36, the less disability.

Relevant parameters commonly tested in clinical trials for the treatment of pain are known in the art and could be readily selected by one of ordinary skill in the art. Examples of such
15 parameters include, but are not limited to NRS; stimulus-evoked NRS; temperature of the painful area; size of the painful area; time to onset of analgesic effect; peak analgesic effect; time to peak analgesic effect; duration of analgesic effect; and/or SF-36 quality of life as described herein. Methods for assessing these parameters are also known in the art and can be carried out by one of ordinary skill using routine methods and procedures.

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Preferably, administration of a polypeptide of the invention increases the subject's SF-36 score post-administration (e.g. from a score of ≤ 50 to a score of ≥ 50) when compared with the subject's SF-36 score pre-administration.

- 25 An inflammatory disorder treated by a polypeptide of the invention may an inflammatory disorder of: the nervous system, the cardiovascular system, the respiratory system, the digestion system, the integumentary system, the musculoskeletal system, the urinary system, the reproductive system, the endocrine system, or the lymphatic system.

- 30 An inflammatory disorder of the nervous system may be one or more selected from the group consisting of: central nervous system inflammation (e.g. encephalitis, myelitis, meningitis, or arachnoiditis), peripheral nervous system inflammation (e.g. neuritis), eye inflammation (e.g. dacryoadenitis, scleritis, episcleritis, keratitis, retinitis, chorioretinitis, blepharitis, conjunctivitis, or uveitis), and ear inflammation (e.g. otitis externa, otitis media, labyrinthitis, and mastoiditis).

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An inflammatory disorder of the cardiovascular system may be one or more selected from the group consisting of: carditis (e.g. endocarditis, myocarditis or pericarditis) and vasculitis (e.g. arteritis, phlebitis or capillaritis).

- 5 An inflammatory disorder of the respiratory system may be one or more selected from the group consisting of: an upper respiratory system inflammatory disorder (e.g. sinusitis, rhinitis, pharyngitis or laryngitis), a lower respiratory system inflammatory disorder (e.g. tracheitis, bronchitis, bronchiolitis, pneumonitis or pleuritis), and mediastinitis.
- 10 An inflammatory disorder of the digestion system may be one or more selected from the group consisting of: mouth inflammation (e.g. stomatitis, gingivitis, gingivostomatitis, glossitis, tonsillitis, sialadenitis/parotitis, cheilitis, pulpitis or gnathitis), gastrointestinal tract inflammation (e.g. esophagitis, gastritis, gastroenteritis, enteritis, colitis, enterocolitis, duodenitis, ileitis, caecitis, appendicitis or proctitis), and inflammation of the accessory digestive organs (e.g.
- 15 hepatitis, ascending cholangitis, cholecystitis, pancreatitis or peritonitis).

An inflammatory disorder of the integumentary system may be one or more selected from the group consisting of: dermatitis (e.g. folliculitis), cellulitis, and hidradenitis.

- 20 An inflammatory disorder of the musculoskeletal system may be one or more selected from the group consisting of: arthritis, dermatomyositis, soft tissue inflammation (e.g. myositis, synovitis/tenosynovitis, bursitis, enthesitis, fasciitis, capsulitis, epicondylitis, tendinitis or panniculitis), osteochondritis, osteitis/osteomyelitis, spondylitis, periostitis, and chondritis.
- 25 An inflammatory disorder of the urinary system may be one or more selected from the group consisting of: nephritis (e.g. glomerulonephritis or pyelonephritis), ureteritis, cystitis, and urethritis.

- 30 An inflammatory disorder of the reproductive system may be one or more selected from the group consisting of: inflammation of the female reproductive system (e.g. oophoritis, salpingitis, endometritis, parametritis, cervicitis, vaginitis, vulvitis or mastitis), inflammation of the male reproductive system (e.g. orchitis, epididymitis, prostatitis, seminal vesiculitis, balanitis, posthitis or balanoposthitis), and inflammation associated with pregnancy, birth and/or the new-born (e.g. chorioamnionitis, funisitis or omphalitis).

An inflammatory disorder of the endocrine system may be one or more selected from the group consisting of: insulinitis, hypophysitis, thyroiditis, parathyroiditis, and adrenalitis.

5 An inflammatory disorder of the lymphatic system may be one or more selected from the group consisting of: lymphangitis and lymphadenitis.

Preferably, an inflammatory disorder is one or more selected from: complex regional pain syndrome, endometriosis, rheumatoid arthritis, cystitis, and neuritis. The cystitis is preferably interstitial cystitis. The neuritis is preferably peripheral neuritis.

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Embodiments related to the various therapeutic uses of the invention are intended to be applied equally to methods of treatment, and *vice versa*.

SEQUENCE HOMOLOGY

15 Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, e.g., segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art. Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual
20 residue pairs and by imposing gap penalties. Non-limiting methods include, e.g., CLUSTAL W, see, e.g., Julie D. Thompson et al., CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position- Specific Gap Penalties and Weight Matrix Choice, 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, e.g., Osamu Gotoh, Significant Improvement in Accuracy of Multiple Protein.
25 Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments, 264(4) J. Mol. Biol. 823-838 (1996). Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, e.g., Match-box, see, e.g., Eric Depiereux and Ernest Feytmans, Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences,
30 8(5) CABIOS 501 -509 (1992); Gibbs sampling, see, e.g., C. E. Lawrence et al., Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment, 262(5131) Science 208-214 (1993); Align-M, see, e.g., Ivo Van Walle et al., Align-M - A New Algorithm for Multiple Alignment of Highly Divergent Sequences, 20(9) Bioinformatics:1428-1435 (2004).

35 Thus, percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad.

Sci. USA 89:10915-19, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown below (amino acids are indicated by the standard one-letter codes); preferably this method is used to align a sequence
5 with a SEQ ID NO described herein to define amino acid position numbering, as described herein.

The "percent sequence identity" between two or more nucleic acid or amino acid sequences is a function of the number of identical positions shared by the sequences. Thus, % identity
10 may be calculated as the number of identical nucleotides / amino acids divided by the total number of nucleotides / amino acids, multiplied by 100. Calculations of % sequence identity may also take into account the number of gaps, and the length of each gap that needs to be introduced to optimize alignment of two or more sequences. Sequence comparisons and the determination of percent identity between two or more sequences can be carried out using
15 specific mathematical algorithms, such as BLAST, which will be familiar to a skilled person.

ALIGNMENT SCORES FOR DETERMINING SEQUENCE IDENTITY

A R N D C Q E G H I L K M F P S T W Y V

A 4

R -1 5

5 N -2 0 6

D -2 -2 1 6

C 0 -3 -3 -3 9

Q -1 1 0 0 -3 5

E -1 0 0 2 -4 2 5

10 G 0 -2 0 -1 -3 -2 -2 6

H -2 0 1 -1 -3 0 0 -2 8

I -1 -3 -3 -3 -1 -3 -3 -4 -3 4

L -1 -2 -3 -4 -1 -2 -3 -4 -3 2 4

K -1 2 0 -1 -3 1 1 -2 -1 -3 -2 5

15 M -1 -1 -2 -3 -1 0 -2 -3 -2 1 2 -1 5

F -2 -3 -3 -3 -2 -3 -3 -3 -1 0 0 -3 0 6

P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3 -3 -1 -2 -4 7

S 1 -1 1 0 -1 0 0 0 -1 -2 -2 0 -1 -2 -1 4

T 0 -1 0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1 1 5

20 W -3 -3 -4 -4 -2 -2 -3 -2 -2 -3 -2 -3 -1 1 -4 -3 -2 11

Y -2 -2 -2 -3 -2 -1 -2 -3 2 -1 -1 -2 -1 3 -3 -2 -2 2 7

V 0 -3 -3 -3 -1 -2 -2 -3 -3 3 1 -2 1 -1 -2 -2 0 -3 -1 4

The percent identity is then calculated as:

25

Total number of identical matches

 x 100

[length of the longer sequence plus the

number of gaps introduced into the longer

30 sequence in order to align the two sequences]

Substantially homologous polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see below) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-

35

terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag.

CONSERVATIVE AMINO ACID SUBSTITUTIONS

5	Basic:	arginine
		lysine
		histidine
	Acidic:	glutamic acid
		aspartic acid
10	Polar:	glutamine
		asparagine
	Hydrophobic:	leucine
isoleucine		
valine		
15	Aromatic:	phenylalanine
		tryptophan
		tyrosine
	Small:	glycine
		alanine
20		
		threonine
		methionine

In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-N-methyl lysine, 2-aminoisobutyric acid, isovaline and α -methyl serine) may be substituted for amino acid residues of the polypeptides of the present invention. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for polypeptide amino acid residues. The polypeptides of the present invention can also comprise non-naturally occurring amino acid residues.

Non-naturally occurring amino acids include, without limitation, trans-3-methylproline, 2,4-methano-proline, cis-4-hydroxyproline, trans-4-hydroxy-proline, N-methylglycine, allo-threonine, methyl-threonine, hydroxy-ethylcysteine, hydroxyethylhomo-cysteine, nitro-glutamine, homoglutamine, pipecolic acid, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenyl-alanine, 4-azaphenyl-alanine, and 4-fluorophenylalanine. Several methods are

known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an in vitro system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing
5 nonsense mutations is carried out in a cell free system comprising an E. coli S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in Xenopus
10 oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, E. coli cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally
15 occurring amino acid is incorporated into the polypeptide in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by in vitro chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

20 A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for amino acid residues of polypeptides of the present invention.

25 Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-5, 1989). Sites of biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity
30 labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-12, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related components (e.g. the translocation or protease components) of the polypeptides of the present invention.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-7, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-6, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-7, 1991; Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Singleton, et al., DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 20 ED., John Wiley and Sons, New York (1994), and Hale & Marham, THE HARPER COLLINS DICTIONARY OF BIOLOGY, Harper Perennial, NY (1991) provide the skilled person with a general dictionary of many of the terms used in this disclosure.

This disclosure is not limited by the exemplary methods and materials disclosed herein, and any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of this disclosure. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

The headings provided herein are not limitations of the various aspects or embodiments of this disclosure.

Amino acids are referred to herein using the name of the amino acid, the three letter abbreviation or the single letter abbreviation. The term "protein", as used herein, includes proteins, polypeptides, and peptides. As used herein, the term "amino acid sequence" is synonymous with the term "polypeptide" and/or the term "protein". In some instances, the term "amino acid sequence" is synonymous with the term "peptide". In some instances, the term "amino acid sequence" is synonymous with the term "enzyme". The terms "protein" and "polypeptide" are used interchangeably herein. In the present disclosure and claims, the conventional one-letter and three-letter codes for amino acid residues may be used. The 3-

letter code for amino acids as defined in conformity with the IUPACIUB Joint Commission on Biochemical Nomenclature (JCBN). It is also understood that a polypeptide may be coded for by more than one nucleotide sequence due to the degeneracy of the genetic code.

5 Other definitions of terms may appear throughout the specification. Before the exemplary embodiments are described in more detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be defined only
10 by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any
15 stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within this disclosure. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within this disclosure, subject to any specifically excluded limit in the stated range. Where the stated
20 range includes one or both of the limits, ranges excluding either or both of those included limits are also included in this disclosure.

It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for
25 example, reference to “a clostridial neurotoxin” includes a plurality of such candidate agents and reference to “the clostridial neurotoxin” includes reference to one or more clostridial neurotoxins and equivalents thereof known to those skilled in the art, and so forth.

The publications discussed herein are provided solely for their disclosure prior to the filing date
30 of the present application. Nothing herein is to be construed as an admission that such publications constitute prior art to the claims appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention will now be described, by way of example only, with reference
35 to the following Figures and Examples.

Figure 1 shows % SNAP25 cleavage in human neuronal cells administered BoNT/A or BoNT/A(0).

Figure 2 shows % SNAP25 cleavage in rat neuronal cells administered BoNT/A or BoNT/A(0).

5

Figure 3 (A) shows the characteristic startle response of mice suspended by the tail. **(B)** shows the scoring used in the Digit Abduction Score (DAS) assay.

Figure 4 (A) presents an experimental schematic for a study using a chronic constriction injury (CCI) model of chronic, neuropathic pain in adult, male Sprague-Dawley rats (220-250 g). Days (D) pre- and post- administration of BoNT/A(0) (60 pg/kg i.pl. administration), BoNT/A (30 pg/kg intraplantar (i.pl.) administration), BoNT/A (60 pg/kg i.pl. administration), vehicle (Gelatine Phosphate Buffer (GPB) i.pl. administration - negative control) or gabapentin (100 mg/kg p.o. administration - positive control) are indicated. Administration occurred on day 0 (D0) and CCI surgery was carried out at D-14. vF indicates that the von Frey test was carried out on the days indicated. **(B)** shows mechanical sensitivity (measured via the von Frey test) in the ipsilateral paw (i.e. the paw to which control compositions, BoNT/A or BoNT/A(0) were administered) over time for animals administered as described in (A). **(C)** shows mechanical sensitivity (measured via the von Frey test) in the contralateral paw (i.e. the paw to which control compositions, BoNT/A or BoNT/A(0) were not administered) over time for animals administered as described in (A). **(D)** shows the bodyweight change for rats administered as described in (A) over time.

Figure 5 (A) presents an experimental schematic for a study using a model of acute oxaliplatin-induced neuropathic pain in adult, male Sprague-Dawley rats. Oxaliplatin (10 mg/kg intraperitoneal (i.p.) administration) and BoNT/A(0) (1000 pg/kg i.pl. administration), BoNT/A (50 pg/kg i.pl. administration), BoNT/A (100 pg/kg i.pl. administration), BoNT/A (160 pg/kg i.pl. administration), or vehicle (GPB i.pl. administration - negative control) were administered on day 0 (D0). As a further negative control for oxaliplatin treatment, a subset of rats were administered 5% glucose i.p. and GBP (i.pl. administration). Duloxetine (100 mg/kg p.o. administration - positive control) was administered 1h before testing on D3. Days and hours post-administration are shown. PI indicates that the paw immersion (cold) test was carried out on the days indicated. **(B)** shows cold sensitivity (measured via the paw immersion test) in the ipsilateral paw (i.e. the paw to which control compositions, BoNT/A or BoNT/A(0) were administered) over time for animals administered as described in (A). **(C)** shows cold sensitivity (measured via the paw immersion test) in the contralateral paw (i.e. the paw to which

control compositions, BoNT/A or BoNT/A(0) were not administered) over time for animals administered as described in (A).

Figure 6 (A) presents an experimental schematic for a study using a model of chronic oxaliplatin-induced neuropathic pain in adult, male Sprague-Dawley rats (180-210 g). Oxaliplatin was administered at day -2 (D-2). BoNT/A(0) (100 pg/kg i.pl. administration), BoNT/A (100 pg/kg i.pl. administration), or vehicle (GPB i.pl. administration - negative control) was administered on day 0 (D0). Pregabalin (30 mg/kg p.o. administration - positive control) on day 3. Days pre- and post-administration are shown. vF and CP indicate that the von Frey and cold plate tests (respectively) were carried out on the days indicated. **(B)** shows mechanical sensitivity (measured via the von Frey test) in the ipsilateral paw (i.e. the paw to which control compositions, BoNT/A or BoNT/A(0) were administered) over time for animals administered as described in (A). **(C)** shows mechanical sensitivity (measured via the von Frey test) in the contralateral paw (i.e. the paw to which control compositions, BoNT/A or BoNT/A(0) were not administered) over time for animals administered as described in (A). **(D)** shows thermal sensitivity (measured via the cold plate test) over time for animals administered as described in (A).

Figure 7 (A) presents an experimental schematic for a study using a model of acute ultraviolet-B (UV-B)-burn induced inflammatory pain in adult, male Wistar rats (180-210 g). BoNT/A(0) (100 pg/kg i.pl. administration), BoNT/A (100 pg/kg i.pl. administration), or vehicle (GPB i.pl. administration - negative control) was administered on day 0 (D0). Indomethacin (5 mg/kg p.o. administration - positive control) was administered 1h before the readout on D3. Rats were exposed to UV-B (500 mJ/cm²) at day 1 (D1). vF indicates that the von Frey test was carried out on the day indicated. **(B)** shows mechanical sensitivity (measured via the von Frey test) for animals administered as described in (A).

Figure 8 shows mechanical sensitivity (measured via the von Frey test) for mice administered vehicle, catalytically active chimeric BoNT/XB (0.3 ng/kg, n=10), catalytically active chimeric BoNT/XB (30 ng/kg, n=10), catalytically inactive chimeric BoNT/XB(0) (0.3 ng/kg, n=10), catalytically inactive chimeric BoNT/XB(0) (30 ng/kg, n=10), BoNT/A (160 pg/kg, n=10) or indomethacin (10 mg/kg, n=9). Sensitivity is shown in non-treated animals (baseline), 2 days after administration of BoNTs or vehicle and prior to Complete Freund's adjuvant (CFA) administration (Day 0 CFA, Day 2) as well as 1 day after CFA administration (Day 1 CFA, Day 3). **P<0.1, ***P<0.01 (Dunnett's multiple comparison vs vehicle after Repeated Measure two-way ANOVA).

SEQUENCE LISTING

Where an initial Met amino acid residue or a corresponding initial codon is indicated in any of the following SEQ ID NOs, said residue/codon is optional.

- 5 **SEQ ID NO: 1** - Nucleotide Sequence of Recombinant Catalytically Inactive BoNT/A (rBoNT/A(0))
- SEQ ID NO: 2** - Polypeptide Sequence of rBoNT/A(0)
- SEQ ID NO: 3** - Nucleotide Sequence of rLH_N/A (light-chain plus translocation domain only).
- SEQ ID NO: 4** - Polypeptide Sequence of rLH_N/A
- SEQ ID NO: 5** - Nucleotide Sequence of rL/A (light-chain only)
- 10 **SEQ ID NO: 6** - Polypeptide Sequence of rL/A
- SEQ ID NO: 7** - Nucleotide Sequence of rH_C/A
- SEQ ID NO: 8** - Polypeptide Sequence of rH_C/A
- SEQ ID NO: 9** - Nucleotide Sequence of rBoNT/B(0)
- SEQ ID NO: 10** - Polypeptide Sequence of rBoNT/B(0)
- 15 **SEQ ID NO: 11** - Nucleotide Sequence of rBoNT/C(0)
- SEQ ID NO: 12** - Polypeptide Sequence of rBoNT/C(0)
- SEQ ID NO: 13** - Nucleotide Sequence of rBoNT/E(0)
- SEQ ID NO: 14** - Polypeptide Sequence of rBoNT/E(0)
- SEQ ID NO: 15** - Nucleotide Sequence of rBoNT/F(0)
- 20 **SEQ ID NO: 16** - Polypeptide Sequence of rBoNT/F(0)
- SEQ ID NO: 17** - Nucleotide Sequence of rBoNT/A(0) (His-tagged)
- SEQ ID NO: 18** - Polypeptide Sequence of rBoNT/A(0) (His-tagged)
- SEQ ID NO: 19** - Nucleotide Sequence of rLH_N/A (His-tagged)
- SEQ ID NO: 20** - Polypeptide Sequence of rLH_N/A (His-tagged)
- 25 **SEQ ID NO: 21** - Nucleotide Sequence of rH_C/A (His-tagged)
- SEQ ID NO: 22** - Polypeptide Sequence of rH_C/A (His-tagged)
- SEQ ID NO: 23** - Nucleotide Sequence of rLC/A (His-tagged)
- SEQ ID NO: 24** - Polypeptide Sequence of rLC/A (His-tagged)
- SEQ ID NO: 25** - Nucleotide Sequence of rBoNT/FA(0) (His-tagged)
- 30 **SEQ ID NO: 26** - Polypeptide Sequence of rBoNT/FA(0) (His-tagged)
- SEQ ID NO: 27** - Nucleotide Sequence of rLH_N/FA (His-tagged)
- SEQ ID NO: 28** - Polypeptide Sequence of rLH_N/FA (His-tagged)
- SEQ ID NO: 29** - Nucleotide Sequence of rH_C/FA (His-tagged)
- SEQ ID NO: 30** - Polypeptide Sequence of rH_C/FA (His-tagged)
- 35 **SEQ ID NO: 31** - Nucleotide Sequence of rLC/FA (His-tagged)
- SEQ ID NO: 32** - Polypeptide Sequence of rLC/FA (His-tagged)

- SEQ ID NO: 33 - Nucleotide Sequence of rBoNT/F(0) (His-tagged)
- SEQ ID NO: 34 - Polypeptide Sequence of rBoNT/F(0) (His-tagged)
- SEQ ID NO: 35 - Nucleotide Sequence of rL_HN/F (His-tagged)
- SEQ ID NO: 36 - Polypeptide Sequence of rL_HN/F (His-tagged)
- 5 SEQ ID NO: 37 - Nucleotide Sequence of rH_C/F (His-tagged)
- SEQ ID NO: 38 - Polypeptide Sequence of rH_C/F (His-tagged)
- SEQ ID NO: 39 - Nucleotide Sequence of rLC/F (His-tagged)
- SEQ ID NO: 40 - Polypeptide Sequence of rLC/F (His-tagged)
- SEQ ID NO: 41 - Nucleotide Sequence of Cationic rH_C/A (His-tagged)
- 10 SEQ ID NO: 42 - Polypeptide Sequence of Cationic rH_C/A (His-tagged)
- SEQ ID NO: 43 - Nucleotide Sequence of rH_C/AB (His-tagged)
- SEQ ID NO: 44 - Polypeptide Sequence of rH_C/AB (His-tagged)
- SEQ ID NO: 45 - Nucleotide Sequence of rH_C/A Variant Y1117V H1253K (His-tagged)
- SEQ ID NO: 46 - Polypeptide Sequence of rH_C/A Variant Y1117V H1253K (His-tagged)
- 15 SEQ ID NO: 47 - Nucleotide Sequence of rH_C/A Variant Y1117V F1252Y H1253K L1278F (His-tagged)
- SEQ ID NO: 48 - Polypeptide Sequence of rH_C/A Variant Y1117V F1252Y H1253K L1278F (His-tagged)
- SEQ ID NO: 49 - Nucleotide Sequence of rH_C/A Variant Y1117V F1252Y H1253K L1278H (His-tagged)
- 20 SEQ ID NO: 50 - Polypeptide Sequence of rH_C/A Variant Y1117V F1252Y H1253K L1278H (His-tagged)
- SEQ ID NO: 51 - Polypeptide Sequence of BoNT/A - UniProt P10845
- SEQ ID NO: 52 - Polypeptide Sequence of BoNT/B - UniProt P10844
- 25 SEQ ID NO: 53 - Polypeptide Sequence of BoNT/C - UniProt P18640
- SEQ ID NO: 54 - Polypeptide Sequence of BoNT/D - UniProt P19321
- SEQ ID NO: 55 - Polypeptide Sequence of BoNT/E - UniProt Q00496
- SEQ ID NO: 56 - Polypeptide Sequence of BoNT/F - UniProt A7GBG3
- SEQ ID NO: 57 - Polypeptide Sequence of BoNT/G - UniProt Q60393
- 30 SEQ ID NO: 58 - Polypeptide Sequence of TeNT – UniProt P04958
- SEQ ID NO: 59 - Polypeptide Sequence of BoNT/X
- SEQ ID NO: 60 – Polypeptide Sequence of Unmodified BoNT/A1
- SEQ ID NO: 61 – Polypeptide Sequence of mrBoNT/AB(0)
- SEQ ID NO: 62 – Polypeptide Sequence of mrBoNT/A(0)
- 35 SEQ ID NO: 63 – Polypeptide Sequence of BoNT/XB(0) (His-tagged)
- SEQ ID NO: 64 – Polypeptide Sequence of BoNT/XB(0)

SEQ ID NO: 65 – Polypeptide Sequence of BoNT/XB(0) Variant (His-tagged)

SEQ ID NO: 66 – Polypeptide Sequence of BoNT/XB(0) Variant

SEQ ID NO: 67 – Polypeptide Sequence of BoNT/XA(0)

SEQ ID NO: 68 – Polypeptide Sequence of BoNT/XA(0) Variant

5 SEQ ID NO: 69 – Polypeptide Sequence of BoNT/XD(0)

SEQ ID NO: 70 – Polypeptide Sequence of BoNT/XF(0)

SEQ ID NO: 71 - C1 Activation Loop Consensus Sequence

SEQ ID NO: 72 - C1 Activation Loop

SEQ ID NO: 73 - C1 Activation Loop Variant

10 **SEQ ID NO: 74** – Polypeptide Sequence of rLC/A(0) (His-tagged)

SEQ ID NO: 75 – Polypeptide Sequence of rLH_N/A(0) (His-tagged)

SEQ ID NO: 76 – Polypeptide Sequence of rLC/X(0)

SEQ ID NO: 77 – PreScission Protease Site

SEQ ID NO: 78 – C1 Activation Loop Variant 2

15

SEQ ID NO: 1 - Nucleotide Sequence of rBoNT/A(0)

ATGCCATTTCGTCAACAAGCAATTCAACTACAAAGACCCAGTCAACGGCGTCGACATCGCATACATCAAGATTCCG
AACGCCGGTCAAATGCAGCCGGTTAAGGCTTTTAAAGATCCACAACAAGATTGGGGTTATCCCGGAGCGTGACACC
TTCACGAACCCGGAAGAAGGCGATCTGAACCCGCCACCGGAAGCGAAGCAAGTCCCTGTCAGCTACTACGATTCTG
ACGTACCTGAGCACGGATAACGAAAAAGATAACTACCTGAAAGGTGTGACCAAGCTGTTCTGAACGTATCTACAGC
ACGGATCTGGGTTCGCATGCTGCTGACTAGCATTGTTTCGCGGTATCCCGTTCTGGGGTGGTAGCACGATTGACACC
GAACTGAAGGTTATCGACACTAACTGCATTAACGTTATTCAACCGGATGGTAGCTATCGTAGCGAAGAGCTGAAT
CTGGTTCATCATTGGCCCCGAGCGCAGACATTATCCAATTGAGTGCAGAGCTTTGGTCACGAGGTTCTGAATCTG
ACCCGCAATGGCTATGGTAGCACCCAGTACATTTCGTTTTTCGCCGGATTTTACCTTCGGCTTTGAAGAGAGCCTG
GAGGTTGATACCAATCCGTTGCTGGGTGCGGGCAAATTCGCTACCGATCCGGCTGTCACGCTGGCCCATCAACTG
ATCTACGCAGGCCACCGCCTGTACGGCATTGCCATCAACCCAAACCGTGTGTTCAAGGTTAATACGAATGCATAC
TACGAGATGAGCGGCCTGGAAGTCAGCTTCGAAGAACTGCGCACCTTCGGTGGCCATGACGCTAAATTCATTGAC
AGCTTGCAAGAGAATGAGTTCGCTCTGTACTACTATAACAAATTCAAAGACATTGCAAGCACGTTGAACAAGGCC
AAAAGCATCGTTGGTACTACCGCGTCGTTGCAGTATATGAAGAAATGTGTTTAAAGAGAAGTACCTGCTGTCCGAG
GATACCTCCGGCAAGTTTAGCGTTGATAAGCTGAAGTTTGACAAACTGTACAAGATGCTGACCGAGATTTACACC
GAGGACAACTTTTGTGAAATTTCTTCAAAGTGTTGAATCGTAAACCTATCTGAATTTTGACAAAGCCGTTTCAAG
ATTAACATCGTGCCGAAGGTGAACTACACCATCTATGACGGTTTTAACCTGCGTAACACCAACCTGGCGGCGAAC
TTTAACGGTCAGAATACGGAAATCAACAACATGAATTTACGAAGTTGAAGAACTTCACGGGTCTGTTTCGAGTTC
TATAAGCTGCTGTGCGTGCGCGGTATCATCACCAGCAAACCAAAGCCTGGACAAAGGCTACAACAAGGCGCTG
AATGACCTGTGCATTAAGGTAAACAATTGGGATCTGTTCTTTTCGCCATCCGAAGATAATTTTACCAACGACCTG
AACAAGGGTGAAGAAATCACCAGCGATACGAATATTGAAGCAGCGGAAGAGAATATCAGCCTGGATCTGATCCAG
CAGTACTATCTGACCTTTAACTTCGACAATGAACCGGAGAACATTAGCATTGAGAATCTGAGCAGCGACATTATC
GGTCAGCTGGAAGTATGCCGAATATCGAACGTTTCCCGAACGGCAAAAAGTACGAGCTGGACAAGTACACTATG
TTCCATTACCTGCGTGACAGGAGTTTGAACACGGTAAAAGCCGTATCGCGCTGACCAACAGCGTTAACGAGGCC
CTGCTGAACCCGAGCCGTGTCTATACCTTCTTCAGCAGCGACTATGTTAAGAAAGTGAACAAAGCCACTGAGGCC
GCGATGTTTCTGGGCTGGGTGGAACAGCTGGTATATGACTTCACGGACGAGACGAGCGAAGTGAGCACTACCGAC
AAAATTGCTGATATTACCATCATTATCCCGTATATTGGTCCGGCACTGAACATTGGCAACATGCTGTACAAAAGAC
GATTTTGTGGGTGCCCTGATCTTCTCCGGTGCCGTGATTCTGCTGGAGTTCATTCCGGAGATTGCGATCCCGGTG
TTGGGTACCTTCGCGCTGGTGTCTTACATCGCGAATAAGGTTCTGACGGTTCAGACCATCGATAACGCGCTGTCTG
AAACGTAATGAAAAATGGGACGAGGTTTACAAATACATTGTTACGAATTGGCTGGCGAAAGTCAATACCCAGATC
GACCTGATCCGTAAGAAAAATGAAAGAGGCGCTGGAGAATCAGGCGGAGGCCACCAAAGCAATTATCAACTACCAA
TACAACAGTACACGGAAGAAGAATAACATTAACCTCAATATCGATGATTTGAGCAGCAAGCTGAATGAA
TCTATTCAACAACAGCGATGATCAATATCAACAAGTTTTGAATCAGTGTAGCGTTTCTGACCTGATGAATAGCATG
ATTCCGTATGGCGTCAAACGTCTGGAGGACTTCGACGCCAGCCTGAAAGATGCGTTGCTGAAATACATTTACGAC
AATCGTGGTACGCTGATTGGCCAAGTTGACCGCTTGAAAGACAAAGTTAACAATACCTTGAGCACCGACATCCCA

TTTCAACTGAGCAAGTATGTTGATAATCAACGCTCTGTTGAGCACTTTACCGAGTATATCAAAAACATCATCAAT
 ACTAGCATTCTGAACCTGCGTTACGAGAGCAATCATCTGATTGATCTGAGCCGTTATGCAAGCAAGATCAACATC
 GGTAGCAAGGTCAATTTTGACCCGATCGATAAGAACCAGATCCAGCTGTTTAAATCTGGAATCGAGCAAAATTGAG
 GTTATCCTGAAAAACGCCATTGTCTACAACCTCCATGTACGAGAATTTCTCCACCAGCTTCTGGATTTCGCATCCCCG
 5 AAATACTTCAACAGCATTAGCCTGAACAACGAGTATACTATCATCAACTGTATGGAGAACAACAGCGGTTGGAAG
 GTGTCTCTGAACTATGGTGAGATCATTTGGACCTTGCAGGACACCCCAAGAGATCAAGCAGCGCGTCTGTTCAG
 TACTCTCAAATGATCAACATTTCCGATTACATTAATCGTTGGATCTTCGTGACCATTACGAATAACCGTCTGAAT
 AACAGCAAGATTTACATCAATGGTCGCTTGATCGATCAGAAACCGATTAGCAACCTGGGTAATATCCACGCAAGC
 AACAACATTATGTTCAAATTGGACGGTTGCCGCGATACCCATCGTTATATCTGGATCAAGTATTTCAACCTGTTT
 10 GATAAAGAAGTGAATGAGAAGGAGATCAAAGATTTGTATGACAACCAATCTAACAGCGGCATTTTGAAGGACTTC
 TGGGGCGATTATCTGCAATACGATAAGCCGTACTATATGCTGAACCTGTATGATCCGAACAAATATGTGGATGTC
 AATAATGTGGGTATTCGTGGTTACATGTATTTGAAGGGTCCGCGTGGCAGCGTTATGACGACCAACATTTACCTG
 AACTCTAGCCTGTACCGTGGTACGAAATTCATCATTAAGAAATATGCCAGCGGCAACAAAGATAACATTGTGCGT
 AATAACGATCGTGTCTACATCAACGTGGTCTGGAAGAATAAAGAGTACCGTCTGGCGACCAACGCTTCGCAGGCG
 15 GGTGTTGAGAAAATTCTGAGCGCGTTGGAGATCCCTGATGTCGGTAATCTGAGCCAAGTCGTGGTTATGAAGAGC
 AAGAACGACCAGGGTATCACTAACAAGTGCAAGATGAACCTGCAAGACAACATGGTAACGACATCGGCTTTATT
 GGTTTCCACCAGTTCAACAATATTGCTAAACTGGTAGCGAGCAATTGGTACAATCGTCAGATTGAGCGCAGCAGC
 CGTACTTTGGGCTGTAGCTGGGAGTTTATCCCGGTCGATGATGGTTGGGGCGAACGTCGCTG

20 **SEQ ID NO: 2 - Polypeptide Sequence of rBoNT/A(0)**

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWVIPERDTFTNPEEGDLNPPPEAKQVPVSYYS
 TYLSTDNEKDNLYLKGVTKLFERIYSTD LGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELN
 LVIIGPSADIIQFECKSFGEVLNLTNRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAAGKFATDPAVTLAHQL
 IYAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYNKFKDIASTLNKA
 25 KSIVGTTASLQYMKNVFKKEYLLSEDTSGKFSVDKLFKDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDAVFK
 INIVPKVNYTIYDGFNLNRLNLAANFNQNTNINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKAL
 NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIEENLSSDII
 GQLELMPNIEFPPNGKKYELDKYTMFHYLRAQEFHEGKSRIALTSVNEALLNPSRVYTFSSDYVKKVNKATEA
 AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIPIYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
 30 LGTFALVSYIANKVLTVQTDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLRKKMKEALENQAEATKAIINYQ
 YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLDFDASLKDALLKYIYD
 NRGTLIGQVDRLKDKVNNLTSTDIPFQLSKYVDNQRLSTFTYIKNIINTSILNRLYESNHLIDLSRYASKINI
 GSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVNSMYENFSTSFWIRIPKYFNSISLNNEYTIINCMENNSGWK
 VSLNYGEIITWLTQDTQEIQRVVFYKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPISNLGNIHAS
 35 NNIMFKLDGCRDTHRYIWIKYFNLFDEKELNEKEIKDLYDNQSNISGILKDFWGDYLYDKPYMLNLYDPNKYVDV
 NNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFIIKKYASGNKDNIVRNNDRVYINVVKNKEYRLATNASQA
 GVEKILSALEIPDVGNLSQVVVMKSKNDQGITNKCKMNLQDNNGNDIGFIGFHQFNNAKLVASNWNRYNRQIERSS
 RTLGCSEWFI PVDDGWGERPL

40 **SEQ ID NO: 3 - Nucleotide Sequence of rLH_N/A**

atggagttcggttaacaaacagttcaactataaagacccagtttaacggtggtgacattgcttacatcaaaatcccg
 aacgctggccagatgcagccggttaaaggcattcaaaatccacaacaaatctgggttatcccggaacgtgatacc
 ttactaaccgggaagaaggtgacctgaacccgccaccggaagcgaaacaggtgcccgtatcttactatgactcc
 acctacctgtctaccgataacgaaaaggacaactacctgaaaggtgttactaaactggttcgagcgtatttactcc
 45 accgacctgggcccgtatgctgctgactagcatcggttcgcggtatcccggtctggtggcggttctaccatcgatacc
 gaactgaaagtaatcgacactaactgcataacggtatttcagccggacggttcctatcggttcgaagaactgaac
 ctggtgatcatcgcccgtctgctgatcatccaggttcgagtgtaagagcttttggtcacgaagttctgaacctc
 acccgtaacggttacggttccactcagtagcatccggttctctccggacttcaccttcggttttgaagaatccctg
 gaagtagacacgaacccactgctgggcgctggtaaatcgcaactgatcctgcggttacccctggtcacgaactg
 50 attcatgcaggccaccgctgtacggtatcgccatcaatccgaaccgtgtcttcaaagttaacaccaacgcgtat
 tacgagatgtccggtctggaagtttagcttcgaagaactgcgtacttttggcggtcacgacgctaaattcatcgac
 tctctgcaagaaaacgagttccgctgtgactactataacaagttcaaagatatcgcatccaccctgaacaaagcg
 aaatccatcggtgggtaccactgcttctctccagtagatgaagaacggtttttaagaaaaatacctgctcagcgaa
 gacacctccggcaaatctctgtagacaagttgaaattcgataaaactttacaaaatgctgactgaaatttacacc
 55 gaagacaacttcgttaagttcttttaagttctgaaccgcaaaacctatctgaacttcgacaaggcagtagttcaaa
 atcaacatcggtgccgaaagttaactacactatctacgatgggttcaacctgcgtaacaccaacctggctgcta
 ttttaacggccagaacacggaaatcaacaacatgaacttcacaaaactgaaaaacttcactggctctgttcgagttt
 tacaagctgctgtgcGTGACGGCATCATTACCTCCAAAACATAATCTGACGATGACGATAAAAACAAAGCGCTG
 AACCTGCAGTgtatcaaggttaacaactgggatttattcttcagccccgagtgaagacaacttcaccaacgacctg
 60 aacaaaggtgaagaaatcacctcagataactaacatcgaagcagccgaagaaaacatctcgctggacctgatccag

cagtactacctgacctttaatttcgacaacgagccggaacacattttctatcgaaaacctgagctctgatatcatc
 ggccagctggaactgatgccgaacatcgaacgtttcccaaccggtaaaaagtagcagctggacaaatataccatg
 ttccactacctgcgcgcgcaggaatttgaacacggcgaatcccgtagcactgactaactccgttaacgaagct
 ctgctcaaccgctcccggtgtatacaccttctctctagcgactacgtgaaaaaggtcaacaaagcgactgaagct
 5 gcaatgttcttgggttgggttgaacagcttgtttatgattttaccgacgagacgtccgaagtatctactaccgac
 aaaattgcgatatactatcatcatcccgtacatcggtccggctctgaacattggcaacatgctgtacaaagac
 gaacttcgttggcgactgatcttctccggtgcggtgatcctgctggagttcatcccggaatcgccatcccggtg
 ctgggcacctttgctctggtttcttacattgcaacaaggttctgactgtacaaaccatcgacaacgcgtgagc
 10 aaacgtaacgaaaaatgggatgaagtttacaatatatcgtgaccaactggctggctaagggttaatactcagatc
 gacctcatccgcaaaaaaatgaaagaagcactggaaaaccaggcggaagctaccaaggcaatcattaactaccag
 tacaaccagtagaccgaggaagaaaaaacaacatcaacttcaacatcgacgatctgtcctctaaactgaacgaa
 tccatcaacaaagctatgatcaacatcaacaagttcctgaaccagtgtctctgtaagctatctgatgaactccatg
 atcccgtagcgtgttaaacgtctggaggacttcgatgcgtctctgaaagacgccctgctgaaatacatttacgac
 15 aaccgtggcactctgatcggtcaggttgatcgtctgaaggacaaaagtgaacaataccttatcgaccgacatccct
 tttcagctcagtaaatatgtcgataaccaacgccttttgtccactctagaagcaCACCATCATCACcaccatcac
 catcaccat

SEQ ID NO: 4 - Polypeptide Sequence of rLH_N/A

MEFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWIWIPERDTFTNPEEGDLNPPPEAKQVPVSYIDS
 20 TYLSTDNEKDNLYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSSYRSEELN
 LVIIGPSADIIQFECKSFGHEVLNLRNGYGSTQYIRFSPDFTFGFEESLEVDNPLLGAGKFATDPAVTLAHEL
 IHAGHRLYGIAPNPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYNKFKDIASTLNKA
 KSIVGTTASLQYMKNVFKKYLSEDTSGKFSDVKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFK
 25 INIVPKVNYTIYDGFNLRNTNLAANFNQNTNINNMNFTKLKNFTGLFEFYKLLCVDGIITSKTSDDDDKKNKAL
 NLQCIKVNNDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIEENLSSDII
 GQLELMPNIEFNPNGKKEYELDKYTMFHYLRAQEFEGHKSRIALNSVNEALLNPSRVYTFSSDYVKKVNKATEA
 AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIPIYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
 LGTFALVSYIANKVLTVQITDNALSKRNEKWDEVYKYIVTNWLAKVNTQIDLRKKMKEALENQAEATKAIINYQ
 30 YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLDFDASLKDALLKYIYD
 NRGTLIGQVDRLLKDKVNNTLSTDIPFQLSKYVDNQRLSTLEAHHHHHHHHHH

SEQ ID NO: 5 - Nucleotide Sequence of rL/A

ATGCCATTTCGTCAACAAGCAATTCAACTACAAAGACCCAGTCAACGGCGTCGACATCGCATACATCAAGATTCCG
 AACGCCGGTCAAATGCAGCCGGTTAAGGCTTTTAAGATCCACAACAAGATTGGGTTATCCCGGAGCGTGACACC
 35 TTCACGAACCCGGAAGGCGATCTGAACCCGCCACCGGAAGCGAAGCAAGTCCCTGTCAGCTACTACGATTCCG
 ACGTACCTGAGCAGGATAACGAAAAAGATAACTACCTGAAAGGTGTGACCAAGCTGTTCGAACGATCTACAGC
 ACGGATCTGGGTGCGATGCTGCTGACTAGCATTGTTTCGGGTATCCCGTTCTGGGGTGGTAGCACGATTGACACC
 GAACTGAAGGTTATCGACACTAACTGCATTAACGTTATTCAACCGGATGGTAGCTATCGTAGCGAAGAGCTGAAT
 CTGGTCATCATTGGCCCCGAGCGCAGACATTATCCAATTTCGAGTGCAAGAGCTTTGGTCACGAGGTTCTGAATCTG
 40 ACCCGCAATGGCTATGGTAGCACCAGTACATTCTGTTTTTCGCCGGATTTTACCTTCGGCTTTGAAGAGAGCCTG
 GAGGTTGATACCAATCCGTTGCTGGGTGCGGGCAAATTCGCTACCGATCCGGCTGTACGCTGGCCCATGAACTG
 ATCCACGCAGGCCACCGCTGTACGGCATTGCCATCAACCCAAACCGTGTGTTCAAGGTTAATACGAATGCATAC
 TACGAGATGAGCGGCTGGAAGTCAGCTTCGAAGAAGTGCACACCTTCGGTGGCCATGACGCTAAATTCATTGAC
 AGCTTGCAAGAGAATGAGTTCCGCTGTACTACTATAACAAATTCAAAGACATTGCAAGCACGTTGAACAAGGCC
 45 AAAAGCATCGTTGGTACTACCGCGTCGTTGCAGTATATGAAGAATGTGTTTAAAGAGAAGTACCTGCTGTCCGAG
 GATACCTCCGGCAAGTTTAGCGTTGATAAGCTGAAGTTTGACAAACTGTACAAGATGCTGACCGAGATTTACACC
 GAGGACAACCTTTGTGAAATTTCTTCAAAGTGTGAAATCGTAAACCTATCTGAATTTTGACAAAGCGGTTTTCAAG
 ATTAACATCGTGCCGAAGGTGAACTACACCATCTATGACGGTTTTAACCTGCGTAACACCAACCTGGCGGCGAAC
 50 TTTAACGGTCAGAATACGGAATCAACAACATGAATTTACGGAAGTTGAAGAACTTCACGGGTCTGTTTCGAGTTC
 TATAAGCTGCTGggtctagaagcaCACCATCATCACcaccatcaccatcaccat

SEQ ID NO: 6 - Polypeptide Sequence of rL/A

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWIWIPERDTFTNPEEGDLNPPPEAKQVPVSYIDS
 55 TYLSTDNEKDNLYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSSYRSEELN
 LVIIGPSADIIQFECKSFGHEVLNLRNGYGSTQYIRFSPDFTFGFEESLEVDNPLLGAGKFATDPAVTLAHEL
 IHAGHRLYGIAPNPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYNKFKDIASTLNKA
 KSIVGTTASLQYMKNVFKKYLSEDTSGKFSDVKLKFDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDKAVFK
 INIVPKVNYTIYDGFNLRNTNLAANFNQNTNINNMNFTKLKNFTGLFEFYKLLGLEAHHHHHHHHHH

SEQ ID NO: 7 - Nucleotide Sequence of rHc/A

ATGCATCATCACCATCACCACAAAAACATCATCAATACTAGCATTCTGAACCTGCGTTACGAGAGCAATCATCTG
 ATTGATCTGAGCCGTTATGCAAGCAAGATCAACATCGGTAGCAAGGTCAATTTTGACCCGATCGATAAGAACCAG
 5 ATCCAGCTGTTTAATCTGGAATCGAGCAAAATTGAGGTTATCCTGAAAAACGCCATTGTCTACAACCTCCATGTAC
 GAGAATTTCTCCACCAGCTTCTGGATTTCGCATCCCGAAATACTTCAACAGCATTAGCCTGAACAACGAGTATACT
 ATCATCAACTGTATGGAGAACAACAGCGGTTGGAAGGTGTCTCTGAACTATGGTGAGATCATTTGGACCTTGCGAG
 GACACCCAAGAGATCAAGCAGCGCGTCGTGTTCAAGTACTCTCAAATGATCAACATTTCCGATTACATTAATCGT
 TGGATCTTCGTGACCATTACGAATAACCGTCTGAATAACAGCAAGATTTACATCAATGGTCGCTTGATCGATCAG
 10 AAACCGATTAGCAACCTGGGTAATATCCACGCAAGCAACAACATTATGTTCAAATTGGACGGTTGCCGCGATACC
 CATCGTTATATCTGGATCAAGTATTTCAACCTGTTTGATAAAAGAACTGAATGAGAAGGAGATCAAAGATTTGTAT
 GACAACCAATCTAACAGCGGCATTTTGAAGGACTTCTGGGGCGATTATCTGCAATACGATAAGCCGTACTATATG
 CTGAACCTGTATGATCCGAACAAATATGTGGATGTCAATAATGTGGGTATTCGTGGTTACATGTATTTGAAGGGT
 CCGCGTGGCAGCGTTATGACGACCAACATTTACCTGAACTCTAGCCTGTACCGTGGTACGAAATTCATCATTAAG
 15 AAATATGCCAGCGGCAACAAAGATAACATTGTGCGTAATAACGATCGTGTCTACATCAACGTGGTCGTGAAGAAT
 AAAGAGTACCGTCTGGCGACCAACGCTTCGACAGGCGGGTGTGAGAAAAATTCTGAGCGCGTTGGAGATCCCTGAT
 GTCGGTAATCTGAGCCAAGTCGTGGTTATGAAGAGCAAGAAGACAGGCTATCATAACAAGTCAAGATGAAC
 CTGCAAGACAACAAATGGTAACGACATCGGCTTTATTGGTTTCCACCAAGTTCAACAATATTGCTAAACTGGTAGCG
 AGCAATTGGTACAATCGTCAGATTGAGCGCAGCAGCCGTACTTTGGGCTGTAGCTGGGAGTTTATCCCGGTCGAT
 20 GATGGTTGGGGCGAACGTCCGCTG

SEQ ID NO: 8 - Polypeptide Sequence of rHc/A

MHHHHHHKNIINTSILNLRYESNHLIDLRSRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVYNSMY
 ENFSTSFWIRIPKYFNSISLNNEYTIINC MENNSGWKVS LNYGEI IWT LQDTQEIKQRVVFKYSQMINISDYINR
 25 WIFVTITNNRLNNSKIYINGRLIDQKPI SNLGN I HASNNIMFKLDGCRDTHRYIWI KYFNLF DKELNEKEIKDLY
 DNQSN SGI LKDFWGDY LQYDKPY YMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFIIK
 KYASGNKDNIVRNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVVMKSKNDQGITNKCKMN
 LQDNNGNDIGFIFGHQFN NIAKLVASNWN RQIERSSRTLGC SWEFIPVDDG WGERPL

SEQ ID NO: 9 - Nucleotide Sequence of rBoNT/B(0)

ATGCCGGTGACGATTAACAACCTTCAACTACAACGACCCGATTGACAACAACAACATTATCATGATGGAACCGCCG
 TTTGCACGCGGCACGGGCGGTTATTACAAAGCGTTTAAAATCACCGATCGTATTTGGATTATCCCGGAACGCTAC
 ACGTTTGGTTATAAACCGGAAGACTTCAACAAAAGCTCTGGCATCTTCAACCGTGATGTTTGCGAATACTACGAT
 35 CCGGACTACCTGAACACCAACGATAAGAAAAACATTTTCTGCAAACGATGATCAAACCTGTTCAATCGCATTA
 AGCAAACCGCTGGGTGAAAACTGCTGGAATGATTATCAATGGCATTCCGTATCTGGGTGATCGTCGCGTGCCG
 CTGGAAGAATTTAACACCAATATCGCGAGTGTTACGGTCAACAAACTGATTTCCAATCCGGGTGAAGTCGAACGT
 AAAAAAGGCATCTTCGCCAACCTGATCATCTTCGGCCCCGGGTCCGGTGTGTAACGAAAAATGAAACCATTGATATC
 GGTATTACAGAACCATTTTGCCTCACGCGAAGGCTTCGGCGGTATTATGCAAATGAAATTTTGCCCGGAATATGTG
 40 TCGGTTTTCAACAATGTTTACAGAAAAACAAAGGTGCAAGCATCTTTAATCGTCGCGGCTATTTCTCTGATCCGGCT
 CTGATCCTGATGCACCAACTGATTATGTGTCTGCACGGCCTGTATGGTATCAAAGTGGATGACCTGCCGATCGTT
 CCGAACGAGAAAAAATTTTTCATGCAGAGCACCGACGCAATTCAAGCTGAAGAACTGTATACGTTTGGCGGTCTAG
 GACCCGTCTATTATCACCCGAGCACCGACAAAAGCATCTACGATAAAGTGCTGCAAAACCTTTCTGGCATTGTT
 GACCGCCTGAATAAAGTCCTGGTGTGTATCTCTGATCCGAACATCAACATCAACATCTACAAAAACAAATTCAAA
 45 GACCAATACAAATTCGTTGAAGATTCTGAAGGCAAAATAGTATTGACGTGCAATCCTTTGATAAACTGTACAAA
 AGTCTGATGTTTCGGTTTTACCGAAACGAACATCGCGGAAAACTACAAAATCAAAACCCGCGCTTCTATTTTCAGC
 GACTCTCTGCCGCCGGTTAAAAATCAAAAATCTGCTGGATAACGAAATTTATACGATCGAAGAAGGTTTCAACATC
 AGCGATAAAGACATGGAAAAAGAATACCGTGGCCAGAATAAAGCAATCAACAAACAGGCGTATGAAGAAATTAGT
 AAAGAACATCTGGCGGTCTACAAAATTCAGATGTGCAAAATCCGTGAAAGCCCCGGGTATTTGTATCGATGTTGAC
 50 AATGAAGACCTGTTTTTCATCGCCGATAAAAAACAGTTTTTCCGATGACCTGTCAAAAAATGAACGCATCGAATAC
 AACACCCAATCGAACTACATCGAAAACGATTTCCCGATCAACGAACTGATTCTGGATACGGACCTGATTAGTAAA
 ATCGAACTGCCGTGAGAAAACACCGAATCGCTGACGGACTTTAATGTTGATGTCCCGGTGTATGAAAAACAGCCG
 GCAATTAAGAAAATTTTACCAGTGA AAAACACGATCTTCCAGTACCTGTACAGCCAAACCTTTCCGCTGGACATT
 CGCGATATCTCTCTGACGAGTTCTTTGATGACGCACTGCTGTTCAGCAACAAAGTGTACTCCTTTTTTCTCAATG
 55 GATTACATCAAAACCGCTAACAAAGTGGTTGAAGCGGGCCTGTTTGCCGGTTGGGTGAAACAGATCGTTAACGAT
 TTCGTTCATCGAAGCCAACAAAAGTAACACGATGGATAAAATGCTGATATCTCCCTGATTGTCCCGTATATTGGC
 CTGGCACTGAATGTGGGTAAACGAAACGGCGAAAGGCAATTTTGAAAAACGCTTCGAAATTCAGGCGCTTCAATC
 CTGCTGGAATTTATTCCGGAACCTGCTGATCCCGGTCTGGGTGCGTTCTTCTGCTGGAATCTTACATCGACAACAAA

AACAAAAATCATCAAAACCATTTGATAACGCGCTGACGAAACGTAACGAAAAATGGTCAGATATGTACGGCCTGATT
 GTTGGCCAGTGGCTGAGCACCGTCAACACGCAATTTTACACCATCAAAGAAGGTATGTACAAAGCGCTGAATTAT
 CAGGCGCAAGCCCTGGAAGAAATCATCAAATACCGCTACAACATCTACAGCGAAAAAGAAAAATCTAACATCAAC
 ATCGACTTTAATGATATCAACAGCAAACTGAACGAAGGTATCAACCAGGCAATCGATAACATCAACAAC'TTCATC
 5 AACGGCTGCTCAGTGTCTGATCTGATGAAGAAAAATGATCCCGCTGGCTGTTGAAAAACTGCTGGATTTTGACAAC
 ACCCTGAAGAAAAACCTGCTGAACTACATCGATGAAAAACAACTGTACCTGATCGGCTCAGCCGAATACGAAAAA
 TCGAAAGTGAACAAATACCTGAAAAACCATCATGCCGTTTGACCTGAGTATTTACACCAACGATACGATCCTGATC
 GAAATGTTCAACAAATACAACCTCCGAAATTCTGAACAATATTATCCTGAACCTGCGTTACAAAGACAACAATCTG
 ATCGATCTGAGCGGCTATGGTGCAAAAGTTGAAGTCTACGACGGTGTGCAACTGAACGATAAAAAACCAGTTCAAA
 10 CTGACCTCATCGGCTAACTCAAAAATTCGTGTGACGCAGAACCAAAACATCATCTTCAACTCGGTCTTTCTGGAC
 TTCAGCGTGTCTTTCTGGATTTCGCATCCCGAAATATAAAAAATGATGGCATCCAGAACTACATCCATAACGAATAC
 ACCATCATCAACTGTATGAAAAACAACAGTGGTTGGAAAAATTTCCATCCGTGGCAACCGCATTATCTGGACCCTG
 ATTGATATCAATGGTAAACGAAAAAGCGTGTTTTTCGAATACAACATCCGTGAAGATATCTCTGAATACATCAAT
 CGCTGGTTTTTTCGTGACCATTACGAACAATCTGAACAATGCGAAAAATCTATATCAACGGCAAACTGGAAAGTAAT
 15 ACCGACATCAAAGATATTCGTGAAGTTATCGCCAACGGTGAAATCATCTTCAAACCTGGATGGCGACATCGATCGC
 ACCCAGTTTCAATTTGGATGAAATACTTCTCCATCTTCAACACGGAACCTGAGTCAGTCCAATATCGAAGAACGCTAC
 AAAATCCAATCATACTCGGAATACCTGAAAGATTTCTGGGGTAACCCGCTGATGTACAACAAAGAATACTACATG
 TTCAACGCGGGGCAACAAAACTCATACTCAAACCTGAAAAAGATTTCGCCGGTGGGTGAAATCCTGACCCGTAGC
 AATACACACCTCAGAGTCTAAATACATCAACTATCGCGATCTGTACATTGGCGAAAAATTTATTATCCGTGCGCAA
 20 AGCAACTCTCAGAGTATTAATGATGACATCGTGCCTAAAGAACTACATCTATCTGGATTTCTTTAATCTTGAAC
 CAAGAATGGCGCGTTTATACCTACAAATACTTCAAAAAAGAAAGAGAACTGTTCTTGGCCCCGATTAGCGAC
 AGCGATGAATTTTACAACACCATCCAGATCAAAGAATACGATGAACAGCCGACGTATAGTTGCCAACTGCTGTTT
 AAAAAAGACGAAGAATCCACCGATGAAATTGGCCTGATTGGTATCCACCGTTTCTATGAAAGCGGTATCGTTTTT
 GAAGAATACAAAGATTACTTCTGTATCTCTAAATGGTATCTGAAAGAAGTCAAACGCAACCGTACAACCTGAAA
 25 CTGGGCTGCAACTGGCAATTTATCCCGAAAGACGAAGGCTGGACCGAA

SEQ ID NO: 10 - Polypeptide Sequence of rBoNT/B(0)

MPVTINNFFNYNDPIDNNNIIMMEPPFARGTGRIYKAFKITDRIWIIPERYTFGYKPEDFNKSSGIFNRDVCEYYD
 PDYLNNTNDKKNIFLQTMIKLFNRKSKPLGEKLLEMIINGIPYLGDRRVPLEEFNTNIASTVNKLI SNPGEVER
 30 KKGIFANLIIFGPGPVLNENETIDIGIQNHFAFREGFGGIMQMKFCPEYVSFVFNQENKGAIFNRRGYFSDPA
 LILMHQLIYVLHGLYGIKVDDLPIVPNEKKFFMQSTDAIQAEELYTFGGQDPSIITPSTDKSIYDKVLQNFGRGIV
 DRLNKVLVCI SDPNININIKYKFKDKYKFVEDSEGKYSIDVESFDKLYKSLMFGFTETNIAENYKIKTRASYFS
 DSLPPVKIKNLLDNEIYTIIEEGFNISDKDMEKEYRGQNKAINKQAYEEISKEHLAVYKI QMCKSVKAPGICIDVD
 NEDLFFIADKNSFSDDLKNERIEYNTQSNYIENDFPINELILDLDLISKIELPSENTESLTDFNVDVPVYEKQP
 35 AIKKIFTDENTIFQYLYSQTFLDIRDISLTSSFDALLFSNKVYSFFSMDYIKTANKVVEAGLFAGWVKQIVND
 FVIEANKSNTMDKIADISLIVPYIGLALNVGNETAKGNFENAFEIAGASILLEFIPELLIPVVGAFLLSYIDNK
 NKIIKTIDNALTKRNEKWSMDYGLIVAQWLSTVNTQFYTIKEGMYKALNYQAQALEEIIKYRYNIYSEKEKSNIN
 IDFNIDINSKLNENINQAI DNINNFINGCSVSYLMMKMIPLAVEKLLDFDNTLKKNLNLYIDENKLYLIGSAEYK
 SKVNKYKLTIMPFDLSIYNTDILIEFMFNKYNSEILNNIILNLRYKDNLLIDLSGYGAKVEVYDGVELNDKNQFK
 40 LTSSANSKIRVTQNNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRIIWT
 IDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDI
 TQFIWMKYFSIFNTELSQSNIERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNYSYIKLKKDSPVGEILTRS
 KYNQNSKYINRYDLIYIGEKFIIRRSNSQSINDDIVRKEDYIYLDFFNLNQEWRYVYTYKFKEEEKLFLAPISD
 SDEFYNTIQIKEYDEQPTYSCQLLFFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKKRPPYNLK
 45 LGCNWQFIPKDEGWTE

SEQ ID NO: 11 - Nucleotide Sequence of rBoNT/C(0)

ATGCCGATCACGATTAATAATTTCAACTATAGCGATCCGGTGGACAATAAGAATATTCTGTATCTGGATACTCAT
 CTGAATACGCTGGCTAACGAACCGGAGAAAGCGTTCCGCATCACAGGCAACATCTGGGTATTATCCCGATCGCTTT
 50 TCACGCAACAGCAACCCTAATCTGAACAAACCTCCTCGTGTACCAGTCTTAAATCCGGTTATTACGACCCAAAC
 TATCTGAGTACGGATAGCGATAAAGATCCCTTTCTGAAAGAGATCATTAAGCTGTTCAAACGCATTAACCTCTCGC
 GAAATTGGGGAAGAGCTGATCTATCGGCTTTTCGACAGATATCCCGTTCCAGGTAACAATAATACCCCGATTAAAT
 ACTTTTCGACTTTGATGTTGATTTCAATTCTGTGGATGTGAAAACGCGTCAAGGCAATAATTGGGTGAAAACCTGGT
 AGCATTAAACCCGAGTGAATTATCACAGGTCCCCGTGAGAACATCATCGACCCGAAACCTCTACCTTCAAGCTG
 55 ACGAACAACACGTTTGTGTCACAGGAAGGGTTTGGTGCCCTGTCAATCATTTCCATCTCACCGCGTTTCATGTTA
 ACCTACTCCAATGCCACAAATGATGTTGGCGAAGGACGTTTTAGCAAATCAGAATTTTGCATGGACCCAATTCTC
 ATTCTGATGgGCaGCTGAACaATGCGATGCACAACTTGATGGCATTGCTATTCCAAACGATCAAACCATTAGC
 TCCGTTACCAGTAATATCTTCTATAGCCAGTATAATGTCAAATTGAGTATGCCGAAATTTACGCCTTTGGAGGC
 CCGACCATTGACCTGATTCCGAAATCTGCACGCAAACTTTCGAAGAAAAAGGCGTTAGATTACTATCGCAGCATC

GCGAAACGCCTGAACTCGATTACCACGGCCAATCCGTCGTCGTTCAACAAATACATTGGTGAATATAAACAGAAA
 CTGATTTCGCAAATATCGGTTTGTCTGTAGAAAAGCTCTGGTGAAGTGACTGTAAACCGCAACAAATTTGTGCAACTC
 TACAACGAGTTGACCCAAATCTTTACCGAGTTTAACTACGCAAAAGATCTATAACGTACAGAACCAGCAAGATTTAT
 CTTAGCAATGTATACACACCGGTTACTGCGAACATCTTAGACGACAATGTGTATGATATTTCAGAATGGCTTTAAC
 5 ATCCCCGAAATCAAATCTGAACGTTCTGTTTATGGGGCCAGAACCTGAGTCGTAATCCAGCACTGCGTAAAGTGAAC
 CCGGAAAATATGCTCTACTTGTGTACCAAATTTTGCCACAAAGCGATTGATGGCCGCTCTCTCTATAACAAAACG
 CTGGATTGTCTGTGAGTTACTTGTGAAGAACTGATTTACCGTTTCATTGGGGATATCTCCGACGTGAAAACCGAT
 ATCTTCTGCGCAAAGACATTAATGAAGAAACGGAAGTCATCTATTACCCCGACAATGTGAGCGTTGATCAGGTC
 10 ATTTTATCGAAGAACACCTCCGAACATGGTCAGTTGGATTGTGTGTACCTTAGCATTGACTCGGAGAGTGAATC
 CTTCCGGGCGAAAATCAAGTGTTTTACGACAACCGTACCCAAAATGTTGATTATTTGAATTTCTTATTACTACCTG
 GAATCTCAGAAATTGAGCGACAATGTGGAAGATTTACGTTTACACGCTCCATTGAGGAAGCGCTGGATAATAGC
 GCGAAAGTGATACGTATTTCCCTACCTTGGCGAATAAAGTAAATGCTGGTGTCCAGGGAGGCTTATTTCTGATG
 TGGGCGAATGATGTGGTAGAAGATTTTACGACCAATATTTTTCGTAAGGACACCTTAGATAAAATTAGCGATGTT
 15 AGCGCCATCATCCCTATATTGGCCAGCACTGAATATCTCGAACTCTGTGCGTCGCGGAAACTTCACCGAAGCA
 TTTGCGGTGACCGGGTTACTATTCTGTTGGAAGCCTTTCCGGAGTTTACTATTCGGGCGCTGGGTGCGTTTGTG
 ATTTATTTCGAAAGTACAAGAACGCAATGAAATTTATCAAAACCATCGATAATTGCCCTGGAACAACGCATTAAACGC
 TGGAAGGATTCTTATGAATGGATGATGGGCACCTGGTTATCCCGTATTATCACACAGTTTAAACAACATCTCGTAT
 CAGATGTACGATTACACTGAACCTACCAAGCAGGGGCGATCAAAGCCAAGATCGACTTAGAATACAAGAAATATTCA
 GGTAGCGATAAAGACAATATTTAAAGCCAGGTTGAAAACCTGAAGAACTCTCTGGATGTCAAAATTTTTCAGAGGCT
 20 ATGAACAACATTAAACAAATTTATCCGCGAATGTAGCGTCACGTATCTGTTTAAAAACATGCTCCCGAAAGTGATT
 GATGAGCTCAACGAGTTTGATCGCAACACAAAGGCCAAACTGATTAACTTGATTGATAGTCACAATATTATTTTA
 GTCGGTGAAGTTGACAAGCTGAAGGCTAAGGTCAATAACAGCTTTTCAGAACACTATTCCGTTTAAATATTTTCTCC
 TATACGAACAATAGTCTGCTGAAAGACATTATCAACGAATACTTCAACAATATTAATGACAGCAAAATTTCTGAGC
 CTGCAGAATCGTAAGAATACGCTGGTAGATACCACTGGATATAATGCGGAAGTCTCAGAAGAGGGTGATGTACAG
 25 CTGAACCCGATCTTTCCGTTTCGACTTTAACTGGGGTCTAGTGGTGAAGATCGCGGTAAAGTGATCGTTACCCAA
 AACGAGAACATTGTGTATAACAGCATGTACGAGAGTTTCTCAATTTCTTTCTGGATTTCGCATCAATAAATGGGTT
 TCTAATTTGCCTGGCTATACCATCATTGATAGCGTCAAAAACAACCTCGGGCTGGTCGATTGGCATTATTAGCAAC
 TTTCTGGTGTTTACCTGAAACAGAATGAGGATTTCGGAACAGAGCATTAACTTCTCCTACGACATCAGCAACAAT
 GCACCAGGGTATAACAAATGGTTCCTTCGTAACGGTGACGAACAATATGATGGGCAATATGAAAATCTACATTAAAC
 30 GGGAACTTATCGACACCATTAAAGTGAAAGAGCTTACTGGGATCAATTTTAGTAAAACCATTACCTTTGAGATC
 AACAAAATTCGGGACACGGGTCTGATTACCTCCGATTTCGGATAATATCAATATGTGGATTTCGCGACTTTTATATC
 TTCGCCAAAGAACTTGATGGCAAAGATATCAACATTTTGTTTAATTCCTGCGATATACCAATGTCTGTTAAGGAC
 TATTGGGGCAATGATCTCCGCTACAATAAAGAATACTACATGGTTAACATCGACTATCTCAATCGCTACATGTAT
 GCTAACTCGCGTCAAATTGTGTTTAAACACACGTCGTAACAACAACGATTTTAAACGAAGTTATAAAATCATTATC
 35 AAACGGATCCGCGGCAATACGAACGATACTCGTGTTTCGTGGCGGTGACATTCTGTATTTTCGACATGACGATTAAT
 AATAAAGCGTACAATCTGTTTCATGAAGAACGAAACCATGTACGCCGATAACCATTCCTGAGATATCTACGCA
 ATCGGACTTCGCGACAGACCAAAAGACATTAACGACAACATCTTTTCAGATTCAACCGATGAATAATACCTAC
 TACTATGCTCCAGATCTTCAAAAGTAATTTCAACGGCGGAAACATTTTCAGGCATTTGCTCAATCGGCACTTAT
 CGGTTCCGGTTAGGTGGTGATTGGTATCGTCACAACCTACCTTGTTCACAGTGAAACAAGGCAACTATGCATCG
 40 CTCTTAGAAAGCACATCTACGCATTGGGGTTTTGTGCCAGTCAGTGAA

SEQ ID NO: 12 - Polypeptide Sequence of rBoNT/C(0)

MPITINNFNYSDPVDNKNILYLDTHLNTLANEPEKAFRITGNIWVIPDRFSRNSNPNLNKPVRVTSPKSGYYDPN
 YLSTDSKDKPFLKEIKLFKRINSREIGEELIYRLSTDIPFPGNNTPIINTFDFDVFNSVDVKTRQGNWVKTG
 45 SINPSVIITGPRENIDPETSTFKLTNNTFAAQEGFGALSIIISIPRFLTYSNATNDVGEGRFSKSEFCMDPIL
 ILMGTLNNAMHNLYGIAIPNDQTISSVTSNIFYSQYNVKLEYAEIYAFGGPTIDLIPKSARKYFEEKALDYRSI
 AKRLNSITTANPSSFNKYIGEYKQKLIRKYRFVVESSGEVTVNRNKFVELYNELTQIFTEFNYAKIYNVQNRKIY
 LSNVYTPVTANILDDNVYDIQNGFNIPKSNLNVLFMGQNLNRPALRKVNPNENMLYLFTKFCHKAIIDGRSLYNKT
 LDCRELLVKNTDLPFIGDISDVKTDFLRKDINEETEVIYYPDNVSVDQVILSKNTSEHGQLDLLYPSIDSESEI
 50 LPGENQVIFYDNRTQNVLDYLSYYYLESQKLSDNVEDFTFTRSIIEALDNSAKVYTYFPTLANKVNAGVQGGFLFM
 WANDVVEDFTTNILRKDTLDKISDVSAIIPYIGPALNISNSVRRGNFTEAFVTVTILLEAFPEFTIPALGAFV
 IYSKVQERNEIIKTIDNCLEQRIKRWKDSYEWMMGTWLSRIITQFNNISYQMYDSLNYQAGAIKAKIDLEYKKYS
 GSDKENIKSQVENLNKSLDVKISEAMNNINKFIRECSVTYLFKNMLPKVIDELNEFDRNTKAKLINLIDSHNIIL
 VGEVDKLKAVNNSFQNTIPFNIFSNTNSSLKDIINEYFNNINDSKILSLQNRKNTLVDTSGYNAEVSEEGDVQ
 55 LNPIFPFDFKLGSSEDRGKVIVTQENIVYNSMYESFSISFWIRINKWVSNLPGYTIIDSVKNSGWSIGIISN
 FLVFTLKLQNEDESDSINFSYDISNAPGYNKWFVTVTNMMGMNMIYINGKLIDTIKVELTGINFSKTITFEI
 NKIPDTGLITSDSDNINMWIRDFYIFAKELDGKIDINLFSNLQYTNVVKDYWGNDLRYNKEYMYMVIDYLNRYMY
 ANSRQIVFNTRRNNNDNNEGKIIKIRIGNTNDRVRGDDILYFDMTINNKAYNLFMKNETMYADNHSTEDIYA
 IGLREQTKDINDNIIFQIQPMNNTYYYASQIFKSNFNGENISGICSIGTYRFRLLGGDWYRHNLYLVPTVKQGNYS
 60 LLESTSTHWGFVPVSE

SEQ ID NO: 13 - Nucleotide Sequence of rBoNT/E(0)

atgcccgaatcaactctttcaactacaacgaccgggttaacgaccgtaccatcctgtatatcaaaccgggtggt
 5 tgcaggaggttctacaaatctttcaacatcatgaaaaacatctggatcatcccgaacgtaacggttatcggtacc
 accccgcaggacttccacccgcccacctctctgaaaaacggtgactcttcttactacgaccggaactacctccag
 tctgacgaagaaaaagaccggttctctgaaaaatcggttaccaaaaatcttcaaccggtatcaacaacaacctgctggt
 ggtatcctgctggaagaactgtctaaagctaaccggtacctgggttaacgacaacaccccggaacacaggttccac
 atcggtgacgcttctgctgttgaaatcaaattctctaacggttctcaggacatcctgctgccgaacggttatcatc
 atgggtgctgaaccggacctgttcgaaaccaactcttctaaccatctctctgctgaacaactacatgccgtctaac
 10 caccggttccggttctatcgctatcggttaccttctctccggaatactctttccggttcaacgacaacagcatgaac
 gagttcatccaggaccggctctgacctgatgaccaaactgatctactctctgcacggtctgtacggtgctaaa
 ggtatcaccaccaaatacaccatcaccagaaaacagaacccgctgatcaccaacatcgtggttaccacatcgaa
 gagttcctgaccttcggtggtaccgacctgaacatcatcacctctgctcagtctaacgacatctacaccaacctg
 ctggctgactacaaaaaaatcgcttctaaactgtctaaagtccaggttcttaaccgctgctgaaccggtacaaa
 15 gacgttttcgaagctaaatacggctctggacaaagacgcttctggtatctactctgttaacatcaaaaattcaac
 gacatcttcaaaaaactgtactctttcaccgagttcgacctggcgaccaaattccaggttaaatgccgtcagacc
 tacatcggtcagtaaaaatacttcaaactgtctaacctgctgaacgactctatctacaacatctctgaagggttac
 aacatcaacaacctgaaagttaacttcggtggtcagaacgctaacctgaaccgctgatcatcaccgcatcacc
 ggtcgtggtctggttaaaaaaatcatccgtttctgcAAGAAATATTGTAAGCGTTAAAGGAATAAGAAAAAGTATC
 20 tgcacgaaatcaacaacgggtgaactgttcttcggtgcttctgaaaaactcttacaacgacgacaacatcaacacc
 ccgaaagaaatcgacgacaccggttacctctaacaacaactacgaaaaacgacctggaccaggttatcctgaacttc
 aactctgaatctgctccgggtctgtctgacgaaaaactgaacctgaccatccagaacgacgcttacatcccga
 tacgactctaacgggtacctctgacatcgaaacgacgacggttaacgaactgaacggtttcttctacctggacgct
 cagaaagtccggaaggtgaaaacaacggttaacctgaccttctctatcgacaccgctctgctggaacagccgaaa
 25 atctacaccttcttctctctgagttcatcaacaacggttaacaaaccggttcaggctgctctgttcggttcttctg
 attcagagggttctggttgacttcaccacgaagctgaaccagaatctaccgttgacaaaactcgtgacatctct
 atcgttggttcgctacggtctggtctggaacatcggttaacgaagctcagaaaggttaacttcaaacgctctg
 gaactgctgggtgctggtatcctgctggaggttcgaaccggaactgctgatcccgaccatcctggttttaccatc
 aaatcttctcgtggttcttctgacaacaaaaacaaagttaataaagctatcaacaacgctctgaaagaacgtgac
 30 gaaaaatggaaagaagtttactctttcatcggttctaaactggatgaccaaatacaaccccagttcaacaaacgt
 aaagaacagatgtaccaggctctccagaaccaggttaacgctatcaaaaccatcatcgaatctaaatacaactct
 tacacctggaagaaaaaaacgaactgaccaacaaatacgacatcaaacagatcgaaaacgaactgaaccagaaa
 gtttctatcgctatgaacaacatcgaccttctgacctgaatcttctatcttctacctgatgaaactcatcaac
 gaagttaaaatcaacaacactgctgaatacgaacgaaaacggttaaaacctacctgctgaactacatcatccagcac
 35 ggttctatcctgggtgaatctcagcaggaactgaactctatggttacggacacctgaacaactctatcccggttc
 aaactgtcttcttacaccgacgacaaaatcctGATCTCTTACTTCAACAAATTTCTTTAAACgcATTAAAGAGTTCA
 TCGGTTctgaatATGCGGTACAAAAATGATAAAatGTGATACTTCTGGATATgatAGCAATATCAACATTAAC
 GGCGACGTGTATAAATATccgACAAATAAAAAACAGTTTGGGATATATAACGACAAGctgTCGGAGGTCAATatt
 TCTCAAAACGACtatATCattTACGATAATaaaTATAAAAACTTTAGCATTAGTtttGGGTTcgtATACCTAAT
 40 tatGACAATAaaattGTAAATGTGAATAACGAGTATACCATTTAAACTGTATGcgcGACAATAACAGTGGTTGG
 AAGGTATCGctgAACATAATGAGATTATCTGGACCctgcagGATAATGcaGGTATAAACGACAAACCTGGCTTTT
 AACTATGGAAACGCAAAATGGGATCTCAGATTACATTaataaaTGatttttGTTaccATTACGACAGTATcgcTTA
 GGCGACTCAAAACTTTATATTAATggcAAATctgATAGATCAGAAATCAATCTTAAATTTGGGCAATATTCTATGTC
 TCTgatAACATCTTGTTCAGATCGTTAATTGCAGTTACACTcgtTATATTGGCATTCTGTACTTTAATATCTTC
 45 gataaaGAActgGACGAGACGGAAATCagACTCTGTATTCAAAACGAGCCCAATACTAATATATTGAAAGATTTT
 TGGGGTAACTATCTTTTATATGATAAAGAATACTATCTCCTGaatGTATTGAAGCCAAACAATTTTCATAGATAGA
 CGCAAGGATAGCACATTAAGTATCAACAATATCAGATCTACTATActgttaGCAAATCGCCTcTACTCCggtATT
 AAAGTGAAGATTcagCGGGTAAATAACTCCAGTACCAATGATAATCTGGTCCGTAAGAACGATCAGGTATACATC
 aatTTCGTGCGAGCAAAACTcatCTCTTCCCGCTTTACGCCgatACAGCTACGACAAACAAGGAAAAAACCAT
 50 AAAATTTCCAGCTCCGGAAACAGATTCAATCAAGTAGTTGTAATGAACTCTGTGGGTaatAATTGTACGATGAAC
 TTTAagAATAACAATGGGAACAATattGGACTTTTGGGCTTcAAAGCCGACACAGTGGTGGCGTCCACCTGGTAT
 TACACGcacATGcggGACCATACGAATTCGAACGGTTGCTTCTGGAACTTTATCTCGGAAGaaCACGGGTGGCAA
 GAAAAA

SEQ ID NO: 14 - Polypeptide Sequence of rBoNT/E(0)

55 MPKINSFNYNDPVNDRITILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDFHPPTSLKNGDSSYYDPNYLQ
 SDEEKDRFLKIVTKIFNRRNNLSGGILLEELSKANPYLGNDNTPDNQFHHGDASAVEIKFSNGSQDILLPNVII
 MGAEPDLFETNSSNISLRNNYMPSNHGFGSIAIVTFSPEYSFRFNDNSMNEFIQDPALTLMHQLIYSLHGLYGAK
 GITTKYTITQKQNPLITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNLLADYKKIASKLSKVQVSNPLLNPNYK
 60 DVFEAKYGLDKDASGIYSVNINKFNDFIKKLYSFTEFDLATKFQVKCRQTYIGQYKYFKLSNLNLSIYNISEGY

NINNLKVNFRGQANLNPRITPITGRGLVKKIIRFCNKIVSVKGIRKSICIEINNNGELFFVASENSYNDDNINT
 PKEIDDTVTSNNNYENDLDQVILNFNSEAPGLSDEKLNLTIQNDAYIPKYDSNGTSDIEQHDVNELNVFFYLDA
 QKVPEGENNVNLTSSIDTALLEQPKIYTFSSSEFINNVNKPVQAALFVSWIQQVLVDFTEANQKSTVDKIADIS
 IVVPYIGLALNIGNEAQKGNFKDALELLGAGILLEFEPELLIPTILVFTIKSFLGSSDNKNKVIKAINNALKERD
 5 EKWKEVYSFIVSNWMTKINTQFNKRKEQMYQALQNVNAIKTIIESKYNSYTLEEKNELTNKYDIKQIENELNQK
 VSIAMNNIDRFLTESSISYLMKLINEVKINKLREYDENVKTYLLNYIIQHGSILGESQOELNSMVTDTLNNSSIPF
 KLSSYTDDKILISYFNKFFKRIKSSSVLNMRYKNDKYVDTSGYDSNININGDVYKYPTNKNQFGIYNDKLSEVNI
 SQNDYIIYDNKYKNFSISFWVRIPNYDNKIVNVNNEYTIINCMRDNNSGWKVS LNHNIEI IWTLQDNAGINQKLAF
 10 NYGNANGISDYINKWIFVTITNDRLGDSKLYINGNLIDQKSILNLGNIHVSNDILFKIVNCSYTRYIGIRYFNIF
 DKELDETEIQTLYSNPNTNLIKDFWGNLYLLYDKEYYLLNLVLPNNFIDRRKSTLSINNIRSTILLANRLYSIGI
 KVKIQRVNNSSNDNLVRKNDQVYINFVASKTHLFPLYADTATTNKEKTIKISSSGNRFNQVVMNSVGNCTMN
 FKNNGNNGNIGLLGFKADTVVASTWYYTHMRDHTNSNGCFWNFI SEEHGWQEK

SEQ ID NO: 15 - Nucleotide Sequence of rBoNT/F(0)

15 ATGCCGGTGGTCATCAACAGCTTCAACTACAACGACCCAGTAAACGACGACGATCCTGTATATGCAAATCCCCG
 TATGAAGAGAAGAGCAAGAAGTACTATAAGGCCTTTGAAATCATGCGCAATGTGTGGATTATTCCGGAGCGTAAT
 ACGATTGGTACTGACCCAAGCGACTTCGATCCACCTGCGTCTTTGGAAAACGGCTCGTCCGCATATTACGACCCG
 AATTACCTGACCACCGATGCGGAGAAAAGATCGTTATTTGAAAACCAACCATCAAGCTGTTCAAACGCATTAACAGC
 AATCCGGCAGGTGAGGTCTGTGCTGCAAGAGATTAGCTACGCAAAAGCCTTATCTGGGTAATGAGCATAACGCTATT
 20 AACGAGTTTACCCGGTTACCCGCACTACCAGCGTTAACATCAAGTCTCGACCAACGTGAAGTCTAGCATTATC
 CTGAACCTGCTGGTTCTGGGTGCCGGTCCGGACATCTTCGAAAACCTAGCTACCCGGTGGTAACTGATGGAT
 AGCGGCGGTGTTTATGACCCGAGCAATGACGGTTTTGGCAGCATCAATATCGTGACGTTTAGCCCGGAGTACGAG
 TACACCTTCAATGATATCAGCGGTGGTTACAATTCTTCTACCGAGAGCTTCATCGCCGACCCGGCGATCAGCCTG
 GCACACCAACTGATCTATGCATTGCATGGCTTGTACGGTGGCCGTGGTGTGACGTATAAAGAGACTATCAAGGTT
 25 AAGCAGGCACCTCTGATGATTGCGGAAAAGCCGATTGCGCTGGAAGAGTTCTTGACCTTCGGCGGTCAAGATTTG
 AACATCATTACCTCGGCCATGAAAGAGAAAAATCTATAACAATTTGCTGGCCAACTATGAAAAGATTGCAACGCGC
 TTGTCTCGTGTTAACTCCGCTCCGCCGGAATACGACATTAATGAGTACAAAAGACTACTTTCAATGGAATATGGC
 CTGGACAAAAATGCGGATGGTTCTTATACCGTGAATGAAAACAAAATCAATGAAATCTACAAGAACTGTACAGC
 TTCACCGAAATCGATCTGGCGAACAAGTTCAAAGTCAAATGTCGTAATACCTACTTCATCAAATATGGCTTCCTG
 30 AAAGTCCCGAACCTGCTGGACGATGACATCTATACCGTCAGCGAAGGCTTCAACATCGGCAATCTGGCCGTGAAT
 AATCGTGGTTCAGAACATCAAATGAATCCGAAAATCATTGACTCCATCCAGACAAGGGCCTGGTTGAGAAAAATC
 GTGAAGTTCTGCAAAAGCGTTATTCCGCGTAAAGGTACGAAAGCACCAGCTCGCCTGTCGCTTCGCTTAAACAAC
 CGTAGGTTGTTCTTTGTGGCATCTGAAAGCAGCTACAACGAGAACGACATCAACACCCCTAAAGAAATTGATGAT
 ACCACGAACCTGAATAACAATTATCGCAACAATCTGGACGAGGTGATCCTGGATTACAATTTCGAAAACCAATCCG
 35 CAAATTAGCAATCAGACGCTGAACACCCCTGGTTACAGGACGATAGCTACGTTCCGCGTTACGACTCCAATGGTACT
 AGCGAGATTGAAGAACACAACGTAAGTGGACTTGAACGTTTTCTTTTATCTGCACGCCAGAAAGTTCCGGAGGGC
 GAAACCAATATTAGCCTGACCAGCTCGATCGACACCGCGCTGTCTGAGGAGAGCCAAGTCTACACCTTTTTTCAGC
 AGCGAGTTTATCAACACTATTAACAAGCCAGTTCATGCTGCATTGTTTATCTCTTGGATTAAACCAGGTGATTCGC
 GACTTTACGACGGAGGCGACCCAGAAGTCTACCTTCGACAAAATTCAGACATCTCCCTGGTCTGCCATACGTC
 40 GGCCTGGCGTTGAATATTGGCAATGAAGTTCAAAAAGAGAACTTCAAAGAAGCGTTCGAGCTGCTGGGTGCAGGC
 ATCCTGCTGGAGTTCTGTCGGGAAGTGTGATCCCGACCATCTGGTGTTCACCATTAAAGAGCTTCATTGGATCC
 TCCGAGAATAAGAACAAGATCATCAAGGCGATCAATAACAGCCTGATGGAGCGTGAAACGAAGTGGAAAGAAATC
 TATAGCTGGATTGTTAGCAATTGGCTGACTCGTATTAACACGCAATTCACAAGCGTAAAGAGCAAATGTACCAA
 GCCCTGCAAAACCAAGTTGACGCCATCAAAAACGGTAATTGAATACAAGTACAACAATTACACGAGCGATGAGCGC
 45 AACCGCCTGGAAAGCGAATACAACATCAACAACATTCGCGAAGAAATGAACAAGAAAGTGAAGCTGGCGATGGAG
 AACATTGAGCGTTTTATCACCGAAAGCAGCATCTTTTACCTGATGAAATTGATTAATGAGGCGAAAGTCTCGAAA
 CTGCGTGAGTACGACGAAGGTGTGAAAGAGTATCTGCTGGATTACATTAGCGAGCACCGTAGCATCTTGGGTAAC
 TCGGTTCAGGAGCTGAACGATCTGGTGACCTCTACCCTGAACAATAGCATCCCGTTTCGAACCTGAGCAGCTATACC
 AATGACAAGATTCTGATTCTGTATTCAATAAACTGTATAAGAAGATCAAGGATAACAGCATTCTGGATATGCGT
 50 TACGAAAACAATAAGTTTATCGACATTTCTGTTACGGCAGCAACATTTCCATCAATGGCGATGTCTACATCTAC
 AGCACCAATCGCAACCAGTTCCGGCATCTACTCTAGCAAAACCGAGCGAAGTTAACATCGCACAGAACAATGATATT
 ATTTATAACGGTCTGTTATCAAAAACCTCTCTATCAGCTTTTGGGTCCGTATCCCGAAGTACTTCAATAAAGTCAAT
 CTGAATAATGAATACACGATCATCGACTGCATTCGCAATAACAACAGCGGTTGGAAAATCAGCCTGAATTACAAC
 AAAATTATTTGGACCCTGCAAGATACGGCGGGTAACAATCAGAAACTGGTGTTTAACTACACGCAAATGATCAGC
 55 ATTTCTGACTATATCAACAAGTGATCTTTGTTACCATCACCAATAATCGTCTGGGCAATAGCCGATTTTACATC
 AACGGTAACCTGATTGATGAGAAAAGCATCAGCAACCTGGGCGATATTACGTCAGCGACAACATTTCTGTTCAAA
 ATTGTTGGTTGTAACGATACCCGTTACGTCGGCATCCGTTATTTCAAGGTTTTTCGATACGGAGCTGGGTAAAACG
 GAAATCGAAACGTTGTACTCCGATGAACCAGATCCGAGCATTTCTGAAGGACTTTTGGGGTAACCTACTTGCTGTAC
 AATAAACGTTACTATCTGCTGAATCTGTTGCGCACCAGACAAGAGCATTACCCAAAACAGCAATTTCTGAACATT

AATCAGCAACGCGGCGTATACCAAAAAACCGAACATCTTCAGCAATACGCGCCTGTATACTGGTGTGGAAGTGATC
 ATTCGTAAGAACGGTAGCACCGACATTAGCAACACGGACAATTTTCGTCCGTAAGAATGACCTGGCGTACATTAAC
 GTCGTGGACCGTGATGTCGAGTATCGTCTGTACGAGACATCAGCATTCGCGAAACCGGAAAAGATTATCAAGCTG
 ATCCGTACCAGCAACAGCAACAACAGCCTGGGTCAGATCATTGTGATGGACAGCATTGGTAATAACTGCACGATG
 5 AACTTCCAGAACAACAATGGTGGTAATATCGGTCTGCTGGGTTTTTCACAGCAATAATCTGGTTGCTTCCAGCTGG
 TACTACAATAACATTCTGTAATAAACAGCTCTAGCAATGGTTGTTTTTGGAGCTTTATCAGCAAAGAGCACGGCTGG
 CAAGAAAAAT

SEQ ID NO: 16 - Polypeptide Sequence of rBoNT/F(0)

MPVVINSFNNDPVNDDTILYMQIPYEEKSKKYYKAFEIMRNVWIIPERNITIGTDPSPDFDPPASLENGSSAYYDP
 NYLTDDAEKDRYLKTTIKLFKRINSNPAGEVLLQEI SYAKPYLGNEHTPINEFHPVTRTTSVNIKSSTNVKSSII
 LNLVLGAGPDIFENS SYPVRKLMDSGGVYDPSNDGFGSINIVTFSPEYETFNDISGGYNSSTESFIADPAISL
 AHQLIYALHGLYGARGVYKETIKVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLANYEKIATR
 LSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTEIDLANKFKVKCRNTYFIKYGFL
 15 KVPNLLDDDIYTVSEGFNIGNLAVNNRQNIKLNPKIIDSIPDKGLVEKIVKFCKSVIPRKGTKAPPRLCIRVNN
 RELFFVASESSYNENDINTPKEIDDTNLNNYRNRLDEVILDYNSETIPQISNQTLLNTLVQDDSYVPRYDSNGT
 SEIEEHNVDLNVFFYLHAQKVPEGETNISLTSSIDTALSEESQVYTFFSSEFINTINKPVHAALFISWINQVIR
 DFTTEATQKSTFDKIADISLVVPYVGLALNIGNEVQKENFKEAFELLGAGILLEFVPELLIPTILVFTIKSFIGS
 SENKNKIIKAINNSLMERETKWKIYSWIVSNWLTRINTQFNKRKEQMYQALQNQVDAIKTVIEYKYNNYTSDER
 20 NRLESEYNINNIREELNKKVSLAMENIERFITESSIFYLMKLINEAKVSKLREYDEGVKEYLLDYISEHRSILGN
 SVQELNDLVSTLNN SIPFELSSYTNDKILILYFNKLYKKIKDNSILDMRYENNKFIDISGYGSNISINGDVYIY
 STNRNQDGIYSSKPSSEVNIAQNNDIYNGRYQNF SIFWVRIPKYFNKVNLLNNEYTIIDCIRNNNSGWKISLNYN
 KIIWTLQDTAGNNQKLVFNFTQMISISDYINKWIFVTITNNRLGNSRIYINGNLIDEKSI SNLGD IHVSDNILFK
 IVGCNDTRYVVGIRYFKVFDTELKGTETIETLYSDEPDPSILKDFWGNLYLLYNKRYLLNLLRDTKSITQNSNFLNI
 25 NQQRGVYQKPNIFSNTRYTGVEVIRKNGSTDISNTDNFVRKNDLAYINVVDRDVEYRLYADISIAPKEIKL
 IRTSNSNNSLGQIIVMDSIGNNCTMNFQNNNGNIGLLGFHSNNLVASSWYNNIRKNTSSNGCFWSFISKEHGW
 QEN

SEQ ID NO: 17 - Nucleotide Sequence of rBoNT/A(0) (His-tagged)

ATGCCGTTTGTGAACAAGCAGTTCAACTATAAAGATCCGGTTAATGGTGTGGATATCGCCTATATCAAAATTCGG
 AATGCAGGTGAGATGCAGCCGGTTAAAGCCTTTAAATCCATAACAAAATTTGGGTGATTCGGGAACGTGATACC
 TTTACCAATCCGGAAGAAGGTGATCTGAATCCGCCTCCGGAAGCAAAACAGGTTCGGGTAGCTATTATGATAGC
 ACCTATCTGAGCACCGATAACGAGAAAGATAACTATCTGAAAGGTGTGACCAAACCTGTTTGAACGCATTTATAGT
 ACCGATCTGGGTGCTATGCTGCTGACCAGCATTGTTTCGTGGTATTCCGTTTTGGGGTGGTAGCACCATTGATACC
 35 GAAGTGAAGTTATTGACACCAACTGCATTAATGTGATTACGCCGATGGTAGCTATCGTAGCGAAGAAGTGAAT
 CTGGTTATTATTGGTCCGAGCGCAGATATCATTAGTTTGAATGTAAAGCTTTGGCCACGAAGTCTGAATCTG
 ACCCGTAATGGTTATGGTAGTACCCAGTATATTCGTTTCAGTCCGGATTTTACCTTTGGCTTTGAAGAAAGCCTG
 GAAGTTGATACAAATCCGCTGTTAGGTGCAGGTAATTTGCAACCGATCCGGCAGTTACCTGGCACACCAGCTG
 ATTTATGCCGGTCATCGTCTGTATGGTATTGCCATTAATCCGAATCGTGTGTTCAAAGTGAATACCAACGCCTAT
 40 TATGAAATGAGCGGTCTGGAAGTGAGTTTTGAAGAACTGCGTACCTTTGGTGGTCATGATGCCAAATTTATCGAT
 AGCCTGCAAGAAAATGAATTCGCCTGTACTACTATAACAAATTCAGGATATTGCGAGCACCTGAATAAAGCC
 AAAAGCATTGTTGGCACCACCGCAAGCCTGCAGTATATGAAAAATGTGTTTAAAGAAAAATATCTGCTGAGCGAA
 GATACCAAGCGGTAAATTTAGCGTTGACAACTGAAATTCGATAAACTGTACAAGATGCTGACCCGAGATTTATACC
 GAAGATAACTTCGTGAAGTTTTTCAAAGTGCTGAACCGCAAAACCTACCTGAACTTTGATAAAGCAGTGTTCAAA
 45 ATCAACATCGTGCCGAAAGTGAAGTATACCATCTATGATGGTTTTAACCTGCGCAATACCAATCTGGCAGCAAAC
 TTTAATGGTCAGAACACCGAAATCAACAACATGAACTTTACCAAACTGAAGAACTTCACCGGTCTGTTTCAATTT
 TACAAACTGCTGTGTGTTTCGTGGCATTATTACCAGCAAAACCAAAAGTCTGGATAAAGGCTACAATAAAGCCCTG
 AATGATCTGTGCATTAAGGTGAATAATTGGGACCTGTTTTTTAGCCCCGAGCGAGGATAATTTACCAACGATCTG
 AACAAAGGCGAAGAAATTACCAGCGATAACCAATATTGAAGCAGCCGAAGAAAACATTAGCCTGGATCTGATTCAG
 50 CAGTATTATCTGACCTTCAACTTCGATAATGAGCCGAAAAATATCAGCATTGAAAACCTGAGCAGCGATATTATT
 GGCCAGCTGGAAGTATGCCGAATATTGAACGTTTTCCGAACGGCAAAAAATACGAGCTGGATAAATACACCATG
 TTCCATTATCTGCGTGCCCAAGAATTTGAACATGGTAAAAGCCGTATTGCACTGACCAATAGCGTTAATGAAGCA
 CTGCTGAACCCGAGCCGTGTTTATACCTTTTTTAGCAGCGATTACGTGAAAAAGGTTAACAAAGCAACCGAAGCA
 GCCATGTTTTTAGGTTGGGTTGAACAGCTGGTTTATGATTTACCCGATGAAACCAGCGAAGTTAGCACCACCGAT
 55 AAAATTGCAGATATTACCATCATCATCCCGTATATCGGTCCGGCACTGAATATTGGCAATATGCTGTATAAAGAC
 GATTTTGTGGGTGCCCTGATTTTTAGCGGTGCAGTTATTCTGCTGGAATTTATTCGGGAAATTGCCATTCCGGTT
 CTGGGCACCTTTGCACTGGTGAGCTATATTGCAATAAAGTTCTGACCGTGCAAGCATCGATAATGCACTGAGC
 AAACGTAACGAAAAATGGGATGAAGTGACAAGTATATCGTGACCAATTGGCTGGCAAAAGTTAACACCCAGATT
 GACCTGATTTCGAAGAAGATGAAAGAAGCACTGGAAAAATCAGGCAGAAGCAACCAAGCCATTATCAACTATCAG
 60 TATAACAGTACACCGAAGAAGAGAAAAATAACATCAACTTCAACATCGACGATCTGTCCAGCAAACCTGAACGAA

AGCATCAACAAAGCCATGATTAACATTAACAAATTTCTGAACCAGTGCAGCGTGAGCTATCTGATGAATAGCATG
 ATTCGGTATGGTGTGAAACGTCTGGAAGATTTTGTATGCAAGCCTGAAAGATGCCCTGCTGAAATATATCTATGAT
 AATCGTGGCACCCTGATTGGTCAGGTTGATCGTCTGAAAGATAAAGTGAACAACACCCTGAGTACCGATATTCCT
 TTTTCAAGTGAAGCAAAATATGTGGATAATCAGCGTCTGCTGTCAACCTTTACCGAATACATTAAGAATCATCAAC
 5 ACCAGCATTCTGAACCTGCGTTATGAAAGCAATCATCTGATTGATCTGAGCCGTTATGCCAGCAAAATCAATATA
 GGCAGCAAGGTTAACTTCGACCCGATTGACAAAAATCAGATACAGCTGTTTAATCTGGAAAGCAGCAAAATTGAG
 GTGATCCTGAAAAACGCCATTGTGTATAATAGCATGTACGAGAATTTCTCGACCAGCTTTTGGATTCTGATCCCCG
 AAATACTTTAATAGCATCAGCCTGAACAACGAGTACACCATTATTAATCTGCATGGAAAAACAATAGCGGCTGGAAA
 GTTAGCCTGAATTATGGCGAAATTATCTGGACCCTGCAGGATACCCAAGAAATCAAACAGCGTGTGGTTTTCAAA
 10 TACAGCCAGATGATTAATATCAGCGACTATATCAACCGCTGGATTTTTGTGACCATTACCAATAATCGCCTGAAT
 AACAGCAAGATCTATATTAACGGTCGTCTGATTGACCAGAAACCGATTAGTAATCTGGGTAATATTTCATGCGAGC
 AACAACATCATGTTTAACTGGATGGTTGTCGTGATACCCATCGTTATATTTGGATCAAGTACTTCAACCTGTTT
 GATAAAGAGTTGAACGAAAAAGAAATTAAGACCTGTATGATAACAGAGCAACAGCGGTATTCTGAAGGATTTT
 TGGGGAGATTATCTGCAGTATGACAAACCGTATTATATGCTGAATCTGTACGACCCGAATAAATACGTGGATGTG
 15 AATAATGTTGGCATCCGTGGTTATATGTACCTGAAAGGTCCGCGTGGTAGCGTTATGACCACAAACATTTATCTG
 AATAGCAGCCTGTATCGCGGAACCAAAATTCATCATTAAGAAATGATGCCAGCGGCAACAAGGATAATATTGTGCGT
 AATAATGATCGCGTGTACATTAACGTTGTGGTGAAGAATAAAGAATATCGCCTGGCAACCAATGCAAGCCAGGCA
 GCGCTTGAAAAAATTTCTGAGTGCCCTGGAAATTCGGATGTTGGTAATCTGAGCCAGGTTGTTGTGATGAAAAGC
 AAAAAATCAGGGCATCACCAACAAGTGCAAAATGAATCTGCAGGACAATAACGGCAACGATATTGGTTTTATT
 20 GGCTTCCACCAGTTCAACAATATTGCGAAATCGTTGCAAGCAATTGGTATAATCGTCAGATTGAACGTAGCAGT
 CGTACCCTGGGTTGTAGCTGGGAATTTATCCCTGTGGATGATGGTTGGGGTGAACGTCCGCTGGAAAACCTGTAT
 TTTCAAGGTGCAAGTCATCATCACCATCACCACCATCATTA

SEQ ID NO: 18 - Polypeptide Sequence of rBoNT/A(0) (His-tagged)

MPFVNKQFNYPKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWIWIPERDFTNPEEGDLNPPPEAKQVPVSYSDS
 TYLSTDNEKDNLYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGYSRSEELN
 LVIIGPSADIIQFECKSFSGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDNPLLGAAGKATDPAVTLAHQL
 IYAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYNKFKDIASTLNKA
 KSIVGTTASLQYMKNVFKKEYLLSEDTSGKFSVDKLFKDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDAVFK
 30 INIVPKVNYTYIDGFNLRLNTNLAANFNQNTNINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKAL
 NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISLENLSSDII
 GQLELMPNIERFPNGKKYELDKYTMFHYLRAQEFEGHKSRIALTNSVNEALLNPSRVYTFSSDYVKKVKNKATEA
 AMFLGWVEQLVYDFDTDETSEVSTTDKIADITIIPIYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
 LGTFALVSYIANKVLTVQIDNALSKRNEKWDEVYKIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQ
 35 YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSMIPYGVKRLDFDASLKDALLKYIYD
 NRGTLIGQVDRLLKDKVNNLTSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNLRYESNHLIDLRYASKINI
 GSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVNSMYENFSTSFWRIPKYFNSISLNNEYTIINCMENNSGWK
 VSLNYGEIITWLTQDTQEIQRVVFYKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPIISNLGNIHAS
 NNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLYDNQNSGILKDFWGDYLYQDKPYMLNLYDPNKYVDV
 40 NNVGIRGYMYLKGPRGSMVTNNIYLNSSLYRGTKFIKKYASGNKDNIVRNNDRVYINVVKNKKEYRLATNASQA
 GVEKILSALEIPDVGNLQVVMKSKNDQGITNCKCMNLQDNNNGNDIGFIGFHQFNNAKLVASNWNRYNRQIERSS
 RTLGCSEWFIIPVDDGWGERPLENLYFQGASHHHHHHHH

SEQ ID NO: 19 - Nucleotide Sequence of rLH_N/A (His-tagged)

ATGCCGTTTGTGAACAAGCAGTTCAACTATAAAGATCCGGTTAATGGTGTGGATATCGCCTATATCAAAATTCGG
 AATGCAGGTGAGATGCAGCCGGTTAAAGCCTTTAAAAATCCATAACAAAAATTTGGGTGATTCGGGAACGTGATACC
 TTTACCAATCCGGAAGAAGGTGATCTGAATCCGCTCCGGAAGCAAAACAGGTTCCGGTTAGCTATTATGATAGC
 ACCTATCTGAGCACCAGATAACGAGAAAGATAACTATCTGAAAGGTGTGACCAAACCTGTTTGAACGCATTTATAGT
 ACCGATCTGGGTGCTATGCTGCTGACCAGCATTGTTCTGTTTCCGTTTTGGGGTGGTAGCACCATTGATACC
 50 GAACTGAAAGTTATTGACACCAACTGCATTAATGTGATTGAGCCGATGGTAGCTATCGTAGCGAAGAACTGAAT
 CTGGTTATTATTGGTCCGAGCGCAGATATCATTCAGTTTGAATGTAAATCCTTTGGCCACGAAGTTCTGAATCTG
 ACCCGTAATGGTTATGGTAGTACCCAGTATATTCGTTTCAGTCCGGATTTTACCTTTGGCTTTGAAGAAAGCCTG
 GAAGTTGATACAAATCCGCTGTTAGGTGCAGGTAATTTGCAACCGATCCGGCAGTTACCTGGCACATGAACCTG
 ATTCATGCCGGTCATCGTCTGTATGGTATTGCAATTAATCCGAACCGTGTGTTCAAAGTGAATACCAACGCATAT
 55 AGCCTGCAAGAAAATGAATTCGCTGTACTACTATAACAAATTCAGGATATTGCGAGCACCCTGAATAAAGCC
 AAAAGCATTGTTGGCACCACCGCAAGCCTGCAGTATATGAAAAATGTGTTTAAAGAAAAATATCTGCTGAGCGAA
 GATACCAGCGGTAATTTAGCGTTGACAAACTGAAATTCGATAAACTGTACAAGATGCTGACCAGAGATTTATACC
 GAAGATAAATTCGTGAAGTTTTTCAAAGTGCTGAACCGCAAAACCTACCTGAACCTTTGATAAAGCGGTGTTCAAA
 60 ATCAACATCGTGCCGAAAGTGAACATATACCATCTATGATGGTTTTAACCTGCGCAATACCAATCTGGCAGCAAAC

TTTAATGGTCAGAACACCGAAATCAACAACATGAACCTTTACCAAACCTGAAGAACTTCACCGGTCTGTTTCAATTT
TACAAACTGCTGTGTGTTTCGTGGCATTATTACCAGCAAAACCAAAAGTCTGGATAAAGGCTACAATAAAGCCCTG
AATGATCTGTGCATTAAAGGTGAATAATTGGGACCTGTTTTTTAGCCCGAGCGAGGATAATTTACCAACGATCTG
AACAAAGGCGAAGAAATTACCAGCGATACCAATATTGAAGCAGCCGAAGAAAACATTAGCCTGGATCTGATTCAG
5 CAGTATTATCTGACCTTCAACTTCGATAATGAGCCGGAATAATCAGCATTGAAAACCTGAGCAGCGATATTATT
GGCCAGCTGGAACCTGATGCCGAATATTGAACGTTTTCCGAACGGCAAAAAATACGAGCTGGATAAATACACCATG
TTCCATTATCTGCGTGCCCAAGAATTTGAACATGGTAAAAAGCCGTATTGCACTGACCAATAGCGTTAATGAAGCA
CTGCTGAACCCGAGCCGTGTTTATACCTTTTTTAGCAGCGATTACGTAAAAAGGTTAACAAAGCAACCGAAGCA
GCCATGTTTTTAGGTTGGGTTGAACAGCTGGTTTATGATTTACCGATGAAACCAGCGAAGTTAGCACCACCGAT
10 AAAATTGCAGATATTACCATCATCATCCCGTATATCGGTCCGGCACTGAATATTGGCAATATGCTGTATAAAGAC
GATTTTGTGGGTGCCCTGATTTTTAGCGGTGCAGTTATTCTGCTGGAATTTATTCGGGAAATTGCCATTCCGGTT
CTGGGCACCTTTGCACTGGTGAGCTATATTGCAAAATAAGTTCTGACCGTGCAGACCATCGATAATGCACTGAGC
AAACGTAACGAAAAATGGGATGAAGTGTAAGTATATCGTGACCAATTGGCTGGCAAAAGTTAACACCCAGATT
GACCTGATTGCAAGAAGATGAAAGAAGCACTGGAAAAATCAGGCAGAAAGCAACCAAGCCATTATCAACTATCAG
15 TATAACAGTACACCGAAGAAGAGAAAAATAACATCAACTTCAACATCGACGATCTGTCCAGCAAACCTGAACGAA
AGCATCAACAAAGCCATGATTAACATTAAACAAATTTCTGAACCAAGTGCAGCGTGAGCTATCTGATGAATAGCATG
ATTCGGTATGGTGTGAAACGTCTGGAAGATTTTGATGCAAGCCTGAAAGATGCCCTGCTGAAATATATCTATGAT
AATCGTGGCACCCTGATTGGTCAGGTTGATCGTCTGAAAGATAAAGTGAACAACACCCTGAGTACCGATATTCCT
TTTCAGCTGAGCAAATATGTGGATAATCAGCGTCTGCTGTCAACCGAAAAATCTGTATTTCCAGGGTGAAGTCAT
20 CATCACCATCACCACCATCATTAA

SEQ ID NO: 20 - Polypeptide Sequence of rLH_M/A (His-tagged)

MPFVNKQFNKYKDPVNGVDIAYIKIPNAGQMOPVKAFFKIHNKIWVIPERDTFTNPPEGDLNPPPEAKQVPVSYIDS
TYLSTDNEKDNLYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELN
25 LVIIGPSADIIQFECKSFSGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAAGKFATDPAVTLAHEL
IHAGHRLYGLAIPNPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYNKFKDIASTLNKA
KSIVGTTASLQYMKNVFEKEYLLSEDTSGKFSVDKLFKDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDAVFK
INIVPKVNYTIYDGFNLNRLNLAANFNQNTNINNMNFTKLKNTGLFEFYKLLCVRGIITSKTSKLDKGYNKAL
NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIEENLSSDII
30 GQLELMPNIEFRFPNGKKYELDKYTMFHYLRAQEFEGHKSRIALTSVNEALLNPSRVYTFSSDYVKKVKNKATEA
AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIPIYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
LGTFAVLSYIANKVLTVQTDNALSKRNEKWDEVYKIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQ
YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYSMLNSMIPYGVKRLEDFFDASLKDALLKYIYD
NRGTLIGQVDRLLKDKVNNLTSTDIPFQLSKYVDNQRLSTENLYFQGASHHHHHHHH
35

SEQ ID NO: 21 - Nucleotide Sequence of rH_C/A (His-tagged)

ATGCATCATCACCATCACCACGAAAAATCTATACTTCCAAGGAAAAAACATCATCAATACTAGCATTCTGAACCTG
CGTTACGAGAGCAATCATCTGATTGATCTGAGCCGTTATGCAAGCAAGATCAACATCGGTAGCAAGTCAATTTT
GACCCGATCGATAAGAACCAGATCCAGCTGTTTAATCTGGAATCGAGCAAAATTGAGGTTATCCTGAAAAACGCC
40 ATTGTCTACAACCTCCATGTACGAGAATTTCTCCACCAGCTTCTGGATTTCGCATCCCGAAATCACTTCAACAGCATT
AGCCTGAACAACAGAGTATACATCATCACTGATGAGAGAACACAGCGGTTGGAAGGTCTCTCGAATGATGAGT
GAGATCATTGACCTTGCAGGACACCAAGAGATCAAGCAGCGCGCTGTTCAAGTACTCTCAAATGATCAAC
ATTTCCGATTACATTAATCGTTGGATCTTCGTGACCATTACGAATAACCGTCTGAATAACAGCAAGATTTACATC
AATGGTCGCTTGATCGATCAGAAACCGATTAGCAACCTGGGTAATATCCACGCAAGCAACAACATTATGTTCAAA
45 TTGGACGGTTGCCGCGATACCCATCGTTATATCTGGATCAAGTATTTCAACCTGTTTGATAAAGAACTGAATGAG
AAGGAGATCAAAGATTTGTATGACAACCAATCTAACAGCGGCATTTTGAAGGACTTCTGGGGCGATTATCTGCAA
TACGATAAGCCGTACTATATGCTGAACCTGTATGATCCGAACAAATATGTGGATGTCAATAATGTGGGTATTCGT
GGTTACATGTATTTGAAGGGTCCGCGTGGCAGCGTTATGACGACCAACATTTACCTGAACCTCTAGCCTGTACCGT
GGTACGAAATTCATCATTAAAGAAATATGCCAGCGGCAACAAAGATAACATTGTGCGTAATAACGATCGTGTCTAC
50 ATCAACGTGGTCGTGAAGAATAAAGAGTACCGTCTGGCGACCAACGCTTCGCAGGCGGGTGTGAGAAAATCTTG
AGCGCGTTGGAGATCCCTGATGTGCGTAATCTGAGCCAAGTCGTGGTTATGAAGAGCAAGAACGACCAGGGTATC
ACTAACAAGTGCAAGATGAACCTGCAAGACAACAATGGTAACGACATCGGCCTTTATTGGTTTTCCACCAGTTCAAC
AATATTGCTAAACTGGTAGCGAGCAATTGGTACAATCGTCAGATTGAGCGCAGCAGCCGTACTTTGGGCTGTAGC
TGGGAGTTTATCCCGGTCGATGATGGTTGGGGCGAACGTCCGCTGTAA
55

SEQ ID NO: 22 - Polypeptide Sequence of rH_C/A (His-tagged)

MHHHHHHENLYFQGKNIINTSILNRLYESNHLIDLSRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNA
IVYNSMYENFSTSFWRIPKYFNSISLNNYEYTIINCMENNSGWKVSILNYGEIIWTLQDTQEIKQRVVFKYSQMIN
ISDYINRWIFVTITNRLNNSKIYINGRLIDQKPIISNLGNIHASNNIMFKLDGCRDTHRYIWIKYFNLFDKELNE
60 KEIKDLYDNQNSGILKDFWGDYLDQYDKPYMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTTNIYLNSSLYR

GTKFIIKKYASGNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVMKSKNDQGI
TNKCKMNLQDNNGNDIGFIGFHQFNNAIKLVASNWYNRQIERSSRTLGCSEWEIFPVDDGWGERPL

5 SEQ ID NO: 23 - Nucleotide Sequence of rLC/A (His-tagged)

ATGCCGTTTGTGAACAAGCAGTTCAACTATAAAGATCCGGTTAATGGTGTGGATATCGCCTATATCAAAATTCCG
AATGCAGGTGAGATGCAGCCGGTTAAAGCCTTTAAATCCATAACAAAATTTGGGTGATTCCGGAACGTGATACC
TTTACCAATCCGGAAGAAGGTGATCTGAATCCGCCTCCGGAAGCAAAACAGGTTCCGGTTAGCTATTATGATAGC
ACCTATCTGAGCACCGATAACGAGAAAGATAACTATCTGAAAGGTGTGACCAAACCTGTTTGAACGCATTTATAGT
10 ACCGATCTGGGTGCTATGCTGCTGACCAGCATTGTTTCGTGGTATTCCGTTTTGGGGTGGTAGCACCATTGATACC
GAACTGAAAGTTATTGACACCAACTGCATTAATGTGATTCAGCCGGATGGTAGCTATCGTAGCGAAGAACTGAAT
CTGGTTATTATTGGTCCGAGCGCAGATATCATTGAGTTTGAATGTAAATCCTTTGGCCACGAAGTTCTGAATCTG
ACCCGTAATGGTTATGGTAGTACCCAGTATATTCGTTTCAGTCCGGATTTTACCTTTGGCTTTGAAGAAAGCCTG
GAAGTTGATACAAATCCGCTGTTAGGTGCAGGTAAATTTGCAACCGATCCGGCAGTTACCTGGCACATGAACCTG
15 ATTCATGCCGGTCATCGTCTGTATGGTATTGCAATTAATCCGAACCGTGTGTTCAAAGTGAATACCAACGCATAT
TATGAAATGAGCGGTCTGGAAGTGTCAATTTGAAGAACTGCGTACCTTTGGTGGTCATGATGCCAAATTTATCGAT
AGCCTGCAAGAAAATGAATTTTCGCTGTACTACTATAACAAATTCAGGATATTGCGAGCACCTGAATAAAGCC
AAAAGCATTGTTGGCACCACCGCAAGCCTGCAGTATATGAAAAATGTGTTTAAAGAAAAATATCTGCTGAGCGAA
GATACCAGCGGTAAATTTAGCGTTGACAACTGAAATTCGATAAACTGTACAAGATGCTGACCGAGATTTATACC
20 GAAGATAACTTCGTGAAGTTTTTCAAAGTGCTGAACCGCAAAACCTACCTGAACCTTTGATAAAGCCGTGTTCAA
ATCAACATCGTGCCGAAAGTGAACATATACCATCTATGATGGTTTTAACCTGCGCAATACCAATCTGGCAGCAAAC
TTTAATGGTCAGAACACCGAAATCAACAACATGAACCTTTACCAAACCTGAAGAACTTCACCGGTCTGTTTGAAGAG
AATCTGTATTTCCAGGGTGCAAGTCATCATCACCATCACCACCATCATTA

25 SEQ ID NO: 24 - Polypeptide Sequence of rLC/A (His-tagged)

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWIWIPERDFTNPEEGDLNPPPEAKQVPVSYDS
TYLSTDNEKDNLYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELN
LVIIGPSADIIQFECKSFHGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAAGKFATDPAVTLAHEL
IHAGHRLYGLIAPNPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYNKFKDIASTLNKA
30 KSIVGTTASLQYMKNVFEKEYLLSEDTSGKFSVDKLFKDKLYKMLTEIYTEDNFVKFKVLNRKTYLNFDAVFK
INIVPKVNYTIYDGFNLNRLNLAANFNGQNTNINNMNFTKLKNFTGLFEENLYFQGASHHHHHHHH

SEQ ID NO: 25 - Nucleotide Sequence of rBoNT/FA(0) (His-tagged)

ATGCCGTTTGTGATTAACAGCTTCAATTATGATGATCCGGTGAACGATAACACCATCATTTATATCCGTCCGCCT
35 TATTATGAAACCAGCAACACCTATTTCAAAGCCTTCCAGATTATGGATAACGTGTGGATTATTCCGGAACGTTAT
CGTCTGGGTATTGATCCGAGCCTGTTTAAATCCGCCTGTTAGCCTGAAAGCAGGTAGTGATGGTTATTTTATGATCCG
AATTATCTGAGCACCAACACCGAGAAAAACAAATACCTGCAGATTATGATCAAGCTGTTCAAACGCATTAATAGC
AAACCGGCAGGTGAGATTCTGCTGGAAGAAAATCAAAAATGCAATTCGGTATCTGGGCAACAGCTATACCCAAGAA
GAACAGTTTACCACCAATAATCGTACCGTGAGCTTTAATGTTAAACTGGCCAATGGTAATATCGTTCAGCAGATG
40 GCAATCTGATTATTTGGGGTCCGGGTCTGATCTGACCACAAAATAAAACCGGTGGTATCATCTATAGCCCGTAT
CAGAGCATGGAAGCAACCCCGTATAAAGATGGTTTTGGTAGCATTATGACCGTGGAATTTAGTCCGGAATATGCA
ACCGCCTTTAACGATATTTCAATTGCAAGCCATAGTCCGTGCTGTTTATCAAAGATCCGGCAGTATTCTGATG
CACCAGTGATTTATGTTCTGCAATGGTCTGTATGGCAGCTATACCGAATACAAAATACCCCGAATGTGGTT
CAGAGCTATATGAAAGTTACCAACCGATTACGAGCGAGAAATTTCTGACCTTTGGTGGTCTGATCGCAATATT
45 GTTCCGCAGAGCATTGAGAGCCAGCTGTATAACAAAGTTCTGAGCGATTATAAACGTATTGCCAGCCGTCTGAAT
AAAGTTAATACCGCAACCGCACTGATCAACATCGATGAATTCAAAAACCTGTACGAGTGGAAATACAGTTTGCC
AAAGATAGCAATGGTGTGTATAGCGTGATCTGAACAAAATTTGAGCAGCTGTACAAAAAATCTATAGCTTCACC
GAATTCACCTGGCCTATGAGTTTAAAATCAAAACCCGTCTGGGTTATCTGGCCGAAAATTTTGGTCCGTTTTAT
CTGCCGAATCTGCTGGATGATAGCATTATACCGAAGTGGATGGTTTTAACATTGGTGCAGTACGATTAACATAT
50 CAGGGTCAGAATATTGGCAGCGATATCAACAGCATCAAAAACTGCAAGGTCAGGGTGTGTTAGCCGTGTTGTT
CGTCTGTGTAGCAATAGCAATACCAAAAACAGCCTGTGCATTACCGTTAATAATCGCGACCTGTTTTTTATCGCA
AGCCAAGAAAGCTATGGCGAGAATACCATTAACACCTATAAAGAGATTGACGATACCACCACACTGGATCCGAGC
TTTGAAGATATTCTGGATAAAGTGATCCTGAACTTCAACGAACAGGTTATTCCGCAGATGCCGAATCGTAATGTT
AGCACCGATATTGAGAAAGACAACCTACATCCCGAAATACGATTATAACCGCACCGACATTATCGATAGCTATGAA
55 GTTGGTGCAGAACTACAACACCTTTTTCTATCTGAATGCCAGAAAATTTAGCCCGAACGAAAGCAATATTACCCGTG
ACCAGCAGCTTTGATACAGGTCTGTTAGAAGGTAGCAAGTGTATACCTTTTTTACGAGCGATTTTATTAAACAAC
ATCAACAAACCGGTTTCAGGCCCTGCTGTTTATTGAATGGGTTAAACAGGTGATTTCGCGATTTTACCACCGAAGCA
ACCAAAACCTCAACCGTTGATAAACTGAAAGATATTAGCCTGGTGGTGCCGTATATTGGTCTGGCACTGAATATT
GGTGATGAGATCTACAAACAGCATTGTCAGAAAGCAGTTGAACTGGTTGGTGCAGGTCTGCTGCTGGAATTTTCA
60 CCGGAATTTCTTATTCCGACGCTGCTGATTTTACCATCAAAGTTATCTGACCGGTAGCATTCCGCGATAAAGAC

AAAATCATTAAACCCCTGGATAACGCCCTGAATGTTCTGTGATCAGAAATGGAAAGAACTGTATCGTTGGGTTGTT
 AGCAAATGGCTGACCACCATTAAATACGCAGTTCAACAAACGCAAAGAACAAATGTACAAAGCCCTGAAAAATCAG
 GCCACCGCCATTAAAAAGATCATCGAGAACAAATATAACAACTATACCACCGATGAAAAAGCAAGATCGATAGC
 AGCTATAACATCAACGAAATTGAACGCACCCTGAACGAAAAAATCAATCTGGCCATGAAAAACATCGAGCAGTTT
 5 ATTACCGAAAGCAGCATTGCCTATCTGATCAATATCATCAACAACGAAACGATCCAGAAACTGAAAAGCTATGAT
 GACCTGGTTTCGTCTTATCTGCTGGGTTATATTCGTAATCATAGCAGCATTCTGGGCAATAGCGTTGAAGAACTG
 AATTCCAAAGTGAACAACCATCTGGATAATGGCATTCCGTTTGAAGTGAAGCAGTTATACCAATGATAGCCTGCTG
 ATCCGCTACTTCAATAAAAACTATGGCGAACTGAAGTACAACCTGCATTCTGAACATCAAATATGAGATGGATCGT
 10 GACAAACTGGTTGATAGCAGCGGTTATCGTAGCCGTATCAATATTGGTACAGGCGTCAAATTTAGCGAGATCGAT
 AAAAATCAAGTGCAGCTGAGCAATCTGGAATCCAGCAAAATGAAGTCATTCTGAATAACGGCGTCATCTATAAC
 AGCATGTATGAAAACCTTTTCGACCAGCTTTTGGATTTCGATTCCGAAATACCTTCGCAACATCAATAACGAGTAC
 AAGATCATCAGCTGTATGCAGAATAATAGCGGTTGGGAAGTGAGCCTGAATTTTAGCAATATGAACTCGAAAATC
 ATCTGGACCCTGCAGGATACCGAAGGTATCAAAAAAACCGTTGTGTTTCAGTACACCCAGAACATTAACATTAGC
 15 GACTATATCAACCGCTGGATCTTTGTGACCATTACAAATAATCGTCTGAGCAACAGCAAAATCTACATTAATGGT
 CGCCTGATCAACGAAGAAAGCATTAGCGATCTGGGTAATATCCATGCCAGCAACAACATTATGTTTAAACTGGAT
 GGTTGCCGTGATCCGCATCGTTATATCTGGATTAAATACTTTAACCTGTTTGACAAAAGAGCTGAACAAGAAAGAA
 ATTAAAGATCTGTACGACAACAGAGCAATAGCGGTATTCTGAAAGATTTCTGGGGTGATTATCTGCAGTATGAC
 AAACCGTATTATGTCTGAATCTGTATGACCCGAATAAGTATCTGGATGTGAATAATGTTGGCATCCGTGGCTAT
 ATGTATCTGAAGGTCGCGGTGGTCTGTATGTGACCACCAACATTTATCTGAATAGCACCTGTATATGGGCACC
 20 AAATTCATCATTAAGAAATATGCCAGCGGCAACAAAGATAACATTGTGCGTAATAATGATCGCGTGTATATTAAC
 GTGGTGGTGAAGAATAAAGAATATCGCCTGGCAACCAATGCAAGCCAGGCAGGCGTTGAAAAAATCTGAGCGCA
 GTTGAAATCCCGGATGTTGGTAATCTGAGCCAGGTTGTTGTGATGAAAAGCGAAAATGATCAGGGCATTTCGCAAC
 AAGTGTAAGTGAATCTGCAAGACAATAACGGCAACGATATTGGCTTTATCGGCTTTTACCAGTTTAATAACATT
 GCAAACTGGTGGCCAGCAACTGGTATAACCGTCAGATTGGTAAAGCAAGCCGTACCTTTGGTTGTAGCTGGGAA
 25 TTTATCCCGGTTGATGATGGTTGGGGTGAAAAGCAGCCTGGAATACTGTATTTCCAGGGTGCCAGTCATCATCAC
 CACCATCACCATCACTGA

SEQ ID NO: 26 - Polypeptide Sequence of rBoNT/FA(0) (His-tagged)

MPVVINSFNYYDDPVNDNTIIYIRPPYYETSNTYFKAFQIMDNVWIIPERYRLGIDPSLFNPPVSLKAGSDGYFDP
 30 NYLSTNTEKNKYLQIMIKLFKRINSKPAGQILLEEIKNAIPYLGNSYTQEEQFTTNRTVSFNVKLANGNIVQQM
 ANLIIWGPDPDLTTNKTGGIIYSPYQSMEATPYKDGFGSMTVEFSPYATAFNDISIASHSPSLFIKDPALILM
 HQLIYVLHGLYGYTITEYKITPNVVQSYMKVTKPITSAEFLTFFGRDRNIVPQSIQSQLYNKVLSDYKRIASRLN
 KVNTATALINIDEFKNLYEWKYQFAKDSNGVYSVDLNFKEQLYKKIYSFTEFNLAYEFKIKTRLGYLAENFGPFY
 35 LPNLLDDSIYTEVDGFNIGALSINYQQNIGSDINSIKKLQGGVVSRRVRLCSNSNTKNSLCITVNNRDLFFIA
 SQESYAGENTINTYKEIDDTTLDPSFEDILDKVLNLFNEQVIPQMPNRNVSTDIQKDNYPKYDYNRTDIIDSYE
 VGRNYNTFFYLNAQKFSFNESNITLTSSFDTLLEGSKVYTFSSDFINNINKPVQALLFIEWVKQVIRDFTEEA
 TKTSTVDKLDISLVVPYIGLALNIGDEIYKQHFABEVELVGAGLLLEFSPEFLIPTLLIFTIKGYLTGSIRDKD
 KIIKTLDNALNVRDQKWKELYRWVVSQWLTTINTQFNKRKEQMYKALKNQATAIKKIIENKYNNTTDEKSKIDS
 40 SYNINEIERTLNEKINLAMKNIEQFITESSIAYLINIINNETIQKLKSYDDLVRRLYLLGYIRNHSSILGNSVEEL
 NSKVVNHLNDNGIPFELSSYTNDSSLIRYFNKNYGEIKYCNILNIKYEMDRDKLVDSSGYRSRINIGTGVKFSEID
 KNQVQLSNLESSKIEVILNNGVIYNSMYENFSTSFWRIPKYFRNINNEYKIIISCMQNNSGWEVSLNFSNMNSKI
 IWLTDQTEGIKKTVVFQYTQININISDYINRWIFVTITNNRLSNSKIYINGRLINEESISDLGNIHASNNIMFKLD
 GCRDPHRYIWIKYFNLFDEKLNKKEIKDLYDNQNSGILKDFWGDYLDQYDKPYMLNLYDPNKYLDVNNVGIRGY
 45 MYLKGPRGRIVTTNIYLNSTLYMGTKFIIKKYASGNKDNIVRNNDRVYINVVKNKEYRLATNASQAGVEKILSA
 VEIPDVGNLQVVMKSENDQGIRNKCKMNLQDNNGNDIGFIFGHQFNNAKLVASNWNRYQIGKASRTFGCSWE
 FIPVDDGWGESSLENLYFQGASHHHHHHHH

SEQ ID NO: 27 - Nucleotide Sequence of rLH_N/FA (His-tagged)

ATGCCGGTTGTGATTAACAGCTTCAATTATGATGATCCGGTGAACGATAACACCATCATTTATATCCGTCCGCCT
 50 TATTATGAAACCAGCAACACCTATTTCAAAGCCTTCCAGATTATGGATAACGTGTGGATTATTCGGAACGTTAT
 CGTCTGGGTATTGATCCGAGCCTGTTTAAATCCGCCTGTAGCCTGAAAGCAGGTAGTGATGGTTATTTTGATCCG
 AATTATCTGAGCACCAACACCGAGAAAAACAAATACCTGCAGATTATGATCAAGCTGTTCAAACGCATTAATAGC
 AAACCGGCAGGTGAGATTCTGCTGGAAGAAAAATCAAAAAATGCAATTCCGTATCTGGGCAACAGCTATACCCAAGAA
 55 GAACAGTTTACCACCAATAATCGTACCGTGAGCTTTAATGTTAAACTGGCCAATGGTAATATCGTTCAGCAGATG
 GCAAACTCTGATTATTTGGGGTCCGGGTCTGATCTGACCACAAATAAAACCGGTGGTATCATCTATAGCCCGTAT
 CAGAGCATGGAAGCAACCCCGTATAAAGATGGTTTTGGTAGCATTATGACCGTGGAATTTAGTCCGGAATATGCA
 ACCGCCTTTAACGATATTTCAATTGCAAGCCATAGTCCGTGCTGTTTATCAAAGATCCGGCACTGATTCTGATG
 CATGAAGTATTCTGATGGTCTGTATGGCACCTATATTACCGAATACAAAAATACCCGAATAGTGGTG
 60 CAGAGCTATATGAAAGTTACCAACCGATTACAGCGCAGAATTTCTGACCTTTGGTGGTGGTATCGCAATATT
 GTTCCGCAGAGCATTACAGAGCCAGCTGTATAACAAAGTTCTGAGCGATTATAAACGTATTGCCAGCCGTCTGAAT

AAAGTTAATACCGCAACCGCACTGATCAACATCGATGAATTCAAAAAACCTGTACGAGTGGAAATACCAGTTTGCC
 AAAGATAGCAATGGTGTGTATAGCGTGGATCTGAACAAAATTTGAGCAGCTGTACAAAAAATCTATAGCTTCACC
 GAATTC AACCTGGCCTATGAGTTTAAAATCAAAACCCGTCTGGGTATCTGGCCGAAAATTTTGGTCCGTTTTAT
 CTGCCGAATCTGCTGGATGATAGCATTTATACCGAAGTGGATGGTTTTAACATTGGTGCAGTGAAGCATTAACTAT
 5 CAGGGTCAGAATATTGGCAGCGATATCAACAGCATCAAAAAACTGCAAGGTCAGGGTGTGTTAGCCGTGTTGTT
 CGTCTGTGTAGCAATAGCAATACCAAAAAACAGCCTGTGCATTACCGTTAATAATCGCGACCTGTTTTTTATCGCA
 AGCCAAGAAAGCTATGGCGAGAATACCATTAACACCTATAAAGAGATTGACGATACCACCACACTGGATCCGAGC
 TTTGAAGATATTCTGGATAAAGTGATCCTGAACTTCAACGAACAGGTTATTCGCGAGATGCCGAATCGTAATGTT
 AGCACCGATATTAGAAAAGACAACACTACATCCCGAAATACGATTATAACCGCACCGACATTATCGATAGCTATGAA
 10 GTTGGTTCGCAACTACAACACCTTTTTCTATCTGAATGCCAGAAAATTTAGCCCGAACGAAAGCAATATTACCCTG
 ACCAGCAGCTTTGATACAGGTCTGTTAGAAGGTAGCAAAGTGTATACCTTTTTTACGAGCGGATTTTATTAACAAC
 ATCAACAAACCGGTTTCAGGCCCTGCTGTTTATTGAATGGGTAAACAGGTGATTCGCGATTTTACCACCGAAGCA
 ACCAAAACCTCAACCGTTGATAAACTGAAAGATATTAGCCTGGTGGTGGCGTATATTGGTCTGGCACTGAATATT
 15 GGTGATGAGATCTACAAACAGCATTTTGCAGAAGCAGTTGAACTGGTGGTGCAGGTCTGCTGCTGGAATTTTCA
 CCGGAATTTCTTATTCCGACGCTGCTGATTTTTTACCATCAAAGGTTATCTGACCGGTAGCATTTCGCGATAAAGAC
 AAAATCATTAAAACCTGGATAACGCCCTGAATGTTTCGTGATCAGAAAATGGAAAGAACTGTATCGTTGGGTGTT
 AGCAAATGGCTGACCACCATTAATACGCAGTTCAACAAACGCAAGAACTGTACAAAGCCCTGAAAAATCAG
 GCCACCGCCATTAAAAAGATCATCGAGAACAAATATAACAACTATACCACCGATGAAAAAGCAAGATCGATAGC
 AGCTATAACATCAAGAAATGAACGCACCTGAACGAAAAAATCAATCTGGCCATGAAAAACATCGAGCAGTTT
 20 ATTACAGAAGCAGCATTGCCTACCTGATCAATATCATCAACGAAACCATTGAGAACTGAAAAGCTATGAT
 GACCTGGTTCGTCGTTATCTGCTGGGTTATATTCTGTAATCATAGCAGCATTTCTGGGCAATAGCGTTGAAGAAGT
 AATTCCAAAGTGAACAACCATCTGGATAATGGCATTCCGTTTGAAGTGAAGCAGTTATACCAATGATAGCCTGCTG
 ATCCGCTACTTCAATAAAAACTATGGCGAAGAGAACCTGTATTTCCAGGGTGCCAGTCATCATCACCACCATCAC
 CATCACTGA

SEQ ID NO: 28 - Polypeptide Sequence of rLH_N/FA (His-tagged)

MPVVINSFNYYDDPVNDNTIIYIRPPYYETSNTYFKAFQIMDNVWIIPERYRLGIDPSLFNPPVSLKAGSDGYFDP
 NYLSTNTEKNKYLQIMIKLFKRINSKPAGQILLEEIKNAIPYLGNSYTQEEQFTTNRTVSFNVKLANGNIVQQM
 ANLIIWGPDPDLTTNKTGGIISPYQSMEATPYKDGFGSINTVEFSPEYATAFNDISIASHSPSLFIKDPALILM
 30 HELIHVLHGLYGYTITEYKITPNVVQSYMKVTKPITSAEFLTFGGRDRNIVPQSIQSQLYNKVLSDYKRIASRLN
 KVNTATALINIDEFNLYEWKYQFAKDSNGVYSVDLNKFEQLYKKIYSFTEFNLAYEFKIKTRLGLAENFGPFY
 LPNLLDDSIYTEVDGFGNIGALSINYQQNIGSDINSIKKLQGGQGVVSRVRLCSNSNTKNSLCITVNNRDLEFFIA
 SQESYAGENTINTYKEIDDTTLLDPSFEDILDKVIILNFNEQVIPQMPNRNVSTDIQKDNYPKYDYNRTDIIDSYE
 VGRNYNTFFYLNAQKFSFNESNITLTSSFDTLLEGSKVYTFSSDFINNINKPVQALLFIEWVKQVIRDFTEEA
 35 TKTSTVDKLDISLVVPYIGLALNIGDEIYKQHF AEAVELVGAGLLLEFSPEFLIPTLLIFTIKGYLTGSIRDKD
 KIIKTLDNALNVRDQKWELRYWVSVKWLTTINTQFNKRKEQMYKALKNQATAIKKIIENKYNNTTDEKSKIDS
 SYNINEIERTLNEKINLAMKNIEQFITESSIAYLINIINNETIQKLKSYDDLVRRLYLLGYIRNHSSILGNSVEEL
 NSKVNHLNDNGIPFELSSYTNDSSLIRYFNKNYGEENLYFQGASHHHHHHHH

SEQ ID NO: 29 - Nucleotide Sequence of rH_C/FA (His-tagged)

ATGCTGAAGTATAACTGCATCCTGAACATCAAAATATGAGATGGATCGTGATAAACTGGTTGATAGCAGCGTTAT
 CGTAGCCGTATCAATATTGGCACCGGTGTGAAATTTAGCGAGATCGATAAAAAATCAGGTGCAGCTGAGCAATCTG
 GAAAGCAGCAAAATTGAAGTGATTCTGAATAACGGCGTGATCTACAATAGCATGTATGAAAACTTTTCGACCAGC
 TTCTGGATTTCGATTCCGAAATACTTTGCAACATCAACAACGAGTACAAGATTATCAGCTGTATGCAGAATAAT
 45 AGCGGTTGGGAAGTTAGCCTGAATTTAGCAATATGAACAGCAAAATCATTGGACCTGCAGGATACCGAAGGT
 ATCAAAAAAACCGTTGTGTTTTCAGTACACCCAGAACATTAACATCAGCGATTACATTAACCGCTGGATCTTTGTG
 ACCATTACCAATAATCGTCTGAGCAACAGCAAGATCTATATTAACGGTCGCCTGATTAACGAAGAGAGCATTAGC
 GATCTGGGTAATATTATGCCAGCAACAACATCATGTTTAACTGGATGGTTGTCGTGATCCGCATCGTTATATT
 TGGATCAAAATACTTCAACCTGTTTGATAAAGAACTGAACAAAAAGAAATCAAAGACCTGTATGATAACCAGAGC
 50 AATAGCGGCATTCTGAAAGATTTTTGGGGTGATTATCTGCAGTATGACAAACCGTATTACATGCTGAATCTGTAC
 GATCCGAACAAATATCTGGATGTGAATAATGTGGGTATCCGTGGCTATATGTATCTGAAAGGTCCGCGTGGTCTG
 ATTGTTACCACCAACATTTATCTGAATAGCACCTGTATATGGGCACCAAAATTCATCATTAAAAAGTATGCCAGC
 GGCAACAAAGATAACATTGTGCGTAATAATGATCGCGTGTATATCAATGTGGTGGTGAAGAATAAAGAATATCGT
 CTGGCCACCAATGCAAGCCAGGCAGGCGTTGAAAAAATCTGAGCGCAGTTGAAATTCGGGATGTTGGTAATCTG
 55 AGCCAGGTTGTTGTTATGAAAAGCGAAAAATGATCAGGGCATTCGCAACAAATGCAAAATGAATCTGCAGGACAAT
 AACGGCAACGATATTGGTTTTATTGGCTTCCACCAGTTCAACAACATTGCAAACTGGTGGCGAGCAATTGGTAT
 AATCGTCAGATTGGTAAAGCAAGCCGTACCTTTGGTTGTAGCTGGGAATTTATTCCGGTTGATGATGGTTGGGGT
 GAAAGCAGCCTGGAATCTGTATTTTTCAGGGTGCAAGTCATCATCACCACCATCACCATCATTA

SEQ ID NO: 30 - Polypeptide Sequence of rHc/FA (His-tagged)

MLKYNCILNIKYEMDRDKLVDSSGYRSRINIGTGVKFSEIDKNQVQLSNLESSKIEVILNNGVIYNSMYENFSTS
FWIRIPKYFRNINNEYKIIISCMQNNSGWEVSLNFSNMNSKIIWTLQDTEGIKKTVVFQYTONINISDYINRWIFV
TITNNRLSNSKIYINGRLINEESISDLGNIHASNNIMFKLDGCRDPHRYIWIKYFNLFDKELNKKEIKDLYDNQS
5 NSGILKDFWGDYLDYDKPYMLNLNLDPNKYLDVNNVGIRGYMYLKGPRGRIVTTNIYLNSTLYMGTKFIIKKYAS
GNKDNIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSAVEIPDVGNLSQVVVMKSENDQGIRNKCKMNLQDN
NGNDIGFIFGHQFNNAKLVASNWYNRQIGKASRTFGCSWEFIPVDDGWGESSLENLYFQGASHHHHHHHH

SEQ ID NO: 31 - Nucleotide Sequence of rLC/FA (His-tagged)

ATGCCGGTTGTGATTAACAGCTTCAATTATGATGATCCGGTGAACGATAACACCATCATTTTATATCCGTCCGCCT
TATTATGAAACCAGCAACACCTATTTCAAAGCCTTCCAGATTATGGATAACGTGTGGATTATTTCCGGAACGTTAT
CGTCTGGGTATTGATCCGAGCCTGTTAATCCGCCTGTTAGCCTGAAAGCAGGTAGTGATGGTTATTTTGATCCG
AATTATCTGAGCACCAACACCGAGAAAAACAAATACCTGCAGATTATGATCAAGCTGTTCAAACGCATTAATAGC
AAACCGGCAGGTGAGATTCTGCTGGAAGAAATCAAAATGCAATTCCGTATCTGGGCAACAGCTATACCCAAGAA
15 GAACAGTTTACCACCAATAATCGTACCGTGAGCTTTAATGTTAACTGGCCAATGGTAATATCGTTCAGCAGATG
GCAAACTCTGATTATTTGGGGTCCGGGTCTGATCTGACCACAAATAAAACCGGTGGTATCATCTATAGCCCGTAT
CAGAGCATGGAAGCAACCCCGTATAAAGATGGTTTTGGTAGCATTATGACCGTGGAATTTAGTCCGGAATATGCA
ACCGCCTTTAACGATATTTCAATTGCAAGCCATAGTCCGTCGCTGTTTATCAAAGATCCGGCACTGATTCTGATG
CATGAAGTGAATTCATGTTCTGCATGGTCTGTATGGCACCTATATTACCGAATACAAAATTACCCCGAATGTGGTG
20 CAGAGCTATATGAAAGTTACCAAACCGATTACCAGCGCAGAATTTCTGACCTTTGGTGGTCTGTATCGCAATATT
GTTCCGCAGAGCATTACAGAGCCAGCTGTATAACAAAGTTCTGAGCGATTATAAACGATTATGCCAGCCGCTCTGAAT
AAAGTTAATACCGCAACCGCACTGATCAACATCGATGAATTCAAAAACCTGTACGAGTGGAAATACCAGTTTGCC
AAAGTATGCAATGGTGTGTATAGCGTGGATCTGAACAAATTTGAGCAGCTGTACAAAAAAATCTATAGCTTCACC
GAATTCAACCTGGCCTATGAGTTTAAATCAAAACCGCTCTGGGTTATCTGGCCGAAAAATTTTGTCGGTTTTTAT
25 CTGCCGAATCTGCTGGATGATAGCATTATACCGAAGTGGATGGTTTTTAACATTGGTGCATGAGCATTAACTAT
CAGGGTCAGAATATTGGCAGCGATATCAACAGCATCAAAAACTGCAAGGTCAGGGTGTGTTAGCCGTGTTGTT
CGTCTGTGTAGCAATAGCGAAAACTGTATTTTCAGGGTGCCAGTCATCATCACCACCATCACCATCACTGA

SEQ ID NO: 32 - Polypeptide Sequence of rLC/FA (His-tagged)

MPVVINSFNYYDDPVNDNTIIYIRPPYYETSNTYFKAQIMDNVWIIPERYRLGIDPSLFNPPVSLKAGSDGYFDP
NYLSTNTEKNKYLQIMIKLFRINSKPAGQILLEEIKNAIPYLGNSYTQEEQFTTNRTVSFNVKLANGNIVQQM
ANLIIWGPDPDLTTNKTGGIISPYQSMEATPYKDGFGSINTVEFSPEYATAFNDISIASHSPSLFIKDPALILM
HELIHVLHGLYGTYTEYKITPNVVQSYMKVTKPITSAEFLTFGGRDRNIVPQSIQSQLYNKVLSDYKRIASRLN
KVNTATALINIDEFNLYEWKYQFAKDSNGVYSVDLNKFEQLYKKIYSFTEFNLAYEFKIKTRLGLAENFGPFY
35 LPNLLDDSIYTEVDGFNIGALSINYQGQNGSDINSIKKLQGGQVVSRRVRLCSNSENLYFQGASHHHHHHHH

SEQ ID NO: 33 - Nucleotide Sequence of rBoNT/F(0) (His-tagged)

ATGCCGGTTGTGATTAACAGCTTCAATTATAACGATCCGGTGAACGATGATACCATCCTGTATATGCAGATTCCG
TATGAAGAGAAAAAGCAAAAAGTACTACAAAGCCTTTGAGATCATGCGCAACGTTTGGATTATTTCCGGAACGTAAT
40 ACCATTGGCACCGATCCGAGCGATTTTGATCCGCCTGCAAGCCTGGAAAAATGGTAGCAGCGCATATTATGATCCG
AATTATCTGACCACCGATGCCGAAAAAGATCGTTATCTGAAAAACCACCATCAAACGTTCAAACGCATTAATAGC
AATCCGGCAGGCGAAGTTCTGCTGCAAGAAATAGCTATGCAAAACCGTATCTGGGCAATGAACATACCCCGATT
AATGAATTTTCATCCGGTTACACGTACCACGAGCGTTAACATTAAAAAGCAGCACCAATGTGAAGTCCAGCATTATT
CTGAATCTGCTGGTTTTAGGTGCAGGTCCGGATATTTTGAATAATCAAGCTATCCGGTGCAGCAACCTGATGGAT
45 AGCGGTGGTGTGTATGATCCGTCAAATGATGTTTTGGCAGCATTAACATTGTGACCTTTAGTCCGGAATATGAA
TACACCTTCAACGATATTAGCGGTGGCTATAATAGCAGCACCGAAAGTTTTATTGCAGATCCGGCAATTAGCCTG
GCACACCAGCTGATTTATGCACTGCATGGTCTGTATGGTGCACGTGGTGTACCTATAAAGAAACCATTAAGTT
AAACAGGCACCGCTGATGATTGCGGAAAAACCGATTTCGCTCGGAAGAATTTCTGACCTTTGGTGGTCAGGATCTG
AACATTATTACCAGCGCAATGAAAGAGAAAAATCTATAATAACCTGCTGGCCAACTATGAGAAAAATTGCAACCCGT
50 CTGAGCCGTGTTAATAGCGCACCTCCTGAATATGATATCAACGAGTATAAAGACTATTTTCAGTGGAAATACGGC
CTGGATAAAAAATGCAGATGGTAGCTATACCGTGAACGAGAAACAAATTTAACGAGATCTACAAAAACCTGTATAGC
TTCACCGAAATCGATCTGGCCAAACAAATTCAAAGTGAAATGCCGCAACACCTACTTCATCAAATATGGCTTTCTG
AAAGTTCCGAACCTGCTTGATGATGATATCTATAACCGTTAGCGAAGGCTTTAACATTGGTAATCTGGCCGTTAAT
AATCGCGGTGAGAACATTAACTGAACCCGAAAAATTCGATAGCATCCCGGATAAAGGCCTGGTTGAAAAAATT
55 GTGAAATTTCTGCAAAAGCGTGATTCCGCGTAAAGGCACCAAGCACCCTCGTCTGTGTATTCTGTGTAATAAT
CGTGAAGTGTTTTTTGTGCAAGCGAGAGCAGCTATAACGAGAATGATATTAAACCCCCGAAAGAGATTGACGAT
ACCACCAATCTGAATAACAATATCGCAACAATCTGGATGAAGTGATCCTGGATTATAACAGCGAAACCATTCGG
CAGATTAGCAATCAGACCCTGAATACCTGGTTCAGGATGATAGCTATGTTCCGCGTTATGATAGCAATGGCACC
AGCGAAATTAAGAACATAATGTGGTTGATCTGAACGTGTTCTTTTATCTGCATGCACAGAAAGTCCGCGAAGGT
60 GAAACCAATATTAGCCTGACCAGCAGCATTGATACCGCACTGAGCGAAGAAAGCCAGGTTTATACCTTTTTTAGC

AGCGAATTCATCAACACCATTAAACAAACCGGTTTCATGCAGCACTGTTTATTAGCTGGATTAAATCAGGTGATTCCG
 GATTTTACCACCGAAGCAACCCAGAAAAAGCACCTTTGATAAAATGCGGATATTAGTCTGGTGGTGCCGTATGTT
 GGTCTGGCACTGAATATTGGTAATGAAGTGCAGAAAAGAGAACTTTAAAGAAGCCTTCGAACCTGTTAGGTGCCGGT
 ATTCTGCTGGAATTTGTGCCGGAAGTCTGATTCCGACCATTCTGGTTTTTACCATTAAAGAGCTTTATTGGCAGC
 5 AGCGAGAACAAGAACAAAATCATTAAAGCCATCAACAACAGCCTGATGGAACGCGAAACCAAATGGAAAGAAATT
 TACAGCTGGATTGTGAGCAATTGGCTGACCCGTATCAATACCCAGTTTAAACAAACGCAAAGAACAAATGTATCAG
 GCCCTGCAGAATCAGGTTGATGCAATTAACACCGTATGCAATACAAATACAAACACTATACCAGCGACGAACGT
 AATCGCCTGGAAAGCGAATACAACATTAATAACATTTCGCGAAGAACTGAACAAAAAAGTGAGCCTGGCAATGGAA
 AACATCGAACGTTTTATTACCGAAAGCAGCATCTTCTACCTGATGAAACTGATTAACGAAGCCAAAGTTAGCAAA
 10 CTGCGCGAATATGATGAAGGCGTTAAAGAATATCTGCTGGACTATATTAGCGAACATCGTAGCATTCTGGGTAAT
 AGCGTTCAAGAGCTGAATGATCTGGTTACCAGCACACTGAATAATAGCATTCCGTTTGAAGTGAAGCAGCTACACC
 AACGATAAAATCCTGATCCTGTACTTCAACAAACTGTACAAGAAGATCAAGGACAACAGCATACTGGATATGCGC
 TATGAAAACAACAAGTTCATTGATATCAGCGGCTATGGTAGCAACATTAGCATTAAATGGTGATGTGTATATCTAC
 AGCACCAACCGCAATCAGTTTGGTATTTATAGCAGCAAAACCGAGCGAAGTTAATATTGCGCAGAATAACGATATC
 15 ATCTACAACGGTCGCTATCAGAACTTTAGCATTAGCTTTTGGGTTCGCATTCCGAAATACTTTAACAAGGTGAAC
 CTGAACAACGAGTACACCATTAATTGATTGCATTGCAATAATAACAGCGGCTGGAAAATCAGCCTGAACATAAC
 AAAATTATCTGGACCCTGCAGGATACCGCAGGTAATAATCAGAACTGGTGTTTAACTACACCCAGATGATTAGC
 ATCAGCGACTATATCAACAAATGGATCTTTGTGACCATTACCAACAATCGTCTGGGTAAACAGCCGCATTATATC
 AATGGCAATCTGATCGACGAGAAAAGCATTTCAAATCTGGGCGATATTCACGTGAGCGATAACATTCTGTTCAAA
 20 ATTGTTGGCTGCAACGATACCCGTTATGTTGGTATTCGTTACTTCAAAGTGTGATACGGAACCTGGGCAAAACG
 GAAATTGAAACCTGTATAGTGATGAACCGGATCCGAGCATCTGAAAAGATTTTGGGGTAATTATCTGCTGTAC
 AACAAACGCTACTATCTGCTGAACCTGCTGCGTACCGATAAAAAGCATTACACAGAATAGCAACTTTCTGAACATC
 AATCAGCAGCGTGGTGTATATCAGAAAACCGAACATTTTTAGCAACACCCGCTGTATACCGGTGTGGAAGTTATT
 ATTCGTAAAAACGGTAGCACCGATATCAGCAACACCGATAACTTTGTGCGTAAAAATGACCTGGCCTATATTAAC
 25 GTTGTGATCGTGATGTTGAGTATCGTCTGTATGCGGATATTAGCATTGCCAAACCGGAAAAGATTATCAAACCTG
 ATCCGTACCAGCAACAGCAATAATCACTGGGTGAGATTATCGTGATGGACAGCATTGGTAACAATTGCACCATG
 AATTTCCAGAACAATAACGGTGGTAATATTGGCCTGCTGGGCTTTTCATAGCAATAATCTGGTTGCAAGCAGCTGG
 TATTACAACAACATCCGTAAAAATACCAGCAGTAATGGTTGCTTTTGGAGCTTTATCAGTAAAGAACATGGCTGG
 CAAGAAAACGAGAACCTGTATTTTCAGGGTGCAAGTCATCATCACCATCACCACCATCATTA

SEQ ID NO: 34 - Polypeptide Sequence of rBoNT/F(0) (His-tagged)

MPVVINSFNNDPVNDDTILYMQIPYEEKSKKYYKAFEMRNVWIIIPERNITGTDPSDFDPPASLENGSSAYYDP
 NYLTTDAEKDRYLKTTIKLFKRINSNPAGEVLLQELISYAKPYLGNEHTPINEFHPVTRTTSVNIKSSTNVKSSII
 LNLVLGAGPDIFENSSYPVRKLMDSGGVYDPSNDGFGSINIVTFSPEYETFNDISGGYNSSTESFIADPAISL
 35 AHQLIYALHGLYGARGVYKETIKVKQAPLMIAEKPIRLEEFLLTFGGQDLNIIITSAMKEKIYNNLLANYEKIATR
 LSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNELIYKKLYSFTEIDLANKFKVKCRNTYFIKYGFL
 KVPNLLDDDIYTVSEGFNIGNLAVNNRQNIKLNPKIIDSIPDKGLVEKIVKFCKSVIPRKGTKAPPRLCIRVNN
 RELFEVASESSYNENDINTPKIIDDITNLNNYRNLDDEVILDYNSETIPQISNQTNLTLVQDDSYVPRYDSNGT
 SEIEEHNVDLNVFFYLHAQKVPEGETNISLTSSIDTALSEESQVYTFSSFEFINTINKPVHAALFISWINQVIR
 40 DFTTEATQKSTFDKIADISLVVPYVGLALNIGNEVQKENFKEAFELLGAGILLEFVPELLIPTILVFTIKSFIGS
 SENKNKIIKAINNSLMERETKWEIYSWIVSNWLTRINTQFNKRKEQMYQALQNQVDAIKTVIEYKYNNTSDER
 NRLESEYNINNIREELNKKVSLAMENIERFITESSIFYLMKLINAEKVKSLREYDEGVKEYLLDYISEHRSILGN
 SVQELNDLVSTLNNISIPFELSSYTNDKILILYFNKLYKKIKDNSILDMRYENNKFIDISGYGSNISINGDVYIY
 STNRNQFGIYSSKPSSEVNIAQNNDIYNGRYQNFISISFWVRIPKYFNKVNLLNNEYTIIDCIRNNNSGWKISLNYN
 45 KIIWTLQDTAGNNQKLVFNYTQMISISDYINKWIFVTITNRLGNSRIYINGNLIDEKISISNLGDIHVSNDILFK
 IVGCNDTRYVGIRYFKVFDTELKTEIETLYSDEPDPSILKDFWGNLYLLNKRYLLNLLRTDKSITQNSNFLNI
 NQQRGVYQKPNIFSNTRLYTGEVVIIRKNGSTDISNTDNFVRKNDLAYINVVDRDVEYRLYADISIAKPEKIIKL
 IRTSNSNNSLGQIIVMDSIGNNCTMNFQNNNGNIGLLGFHSNNLVASSWYNNIRKNTSSNGCFWSFISKEHGW
 QENENLYFQGASHHHHHHHH

SEQ ID NO: 35 - Nucleotide Sequence of rL_HN/F (His-tagged)

ATGCCGGTTGTGATTAAACAGCTTCAATTATAACGATCCGGTGAACGATGATACCATCCTGTATATGCAGATTCCG
 TATGAAGAGAAAAGCAAAAAGTACTACAAAGCCTTTGAGATCATGCGCAACGTTTGGATTATTCCGGAACGTAAT
 ACCATTGGCACCGATCCGAGCGATTTTGATCCGCTGCAAGCCTGGAAAATGGTAGCAGCGCATATTATGATCCG
 55 AATTATCTGACCACCGATGCCGAAAAAGATCGTTATCTGAAAACACCATCAAACCTGTTCAAACGCATTAATAGC
 AATCCGGCAGGCGAAGTTCTGCTGCAAGAAATAGCTATGCAAAACCGTATCTGGGCAATGAACATACCCCGATT
 AATGAATTTTCATCCGTTACACGTACCAGCAGCGTTAACATTAAGCAGCACCAATGTGAAGTCCAGCATTATT
 CTGAATCTGCTGGTTTTAGGTGCAGGTCCGGATATTTTGAAGTATCCGGTGCAGCAACATGATGGAT
 AGCGGTGGTGTATGATCCGTCAAATGATGTTTTGGCAGCATTAACATTGTGACCTTTAGTCCGGAATATGAA
 60 TACACCTTCAACGATATTAGCGGTGGCTATAATAGCAGCACCGAAAGTTTTATTGCAGATCCGGCAATTAGCCTG

GCACATGAACTGATTTCATGCACTGCACTGGTCTGTATGGTGCACGTGGTGTACCTATAAAGAAACCATTAAAGTT
 AAACAGGCACCGCTGATGATTGCGGAAAAACCGATTTCGTCTGGAAGAAATTTCTGACCTTTGGTGGTCAGGATCTG
 AACATTATTACCAGCGCAATGAAAGAGAAAAATCTATAATAACCTGCTGGCCAACCTATGAGAAAAATTGCAACCCGT
 CTGAGCCGTGTTAATAGCGCACCTCCTGAATATGATATCAACGAGTATAAAGACTATTTTTCAGTGGAAATACGGC
 5 CTGGATAAAAAATGCAGATGGTAGCTATAACCGTGAACGAGAACAAAATTTAACGAGATCTACAAAAAACTGTATAGC
 TTCACCGAAATCGATCTGGCCAACAAAATTCAAAAGTGAAATGCCGCAACACCTACTTCATCAAATATGGCTTTCTG
 AAAGTTCCGAACCTGCTTGATGATGATATCTATAACCGTTAGCGAAGGCTTTAACATTGGTAATCTGGCCGTTAAT
 AATCGCGGTGAGAACATTAACTGAACCCGAAAAATTCGATAGCATCCCGGATAAAGGCCTGGTTGAAAAAATT
 GTGAAATTCGCAAAAGCGTGATTCCGCGTAAAGGCACCAAAAGCACCCTCGTCTGTGTATTCTGTGAATAAT
 10 CGTGAACCTGTTTTTTGTTGCAAGCGAGAGCAGCTATAACGAGAATGATATTAACACCCCGAAAGAGATTGACGAT
 ACCACCAATCTGAATAACAACCTATCGCAACAATCTGGATGAAGTGATCCTGGATTATAACAGCGAAACCATTCCG
 CAGATTAGCAATCAGACCCTGAATACCCTGGTTCAGGATGATAGCTATGTTCCGCGTTATGATAGCAATGGCACC
 AGCGAAATTGAAGAACATAATGTGGTTGATCTGAACGTGTTCTTTTATCTGCATGCACAGAAAGTGCCGGAAGGT
 GAAACCAATATTAGCCTGACCAGCAGCATTGATACCGCACTGAGCGAAGAAAGCCAGGTTTATACCTTTTTTAGC
 15 AGCGAATTCATCAACACCATTAACAAACCCGGTTCATGCAGCACTGTTTATTAGCTGGATTAATCAGGTGATTTCGC
 GATTTTACCACCGAAGCAACCCAGAAAAAGCACCTTTGATAAAAATGCGGATATTAGTCTGGTGGTGGCGTATGTT
 GGTCTGGCACTGAATATTGGTAATGAAGTGCAAGAAAGAGAACTTTAAAGAAGCCCTTCGAACGTTAGGTGCCGGT
 ATTCGTGCTGGAATTTGTGCCGGAACCTGCTGATTCCGACCATTCGTGGTTTTTACCATTAAAGAGCTTTATTGGCAGC
 AGCAGAGAACAAGAACAACATCATTAAAGCCATCAACAACAGCCTGATGGAACGCGAAACCAATGGAAAGAAAT
 20 TACAGCTGGATTGTGAGCAATTGGCTGACCCGTCATCAATACCCAGTTTAAACAAACGCAAGAAACAAATGTATCAG
 GCCCTGCAGAATCAGGTTGATGCAATTAACACCGTGATCGAATACAAATACAACAACCTATACCAGCGACGAACGT
 AATCGCCTGGAAAGCGAATACAACATTAATAACATTTCGCGAAGAACTGAACAAAAAAGTGAGCCTGGCAATGGAA
 AACATCGAACGTTTTTATTACCGAAAGCAGCATCTTCTACCTGATGAAACTGATTAACGAAGCCAAAGTTAGCAAA
 CTGCGCGAATATGATGAAGGCGTTAAAGAATATCTGCTGGACTATATTAGCGAACATCGTAGCATTCCTGGGTAAT
 25 AGCGTTCAAGAGCTGAATGATCTGGTTACCAGCACACTGAATAATAGCATTCCGTTTGAAGTGAAGCAGCTACACC
 AACGATAAAATCCTGATCCTGTACTTCAACAACTGTACAAGAAAGAAAACCTGTATTTTCAGGGTGCAAGCCAT
 CATCACCACCATCACCATCATTA

SEQ ID NO: 36 - Polypeptide Sequence of rL_HN/F (His-tagged)

30 MPVVINSFNNDPVNDDTILYMQIPYEEKSKKYYKAFEMRNVWIIIPERNITIGTDPSPDFDPPASLENGSSAYYDP
 NYLTTDAEKDRYLKTTIKLFKRINSNPAGEVLLQELISYAKPYLGNEHTPINEFHPVTRTTSVNIKSSTNVKSSII
 LNLVLVLGAGPDIFENSYPVRKLMDSGGVYDPSNDGFGSINIVTFSPPEYETFNDISGGYNSSTESFIADPAISL
 AHELIHALHGLYGARGVYKETIKVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLANYEKIATR
 LSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTEIDLANKFKVKCRNTYFIKYGFL
 35 KVPNLLDDDIYTVSEGFNIGNLAVNNRQNIKLNPKIIDSIPDKGLVEKIVKFCKSVIPRKGTKAPRLCIRVNN
 RELFFVASESSYNENDINTPKEIDDTNLNNYRNLDDEVILDYNSETIPQISNQTNLTLVQDDSYVPRYDSNGT
 SEIEEHNVDLNVFFYLHAQKVPEGETNISLTSSIDTALSEESQVYTFFSSEFINTINKPVHAALFISWINQVIR
 DFTTEATQKSTFDKDIADISLVVPYVGLALNIGNEVQKENFKAFELLGAGILLEFVPELLIPTILVFTIKSFIGS
 SENKNKIIKAINNSLMERETKWKEIYSWIVSNWLTRINTQFNKRKEQMYQALQNQVDAIKTVIEYKYNNYTSDER
 40 NRLESEYNINNIREELNKKVSLAMENIERFITESSIFYLMKLINAEKVKSLREYDEGVKEYLLDYISEHRSILGN
 SVQELNDLVTSTLNNIPFELSSYTNDKILILYFNKLYKKENLYFQGASHHHHHHHH

SEQ ID NO: 37 - Nucleotide Sequence of rH_C/F (His-tagged)

ATGATCAAGGATAACAGCATTCTGGATATGCGCTATGAGAACAAACAAATTCATTGATATTAGCGGCTATGGCAGC
 45 AACATTAGCATTAATGGTGATGTGTATATCTACAGCACCAACCGTAATCAGTTTGGCATTATAGCAGCAAACCG
 AGCGAAGTTAATATTGCCAGAACACGATATCATCTATAACGGTCGCTATCAGAACTTCAGCATTAGCTTTTGG
 GTTCGCATTCCGAAATACTTCAATAAGGTGAACCTGAACAACGAGTATACCATCATTGATTGCATTTCGCAATAAT
 AACAGCGGCTGGAAAATTAGCCTGAACCTACAACAAAATTTATCTGGACCCTGCAGGATACCGCAGGTAATAATCAG
 AAACCTGGTGTTTAACTACACCCAGATGATTAGCATCAGCGACTATATCAACAAATGGATCTTTGTGACCATTACC
 50 AATAATCGCCTGGGTAATAGCCGCATTTATATCAATGGTAACCTGATCGATGAGAAAAGCATTAGCAATCTGGGT
 GATATTTCATGTGAGCGATAACATCCTGTTTAAAATCGTGGGTGTGAACGATACCCGTTATGTTGGTATTTCGCTAC
 TTCAAAGTGTGTTGATACCGAACTGGGTAAAACCGAAAATGAAAACCTGTATAGTGATGAACCGGATCCGAGCATT
 CTGAAAGATTTTTTGGGGTAATTATCTGCTGTACAACAAACGCTACTATCTGCTGAATCTGCTGCGTACCGATAAA
 TCAATTACCCAGAATAGCAACTTCTGAAACATTAATCAGCAGCGTGGTGTATTATCAGAAACCGAACATTTTTTAGC
 55 AACACCCGCTCTGTATACCGGTGTGGAAGTTATTATTCGTAAAAATGGCAGCACCGATATCAGCAACACCGATAAC
 TTTGTTTCGCAAAAATGATCTGGCGTATATCAACGTTGTTGATCGTGATGTTGAATATCGTCTGTATGCCGATATT
 AGCATTGCCAAACCGGAAAAAATCATCAAACTGATCCGTACCAGCAACAGCAATAATTCACCTGGGTGAGATTATT
 GTGATGGATAGCATTGGTAATAACTGCACCATGAACCTTCAGAACAATAACGGTGGTAATATTGGTCTGCTGGGC
 TTTCATAGTAATAATCTGGTTGCAAGCAGCTGGTATTATAACAACATCCGTAAAAATACCAGCAGCAATGGTTGC

TTTTGGAGCTTTATTAGCAAAGAACATGGCTGGCAAGAAAACGAGAATCTGTATTTTCAGGGTGCAAGTCATCAT
CACCACCATCACCATCATTA

SEQ ID NO: 38 - Polypeptide Sequence of rH_c/F (His-tagged)

MIKDNSILDMRYENNKFIDISGYGSNISINGDVYIYSTNRNQFGIYSSKPSEVNIAQNNDIIYNGRYQNFESISFW
VRIPKYFNKVNLLNNEYTIIDCIRNNNSGWKISLNYNKLIWTLQDTAGNNQKLVFNQYTMISISDYINKWIFVTIT
NNRLGNSRIYINGNLIDEKISISNLGDIHVSDNLFKIVGCNDTRYVGIYFKVFDTELGKTEIETLYSDEPDPSI
LKDFWGNLYLLNKRYLLNLLRDTKSITQNSNLFNINQQRGVYQKPNIFSNTRLYTGVEVIIRKNGSTDISNTDN
FVRKNDLAYINVVDRDVEYRLYADISIAKPEKIIKLIRTSNSNNSLGQIIIVMDSIGNNCTMNFQNNNGGNIGLLG
FHSNNLVASSWYYNNIRKNTSSNGCFWSFISKEHGWQENENLYFQGASHHHHHHHH

SEQ ID NO: 39 - Nucleotide Sequence of rLC/F (His-tagged)

ATGCCGGTTGTGATTAACAGCTTCAATTATAACGATCCGGTGAACGATGATACCATCCTGTATATGCAGATTCCG
TATGAAGAGAAAAGCAAAAAGTACTACAAAGCCTTTGAGATCATGCGCAACGTTTGGATTATTCGGAACGTAAT
ACCATTGGCACCAGATCCGAGCGATTTTGTATCCGCTGCAAGCCTGAAAAATGGTAGCAGCGCATATTATGATCCG
AATTATCTGACCACCGATGCCGAAAAAGATCGTTATCTGAAAACCCACCATCAAACCTGTTCAAACGCATTAATAGC
AATCCGGCAGGCGAAGTTCTGCTGCAAGAAATTAGCTATGCAAAAACCGTATCTGGGCAATGAACATACCCCGATT
AATGAATTTTCATCCGGTTACACGTACCACGAGCGTTAACATTAAAAAGCAGCACCAGTGTGAAGTCCAGCATTATT
CTGAATCTGCTGGTTTTAGGTGCAGGTCCGGATATTTTTGAAAAATTCAAGCTATCCGGTGCAGCAACCTGATGGAT
AGCGGTGGTGTGTATGATCCGTCAAATGATGGTTTTGGCAGCATTAACATTGTGACCTTTAGTCCGGAATATGAA
TACACCTTCAACGATATTAGCGGTGGCTATAATAGCAGCACCAGAAAGTTTTATTGCAGATCCGGCAATTAGCCTG
GCACATGAACCTGATTCATGCACTGCATGGTCTGTATGGTGCACGTGGTGTACCTATAAAGAAACCATTAAGTT
AAACAGGCACCCGTGATGATTGCGGAAAAACCGATTCTGCTGGAAGAATTTCTGACCTTTGGTGGTCAGGATCTG
AACATTATTACACAGCGCAATGAAAGAGAAAAATCTATAATAACCTGCTGGCCAACTATGAGAAAAATTGCAACCCGT
CTGAGCCGTGTTAATAGCGCACCTCCTGAATATGATATCAACGAGTATAAAGACTATTTTTCAGTGGAAATACGGC
CTGGATAAAAAATGCAGATGGTAGCTATACCGTGAACGAGAACAAAATTTAACGAGATCTACAAAAAATGTATAGC
TTCACCGAAATCGATCTGGCCAAACAAAATCAAAAGTGAATGCCGCAACACCTACTTCATCAAATATGGCTTTCTG
AAAGTTCCGAACCTGCTTGATGATGATATCTATACCGTTAGCGAAGGCTTTAACATTGGTAATCTGGCCGTTAAT
AATCGCGGTGAGAACATTAACTGAACCCGAAAAATTATCGATAGCATCCCGGATAAAGGCTGGTTGAAAAAATT
GTGAAATTTCTGCAAAAGCGAGAACCTGTATTTTCAGGGTGCAAGTCATCATCACCATCACCACCATCATTA

SEQ ID NO: 40 - Polypeptide Sequence of rLC/F (His-tagged)

MPVVINSFNNDPVNDDTILYMQIPYEEKSKKYYKAFEMIRNVWIIIPERNITIGTDPSPDFDPPASLENGSSAYYDP
NYLTDDAEKDRYLKTTIKLFRKINSNPAGEVLLQELISYAKPYLGNEHTPINEFHPVTRTTSVNIKSSTNVKSSII
LNLVLVLAGPDIFENSSYPVRKLMDSGGVYDPSNDGFGSINIVTFSPYEYTFNDISGGYNSSTESFIADPAISL
AHELIHALHGLYGARGVYKETIKVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLANYEKIATR
LSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTEIDLANKFKVKCRNTYFIKYGFL
KVPNLLDDDIYTVSEGFNIGNLAVNNRQGNIKLNPKIIDSIPDKGLVEKIVKFCKSENLYFQGASHHHHHHHH

SEQ ID NO: 41 - Nucleotide Sequence of Cationic rH_c/A (His-tagged)

ATGATCATCAACACCAGCATTCTGAACCTGCGTTATGAAAGCAAAACATCTGATTGATCTGAGCCGTTATGCCAGC
AAAATCAATATAGGCAGCAAGGTTAACTTCGACCCGATTGACAAAAATCAGATACAGCTGTTTAATCTGGAAAGC
AGCAAAATTGAGGTGATCCTGAAAAAAGCGATCGTGTATAATAGCATGTACGAGAATTTTTTCGACCAGCTTTTGG
ATTCGCATCCCGAAATACTTTAACAAGATTAGCCTGAACAACGAGTATACCATCATTAACCTGCATGGAAAAACAAT
AGCGGTTGGAAAGTCAGCCTGAATTATGGCGAAATTATCTGGACCCCTGCAGGATACCAAAGAAATCAAACAGCGT
GTGGTGTTCAAATACAGCCAGATGATTAATATCAGCGACTATATCAACCGCTGGATTTTTGTGACCATTACCAAT
AATCGGCTGAACAAGAGCAAGATCTATATTAACGGTCTGCTGATTGACCAGAAACCGATTAGTAATCTGGGTAAT
ATTCATGCGAGCAACAAAATCATGTTTAACTGGATGGTTGCCGTGATACCCATCGTTATATTTGGATCAAATAC
TTCAACCTGTTTCGATAAAGAGTTGAACGAAAAAGAAATTAAGACCTGTACGATAACCAGAGCAATAGCGGCATA
CTGAAAGATTTTTGGGGAGATTATCTGCAGTATGACAAACCGTATTATATGCTGAATCTGTACGACCCGAATAAA
TACGTGGATGTTAATAATGTGGGCATCCGTGGTTATATGTACCTGAAAGGTCCGCGTGGTAGCGTTATGACCACA
AACATTTATCTGAATAGCAGCCTGTATCGCGGAACCAAAATTCATCATTAAGAAAGTATGCCAGCGGCAACAAGGAT
AATATTGTGCGTAATAATGATCGCGTGTACATTAACGTTGTGGTGAAGAATAAAGAATATCGCCTGGCAACCAAT
GCAAGCCAGGCAGGCGTTGAAAAAATCTGAGTGCCCTGGAAATTCGGGATGTTGGTAATCTGAGCCAGGTTGTT
GTGATGAAAAGCAAAAACGATAAAGGCATCACCAACAAAATGCAAGATGAATCTGCAGGACAATAACGGCAATGAT
ATTGGCTTCATTGGCTTTACACAGTTTAAACAACATTGCAAACTGGTTGCCGAGCAATTGGTATAATCGTCAGATT
GAACGTAGCAGTCGTACCCTGGGTTGTAGCTGGGAATTTATCCCTGTGGATGATGGTTGGGGTGAACGTCCGCTG
AAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCTGA

SEQ ID NO: 42 - Polypeptide Sequence of rHc/A (His-tagged)

MIINTSILNLRYESKHLIDL SRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKKAIVYNSMYENFSTSFW
IRIPKYFNKISLNNEYTIINC MENNSGWKVS LNYGEI IWT LQDTKEIKQRVVF KYSQMINISDYINRWIFVTITN
NRLNKSIIYINGRLIDQKPI SNLGNIHASNKIMFKLDGCRDTHRYIWI KYFNLF DKELNEKEIKDLYDNQNSNGI
5 LKDFWGDYLDQYDKPYMLNLYDPNKYVDVNNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFIIKKYASGNKD
NIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVVMKSKNDKGITNKCKMNLQDNNGND
IGFIFGHQFNNI AKLVASNWYNRQIERSSRTLGC SWEFIPVDDGWGERPLKLAAALEHHHHHH

SEQ ID NO: 43 - Nucleotide Sequence of rHc/AB (His-tagged)

ATGATTCTGAACAATATTATCCTGAACCTGCGTTACAAAAGACAACAATCTGATCGATCTGAGCGGCTATGGTGCA
AAAGTTGAAGTCTACGACGGTGTGGAACCTGAACGATAAAAAACCAGTTCAAACCTGACCTCATCGGCTAACTCAAAA
ATTCGTGTGACGCAGAACCAAAACATCATCTTCAACTCGGTCTTTCTGGACTTCAGCGTGTCTTTCTGGATTCCGC
ATCCCCGAAATATAAAAAATGATGGCATCCAGAACTACATCCATAACGAATACACCATCATCAACTGTATGAAAAAC
AACAGTGGTTGGAAAATTTCCATCCGTGGCAACCGCATTATCTGGACCCTGATTGATATCAATGGTAAAACGAAA
15 AGCGTGTTTTTTCGAATACAACATCCGTGAAGATATCTCTGAATACATCAATCGCTGGTTTTCTCGTGACCATTACG
AACAATCTGAACAATGCGAAAAATCTATATCAACGGCAAACTGGAAAGTAATACCGACATCAAAGATATTCTGTGAA
GTTATCGCCAACGGTGAAAATCATCTTCAAACCTGGATGGCGACATCGATCGCACCCAGTTTCATTTGGATGAAATAC
TTCTCCATCTTCAACACGGAACCTGAGTCAGTCCAATATCGAAGAACGCTACAAAATCCAATCATACTCGGAATAC
CTGAAAGATTTCTGGGGTAACCCGCTGATGTACAACAAAAGAATACTACATGTTCAACGCGGGCAACAAAACCTCA
20 TACATCAAACCTGAAAAAAGATTTCGCCGGTGGGTGAAATCCTGACCCGTAGCAAATACAACCAGAACTCTAAATAC
ATCAACTATCGCGATCTGTACATTGGCGAAAAATTTATTATCCGTGCGAAAAAGCAACTCTCAGAGTATTAATGAT
GACATCGTGCGTAAAGAAGACTACATCTATCTGGATTCTTTAATCTGAACCAAGAATGGCGCGTTTATACCTAC
AAATACTTCAAAAAAGAAGAAATGAAACTGTTCTGGCCCCGATTACGACAGCGATGAATTTTACAACACCATC
CAGATCAAAGAATACGATGAACAGCCGACGTATAGTTGCCAACTGCTGTTCAAAAAAGACGAAGAATCCACCGAT
25 GAAATTGGCCTGATTGGTATCCACCGTTTCTATGAAAGCGGTATCGTTTTTCGAAGAATACAAAGATTACTTCTGT
ATCTCTAAATGGTATCTGAAAGAAGTCAAACGCAAAACCGTACAACCTGAAACTGGGCTGCAACTGGCAATTTATC
CCGAAAGACGAAGGCTGGACCGAAAAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCTGA

SEQ ID NO: 44 - Polypeptide Sequence of rHc/AB (His-tagged)

MILNNIILNLRKYDNNLIDL SGYGAKVEVYDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIR
IPKYKNDGIQNYIHNEYTIINC MKNNSGWKISIRGNRIIWT LIDINGKTKSVFFEYNIREDISEYINRWFFVTIT
NNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSYSEY
LKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRKSNQSQSIND
DIVRKEDIYIYLDFFNLNQEW RVYTYKYFKKEEMKFLFAP IYDSDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTD
35 EIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTEKLAAALEHHHHHHH

SEQ ID NO: 45 - Nucleotide Sequence of rHc/A Variant Y1117V H1253K (His-tagged)

ATGATCATCAATACTAGCATTCTGAACCTGCGTTACGAGAGCAATCATCTGATTGATCTGAGCCGTTATGCAAGC
AAGATCAACATCGGTAGCAAGGTCAATTTTGACCCGATCGATAAGAACCAGATCCAGCTGTTTAATCTGGAATCG
40 AGCAAAATTGAGGTTATCCTGAAAAACGCCATTGTCTACAACCTCCATGTACGAGAATTTCTCCACCAGCTTCTGG
ATTCGCATCCCGAAATACTTCAACAGCATTAGCCTGAACAACGAGTATACTATCATCAACTGTATGGAGAACAAC
AGCGGTTGGAAGGTGTCTCTGAACTATGGTGAGATCATTTGGACCTTGCCAGGACACCCAAGAGATCAAGCAGCGC
GTCGTGTTCAAGTACTCTCAAATGATCAACATTTCCGATTACATTAATCGTTGGATCTTCGTGACCATTACGAAT
AACCCTCTGAATAACAACGAAGATTTACATCAATGGTCGCTTGATCGATCAGAAACCGATTAGCAACCTGGGTAAAT
45 ATCCACGCAAGCAACAACATTATGTTCAAATTGGACGGTTGCCGCGATACCCATCGTTATATCTGGATCAAGTAT
TTCAACCTGTTTGATAAAGAACTGAATGAGAAGGAGATCAAAGATTTGTATGACAACCAATCTAACAGCGGCATT
TTGAAGGACTTCTGGGGCGATTATCTGCAATACGATAAGCCGTACTATATGCTGAACCTGgtTGATCCGAACAAA
TATGTGGATGTCAATAATGTGGGTATTCTGTGTTACATGTATTTGAAGGGTCCGCGTGGCAGCGTTATGACGACC
AACATTTACCTGAACCTTAGCCTGTACCGTGGTACGAAATTCATCATTAAGAAATATGCCAGCGGCAACAAAGAT
50 AACATTGTGCGTAATAACGATCGTGTCTACATCAACGTGGTCGTGAAGAATAAAGAGTACCGTCTGGCGACCAAC
GCTTCGACGGCGGGTGTGAGAAAAATTCTGAGCGCGTTGGAGATCCCTGATGTCCGTAATCTGAGCCAAGTCGTG
GTTATGAAGAGCAAGAACGACGAGGGTATCACTAACAAGTGCAAGATGAACCTGCAAGACAACAATGGTAACGAC
ATCGGCTTTATTGGTTTTCaAaCAGTTCAACAATATTGCTAAACTGGTAGCGAGCAATTGGTACAATCGTCAGATT
GAGCGCAGCAGCCGTACTTTGGGCTGTAGCTGGGAGTTTATCCCGGTCGATGATGGTTGGGGCGAACGTCCGCTG
55 CACCATCACCATCACCATCACCATCACCATT

SEQ ID NO: 46 - Polypeptide Sequence of rHc/A Variant Y1117V H1253K (His-tagged)

MIINTSILNLRYESNHLIDL SRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVYNSMYENFSTSFW
IRIPKYFNKISLNNEYTIINC MENNSGWKVS LNYGEI IWT LQDTQEI KQRVVF KYSQMINISDYINRWIFVTITN
60 NRLNNSKIYINGRLIDQKPI SNLGNIHASNIMFKLDGCRDTHRYIWI KYFNLF DKELNEKEIKDLYDNQNSNGI

LKDFWGDYLYQDKPYMYMLNLVDPNKYVDVNNVGIRGYMYLKGRGSVMTTNIYLNSSLYRGTKFIIKKYASGNKD
NIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVMKSKNDQGITNKCKMNLQDNNNGND
IGFIGFKQFNNI AKLVASNWYNRQIERSSRTLGCSEWFIPVDDGWGERPLHHHHHHHHHHH

5 SEQ ID NO: 47 - Nucleotide Sequence of rH_c/A Variant Y1117V F1252Y H1253K L1278F (His-tagged)

ATGATCATCAATACTAGCATTCTGAACCTGCGTTACGAGAGCAATCATCTGATTGATCTGAGCCGTTATGCAAGC
AAGATCAACATCGGTAGCAAGGTCAATTTTGACCCGATCGATAAGAACCAGATCCAGCTGTTTAATCTGGAATCG
AGCAAAATTGAGGTTATCTTGAAAAACGCCATTGTCTACAACCTCCATGTACGAGAATTTCTCCACCAGCTTCTGG
10 ATTCGCATCCCCGAAATACTTCAACAGCATTAGCCTGAACAACGAGTATACTATCATCAACTGTATGGAGAACAAC
AGCGGTTGGAAGGTGTCTCTGAACATATGGTGAGATCATTTGGACCTTGCCAGGACACCCAAGAGATCAAGCAGCGC
GTCGTGTTCAAGTACTCTCAAATGATCAACATTTCCGATTACATTAATCGTTGGATCTTCTGTGACCATTACGAAT
AACCGTCTGAATAACAGCAAGATTTACATCAATGGTCGCTTGATCGATCAGAAACCGATTAGCAACCTGGGTAAT
ATCCACGCAAGCAACAACATTATGTTCAAATTTGGACGGTTGCCGCGATACCCATCGTTATATCTGGATCAAGTAT
15 TTCAACCTGTTTGATAAAGAACTGAATGAGAAGGAGATCAAAGATTTGTATGACAACCAATCTAACAGCGGCATT
TTGAAGGACTTCTGGGGCGATTATCTGCAATACGATAAGCCGTACTATATGCTGAACCTGgtTGATCCGAACAAA
TATGTGGATGTCAATAATGTGGGTATTCTGTGGTTACATGTATTTGAAGGGTCCGCGTGGCAGCGTTATGACGACC
AACATTTACCTGAACCTTAGCCTGTACCGTGGTACGAAATTCATCATTAAGAAATATGCCAGCGGCAACAAAGAT
AACATTGTGCGTAATAACGATCGTGTCTACATCAACGTGGTTCGTGAAGAATAAAGAGTACCGTCTGGCGACCAAC
20 GCTTCGCAGGCGGGTGTGAGAAAAATCTGAGCGCGTTGGAGATCCCTGATGTCCGTAATCTGAGCCAAGTCGTG
GTTATGAAGAGCAAGAACGACCGAGGGTATCACTAACAAGTGCAAGATGAACCTGCAAGACAACAATGGTAACGAC
ATCGGCTTTATTGGTTaCaAaCAGTTCAACAATATTGCTAAACTGGTAGCGAGCAATTGGTACAATCGTCAGATT
GAGCGCAGCAGCCGTACTTTtGGCTGTAGCTGGGAGTTTATCCCGGTCGATGATGGTTGGGGCGAACGTCCGCTG
CACCATCACCATCACCATCACCATCACCATTAA

25 SEQ ID NO: 48 - Polypeptide Sequence of rH_c/A Variant Y1117V F1252Y H1253K L1278F (His-tagged)

MIINTSILNLRYESNHLIDLRLRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVYNSMYENFSTSF
IRIPKYFNISISLNNEYTIINC MENNSGWKVS LNYGEI IWLQDTQEIKQRVVF KYSQMINISDYINRWIFVTITN
30 NRLNNSKIYINGRLIDQKPI SNLGNIHASNNIMFKLDGCRDTHRYIWIKYFNLF DKELNEKEIKDLYDNQSN SGI
LKDFWGDYLYQDKPYMYMLNLVDPNKYVDVNNVGIRGYMYLKGRGSVMTTNIYLNSSLYRGTKFIIKKYASGNKD
NIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVMKSKNDQGITNKCKMNLQDNNNGND
IGFIGYKQFNNI AKLVASNWYNRQIERSSRTFGCSWEFIPVDDGWGERPLHHHHHHHHHHH

35 SEQ ID NO: 49 - Nucleotide Sequence of rH_c/A Variant Y1117V F1252Y H1253K L1278H (His-tagged)

ATGATCATCAATACTAGCATTCTGAACCTGCGTTACGAGAGCAATCATCTGATTGATCTGAGCCGTTATGCAAGC
AAGATCAACATCGGTAGCAAGGTCAATTTTGACCCGATCGATAAGAACCAGATCCAGCTGTTTAATCTGGAATCG
AGCAAAATTGAGGTTATCTTGAAAAACGCCATTGTCTACAACCTCCATGTACGAGAATTTCTCCACCAGCTTCTGG
40 ATTCGCATCCCCGAAATACTTCAACAGCATTAGCCTGAACAACGAGTATACTATCATCAACTGTATGGAGAACAAC
AGCGGTTGGAAGGTGTCTCTGAACATATGGTGAGATCATTTGGACCTTGCCAGGACACCCAAGAGATCAAGCAGCGC
GTCGTGTTCAAGTACTCTCAAATGATCAACATTTCCGATTACATTAATCGTTGGATCTTCTGTGACCATTACGAAT
AACCGTCTGAATAACAGCAAGATTTACATCAATGGTCGCTTGATCGATCAGAAACCGATTAGCAACCTGGGTAAT
ATCCACGCAAGCAACAACATTATGTTCAAATTTGGACGGTTGCCGCGATACCCATCGTTATATCTGGATCAAGTAT
45 TTCAACCTGTTTGATAAAGAACTGAATGAGAAGGAGATCAAAGATTTGTATGACAACCAATCTAACAGCGGCATT
TTGAAGGACTTCTGGGGCGATTATCTGCAATACGATAAGCCGTACTATATGCTGAACCTGgtTGATCCGAACAAA
TATGTGGATGTCAATAATGTGGGTATTCTGTGGTTACATGTATTTGAAGGGTCCGCGTGGCAGCGTTATGACGACC
AACATTTACCTGAACCTTAGCCTGTACCGTGGTACGAAATTCATCATTAAGAAAATAGCCAGCGGCAACAAAGAT
AACATTGTGCGTAATAACGATCGTGTCTACATCAACGTGGTTCGTGAAGAATAAAGAGTACCGTCTGGCGACCAAC
50 GCTTCGCAGGCGGGTGTGAGAAAAATCTGAGCGCGTTGGAGATCCCTGATGTCCGTAATCTGAGCCAAGTCGTG
GTTATGAAGAGCAAGAACGACCGAGGGTATCACTAACAAGTGCAAGATGAACCTGCAAGACAACAATGGTAACGAC
ATCGGCTTTATTGGTTaCaAaCAGTTCAACAATATTGCTAAACTGGTAGCGAGCAATTGGTACAATCGTCAGATT
GAGCGCAGCAGCCGTACTcatGGCTGTAGCTGGGAGTTTATCCCGGTCGATGATGGTTGGGGCGAACGTCCGCTG
CACCATCACCATCACCAT

55 SEQ ID NO: 50 - Polypeptide Sequence of rH_c/A Variant Y1117V F1252Y H1253K L1278H (His-tagged)

MIINTSILNLRYESNHLIDLRLRYASKINIGSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVYNSMYENFSTSF
IRIPKYFNISISLNNEYTIINC MENNSGWKVS LNYGEI IWLQDTQEIKQRVVF KYSQMINISDYINRWIFVTITN

NRLNNSKIYINGRLIDQKPI SNLGNHASNNIMFKLDGCRDTHRYIWI KYFNLFDKELNEKEIKDLYDNQNSNGI
LKDFWGDYLDQYDKPYMLNLVDPNKYVDVNNVGIRGYMYLKGP RGSVMTTNIYLNSSLYRGTKFIIKKYASGNKD
NIVRNNDRVYINVVVKNKEYRLATNASQAGVEKILSALEIPDVGNLSQVVVMKSKNDQGITNKCKMNLQDNNNGND
IGFIGYKQFNNI AKLVASNWYNRQIERSSRTHGCSWEFIPVDDGWGERPLHHHHHH

5

SEQ ID NO: 51 - Polypeptide Sequence of BoNT/A - UniProt P10845

MPFVNKQFNYKDPVNGVDIAYIKIPNVGQM QPVKAFKIH NKI WVIPERDTFTNPEEGDLN
PPPEAKQVPVSYDYDSTYLSTDNEKDNYLKGVTKL FERIYSTDLGRMLLTSIVRGIPFWGG
STIDTELKVIDTNCINVIQPDGSYRSEELNLVIIGPSADIIQFECKSFGHEVLNLTRNGY
10 GSTQYIRFSPDFTFGFEESLEVDTNPLL GAGKFATDPAVTLAHEL IHAGHRLYGI AINPN
RVFKVNTNAYYEMSGLEVSFEELRTFGGHD AKFIDSLQENEFRLYYYNKF KDIAS TLNKA
KSIVGTTASLQYMKNVFKEKYLLEDTS GKFSDVKLKF DKLKMLTEIYTEDNFVKFFKV
LNRKTYLNFDAVFKINIVPKVNYTIYDGFNL RNTNLAANFNGQNT EINNMF TKLKNFT
GLFEFYKLLCVRGIITSKTKSLDKGYNKALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEE
15 ITSDTNIEAAEENISLDLIQYYLTFNFDNEPENISIENLSSDIIGQLELMPNIERFPNG
KKYELDKYTMFHYLRAQEFEGHKSRIAL TNSVNEALLNPSRVYTFSSDYVKKVNKATEA
AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSG
AVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALS KRNEKWDEVYKYIVTNWLAK
VNTQIDLIRKKMKEALENQAEATKAIINYQYNQYTEEEKNNINFNIDDLSSKLNESINKA
20 MININKFLNQCSVSYLMNSMIPYGVKRL EDFDASLKDALLKYIYDNRGTLIGQVDR LKDK
VNNTLSTDIPFQLSKYVDNQRLSTFT EYIKNIINTSILNLRYESNHLIDL SRYASKINI
GSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVNSMYENFSTSFWIRIPKYFNSISLNN
EYTIINCMENNSGWKVS LNYGEIIWTLQDTQEIKQRVVF KYSQMINISDYINRWIFVTIT
NNRLNNSKIYINGRLIDQKPI SNLGNHASNNIMFKLDGCRDTHRYIWI KYFNLFDKELN
25 EKEIKDLYDNQNSNGILKDFWGDYLDQYDKPYMLNLVDPNKYVDVNNVGIRGYMYLKGP
GSVMTTNIYLNSSLYRGTKFIIKKYASGNKD NIVRNNDRVYINVVVKNKEYRLATNASQA
GVEKILSALEIPDVGNLSQVVVMKSKNDQGITNKCKMNLQDNNNGNDIGFIGFHQFN NIAK
LVASNWYNRQIERSSRTLGC SWEFIPVDDGWGERPL

30 SEQ ID NO: 52 - Polypeptide Sequence of BoNT/B - UniProt P10844

MPVTINNFNYNPDIDNNNIIMMEPPFARGTG RYKAFKITDRIWIIPERYTFGYKPEDFN
KSSGIFNRDVCEYYDPDYLTNDKKNIFLQTMIKLFNR IKS KPLGEKLLEMIINGIPYLG
DRRVPLEEFNTNIASVTVNKLISNPGEVERKKGIFANLIIFGPGPVLNENETIDIGIQNH
FASREGFGGIMQMKFCPEYVSFVNNVQENKGASIFNRRGYFSDPALILMH ELIHVLHGLY
35 GIKVDDLPIVPNEKKFFMQSTDAIQAEELYTFGGQDPSIITPSTDKSIYDKVLQNF RGIV
DRLNKVLVCISDPNININIKNFKDKYKFVEDSE GKYSIDVESFDKLYKSLMFGFTETN
IAENYKIKTRASYFSDSLPPVKIKNLLDNEIY TIEEGFNISDKDMEKEYRGQNKAINKQA
YEEISKEHLAVYKIOMCKSVKAPGICIDVDNEDLFFIADKNSFSDDL SKNERIEYNTQSN
YIENDFPINELILD TDLISKIELPSENTESLTDFNVDVPVYEKQPAIKKIFTDENTIFQY
40 LYSQTFPLDIRDISLTSSFD DALLFSNKVYSFFSMDYIKTANKVVEAGLFAGWVKQIVND
FVIEANKSNTMDKIADISLIVPYIGLALNVGNETAKGNFENAF EIA GASILLEFIPELLI
PVVGAFLLESYIDNKNKIIKTIDNALTKRNEKWS DMYGLIVAQWLSTVNTQFYTIKEGMY
KALNYQAQALEEIIKYRYNIYSEKEKSNINIDFNDINSKLN EGINQAIDNINNFIN GCSV
SYLMKKMIPLAVEKLLDFDNTLKKNLLNYIDENKLYLIGSAEY ESKSVNKYLKTIMPFDL
45 SIYTNDTILIEFMNKYNSEILNNIILNLR YKDNNLIDLSGYGAKVEVYDGV ELNDKNQFK
LTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNS
GWKISIRGNRIIWT LIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYING
KLESNTDIKDIREVIANG EIIFKLDGDI DRTQFIWMKYFSIFNTELSQS NIEERYKIQSY
SEY LKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYIN YRDLY
50 IGEKFIIRKSNSQSINDDIVRKEDIYLDFFNLNQEW RVYTYKYFKKEEEKLFLAPISD
SDEFYNTIQIKEYDEQPTYSCQLLFFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCIS
KWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE

SEQ ID NO: 53 - Polypeptide Sequence of BoNT/C - UniProt P18640

MPITINNFNYSDFVDNKNILYLDTHLNTLANEPEKAFRITGNIWVIPDRFSRNSNPNLNK
 PPRVTSKSGYYDPNYLSTDSKDPFLKEIKLFRKINSREIGEELIYRLSTDIPFPGNN
 NTPINTFDFDVFNSVDVKTRQGNNWVKTSINPSVIITGPRENIIIDPETSTFKLTNNTF
 5 AAQEGFGALSIIISISPRFMLTYSNATNDVGEGRFSKSEFCMDPILILMHENHAMHNLGY
 IAIPNDQTISSVTSNIFYSQYNVKLEYAEIYAFGGPTIDLIPKSARKYFEELKALDYYSI
 AKRLNSITTANPSSFNKYIGEYKQKLIRKYRFVVESSGEVTVNRNKFVELYNELTQIFTE
 FNYAKIYNVQNRKIYLSNVYTPVTANILDDNVYDIQNGFNIPKSNLNVLFMGQNLNRNPA
 LRKVNPNENMLYLFTKFKHKAIDGRSLYNKTLDCRELLVKNTDLPFIGDISDVKTDIFLRK
 10 DINEETEVIYYPDNVSVDQVILSKNTSEHGQDLLEYPSIDSESEILPGENQVFYDNRTQN
 VDYLNSYYLESQKLSDNVEDFTFTRSIIEEALDNSAKVYTYFPTLANKVNAGVQGGFLM
 WANDVVEDFTTNILRKDTLDKISDVSAIIPYIGPALNISNSVRRGNFTEAFAVTGVITLL
 EAFPEFTIPALGAFVIYSKVQERNEIKTIDNCLEQRIKRWKDSYEWMMGTWLSRIITQF
 NNISYQMYDSLNYQAGAIKAKIDLEYKKYSGSDKENIKSQVENLNKSLDVKISEAMNNIN
 15 KFIRECSVTYLFKNMLPKVIDELNEFDRNTKAKLINLIDSHNIIILVGEVDKLKAKVNNFS
 QNTIPFNIFSNTNSSLKDIINEYFNNINDSKILSLQNRKNTLVDTSGYNAEVSEEGDVQ
 LNPIFPDFDKLGSSGEDRGKVIIVTQENINIVNSMYESFSISFWIRINKWVSNLPGYTIID
 SVKNNSGWSIGIISNFLVFTLKQNEDEQSINFSDISNAPGYNKWFFVTVTNNMMGMN
 KIYINGKLIDTIKVKELTGINFSKTIITFEINKIPDTGLITSDSDNINMWIRDFYIFAKEL
 20 DGKDINILFNSLQYTNVVKDYWGNDLRYNKEYYMNIDYLNRYMYANSRQIVFNTRNNN
 DFNEGYKIIIKRIRGNTNDTRVRGGDILYFDMTINNKAYNLFMKNETMYADNHSTEDIYA
 IGLREQTKDINDNIIFQIQPMNNTYYYASQIFKSNFNGENISGICSIGTYRFLGGDWYR
 HNYLVPTVKQGNYSLLLESTSTHWGFVPVSE

25 SEQ ID NO: 54 - Polypeptide Sequence of BoNT/D - UniProt P19321

MTWPVKDFNYSDFVNDNDILYLRIQNKLIITPVKAFMITQNIWVIPERFSSDTNPSLSK
 PPRPTSKYQSYDPSYLSSTDEQKDTFLKGIKLFKRINERDIGKKLINYLVGSPFMGDS
 STPEDTFDFTRHTTNIAVEKFENGSWKVTNIIITPSVLIFGPLPNILDYASLTLLQGQQSN
 PSFEGFGTSLILKVAPEFLLTFSDVTSNQSSAVLGKSIFCMDPVIALMHETHSLHQLYG
 30 INIPSDKRIRPQVSEGGFSQDGPVQFEELYTFGGDLVEIIPQIERSQLREKALGHYKDI
 AKRLNNINKTIPSSWISNIDKYKKIFSEKYNFDKDNLTGNFVVDKFNLSYDLTNVMSE
 VVYSSQYNVKNRTHYFSRHYLPVFANILDDNIYTIRDGFNLTKGFNIENSGQNIERNPA
 LQKLSSSVVDLFTKVCLRLTKNSRDDSTCIKVKNRRLPYVADKDSISQEIFENKIIITDE
 TNVQNYSDKFSLDESILDGQVPINPEIVDPLLPNVNMEPLNLPGEIIVFYDDITKYVDYL
 35 NSYYLESQKLSNNVENITLTTSVEEALGYSNKIYTFPLSLAEKVNKGVQAGLFLNWANE
 VVEDFTTNIMKKDTLDKISDVSVIIPYIGPALNIGNSALRGNFNQAFAATAGVAFLLLEGFP
 EFTIPALGVFTFYSSIQEREKIIKTIENTCLEQRVKRWKDSYQWMVSNWLSRITTQFNHIN
 YQMYDSLQYQADAIKAKIDLEYKKYSGSDKENIKSQVENLNKSLDVKISEAMNNINKFIR
 ECSVTYLFKNMLPKVIDELNKFDLRTKTELINLIDSHNIIILVGEVDRLKAKVNESFENTM
 40 PFNIFSNTNSSLKDIINEYFNSINDSKILSLQNKKNALVDTSGYNAEVRVGDVQNLNTI
 YTNDFKLSSSGDKIIVNLNNNIIYSAIYENSSVSFWIKISKDLTNSHNEYTIINSIEQNS
 GWKLCIRNGNIEWILQDVNRKYKSLIFDYSESLSHTGYTNKWFFVTITNNIMGYMKLYIN
 GELKQSQKIEDLDEVKLDKTIVFGIDENIDENQMLWIRDFNIFSKELSNEDINIVYEQI
 LRNVIKDYWGPNLKFDTYYIINDNYIDRYIAPESNVLVLVQYPDRSKLYTGNPITIKSV
 45 SDKNPYSRILNGDNIILHMLYNSRKYMIIRDTDTIYATQGGECSSQNCVYALKLQSNLGN
 GIGIFSINKNIVSKNKYCSQIFSSFRENTMLLADIYKPWRFSFKNAYTPVAVTNYETKLLS
 TSSFWKFISRDPGWVE

SEQ ID NO: 55 - Polypeptide Sequence of BoNT/E - UniProt Q00496

50 MPKINSFNYPNDPVNDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDFHPPTS
 LKNGDSSYYDPNYLQSDDEKDRFLKIVTKIFNRINNNLSGGILLEELSKANPYLGNDNTP
 DNQFHIGDASAVEIKFSNGSQDILLPNVIIMGAEPDLFETNSSNISLRNNYMPSNHRFGS

IAIVTFSPEYSFRFNDNCMNEFIQDPALTLMHელიHSLHGLYGAKGITTKYTITQKQNPL
 ITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNNLADYKKIASKLSKVQVSNPLLNPKYK
 DVFEAKYGLDKDASGIYSVNINKFNDIFKKLYSFTEFDLRTKFQVKCRQTYIGQYKYFKL
 SNLLNDSIYNISEGYNNLNKVNFRGQNANLNPRIITPITGRGLVKKIIRFCKNIVSVKG
 5 IRKSICIEINNGELFFVASSENSYNDNINTPKEIDDTVTSNNNYENDLDQVILNFNSES
 PGLSDEKLNLTIQNDAYIPKYDSNGTSDIEQHDVNELNVFFYLDAQKVPEGENNVNLTSS
 IDTALLEQPKIYTFFSSEFINNVNKPVQAALFVSWIQQVLVDFTTEANQKSTVDKIADIS
 IVVPYIGLALNIGNEAQKGNFKDALELLGAGILLEFEPELLIPTILVFTIKSFLGSSDNK
 NKVIKAINNALKERDEKWKVEYSFIVSNWMTKINTQFNKRKEQMYQALQNQVNAIKTIE
 10 SKYNSYTLLEKNELTNKYDIKQIENELNQKVSIAMNNIDRFLTESSISYLMKIINEVKIN
 KLREYDENVKTYLLNYIIQHGSILGESQQELNSMVTDTLNN SIPFKLSSYTDDKILISYF
 NKFFKRIKSSSVLNMRYKNDKYVDTSGYDSNININGDVYKYPTNKNQFGIYNDKLSEVNI
 SQNDYIIYDNKYKNFSISFWVRIPNYDNKIVNVNNEYTIINCMRDNNSGWKVSLNHNEII
 WTFEDNRGINQKLAFFNYGNANGISDYINKWIFVTITNDRLGDSKLYINGNLIDQKSILNL
 15 GNIHVDNLFKIVNCSTYTRYIGIRYFNIFDKELDETEIQTLYSNEPNTNLIKDFWGNLY
 LYDKEYYLLNVLKPNFIDRRKDSTLSINNIRSTILLANRLYSGIKVKIQRVNNSTNDN
 LVRKNDQVYINFVASKTHLFLYADTATTNKEKTIKISSSGNRFNQVVVMNSVGNCTMNF
 KNNNGNIGLLGFKADTVVASTWYYTHMRDHTNSNGCFWNFISEEHWQEK

20 **SEQ ID NO: 56 - Polypeptide Sequence of BoNT/F - UniProt A7GBG3**

MPVVINSFNNDPVNDDTILYMQIPYEEKSKKYYKAFEIMRNVWIIIPERNITIGTDPDSDFD
 PPASLENGSSAYYDPNYLTDAEKDRYLKTTIKLFRINSNPAGEVLLQEISYAKPYLGN
 EHTPINEFHPVTRTTSVNIKSSTNVKSSIIILNLLVLGAGPDIFENSSTPVRKLMDSGGVY
 25 DPSNDGFGSINIVTFSPEYETFDNDISGGYNSSTESFIADPAISLAHELIHALHGLYGAR
 GVTYKETIKVKQAPLMIAEKPIRLEEFLTFGGQDLNIITSAKKEKIYNNLLANYEKIATR
 LSRVNSAPPEYDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTEIDLANKF
 KVKCRNTYFIKYGFLKVPNLLDDDIYTVSEGFNIGNLAVNNRGQNIKLNPKIIDSIPDKG
 LVEKIVKFKSVIPRKGTKAPPRLCIRVNNRELFVASESSYNENDINTPKEIDDTNLN
 NNYRNNLDEVILDYNSETIPQISNQTLNLTIVQDDSYVPRYDSNGTSEIEEHNVDLVNFF
 30 YLHAQKVPEGETNISLTSSIDTALSEEVSQVYTFFSSEFININKPVHAALFISWINQVIR
 DFTTEATQKSTFDKIADISLVVPYVGLALNIGNEVQKENFKEAFELLGAGILLEFVPELL
 IPTILVFTIKSFIGSSENKNKIIKAINNSLMERETKWKEIYSWIVSNWLTRINTQFNKRK
 EQMYQALQNQVDAIKTVIEYKYNNTSDENRLESEYNINNIREELNKKVSLAMENIERF
 ITESSIFYLMKLINAEKVKSLREYDEGVKEYLLDYISEHRSILGNSVQELNDLVTSTLNN
 35 SIPFELSSYTNDKILILYFNKLYKKIKDNSILDMRYENNKFIDISGYGSNISINGDVYIY
 STNRNQFGIYSSKPSSEVNIQAQNDIIYNGRYQNFISISFWVRIPKYFNKVNLNNEYTIIDC
 IRNNNSGWKISLNYNKIIWTLQDTAGNNQKLVFNQYTMISISDYINKWIFVTITNNRLGN
 SRIYINGNLIDEKSSISNLGDIHVSDNLFKIVGCNDTRYVGIRYFKVFDTELGKTEIETL
 YSDEPDPSILKDFWGNLYLLNKRYYLLNLLRTDKSITQNSNFLNINQQRGVYQKPNIFSN
 40 TRLYTGVEVIIRKNGSTDISNTDNFVRKNDLAYINVVDRDVEYRLYADISIAKPEKIIKL
 IRTSNSNNSLGQIIVMDSIGNNCTMNFQNNNGNIGLLGFHSNNLVASSWYNNIRKNTS
 SNGCFWSFISKEHWQEN

SEQ ID NO: 57 - Polypeptide Sequence of BoNT/G - UniProt Q60393

MPVNIKXFNYNDPINDDIIMMEPFNDPGPGTYKAFRIIDRIWIVPERFTYGFQPDQFN
 ASTGVFSKDVYEEYDPTYLKTDAEKDKFLKTMIKLFNRINSKPSGQRLLDMIVDAIPYLG
 NASTPPDKFAANVANVSINKKIIQPGAEDQIKGLMTNLIIFGPGPVLSDNFTDSMIMNGH
 SPISEGFGARMMIRFCPSCLNVFNQENKDTISFRRAYFADPALTLMHელიHVLHGLY
 GIKISNLPITPNTKEFFMQHSDPVQAEELYTFGGHDPSVISPSTDMNIYNKALQNFQDIA
 50 NRLNIVSSAQGSIDISLYKQIYKNKYDFVEDPNGKYSVDKDKFDKLYKALMFGFTETNL
 AGEYGIKTRYSYFSEYLPPIKTEKLLDNTIYTQNEGFNIASKNLKTEFNGQNKAVNKEAY
 EEISLEHLVIYRIAMCKPVMYKNTGKSEQCIIVNNEDLFFIANKDSFSKDLAKAETIAYN
 TQNTIENNFSIDQLILDNDLSSGIDLNPENTEPFTNFDDIDIPVYIKQSALKKIFVDGD
 SLFEYLHAQTFSNIENLQLTNSLNDALRNNNKVYTFSTNLVEKANTVVGASLFVNWVK
 55 GVIDDFTSESTQKSTIDKVSVDVSIIPYIGPALNVGNETAKENFKNAFEIGGAAILMEFI

PELIVPIVGFFTTLESYVGNKGHIIMTISNALKKRDQKWTDMYGLIVSQWLSTVNTQFYTI
 KERMYNALNNQSAIEKIIEDQYNRYSEEDKMNINIDFNDIDFKLNQNSINLAINNIDDFI
 NQCSISYLMNRMIPLAVKKLKDFDDNLKRDLLEYIDTNELYLLDEVNLIKSKVNRHLKDS
 5 IPFDLSLYTKDTILIQVFNNYISNISSNAILSLSYRGGRLIDSSGYGATMNVGSDVIFND
 IGNGQFKLNSENSENITAHQSKFVVYDSMFDNFSINFWVRTPKYNNNDIQTYLQNEYTII
 SCIKNDSGWKVSIKGNRIIWTLIDVNAKSKSIFFEYSIKDNISDYINKWFSITITNDRLG
 NANIYINGSLKKSEKILNLDNRINSSNDIDFKLINCTDTTKFVWIKDFNIFGRELNATEVS
 SLYWIQSSTNTLKDFWGNPLRYDTQYYLFNQGMQNIYIKYFSKASMGETAPRTNFNNAAI
 NYQNLYLGLRFIIKKASNSRNINNDNIVREGDYIYLNIDNISDESRYVYVLVNSKEIQTQ
 10 LFLAPINDDPTFYDVLQIKKYYEKTYYNCQILCEKDTKTFGLFGIGKFVKDYGYVWDTYD
 NYFCISQWYLRRRISENINKLRLGCNWQFIPVDEGWTE

SEQ ID NO: 58 - Polypeptide Sequence of TeNT – UniProt P04958

MPITINNFRYSDPVNNDTIIMMEPPYCKGLDIYYKAFKITDRIWIVPERYEFGTKPEDFN
 15 PPSSSLIEGASEYYDPNYLRDSDKDRFLQTMVKLFNRKNNVAGEALLDKIINAIPYLG
 SYSLLDKFDTNNSVSVFNLEQDPSGATTKSAMLNLIIFGPGPVLNKNEVRGIVLRVDN
 KNYFPCRDPGFGSIMQMAFCPEYVPTFDNVIENITSLTIGKSKYFQDPALLMHელიHVLH
 GLYGMQVSSHEIIPSKQEIYMQHTYPIAEELETFGGQDANLISIDIKNDLYEKTLDNDYK
 AIANKLSQVTSNDPNIDIDSYKQIYQKYQFDKDSNGQYIVNEDKFQILYNSIMYGFT
 20 IELGKKFNIKTRLSYFSMNHPVKIPNLLDDTIYNDTEGFNIESKDLKSEYKGQNMVRNT
 NAFRNVDSGLVSKLIGLCKKIIPTTNIRENLYNRTASLTDLGGELCIKIKNEDLTFIAE
 KNSFSEEPFQDEIVSYNTKNKPLNFNYSLDKIIVDYNLQSKITLPNDRTTPVTKGIPYAP
 EYKSNAASTIEIHNDNTIYQYLYAQKSPTTLQRITMTNSVDDALINSTKIYSYFPSVI
 SKVNQGAQGILFLQWVRDIIDDFTNESQKTTIDKISDVSTIVPYIGPALNIVKQGYEGN
 25 FIGALETTGVVLLLEYIPEITLPVIAALSIAESSTQKEKIIKTIDNFLEKRYEKWIEVYK
 LVKAKWLGTVNTQFQKRSYQMYRSLEYQVDAIKKIIDYEYKIYSGPDKEQIADEINNLKN
 KLEEKANKAMININIFMRESSRSFLVNQMINEAKKQLEFDTQSKNILMQYIKANSKFIG
 ITELKKLESKINKVFSTPIPFSSYKNLDCWVDNEEDIDVILKKSTILNLDINNDIISDIS
 GFNSSVITYPDAQLVPGINGKAIHLVNNESEVIVHKAMDIEYNDMFNNFTVSFWLRVPK
 30 VSASHLEQYGTNEYSIISSMKKHSLSIGSGWSVSLKGNLIWTLKDSAGEVRQITFRDLP
 DKFNAYLANWKVFIITITNDRSSANLYINGVLMGSAEITGLGAIREDDNITLKLDRCNN
 NQYVSIDKFRIFCKALNPKEIEKLYTSYLSITFLRDFWGNPLRYDTEYYLIPVASSSKDV
 QLKNTIDMYLTNAPSNTGKLNIIYRRLYNGLKFIKRYTPNNEIDSFVKSGDFIKLYV
 SYNNEHIVGYPKDGNFNNLDRILRVGYNAPGIPLYKKMEAVKLRLDLKTYSVQLKLYDD
 35 KNASLGLVGTHNGQIGNDPNRDILIASNWYFNHLKDKILGCDWYFVPTDEGWTD

SEQ ID NO: 59 - Polypeptide Sequence of BoNT/X

MKLEINKFNYNPDIDGINVITMRPPRHSDKINKGKGPFAFQVIKNIWIVPERYNFTNNT
 NDLNIPSEPIMEADAIYNPNYLNTPSEKDEFLQGVIVLERIKSKPEGEKLELIISSSIP
 40 LPLVSNAGALTSDNETIAYQENNNIVSNLQANLVIYGPDPDIANNATYGLYSTPISNGEG
 TLSEVSFSFPFYLPFDESIGNYRSLVNIVNKFVKREFAPDPASTLMHVELVHVTHNLYGIS
 NRNFYYNFDTGKIETSRQNSLIFEELLTFGGIDSKAIISSLIKKIIEAKNNYTTLISE
 RLNTVTVENDLLKYIKNKIPVQGRGLGNFKLDTAEFKKLNTILFVLNENSLAQRFSILVR
 KHYLKERPIDPIYVNILDDNSYSTLEGFNISSQGSNDFQGLLESSYFEKIESNALRAFI
 45 KICPRNGLLYNAIYRNSKNYLNIDLEDKKTTSKTNVSYPCSLNGCIEVENKDLFLISN
 KDSLNDINLSEEKIKPETTVFFKDKLPPQDITLSNYDFTEANSIPSISQQNILERNEELY
 EPIRNSLFIEKTIYVDKLTTFHFLEAQNIDESIDSSKIRVELTDSVDEALSNPNKVYSPF
 KNMSNTINSIETGITSTYIFYQWLRISIVKDFSDETGKIDVIDKSSDTLAIVPYIGPLNI
 GNDIRHGDFVGAIELAGITALLEYVPEFTIPIILVGLEVIGGELAREQVEAIVNNALDKRD
 50 QKWAEVYNITKAQWWGTIHLQINTRLAHTYKALSRQANAIIKMNMEFQLANYKGNIDDKAK
 IKNAISETTEILLNKSVEQAMKNTKFMIKLSNSYLTKEMIPKVQDNLKNFDLETKKTLDK
 FIKEKEDILGTNLSSSLRRKVSIRLNKNIAFDINDIPFSEFDDLINQYKNEIEDYEVLNL
 GAEDGKIKDLSGTSDINIGSDIELADGRENKAIKIKGSENSTIKIAMNKYLRFSATDNF
 SISFWIKHPKPTNLLNGIEYTLVENFNQRGWKISIQDSKLIWYLRDHNNSIKIVTPDYI
 55 AFNGWNLITITNNRSKGSIVYVNGSKIEEKDISSIWNTEVDDPIIFRLKNNRDTQAFTLL

DQFSIYRKELNQNEVVKLYNYFFNSNYIRDIWGNPLQYNKKYYLQTQDKPGKGLIREYWS
 SFGYDYVILSDSKTITFPNNIRYGALYNGSKVLIKNSKKLDGLVRNKDFIQLEIDGYNMG
 ISADRFNEDTNYIGTTYGTTDHLTTDFEIIQRQEKYRNYCQLKTPYNIFHKSGLMSTETS
 KPTFHDYRDWVYSSAWYFQNYENLNLKHKTKTNWYFIPKDEGWDED

5

SEQ ID NO: 60 – Polypeptide Sequence of Unmodified BoNT/A1

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWWIPERDTFTNPEEGDLNPPPEAKQVPVSYSDS
 TYLSTDNEKDNLYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGYSRSEELN
 LVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAAGKFATDPAVTLAHEL
 IHAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
 KSIVGTTASLQYMKNVFKKEYLLSEDTSGKFSVDKLFKDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDAVFK
 INIVPKVNYTIYDGFNLNRNTNLAANFNGQNTNINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKAL
 NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIEENLSSDII
 GQLELMPNIEFNPNGKKEYLDKYTMFHYLRAQEFEGHKSRIALTNSVNEALLNPSRVYTFSSDYVKKVKNKATEA
 AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
 LGTFALVSYIANKVLTVQTIDNALSKEKWEDEVYKIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQ
 YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYSMLNMSMIPYGVKRLDFDASLKDALLKYIYD
 NRGTLIGQVDRKDKVNNLTSTDIPFQLSKYVDNQRLLSTFTEYIKNIINTSILNRLYESNHLIDLSRYASKINI
 GSKVNFDPIDKNQIQLFNLESSKIEVILKNAIVNSMYENFSTSFWRIPKYFNSISLNNEYTIINCMENNSGWK
 VSLNYGEIITWLTQDTQEIQRVVFYKYSQMINISDYINRWIFVTITNNRLNNSKIYINGRLIDQKPIISNLGNIHAS
 NNIMFKLDGCRDTHRYIWIKYFNLFDEKELNEKEIKDLYDNQNSGILKDFWGDYLYQDKPYMLNLYDPNKYVDV
 NNVGIRGYMYLKGPRGSVMTTNIYLNSSLYRGTKFLIKKYASGNKDNIVRNNDRVYINVVVKNEYRLATNASQA
 GVEKILSALEIPDVGNLSQVVVMKSKNDQGITNKCKMNLQDNNGNDIGFIFGHQFNNIKLVASNWNRYNRQIERSS
 RTLGCSEWEIFPVDDGWGERPL

25

SEQ ID NO: 61 – Polypeptide Sequence of mrBoNT/AB(0)

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWWIPERDTFTNPEEGDLNPPPEAKQVPVSYSDS
 TYLSTDNEKDNLYLKGVTKLFERIYSTDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGYSRSEELN
 LVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESLEVDTNPLLGAAGKFATDPAVTLAHL
 IYAGHRLYGIAINPNRVFKVNTNAYYEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
 KSIVGTTASLQYMKNVFKKEYLLSEDTSGKFSVDKLFKDKLYKMLTEIYTEDNFVKFFKVLNRKTYLNFDAVFK
 INIVPKVNYTIYDGFNLNRNTNLAANFNGQNTNINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKAL
 NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQQYYLTFNFDNEPENISIEENLSSDII
 GQLELMPNIEFNPNGKKEYLDKYTMFHYLRAQEFEGHKSRIALTNSVNEALLNPSRVYTFSSDYVKKVKNKATEA
 AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKDDFVGALIFSGAVILLEFIPEIAIPV
 LGTFALVSYIANKVLTVQTIDNALSKEKWEDEVYKIVTNWLAKVNTQIDLIRKKMKEALENQAEATKAIINYQ
 YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYSMLNMSMIPYGVKRLDFDASLKDALLKYIYD
 NRGTLIGQVDRKDKVNNLTSTDIPFQLSKYVDNQRLLSTFTEYIKNIILNLIILNRLYKDNLDLSGYGAKVEV
 YDGVELNDKNQFKLTSSANSKIRVTQONQIIFNSVFLDFSVSFWRIPKYKNDGIQNYIHNEYTIINCMKNNSGW
 KISIRGNRIITWLTIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIAN
 GEIIFKLDGDDIDRTQFIWMKYFSIFNTELSQSNIERYKIQSYSEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKL
 KKDSPLVEILTRSKYNNQSKYINRYDLYIGKFLIRKKSNSQSINDDIVRKEDYIYLDFFNLNQEWRYVYTYKYFK
 KEEMKFLAPIDYDSEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKW
 YLKEVKRKPYNLKLGCNWQFIPKDEGWTE

45

SEQ ID NO: 62 – Polypeptide Sequence of mrBoNT/A(0)

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIHNKIWWIPERDT
 FTNPEEGDLNPPPEAKQVPVSYSDSTYLSTDNEKDNLYLKGVTKLFERIYS
 TDLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGYSRSEELN
 LVIIGPSADIIQFECKSFGHEVLNLTRNGYGSTQYIRFSPDFTFGFEESL
 EVDNTPLLGAGKFATDPAVTLAHLIYAGHRLYGIAINPNRVFKVNTNAY
 YEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
 KSIVGTTASLQYMKNVFKKEYLLSEDTSGKFSVDKLFKDKLYKMLTEIYT
 EDNFVKFFKVLNRKTYLNFDAVFKINIVPKVNYTIYDGFNLNRNTNLAAN
 FNGQNTNINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDKGYNKAL
 NDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENISLDLIQ
 QYYLTFNFDNEPENISIEENLSSDIIIGQLELMPNIEFNPNGKKEYLDKYTM
 FHYLRAQEFEGHKSRIALTNSVNEALLNPSRVYTFSSDYVKKVKNKATEA

55

AMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIGNMLYKD
 DFGALIFSGAVILLEFIPEIAIPVLGTFALVSYIANKVLTVQTIDNALS
 KRNEKWDEVYKYIVTNWLAKVNTQIDLRKKMKEALENQAEATKAIINYQ
 YNQYTEEEKNNINFNIDDLSSKLNESINKAMININKFLNQCSVSYLMNSM
 5 IPYGVKRLEDFDASLKDALLKYIYDNRGTLIGQVDRDKDKVNNLTSTDIP
 FQLSKYVDNQRLSTFTTEYIKNIINTSILNLRYESKHLIDLRYASKINI
 GSKVNFDPIDKNQIQLFNLESSKIEVILKKAIVNSMYENFSTSFWRIP
 KYFNKISLNNEYTIINC MENNSGWKVS LNYGEIIWTLQDTKEIKQRVVK
 10 YSQMINISDYINRWIFVTITNNRLNKS KIYINGRLIDQKPISNLGNIHAS
 NKIMFKLDGCRDTHRYIWI KYFNLFDKELNEKEIKDLYDNQSN SGILKDF
 WGDYLYQDKPYMLNLYDPNKYVDVNNV GIRGYMYLKGRGSMVTNIYL
 NSSLYRGTKFLIKKYASGNKDNIVRNDRVYINVVVKNEYRLATNASQA
 GVEKILSALEIPDVG NLSQVVVMKSKNDKGITNKCKMNLQDNNGNDIGFI
 GFHQFNNAIKLVASNWYNRQIERSSRTLGC SWEFIPVDDGWGERPL

SEQ ID NO: 63 – Polypeptide Sequence of BoNT/XB(0) (His-tagged)

MGSMKLEINKFNYNPDIDGINVITMRPPRH SDKINKGKGPFKAFQVIKNIWIVPERYNT
 NNTNDLNIPSEPIMEADAIYNPNYLNT PSE KDEF LQGV I KVLERIKSKPEGEKLELLEISS
 SIPLPLVSN GALT L S DNETIAYQENNNIVS NLQANLVIYGP GPD IANNATYGLYSTPISN
 20 GEGTLSEVSFS PFY LKPFDES YGNYSRLVN IVNKFVKREFAPDPASTLMHQLVYVTHNLY
 GISNRNFYFNFDTGKIETSRQQNSLIFEEL LTFGGIDSKAISSLIKKI IETAKNNYTTL
 ISERLNTVTVDNDLLKYIKNKIPVQGR LGN FKLDTAEF EKKLNTILFVLNESNLAQRFSI
 LVRKH YLKERPIDPIYVNILDDNSYS TLEG FNISSQGSNDFQGGQLLESSYFEKIESNALR
 AFIKICHKAIDGRSLYNKTLDCIEVENKDL FLISNKDSLNDINLSEEKIKPETTVFFKDK
 25 LPPQDITLSNYDFTEANSIPSISQQNILER NEELYEPIRNSLFEIKTIYVDKLTTFHFLE
 AQNIDESIDSSKIRVELTDSVDEALS NPNK VYSPFKNMSNTINSIETGITSTYIFYQWLR
 SIVKDFSDETGKIDVIDKSSDTLAIVPYIG PLLNIGNDIRHGDFVGAIELAGITALLEYV
 PEFTTIPILVGLLEVIGGELAREQVEAIVNNA LDKRDQKWADEVYNITKAQWWGTIHLQINTR
 LAHTYKALS RQANA I KMMEFQLANYKGNI DDKAKIKNAISET EILLNKSVEQAMKNTEK
 30 FMIKLSNSYLTKE MIPKVQDN LKNFDLETK KTLDKF I KEKEDILGTNLSSSLRRKVSIRL
 NKNIAFDINDIPFSEFDDLINQYKNEIEDY EVLNLGAEDGKIKDLSGTTSDINIGSDIEI
 LNNIILNLRYKDNNDLIDLSGYGAKVEVYDG VELNDKNQFKLTSSANSKIRVTQNQNIIFN
 SVFLDFSVSFWIRIPKYKNDGIQNYIHNEY TIINCMKNNSGWKISIRGNRIIWTLDING
 KTKSVFFEYNIREDISEYINRWFFVTITNN LNNAKIYINGKLESNTDIKDIREVIANGEI
 35 IFKLDGDI DRTQFIWMKYFSIFNTELSQSN IEERYKIQSYSEY LKDFWGNPLMYNKEYYM
 FNAGNKNSYIKLKKDSPVGEILTRSKYNQN SKYINRYRDLYIGEKF IIRRSNSQSINDDI
 VRKEDIYIYLDFFNLNQEW RVYTYKYFKKEE MKLFLAPIYDSDEFYNTIQIKEYDEQPTYS
 CQLLFKKDEESTDEIGLIGIHRFYESGIVF EEYKDYFCISKWYLKEVKKRPYNLKLGCNW
 40 QFIPKDEGWTEHHHHHHHHHHH

SEQ ID NO: 64 – Polypeptide Sequence of BoNT/XB(0)

MGSMKLEINKFNYNPDIDGINVITMRPPRH SDKINKGKGPFKAFQVIKNIWIVPERYNT
 NNTNDLNIPSEPIMEADAIYNPNYLNT PSE KDEF LQGV I KVLERIKSKPEGEKLELLEISS
 SIPLPLVSN GALT L S DNETIAYQENNNIVS NLQANLVIYGP GPD IANNATYGLYSTPISN
 45 GEGTLSEVSFS PFY LKPFDES YGNYSRLVN IVNKFVKREFAPDPASTLMHQLVYVTHNLY
 GISNRNFYFNFDTGKIETSRQQNSLIFEEL LTFGGIDSKAISSLIKKI IETAKNNYTTL
 ISERLNTVTVDNDLLKYIKNKIPVQGR LGN FKLDTAEF EKKLNTILFVLNESNLAQRFSI
 LVRKH YLKERPIDPIYVNILDDNSYS TLEG FNISSQGSNDFQGGQLLESSYFEKIESNALR
 AFIKICHKAIDGRSLYNKTLDCIEVENKDL FLISNKDSLNDINLSEEKIKPETTVFFKDK
 50 LPPQDITLSNYDFTEANSIPSISQQNILER NEELYEPIRNSLFEIKTIYVDKLTTFHFLE
 AQNIDESIDSSKIRVELTDSVDEALS NPNK VYSPFKNMSNTINSIETGITSTYIFYQWLR
 SIVKDFSDETGKIDVIDKSSDTLAIVPYIG PLLNIGNDIRHGDFVGAIELAGITALLEYV
 PEFTTIPILVGLLEVIGGELAREQVEAIVNNA LDKRDQKWADEVYNITKAQWWGTIHLQINTR
 LAHTYKALS RQANA I KMMEFQLANYKGNI DDKAKIKNAISET EILLNKSVEQAMKNTEK
 55 FMIKLSNSYLTKE MIPKVQDN LKNFDLETK KTLDKF I KEKEDILGTNLSSSLRRKVSIRL
 NKNIAFDINDIPFSEFDDLINQYKNEIEDY EVLNLGAEDGKIKDLSGTTSDINIGSDIEI
 LNNIILNLRYKDNNDLIDLSGYGAKVEVYDG VELNDKNQFKLTSSANSKIRVTQNQNIIFN
 SVFLDFSVSFWIRIPKYKNDGIQNYIHNEY TIINCMKNNSGWKISIRGNRIIWTLDING
 KTKSVFFEYNIREDISEYINRWFFVTITNN LNNAKIYINGKLESNTDIKDIREVIANGEI
 60 IFKLDGDI DRTQFIWMKYFSIFNTELSQSN IEERYKIQSYSEY LKDFWGNPLMYNKEYYM

FNAGNKNSYIKLKKDSPVGEILTRSKYNQN SKYINYRDLYIGEKFIIIRKSNSQSINDDI
VRKEDIYILDFFNLNQEWVYTYKYFKKEE MKLFLAPIYDSDEFYNTIQIKEYDEQPTY
CQLLFKKDEESTDEIGLIGIHRFYESGIVF EEYKDYFCISKWYLKEVKKRPYNLKLGCNW
QFIPKDEGWTE

5

SEQ ID NO: 65 – Polypeptide Sequence of BoNT/XB(0) Variant (His-tagged)

MGSMKLEINKFNNDPIDGINVITMRPPRH SDKINKGKGPFAFQVIKNIWIVPERYNFT
NNTNDLNIPSEPIMEADAIYNPYNLTNPSE KDEFLLQGVIVLERIKSKPEGEKLELLELISS
SIPLPLVSNAGALTSDNETIAYQENNNIVS NLQANLVIYGPDPDIANNATYGLYSTPISN
10 GEGTLSEVSFSPPYFKPFDESIGNYRSLVN IVNKFVKREFAPDPASTLMHQLVYVTHNLY
GISNRNFYFNFTDGTGKIETSRQQNSLIFEEL LTFGGIDSKAIISSLIKKIETAKNNYTTL
ISERLNTVTVENDLLKYIKNKIPVQGR LGN FKLDTAEFKKLNTILFVLNESNLAQRFSI
LVRKHLYKERPIDPIYVNILDDNSYSTLEG FNISSQGSNDFQQLLESSYFEKIESNALR
AFIKICHKAIDGRSLYNKTLDCIEVENKDL FLISNKDSLNDINLSEEKIKPETTVFFKDK
15 LPPQDITLSNYDFTEANSIPSISQQNILER NEELYEPIRNSLFEIKTIYVDKLTTFHFLE
AQNIDESIDSSKIRVELTDSVDEALSNNPK VYSPFKNMSNTINSIETGITSTYIFYQWLR
SIVKDFSDETGGKIDVIDKSSDTLAIVPYIG PLLNIGNDIRHGDFVGAIELAGITALLEYV
PEFTTIPILVGLVIGGELAREQVEAIVNNA LDKRDQKWADEVYNITKAQWWGTIHLQINTR
LAHTYKALSRQANAIAKMNMEFQLANYKGN I DDKAKIKNAISETTEILLNKSVEQAMKNTK
20 FMIKLSNSYLTKEMIPKVQDNLKNFDLETK KTLDKFIEKEDILGTNLSSSLRRKVSIRL
NKNIAFDINDIPFSEFDDLINQYKNEILNN IILNLRYKDNNDLIDLSGYGAKVEVYDGVEL
NDKNQFKLTSSANSKIRVTQONQNIIFNSVF LDFSVSFWIRIPKYKNDGIQNYIHNEYTII
NCMKNSGKWSIRGNRIIWTLDINGKTK SVFFEYNIREDISEYINRWFFVTITNNLNN
AKIYINGKLESNTDIKDIREVIANGEIIFK LDGIDIDRTQFIWMKYFSIFNTELSQSNIEE
25 RYKIQSYSEYKDFWGNPLMYNKEYYMFNA GNKNSYIKLKKDSPVGEILTRSKYNQNSKY
INYRDLYIGEKFIIIRKSNSQSINDDIVRK EDYIYLDFFNLNQEWVYTYKYFKKEEMKL
FLAPIYDSDEFYNTIQIKEYDEQPTYSCQL LFKKDEESTDEIGLIGIHRFYESGIVFEEY
KDYFCISKWYLKEVKKRPYNLKLGCNWQFI PKDEGWTEHHHHHHHHHHH

SEQ ID NO: 66 – Polypeptide Sequence of BoNT/XB(0) Variant

MGSMKLEINKFNNDPIDGINVITMRPPRH SDKINKGKGPFAFQVIKNIWIVPERYNFT
NNTNDLNIPSEPIMEADAIYNPYNLTNPSE KDEFLLQGVIVLERIKSKPEGEKLELLELISS
SIPLPLVSNAGALTSDNETIAYQENNNIVS NLQANLVIYGPDPDIANNATYGLYSTPISN
35 GEGTLSEVSFSPPYFKPFDESIGNYRSLVN IVNKFVKREFAPDPASTLMHQLVYVTHNLY
GISNRNFYFNFTDGTGKIETSRQQNSLIFEEL LTFGGIDSKAIISSLIKKIETAKNNYTTL
ISERLNTVTVENDLLKYIKNKIPVQGR LGN FKLDTAEFKKLNTILFVLNESNLAQRFSI
LVRKHLYKERPIDPIYVNILDDNSYSTLEG FNISSQGSNDFQQLLESSYFEKIESNALR
AFIKICHKAIDGRSLYNKTLDCIEVENKDL FLISNKDSLNDINLSEEKIKPETTVFFKDK
40 LPPQDITLSNYDFTEANSIPSISQQNILER NEELYEPIRNSLFEIKTIYVDKLTTFHFLE
AQNIDESIDSSKIRVELTDSVDEALSNNPK VYSPFKNMSNTINSIETGITSTYIFYQWLR
SIVKDFSDETGGKIDVIDKSSDTLAIVPYIG PLLNIGNDIRHGDFVGAIELAGITALLEYV
PEFTTIPILVGLVIGGELAREQVEAIVNNA LDKRDQKWADEVYNITKAQWWGTIHLQINTR
LAHTYKALSRQANAIAKMNMEFQLANYKGN I DDKAKIKNAISETTEILLNKSVEQAMKNTK
45 FMIKLSNSYLTKEMIPKVQDNLKNFDLETK KTLDKFIEKEDILGTNLSSSLRRKVSIRL
NKNIAFDINDIPFSEFDDLINQYKNEILNN IILNLRYKDNNDLIDLSGYGAKVEVYDGVEL
NDKNQFKLTSSANSKIRVTQONQNIIFNSVF LDFSVSFWIRIPKYKNDGIQNYIHNEYTII
NCMKNSGKWSIRGNRIIWTLDINGKTK SVFFEYNIREDISEYINRWFFVTITNNLNN
AKIYINGKLESNTDIKDIREVIANGEIIFK LDGIDIDRTQFIWMKYFSIFNTELSQSNIEE
50 RYKIQSYSEYKDFWGNPLMYNKEYYMFNA GNKNSYIKLKKDSPVGEILTRSKYNQNSKY
INYRDLYIGEKFIIIRKSNSQSINDDIVRK EDYIYLDFFNLNQEWVYTYKYFKKEEMKL
FLAPIYDSDEFYNTIQIKEYDEQPTYSCQL LFKKDEESTDEIGLIGIHRFYESGIVFEEY
KDYFCISKWYLKEVKKRPYNLKLGCNWQFI PKDEGWTE

SEQ ID NO: 67 – Polypeptide Sequence of BoNT/XA(0)

MGSMKLEINKFNNDPIDGINVITMRPPRH SDKINKGKGPFAFQVIKNIWIVPERYNFT
NNTNDLNIPSEPIMEADAIYNPYNLTNPSE KDEFLLQGVIVLERIKSKPEGEKLELLELISS
SIPLPLVSNAGALTSDNETIAYQENNNIVS NLQANLVIYGPDPDIANNATYGLYSTPISN
55 GEGTLSEVSFSPPYFKPFDESIGNYRSLVN IVNKFVKREFAPDPASTLMHQLVYVTHNLY
GISNRNFYFNFTDGTGKIETSRQQNSLIFEEL LTFGGIDSKAIISSLIKKIETAKNNYTTL
ISERLNTVTVENDLLKYIKNKIPVQGR LGN FKLDTAEFKKLNTILFVLNESNLAQRFSI

60

LVRKHYLKERPIDPIYVNILDDNSYSTLEG FNISSQGSNDFQGQLLESSYFEKIESNALR
 AFIKICHKAIDGRSLEVLFGQPLYNKTLDCEVENKDLFLISNKDSLNDINLSEEKIKPE
 TTFFFKDKLPPQDITLSNYDFTEANSIPSI SQQNILERNEELYEPIRNSLFEIKTIYVDK
 LTTFFHFLEAQNIDESIDSSKIRVELTDSVD EALSNNPKVYSPFKNMSNTINSIETGITST
 5 YIFYQWLRISIVKDFSDETGKIDVIDKSSDT LAIVPYIGPLLNIGNDIRHGDFVGAIELAG
 ITALLEYVPEFTTIPILVGLLEVIGGELAREQ VEAIVNNALDKRDQKWADEVYNITKAQWWGT
 IHLQINTRLAHTYKALSQANAIKMNMEFQ LANYKGNIDDKAKIKNAISETTEILLNKSVE
 QAMKNTEKFMIKLSNSYLTKEMIPKVQDNL KNFDLETKKTLDKFKEKEDILGTNLSSSL
 RRKVSIRLNKNIAFDINDIPFSEFDDLINQ YKNEIEDYEVLNLGAEDGKIKDLSGTTSDI
 10 NIGSDIEIINTSILNLRYESNHLIDLDSRYA SKINIGSKVNFDPIDKNQIQLFNLESSKIE
 VILKNAIVYNSMYENFSTSFWIRIPKYFNS ISLNNEYTIINC MENNSGWKVS LNYGEI IW
 TLQDTQEIKQRVVFKYSQMINISDYINRWI FVTITNNRLNNSKIYINGRLIDQKPISNLG
 NIHASNNIMFKLDGCRDTHRYIWIKYFNLFDKELNEKEIKDLYDNQSN SGILKDFWGDYDYL
 QYDKPYYMLNLYDPNKYVDVNNVGIRGYMY LKGPRGSMVTNIYLNSSLYRGTKFIIKKY
 15 ASGNKDNIVRNDRVYINVVVKNKEYRLAT NASQAGVEKILSALEIPDVGNLSQVVMKS
 KNDQGITNKCKMNLQDNNNGNDIGFIGFHQF NNI AKLVASNWYNRQIERSSRTLGC SWEFI
 PVDDGGERPL

SEQ ID NO: 68 – Polypeptide Sequence of BoNT/XA(0) Variant

20 MGSMKLEINKFNNDPIDGINVITMRPPRH SDKINKGKGPFKAFQVIKNIWIVPERYNT
 NNTNDLNIPSEPIMEADAIYNPNYLNTPSE KDEFLQGVIVLERIKSKPEGEKLELLEISS
 SIPLPLVSN GALTLS DNETIAYQENNNIVS NLQANLVIYGP GPDIAN NATYGLYSTPISN
 GEGTLSEVSFSPFYLPKPFDES YGNYRSLVN IVNKFVKREFAPDPASTLMHQLVVYVTHNLY
 GISNRNFYFNFDTGKIETSRQQNSLIFEEL LTFGGIDSKAISSLIKKIETAKNNYTTL
 25 ISERLNTVTVENDLLKYIKNKIPVQGR LGN FKLDTAEF EKKLNTILFVLNESNL AQRF SI
 LVRKHYLKERPIDPIYVNILDDNSYSTLEG FNISSQGSNDFQGQLLESSYFEKIESNALR
 AFIKICHKAIDGRSLEVLFGQPLYNKTLDCEVENKDLFLISNKDSLNDINLSEEKIKPE
 TTFFFKDKLPPQDITLSNYDFTEANSIPSI SQQNILERNEELYEPIRNSLFEIKTIYVDK
 LTTFFHFLEAQNIDESIDSSKIRVELTDSVD EALSNNPKVYSPFKNMSNTINSIETGITST
 30 YIFYQWLRISIVKDFSDETGKIDVIDKSSDT LAIVPYIGPLLNIGNDIRHGDFVGAIELAG
 ITALLEYVPEFTTIPILVGLLEVIGGELAREQ VEAIVNNALDKRDQKWADEVYNITKAQWWGT
 IHLQINTRLAHTYKALSQANAIKMNMEFQ LANYKGNIDDKAKIKNAISETTEILLNKSVE
 QAMKNTEKFMIKLSNSYLTKEMIPKVQDNL KNFDLETKKTLDKFKEKEDILGTNLSSSL
 RRKVSIRLNKNIAFDINDIPFSEFDDLINQ YKNEIINTSILNLRYESNHLIDLDSRYASKI
 35 NIGSKVNFDPIDKNQIQLFNLESSKIEVIL KNAIVYNSMYENFSTSFWIRIPKYFNSISL
 NNEYTIINC MENNSGWKVS LNYGEI IWTLQ DTQEIKQRVVFKYSQMINISDYINRWIFVT
 ITNNRLNNSKIYINGRLIDQKPISNLGN IHN LNEKEIKDLYDNQSN SGILKDFWGDYDYLQYD
 PRGSMVTNIYLNSSLYRGTKFIIKKYASG KPYMLNLYDPNKYVDVNNVGIRGYMYLKG
 NKDNIVRNDRVYINVVVKNKEYRLATNAS QGITNKCKMNLQDNNNGNDIGFIGFHQFN NI
 40 QAGVEKILSALEIPDVGNLSQVVMKSKND QGITNKCKMNLQDNNNGNDIGFIGFHQFN NI
 AKLVASNWYNRQIERSSRTLGC SWEFI PVD D G W G E R P L

SEQ ID NO: 69 – Polypeptide Sequence of BoNT/XD(0)

45 MGSMKLEINKFNNDPIDGINVITMRPPRH SDKINKGKGPFKAFQVIKNIWIVPERYNT
 NNTNDLNIPSEPIMEADAIYNPNYLNTPSE KDEFLQGVIVLERIKSKPEGEKLELLEISS
 SIPLPLVSN GALTLS DNETIAYQENNNIVS NLQANLVIYGP GPDIAN NATYGLYSTPISN
 GEGTLSEVSFSPFYLPKPFDES YGNYRSLVN IVNKFVKREFAPDPASTLMHQLVVYVTHNLY
 GISNRNFYFNFDTGKIETSRQQNSLIFEEL LTFGGIDSKAISSLIKKIETAKNNYTTL
 50 ISERLNTVTVENDLLKYIKNKIPVQGR LGN FKLDTAEF EKKLNTILFVLNESNL AQRF SI
 LVRKHYLKERPIDPIYVNILDDNSYSTLEG FNISSQGSNDFQGQLLESSYFEKIESNALR
 AFIKICHKAIDGRSLYNKTLDCEVENKDL FLISNKDSLNDINLSEEKIKPETTVFFKDK
 LPPQDITLSNYDFTEANSIPSI SQQNILER NEELYEPIRNSLFEIKTIYVDKLTTFHFLE
 AQNIDESIDSSKIRVELTDSVDEALSNNPK VYSPFKNMSNTINSIETGITSTYIFYQWLR
 55 SIVKDFSDETGKIDVIDKSSDTLAIVPYIG PLLNIGNDIRHGDFVGAIELAGITALLEYV
 PEFTTIPILVGLLEVIGGELAREQVEAIVNNA LDKRDQKWADEVYNITKAQWWGTIHLQINTR
 LAHTYKALSQANAIKMNMEFQLANYKGN I DDKAKIKNAISETTEILLNKSVEQAMKNTEK
 FMIKLSNSYLTKEMIPKVQDNLKNFDLET KTLDKFKEKEDILGTNLSSSLRRKVSIRL
 NKNI AFDINDIPFSEFDDLINQYKNEIEDY EVLNLGAEDGKIKDLSGTTSDINIGSDIEN
 DSKILSLQNKKNALVDTSGYNAEVRVGDNV QLNTIYTND FKLSSSGDKIIVNLNNN ILYS
 60 AIYENSSVSFWIKISKDLTNSHNEYTIINS IEQNSGWKLCIRNGNIEWILQDVNRKYKSL

IFDYSESLSHTGYTNKWWFFVTITNNIMGYM KLYINGELKQSQKIEDLDEVKLDKTIVFGI
 DENIDENQMLWIRDFNIFSKELSNEDINIV YEGQILRNVIKDYWGNPLKFDTEYYIINDN
 YIDRYIAPESNVLVLVQYPDRSKLYTGNPI TIKSVSDKNPYSRILNGDNIIILHMLYNSRK
 YMIIRDTDITYATQGGECSSQNCVYALKLQS NLGNYGIGIFSIKNIVSKNKYCSQIFSSFR
 5 ENTMLLADIYKPWRFSFKNAYTPVAVTNYE TKLLSTSSFWKFISRDPGWVE

SEQ ID NO: 70 – Polypeptide Sequence of BoNT/XF(0)

MGSMKLEINKFNNDPIDGINVITMRPPRH SDKINKGKGPFAFQVIKNIWIVPERYNFT
 NNTNDLNIPSEPIMEADAIYNPNYLNTNSE KDEFLLQGVIVLERIKSKPEGEKLELLEISS
 10 SIPLPLVSNAGALTSDNETIAYQENNNIVS NLQANLVIYGPDPDIANNATYGLYSTPISN
 GEGTLSEVSFSFPYLKPFDESYGNYSRLVN IVNKFVKREFAPDPASTLMHQLVYVTHNLY
 GISNRNFYFNFDTGKIETSRQONSLIFEEL LTFGGIDSKAISSLIKKIETAKNNYTTL
 ISERLNTVTVENDLLKYIKNKIPVQGRNGN FKLDTAEFKKLNTILFVLNESNLAQRFSI
 15 LVRKHYLKERPIDPIYVNILDDNSYSTLEG FNISQGSNDFQGGLESSEYFEKIESNALR
 AFIKICHKAIDGRSLYNKTLDCIEVENKDL FLISNKDSLNDINLSEEKIKPETTVFFKDK
 LPPQDITLSNYDFTEANSIPSISQQNILER NEELYEPIRNSLFEIKTIYVDKLTTFHFLE
 AQNIDESIDSSKIRVELTDSVDEALSNNPK VYSPFKNMSNTINSIETGITSTYIFYQWLR
 SIVKDFSDETGKIDVIDKSSDTLAIVPYIG PLLNIGNDIRHGDVFGAIELAGITALLEYV
 PEFTTIPILVGLLEVIGGELAREQVEAIVNNA LDKRDQKWAEVYNITKAQWWGTIHLQINTR
 20 LAHTYKALSQANAIKMNMEFQLANYKGNL DDKAKIKNAISETEILLNKSVEQAMKNTK
 FMIKLSNSYLTKEKIPKVQDNLKNFDLETK KTLDFIKEKEDILGTNLSSSLRRKVSIRL
 NKNIAFDINDIPFSEFDDLINQYKNEIEDY EVLNLGAEDGKIKDLSGTTSDINIGSDIEI
 KDNSILDMMRYENNNKFDISGYGSNISIND VYIYSTNRNQFGIYSSKPSEVNIAQNNNDII
 YNGRYQNFYSISFWVRIPKYFNKVNLNNEYT IIDCIRNNNSGWKISLNYNKIIWTLQDQTAG
 25 NNQKLVFNFTQMSISDSYINKWIFVTITNN RLGNRSRIYINGNLIDEKSI SNLGDHVSNDN
 ILFKIVGCNDTRYVVGIRYFKVFDTELKTE IETLYSDEPDPSILKDFWGNLYLLYNKRYYL
 LNLRLTDKSIQNSNFLNINQQRGVYQKPN IFSNTRLYTGVEVIIRKNGSTDISNTDNFV
 RKNDLAYINVVDREVEYRLYADISIAKPEK IIKLIRTSNSNNSLGGIIVMDSIGNNCTMN
 FQNNNGGNIGLLGFKSNNLVASSWYNNIR KNTSSNGCFWSFISKEHGWQEN
 30

SEQ ID NO: 71 - C1 Activation Loop Consensus Sequence

Cys-(Xaa)_a-Ile-Asp/Glu-Gly-Arg-(Yaa)_b-Cys, wherein a = 1-10 and b = 4-15

SEQ ID NO: 72 - C1 Activation Loop

35 CHKAIDGRSLYNKTLDC

SEQ ID NO: 73 - C1 Activation Loop Variant

CHKAIEGRSLYNKTLDC

SEQ ID NO: 74 – Polypeptide Sequence of rLC/A(0) (His-tagged)

MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT FTNPEEGDLN
 PPPEAKQVPV SYDSTYLST DNEKDNYLKG VTKLFERIYS TDLGRMLLTS IVRGIPFWGG
 STIDTELKVI DTNCINVIQD DGSYRSEELN LVIIGPSADI IQFECKSFSGH EVLNLTRNGY
 GSTQYIRFSP DFTFGFEESL EVDTNPLLGA GKFAIDPAVT LAHQLIYAGH RLYGIAINPN
 45 RVFKVNTNAY YEMSGLEVSE EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIASLTNKA
 KSIVGTTASL QYMKNVFKEK YLLSEDTSGK FSVDKLKFDK LYKMLTEIYT EDNFVKFFKV
 LNRKTYLNFV KAVFKINIVP KVNITYYDGF NLRNTNLAAN FNGQNTINN MNFTKLKNFT
 GLFEENLYFQ GASHHHHHHH H

SEQ ID NO: 75 – Polypeptide Sequence of rLH_N/A(0) (His-tagged)

MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT FTNPEEGDLN
 PPPEAKQVPV SYDSTYLST DNEKDNYLKG VTKLFERIYS TDLGRMLLTS IVRGIPFWGG
 STIDTELKVI DTNCINVIQD DGSYRSEELN LVIIGPSADI IQFECKSFSGH EVLNLTRNGY
 GSTQYIRFSP DFTFGFEESL EVDTNPLLGA GKFAIDPAVT LAHQLIYAGH RLYGIAINPN
 55 RVFKVNTNAY YEMSGLEVSE EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIASLTNKA
 KSIVGTTASL QYMKNVFKEK YLLSEDTSGK FSVDKLKFDK LYKMLTEIYT EDNFVKFFKV
 LNRKTYLNFV KAVFKINIVP KVNITYYDGF NLRNTNLAAN FNGQNTINN MNFTKLKNFT
 GLFEFYKLLC VRGIITSTK SLDKGYNKAL NDLCIKVNNW DLFFSPSEDN FTNDLNKGEE
 ITSDTNIEAA EENISLDLIQ QYYLTFNFNDN EPENISIEENL SSDIIGQLEL MPNIERFPNG

KKYELDKYTM FHYLRAQEFE HGKSRIALTN SVNEALLNPS RVYTFSSDY VKKVNKATEA
 AMFLGWVEQL VYDFTDETSE VSTTDKIADI TIIIPYIGPA LNIGNMLYKD DFVGALIFSG
 AVILLEFIPE IAIPVLGTFA LVSYIANKVL TVQTIDNALS KRNEKWDEVY KYIVTNWLAK
 VNTQIDLIRK KMKEALENQA EATKAIINYQ YNQYTEEEKN NINFNIDDL SSKNESINKA
 5 MININKFLNQ CSVSYLMNSM IPYGVKRLD FDASLKDALL KYIYDNRGTL IGQVDRCLKDK
 VNNTLSTDIP FQLSKYVDNQ RLLSTENLYF QGASHHHHHH HH

SEQ ID NO: 76 – Polypeptide Sequence of rLC/X(0)

MKLEINKFNY NDPIDGINVI TMRPPRHSDK INKGKGPFA FQVIKNIWIV PERYNFTNNT
 10 NDLNIPSEPI MEADAIYNPN YLNTPEKDE FLQGVIVLE RIKSKPEGEK LLELISSIP
 LPLVSNAGLT LSDNETIAYQ ENNNIVSNLQ ANLVIYGGP DIANNATYGL YSTPISNGEG
 TLSEVSFSPF YLKPFDSEY NYRSLVNIVN KFKREFAPD PASTLMHQLV YVTHNLYGIS
 NNNFYNFDT GKIETSRQON SLIFEELLTF GGIDSKAISS LIIKKIETA KNNYTTLISE
 RLNTVTVEND LLKYIKNKIP VQGR LGNFKL DTAEFEKKLN TILFVLNESN LAQRFSILVR
 15 KHYLKERPID PIYVNILDDN SYSTLEGFNI SSQGSNDFQG QLLESSYFEK IESNALRAFI
 KIAPRNGLLY NAIYRNSK

SEQ ID NO: 77 – PreScission Protease Site

LEVLFQGP

20

SEQ ID NO: 78 – C1 Activation Loop Variant 2

CHKAIDGRSLEVLFQGPLYNKTLDC

EXAMPLES**EXAMPLE 1****BoNT/A(0) is Catalytically Inactive *in vitro* and *in vivo***

- 5 Catalytic activity of BoNT/A(0) (SEQ ID NO: 2) was tested in *in vitro* cell-based models, which measure cleavage of SNAP25, the BoNT/A target SNARE protein. Figure 1 shows that, in contrast to wild-type BoNT/A (SEQ ID NO: 60), BoNT/A(0) does not cleave SNAP25 in a human neuronal assay. Figure 2 confirms this result in a rat neuronal assay.
- 10 By way of confirmation, an *in vivo* DAS assay was carried out using BoNT/A and BoNT/A(0). The DAS assay was performed by injection of 20µl of clostridial toxin, formulated in Gelatine Phosphate Buffer, into the mouse gastrocnemius/soleus complex, followed by assessment of Digital Abduction Score using the method of Aoki (Aoki KR, Toxicon 39: 1815-1820; 2001). In the DAS assay, mice were suspended briefly by the tail in order to elicit a characteristic startle
- 15 response (Figure 3A) in which the mouse extends its hind limbs and abducts its hind digits. Following clostridial toxin injection, the varying degrees of digit abduction were scored on a five-point scale (0=normal to 4=maximal reduction in digit abduction— Figure 3B). This provides a functional measure of the paralysis induced by the neurotoxin's activity in the neuro-muscular junction. In addition, bodyweight change was assessed in the mice within seven days of
- 20 administration. This provides a measure of toxicity and the undesired effects of toxin diffusion away from the site of administration. Results are presented in Table 1, below.

Table 1. DAS score (after 24 hours) and bodyweight change following administration of BoNT/A(0) or BoNT/A.

	DAS Score	Bodyweight Change Observed
BoNT/A	4	Yes
BoNT/A(0)	0	No

25 The results confirm that BoNT/A(0) is catalytically inactive *in vivo* and does not result in any symptoms of toxicity. Thus, BoNT/A(0) is a safe, substantially non-toxic therapeutic.

EXAMPLE 2

30 **Treatment of Chronic Neuropathic Pain (Chronic Constriction Injury (CCI) Rat Model) Using Catalytically Inactive BoNT**

Materials & Methods

The chronic constriction injury (CCI) was performed as previously described by Bennett and Xie (1988), Pain, 33(1):87-107. On day – 14, adult, male Sprague-Dawley rats (220-250 g)

were anesthetized before a segment of the left sciatic nerve was exposed and four loose ligations of the silk suture were placed on the nerve. On day 0 (D0) the rats were injected with either BoNT/A (30 pg/kg), BoNT/A (60 pg/kg), BoNT/A(0) (60 pg/kg) or vehicle (GPB) administered via intraplantar (i.pl.) route (n=10-11/group), whereas a positive control, gabapentin (100 mg/kg) was administered via oral (p.o.) route (n=8/group). Animals treated with gabapentin were tested 1, 2 and 4 h after treatment. BoNT/A, BoNT/A(0) or vehicle-treated animals were tested on days 3, 5, and 9. Animals were evaluated for mechanical sensitivity in the von Frey test.

Results

The experiments showed that by administering catalytically inactive BoNT (BoNT/A(0)), mechanical sensitivity in the ipsilateral paw was reduced (Figure 4B). Moreover, BoNT/A(0) was more effective at reducing mechanical sensitivity when compared to equivalently dosed BoNT/A. At later time points, BoNT/A(0) was also more effective than gabapentin. To confirm that the reduced sensitivity was as a result of BoNT/A(0) administration, mechanical sensitivity of the contralateral paw was also tested. Results showed that differences in mechanical sensitivity of the contralateral paw among conditions were non-significant (Figure 4C). Furthermore, as confirmation of the substantial non-toxicity of BoNT/A(0), bodyweight change over time was equivalent to that of rats administered vehicle or gabapentin (Figure 4D). This is in contrast to the change observed in rats administered catalytically active BoNT/A, which was statistically significantly different at day 9.

In conclusion, catalytically inactive clostridial neurotoxins are surprisingly capable of reducing pain (e.g. chronic neuropathic pain), thereby suggesting that such neurotoxins are suitable pain therapeutics.

EXAMPLE 3

Treatment of Acute Neuropathic Pain (Oxaliplatin Rat Model) Using Catalytically Inactive BoNT

Materials & Methods

The experimental model of oxaliplatin-induced peripheral sensory neuropathy was induced by intraperitoneal injection of oxaliplatin (Ling *et al* (2007), Pain, 128(3):225-234; Ling *et al* (2007), Toxicology, 20;234(3):176-84). On day 0, adult, male Sprague-Dawley rats (100-133 g) received an i.p. injection of a sham-treatment (5% glucose) or oxaliplatin (10 mg/kg). Immediately after, sham-treated animals received an i.pl. injection of vehicle, whereas oxaliplatin-treated animals received an i.pl. injection of BoNT/A(0) (1000 pg/kg), BoNT/A (50

pg/kg), BoNT/A (100 pg/kg), BoNT/A (160 pg/kg) or vehicle (GPB; n=10/group). On D3 a positive control, duloxetine (100 mg/kg) was given via p.o. route. Animals were evaluated for thermal (cold) sensitivity on D3 and D5.

5 Results

The experiments showed that by administering catalytically inactive BoNT (BoNT/A(0)) cold sensitivity in the ipsilateral paw was reduced (Figure 5B). There was no difference in thermal sensitivity of the contralateral paw across the groups treated with BoNT/A, BoNT/A(0) or vehicle (Figure 5C).

10

In conclusion, catalytically inactive clostridial neurotoxins are surprisingly capable of reducing acute neuropathic pain, thereby suggesting that such neurotoxins have generic application for the treatment of pain.

15 EXAMPLE 4

Treatment of Chronic Neuropathic Pain (Oxaliplatin Rat Model) Using Catalytically Inactive BoNT

Materials & Methods

On day -2 (D-2) adult, male Sprague-Dawley rats (180 – 210 g) received i.p. injection of oxaliplatin (10 mg/kg), before being treated with BoNT/A (100 pg/kg), BoNT/A(0) (100 pg/kg) or vehicle (GPB) via i.pl. route on day 0 (n=11-12/group). A positive control, pregabalin was administered on D3 (n=12). Animals were tested for mechanical sensitivity (von Frey test) and thermal (cold) sensitivity (cold plate test) on days 3, 6 and 9.

25 Results

The experiments showed that by administering catalytically inactive BoNT (BoNT/A(0)) mechanical sensitivity in the ipsilateral paw (Figure 6B) and cold sensitivity (Figure 6D) was reduced.

30 In conclusion, catalytically inactive clostridial neurotoxins are surprisingly capable of reducing chronic neuropathic pain in a different chemotherapy-induced pain model.

EXAMPLE 5**Treatment of Inflammatory Pain (UV-B Burn Rat Model) Using Catalytically Inactive BoNT****Materials & Methods**

5 In humans, as well as in a rodent model ultraviolet (UV)-B radiation results in both mechanical and thermal hyperalgesia. Adult, male Wistar rats (180-210g) were administered BoNT/A (100 pg/kg), BoNT/A(0) (100 pg/kg) or vehicle (GPB; n=12/group) by i.pl. injection. 24 hours later, the plantar surface of the ipsilateral paw was exposed to ultraviolet-B (UVB) irradiation for approximately 5 min, receiving a dose of 500 mJ/cm². 48 hours after UVB and 72 hours after
10 BoNT/A, BoNT/A(0) or vehicle injection, animals were tested for mechanical sensitivity in the von Frey test. An additional group of UVB-exposed animals were injected with a positive control, indomethacin 48 hours later and tested in the von Frey test 1 hour after the injection (n=12/group).

Results

The experiments showed that by administering catalytically inactive BoNT (BoNT/A(0)), mechanical sensitivity was reduced (Figure 7B). In conclusion, catalytically inactive clostridial neurotoxins are surprisingly capable of reducing inflammatory pain (e.g. acute inflammatory pain), thereby confirming that such neurotoxins have generic application for the treatment of
20 pain.

The surprising finding that a catalytically inactive clostridial neurotoxin reduced inflammatory pain indicated that it finds utility in treating the underlying inflammatory conditions (e.g. including treating at least one symptom of the inflammatory condition, i.e. associated pain).
25 Thus, it was considered credible that catalytically inactive clostridial neurotoxin can be used to treat inflammatory conditions.

EXAMPLE 6**Treatment of Inflammatory Pain (CFA-Induced Inflammatory Pain Model) Using a Catalytically Inactive Chimeric BoNT****Materials & Methods**

Prior to BoNT or vehicle dosing, paw withdrawal threshold (PWT, g) of 70 adult male C57/BL6 mice (22-26g) was assessed on 3 consecutive days using graduated von Frey filaments of increasing force. The mean of the last 2 days was considered as the baseline. On Day 0, under
35 gas anesthesia, BoNT/XB (0.3 and 30 ng/kg), BoNT/XB(0) (0.3 and 30 ng/kg), BoNT/A (160 pg/kg) or vehicle (840 µl/kg) were injected into the intraplantar footpad of the left hindpaw

(n=10/group). On day 2, prior to CFA injection, PWT was reassessed. Then under isoflurane anesthesia, a fixed 20 μ L volume of CFA (1.5 mg/mL) was injected into the same hindpaw. On day 3 (day 1 post CFA), 1 hour prior to PWT assessment, animals allocated to the indomethacin group were orally dosed with indomethacin (10 mg/kg, n=9).

5

Results

The experiments showed that a catalytically inactive chimeric BoNT (BoNT/XB(0)) comprising a catalytically inactive BoNT/X L-chain and translocation domain (BoNT/X LH_N) and a BoNT/B receptor binding domain BoNT/X (H_C domain) was effective at treating inflammatory pain. In more detail, Figure 8 shows that mechanical sensitivity following CFA-induction of inflammatory pain was reduced in mice administered catalytically active BoNT/XB and catalytically inactive BoNT/XB(0) at a dose of 30 ng/kg. The reduction in sensitivity was equivalent to that of BoNT/A or positive control indomethacin.

10

15

The surprising finding that BoNT/XB(0) reduced inflammatory pain indicated that it finds utility in treating the underlying inflammatory conditions (e.g. including treating at least one symptom of the inflammatory condition, i.e. associated pain). Thus, it was considered further evidence of the credibility of catalytically inactive clostridial neurotoxin for use in treating inflammatory conditions.

20

EXAMPLE 7

Treatment of Atopic Dermatitis Using a Catalytically Inactive Chimeric BoNT

Vehicle or BoNT/XB(0) (40 pg/mouse, 100 pg/mouse or 400 pg/mouse) is administered subcutaneously on the medial part of the back of adult C57/BL6 mice one day prior to exposure to Calcipotriol. Mice are then treated with calcipotriol on 5 consecutive days. At the end of the study, animals are euthanized, the skin of the back is collected, fixed and treated for histological analysis. After Hematoxylin and eosin staining, the epidermal thickness is evaluated. Immunolabelling is performed to evidence CD45+ cells .

25

30

The experiment shows that a catalytically inactive chimeric BoNT (BoNT/XB(0)) comprising a catalytically inactive BoNT/X L-chain and translocation domain (BoNT/X LH_N) and a BoNT/B receptor binding domain BoNT/X (H_C domain) is effective at treating atopic dermatitis, a model inflammatory condition. The results show an improvement in dermal thickness following administration of BoNT/XB(0). Dermal thickness is indicative of fibrosis, an inflammatory response to calcipotriol and is shown to be statistically-significant reduced in the BoNT/XB(0) treated animals. Moreover, the anti-inflammatory effect of BoNT/XB(0) is confirmed by a

35

reduction in the number of CD45-positive cells in the BoNT/XB(0) treated animals (CD45 transduces activation signals in inflammatory cells).

Thus, it is concluded that, BoNT/XB(0) has anti-inflammatory properties, thus finding utility in
5 treating inflammatory disorders.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit
10 of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the
15 following claims.

CLAIMS

1. A polypeptide for use in treating pain, wherein the polypeptide comprises a clostridial
5 neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain)
and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the
polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.
2. A method for treating pain, the method comprising administering a polypeptide to a
10 subject, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a
clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin
receptor binding domain (H_C domain), wherein when the polypeptide comprises a clostridial
neurotoxin L-chain, the L-chain is catalytically inactive.
- 15 3. Use of a polypeptide in the manufacture of a medicament for treating pain, wherein the
polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin
translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain
(H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the
L-chain is catalytically inactive.
20
4. The polypeptide for use according to claim 1, the method according to claim 2 or the
use according to claim 3, wherein the polypeptide does not treat pain by any of the
following: by promoting neuronal growth, by promoting neuronal repair, or by promoting
neuronal growth and repair.
25
5. A polypeptide for use in treating an inflammatory disorder, wherein the polypeptide
comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin
translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain
(H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the
30 L-chain is catalytically inactive.
6. A method for treating an inflammatory disorder, the method comprising administering
a polypeptide to a subject, wherein the polypeptide comprises a clostridial neurotoxin light-
chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a
35 clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide
comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

7. Use of a polypeptide in the manufacture of a medicament for treating an inflammatory disorder, wherein the polypeptide comprises a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.
8. The polypeptide for use according to claim 5, the method according to claim 6 or the use according to claim 7, wherein the polypeptide does not treat the inflammatory condition by any of the following: by promoting neuronal growth, by promoting neuronal repair, or by promoting neuronal growth and repair.
9. The polypeptide for use, the method or the use according to any one of the preceding claims, wherein the polypeptide does not comprise a further catalytically active domain.
10. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide does not comprise a therapeutic or diagnostic agent (e.g. a covalently or non-covalently associated therapeutic or diagnostic agent) additional to the clostridial neurotoxin L-chain, H_N domain and/or H_C domain.
11. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide is not administered (e.g. sequentially or subsequently) with a further therapeutic or diagnostic agent.
12. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide comprises a catalytically inactive clostridial neurotoxin L-chain.
13. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide comprises a clostridial neurotoxin L-chain, H_N domain and an H_C domain, wherein the L-chain is catalytically inactive.
14. The polypeptide for use, method or use according to any one of claims 1-12, wherein the polypeptide consists essentially of a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises or consists essentially of a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

15. The polypeptide for use, method or use according to any one of claims 1-12 or 14, wherein the polypeptide consists essentially of a clostridial neurotoxin light-chain (L-chain) and a clostridial neurotoxin translocation domain (H_N domain), wherein the L-chain is catalytically inactive.
16. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide consists essentially of a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and a clostridial neurotoxin receptor binding domain (H_C domain), wherein the L-chain is catalytically inactive.
17. The polypeptide for use, method or use according to any one of claims 1-12 or 14, wherein the polypeptide consists of a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and/or a clostridial neurotoxin receptor binding domain (H_C domain), wherein when the polypeptide comprises or consists of a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.
18. The polypeptide for use, method or use according to any one of claims 1-12, 15 or 17, wherein the polypeptide consists of a clostridial neurotoxin light-chain (L-chain) and a clostridial neurotoxin translocation domain (H_N domain), wherein the L-chain is catalytically inactive.
19. The polypeptide for use, method or use according to any one of claims 1-13, 14, 16 or 17, wherein the polypeptide consists of a clostridial neurotoxin light-chain (L-chain), a clostridial neurotoxin translocation domain (H_N domain) and a clostridial neurotoxin receptor binding domain (H_C domain), wherein the L-chain is catalytically inactive.
20. The polypeptide for use, method or use according to any one of claims 1-12, 14, 15, 17 or 18, wherein the polypeptide does not comprise both a clostridial neurotoxin H_N domain and H_C domain.
21. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide does not further comprise a non-clostridial catalytic domain.
22. The polypeptide for use, method or use according to any one of the preceding claims, wherein the pain is chronic pain.

23. The polypeptide for use, method or use according to any one of claims 1-21, wherein the pain is acute pain.
24. The polypeptide for use, method or use according to any one of the preceding claims,
5 wherein the pain is inflammatory pain.
25. The polypeptide for use, method or use according to claim 24, wherein the inflammatory pain is caused by or associated with sunburn, UV-induced damage, an arthritic disorder, an autoimmune disease, a connective tissue disorder, an injury, an infection, neuritis, joint
10 inflammation or a headache (preferably a muscular/myogenic headache, a vascular headache, a high blood pressure headache, a hormone headache, a rebound headache, a chronic sinusitis headache, an organic headache, or an ictal headache).
26. The polypeptide for use, method or use according to any one of claims 1-23, wherein
15 the pain is neuropathic pain.
27. The polypeptide for use, method or use according to claim 26, wherein the neuropathic pain is (or is caused by or associated with) neuralgia, deafferentation, a complex regional pain syndrome (CRPS), or a neuropathy (e.g. a central or peripheral neuropathy).
20
28. The polypeptide for use, method or use according to any one of claims 1-23, wherein the pain is mixed pain.
29. The polypeptide for use, method or use according to any one of claims 1-23, wherein
25 the pain is allodynia.
30. The polypeptide for use, method or use according to any one of claims 1-23, wherein the pain is visceral pain.
- 30 31. The polypeptide for use, method or use according to claim 30, wherein the visceral pain is (or is caused by or associated with) functional visceral pain, chronic gastrointestinal inflammation, autoimmune pain, organic visceral pain, or treatment-induced visceral pain.
32. The polypeptide for use, method or use according to any one of claims 1-23, wherein
35 the pain is headache pain (e.g. a migraine).

33. The polypeptide for use, method or use according to claim 32, wherein the pain is migraine pain.

34. The polypeptide for use, method or use according to claim 32, wherein the headache
5 pain is caused by or associated with a muscular/myogenic headache, a vascular headache, a high blood pressure headache, a hormone headache, a rebound headache, a chronic sinusitis headache, an organic headache, or an ictal headache.

35. The polypeptide for use, method or use according to any one of claims 1-23, wherein
10 the pain is post-operative pain.

36. The polypeptide for use, method or use according to any one of claims 1-23, wherein the pain is referred pain.

37. The polypeptide for use, method or use according to any one of claims 1-23, wherein
15 the pain is somatic pain.

38. The polypeptide for use, method or use according to claim 37, wherein the pain is
20 somatic pain caused by or associated with excessive muscle tension, a repetitive motion disorder, a muscle disorder, myalgia, an infection, or drugs.

39. The polypeptide for use, method or use according to any one of claims 1-23, wherein
the pain is bladder pain syndrome, preferably wherein the bladder pain is caused by or
associated with interstitial cystitis.

40. The polypeptide for use, method or use according to any one of claims 1-23, wherein
the pain is phantom limb pain.

41. The polypeptide for use, method or use according to any one of claims 5-21, wherein
30 the inflammatory disorder is one or more selected from: cystitis, endometriosis, rheumatoid arthritis, complex regional pain syndrome, and neuritis.

42. The polypeptide for use, method or use according to claim 41, wherein the cystitis is
interstitial cystitis.

43. The polypeptide for use, method or use according to claim 41, wherein the neuritis is peripheral neuritis.

44. The polypeptide for use, method or use according to any one of the preceding claims,
5 wherein a single dose of the polypeptide administered is greater than 250 µg.

45. The polypeptide for use, method or use according to any one of the preceding claims,
wherein a single dose of the polypeptide administered is 251 µg to 10 g.

10 46. The polypeptide for use, method or use according to any one of the preceding claims,
wherein a single dose of the polypeptide administered is 251 µg to 1 g.

47. The polypeptide for use, method or use according to any one of the preceding claims,
wherein a single dose of the polypeptide administered is 251-1000 µg.

15 48. The polypeptide for use, method or use according to any one of the preceding claims,
wherein the polypeptide is administered iteratively (e.g. as part of a pain treatment
regimen).

20 49. The polypeptide for use, method or use according to any one of the preceding claims,
wherein the polypeptide is administered intradermally.

50. The polypeptide for use, method or use according to any one of the preceding claims,
wherein the polypeptide comprises a polypeptide sequence having at least 70% sequence
25 identity to any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32,
34, 36, 38, 40, 42, 44, 46, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65,
66, 67, 68, 69, 70, 74, 75 or 76 with the proviso that when the polypeptide comprises a
clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

30 51. The polypeptide for use, method or use according to any one of the preceding claims,
wherein the polypeptide comprises a polypeptide sequence having at least 80% sequence
identity to any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32,
34, 36, 38, 40, 42, 44, 46, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65,
66, 67, 68, 69, 70, 74, 75 or 76 with the proviso that when the polypeptide comprises a
35 clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

52. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide comprises a polypeptide sequence having at least 90% sequence identity to any one of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 74, 75 or 76 with the proviso that when the polypeptide comprises a clostridial neurotoxin L-chain, the L-chain is catalytically inactive.

53. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide comprises a polypeptide sequence of any one of SEQ ID NOs: 2, 8, 10, 12, 14, 16, 18, 22, 26, 30, 34, 38, 42, 44, 46, 48, 50, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 74, 75 or 76.

54. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide is a catalytically inactive BoNT/A.

55. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide is a modified clostridial neurotoxin, such as a chimeric clostridial neurotoxin or a hybrid clostridial neurotoxin, preferably wherein the polypeptide does not comprise a native clostridial neurotoxin H-chain.

56. The polypeptide for use, method or use according to any one of claims 1-53 or 55, wherein the polypeptide lacks a functional H_{CC} domain or H_C domain of a clostridial neurotoxin.

57. The polypeptide for use, method or use according to any one of claims 1-53 or 55-56, wherein the polypeptide is a retargeted clostridial neurotoxin comprising a non-clostridial Targeting Moiety (TM).

58. The polypeptide for use, method or use according to any one of claims 1-53 or 55-56, wherein the polypeptide lacks a functional H_C domain of a clostridial neurotoxin and also lacks any functionally equivalent exogenous ligand Targeting Moiety (TM).

59. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide is not expressed in a cell of the subject, e.g. wherein the use or method does not comprise expressing a nucleic acid encoding the polypeptide in a cell of the subject.

60. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide further comprises one or more non-clostridial neurotoxin sequences.

61. The polypeptide for use, method or use according to claim 60, wherein the one or more non-clostridial neurotoxin sequence(s) do(es) not bind to a cellular receptor.

62. The polypeptide for use, method or use according to claim 60 or 61, wherein the one or more non-clostridial neurotoxin sequence(s) do(es) not comprise a ligand for a cellular receptor.

63. The polypeptide for use, method or use according to any one of claims 1-53, 55 or 59-62, wherein the polypeptide is a chimeric botulinum neurotoxin (BoNT) comprising a catalytically inactive BoNT/A light-chain and translocation domain, and a BoNT/B receptor binding domain (H_C domain).

64. The polypeptide for use, method or use according to any one of claims 1-55 or 59-62, wherein the polypeptide comprises a modified BoNT/A H_C domain comprising a modification at one or more amino acid residue(s) selected from: ASN 886, ASN 905, GLN 915, ASN 918, GLU 920, ASN 930, ASN 954, SER 955, GLN 991, GLU 992, GLN 995, ASN 1006, ASN 1025, ASN 1026, ASN 1032, ASN 1043, ASN 1046, ASN 1052, ASP 1058, HIS 1064, ASN 1080, GLU 1081, GLU 1083, ASP 1086, ASN 1188, ASP 1213, GLY 1215, ASN 1216, GLN 1229, ASN 1242, ASN 1243, SER 1274, and THR 1277, wherein the modification is selected from:

- i. substitution of an acidic surface exposed amino acid residue with a basic amino acid residue;
- ii. substitution of an acidic surface exposed amino acid residue with an uncharged amino acid residue;
- iii. substitution of an uncharged surface exposed amino acid residue with a basic amino acid residue;
- iv. insertion of a basic amino acid residue; and
- v. deletion of an acidic surface exposed amino acid residue.

65. The polypeptide for use, method or use according to any one of claims 1-53 or 55-62, wherein the polypeptide comprises a catalytically inactive botulinum neurotoxin serotype X (BoNT/X) L-chain, a BoNT/X H_N domain, and/or a BoNT/X H_C domain.

66. The polypeptide for use, method or use according to any one of claims 1-53, 59-62 or 65, wherein the polypeptide is a chimeric botulinum neurotoxin (BoNT) comprising a catalytically inactive BoNT/X light-chain and translocation domain, and a receptor binding domain (H_C domain) from a different (i.e. non-BoNT/X) clostridial neurotoxin.
67. The polypeptide for use, method or use according to any one of claims 1-53, 59-62 or 65-66, wherein the polypeptide is a chimeric botulinum neurotoxin (BoNT) comprising a catalytically inactive BoNT/X light-chain and translocation domain, and a BoNT/B receptor binding domain (H_C domain).
68. The polypeptide for use, method or use according to any one of claims 65-67, wherein the pain is inflammatory pain.
69. The polypeptide for use, method or use according to any one of the preceding claims, wherein the polypeptide comprises Cys-(Xaa)_a-Ile-Asp/Glu-Gly-Arg-(Yaa)_b-Cys (SEQ ID NO: 71), wherein a = 1-10 and b = 4-15.

FIGURE 1

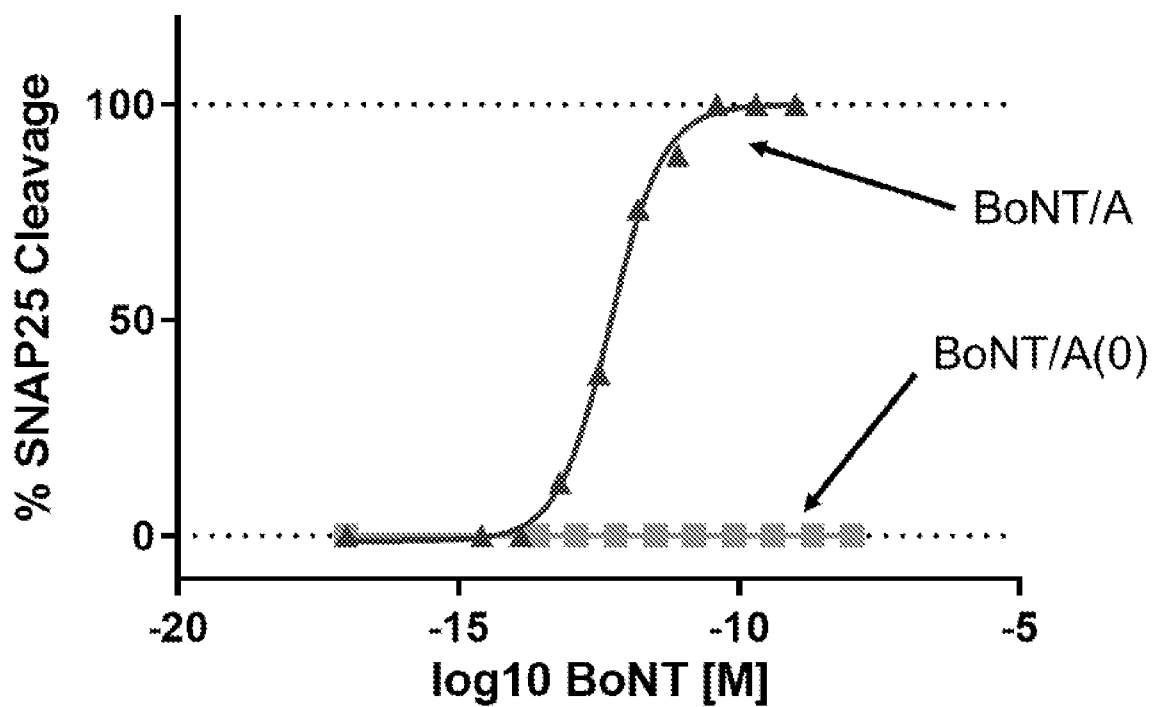
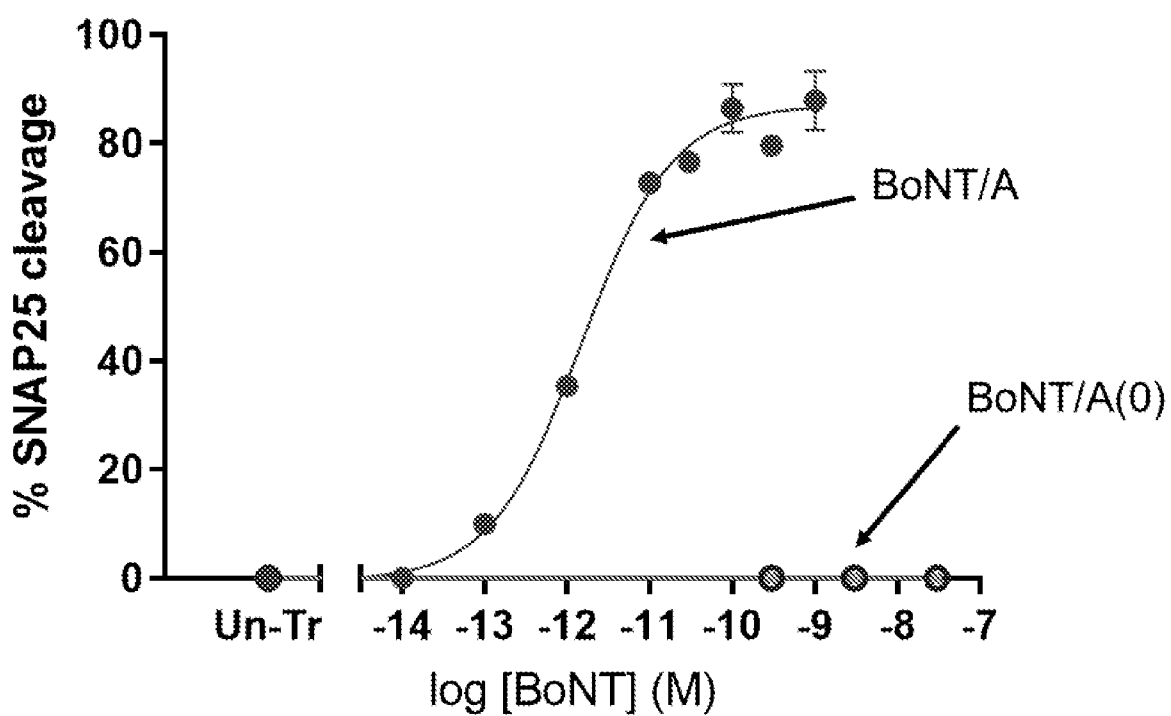
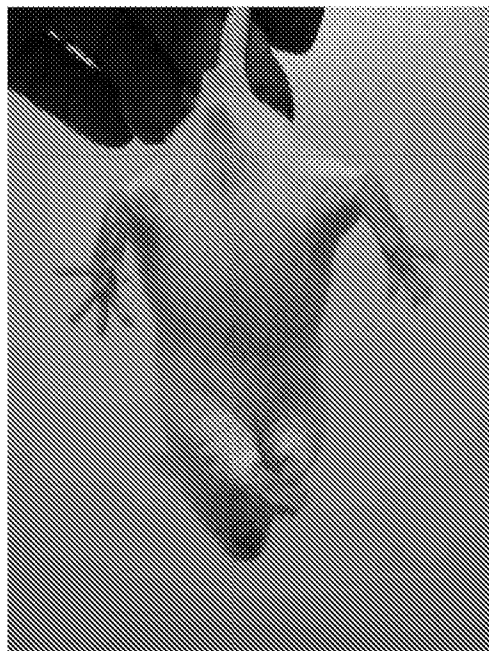
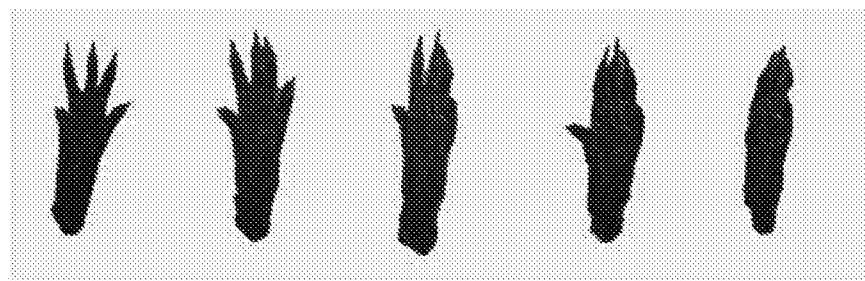


FIGURE 2



2 / 16

FIGURE 3

A**B**

DAS 0 DAS 1 DAS 2 DAS 3 DAS 4

FIGURE 4

A

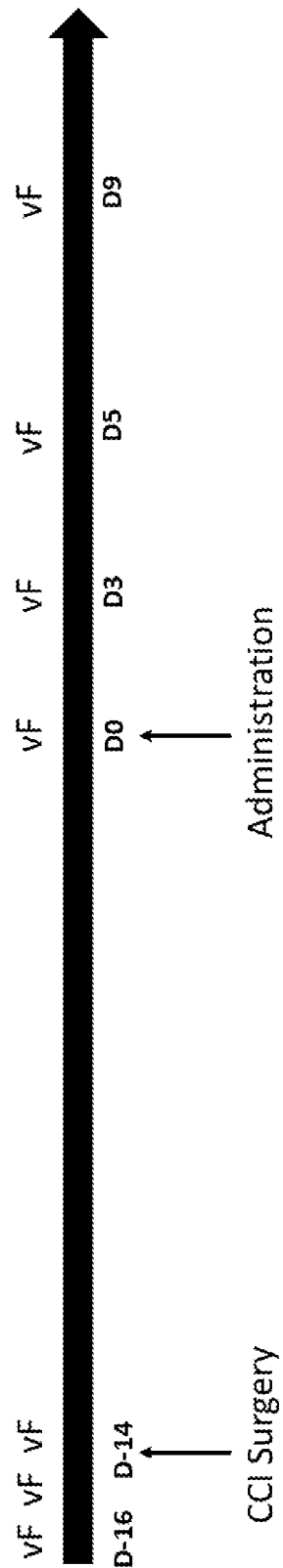


FIGURE 4 CONTINUED

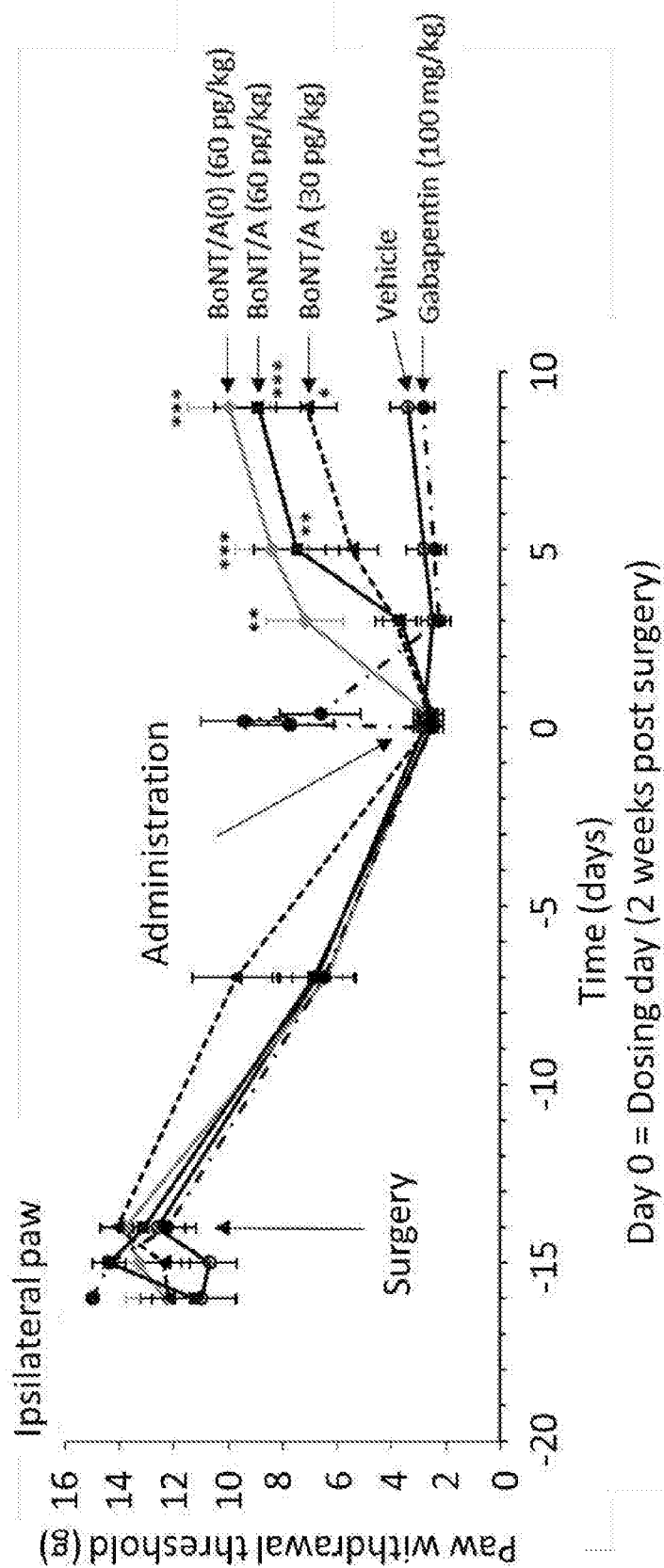
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FIGURE 4 CONTINUED

C

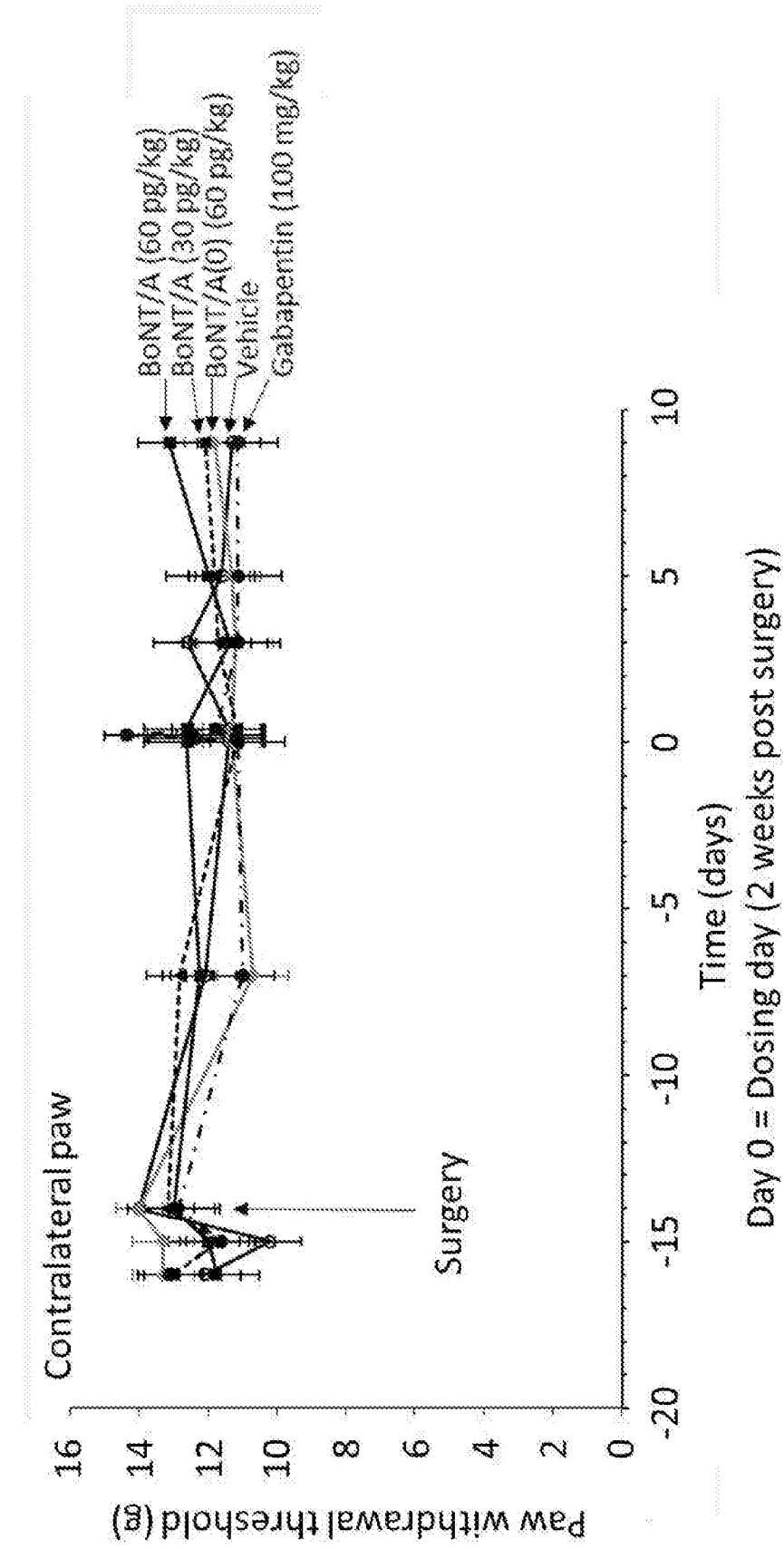


FIGURE 4 CONTINUED

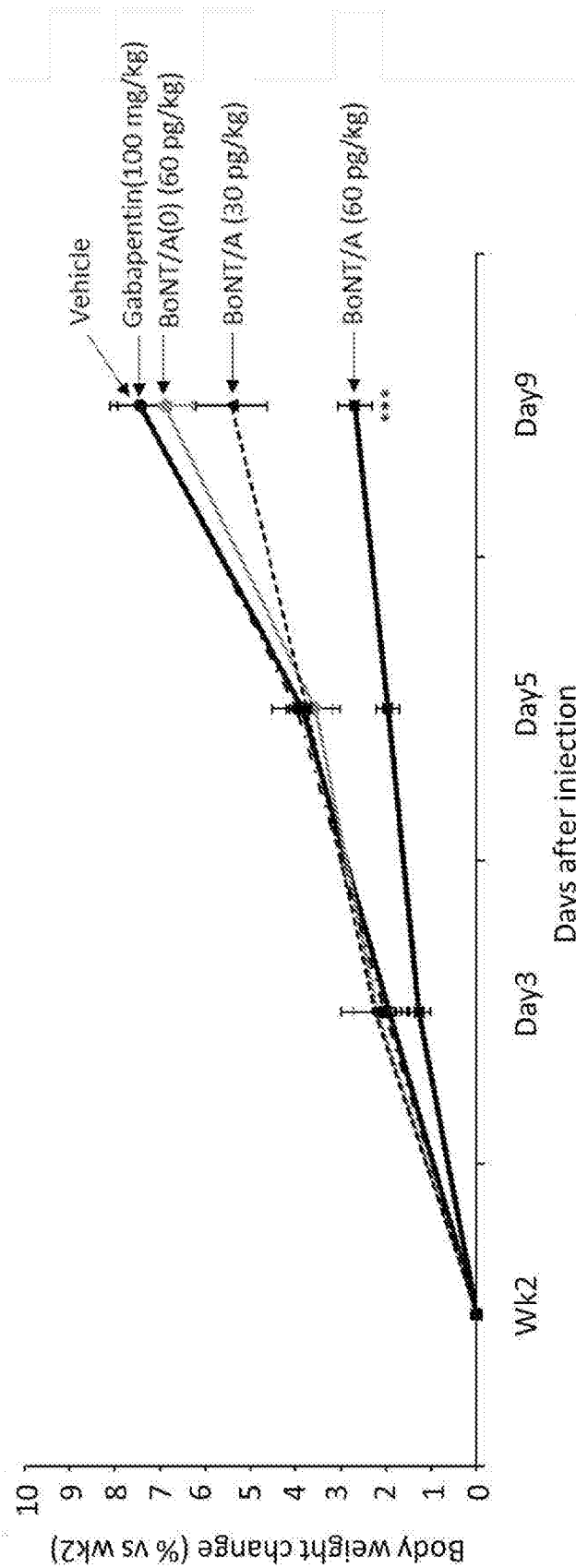
D

FIGURE 5

A



FIGURE 5 CONTINUED

B

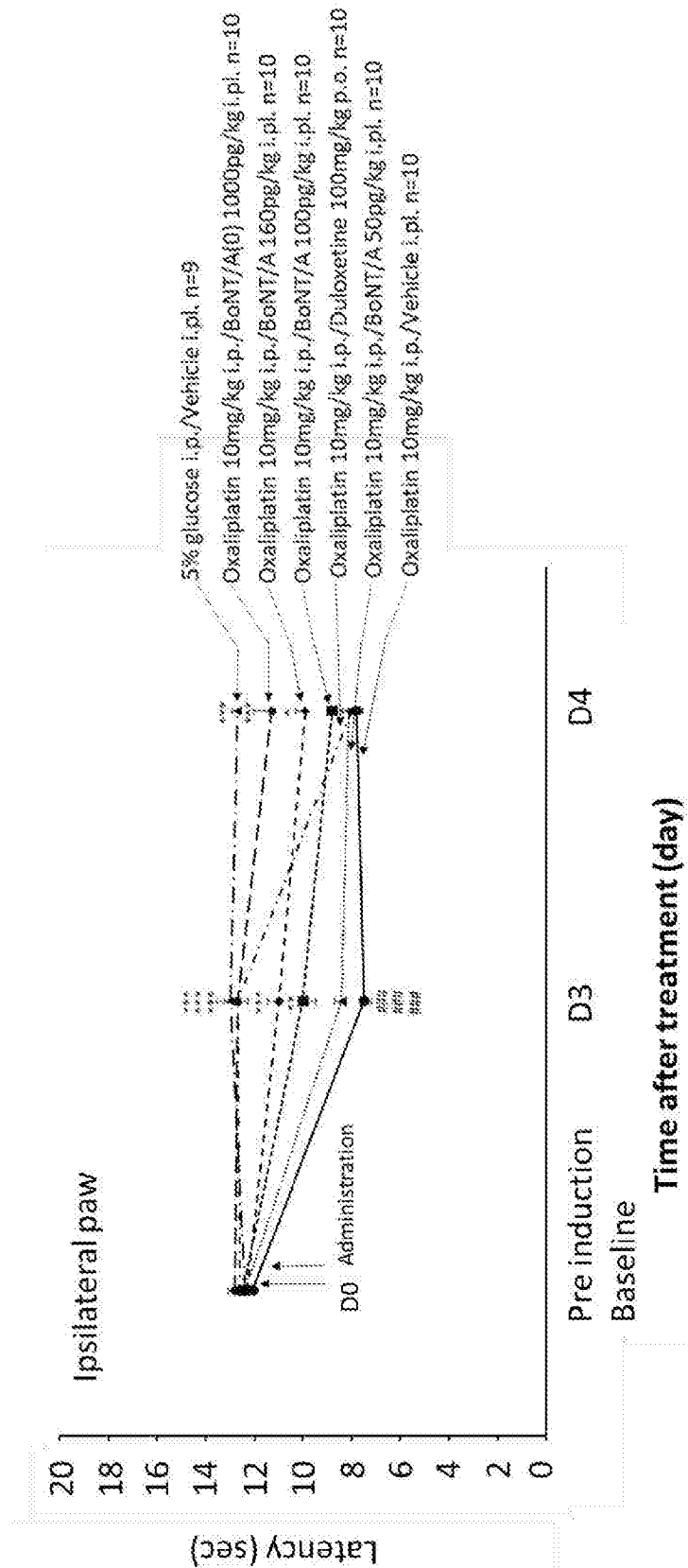


FIGURE 5 CONTINUED
C

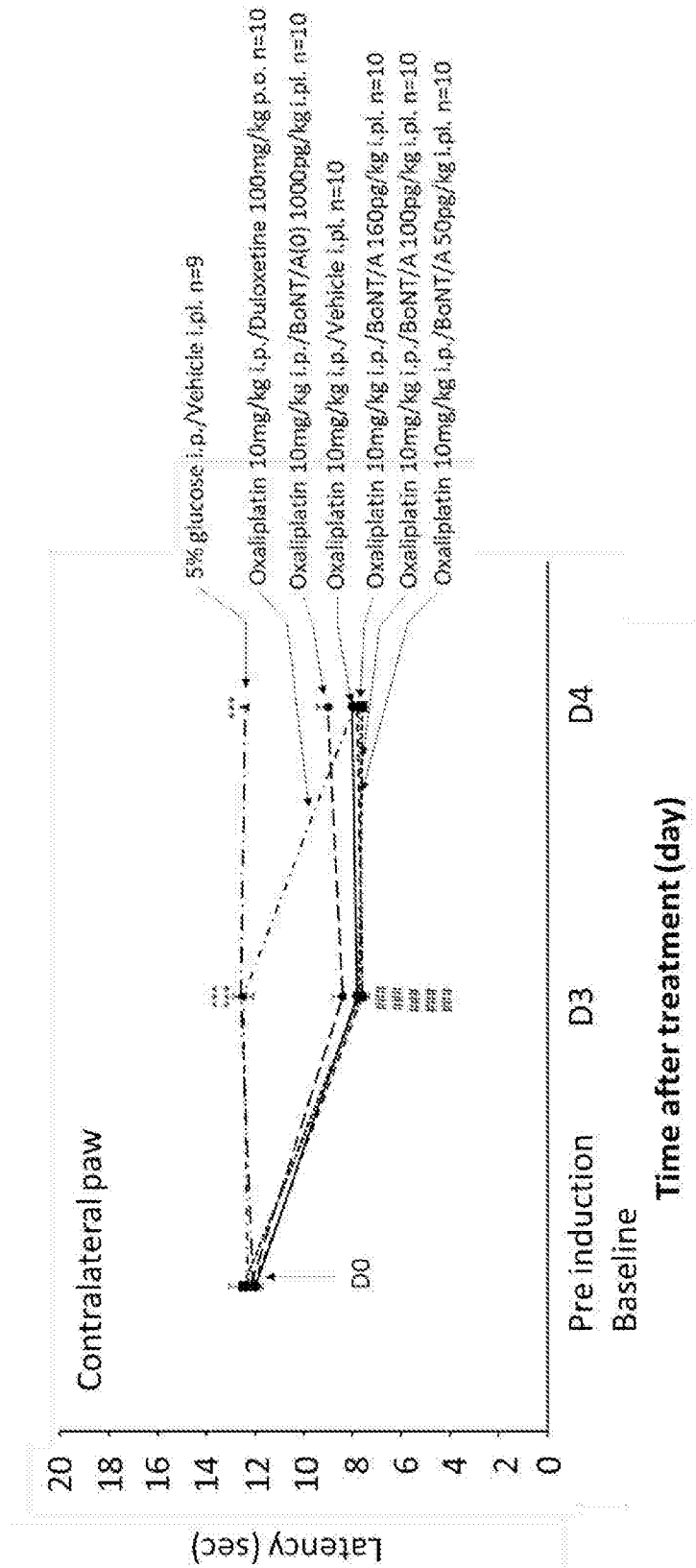


FIGURE 6

A

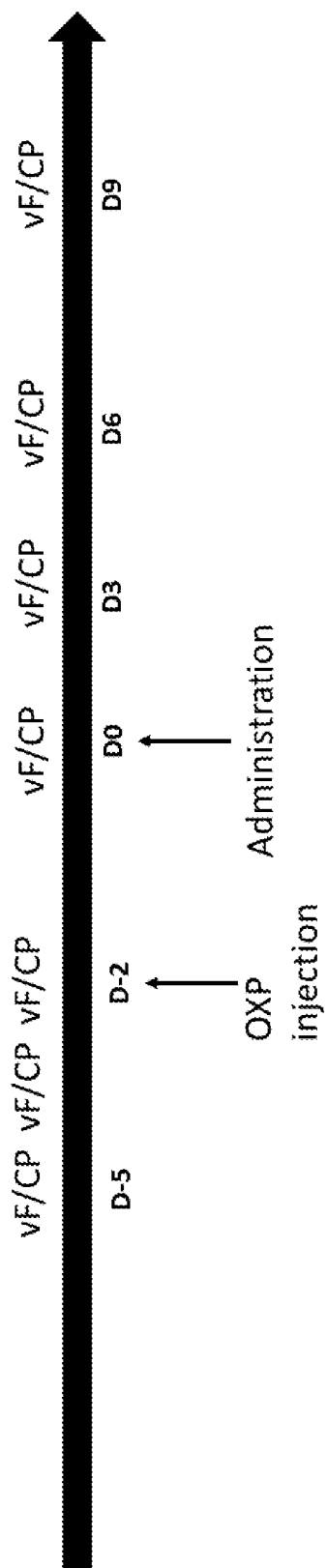


FIGURE 6 CONTINUED

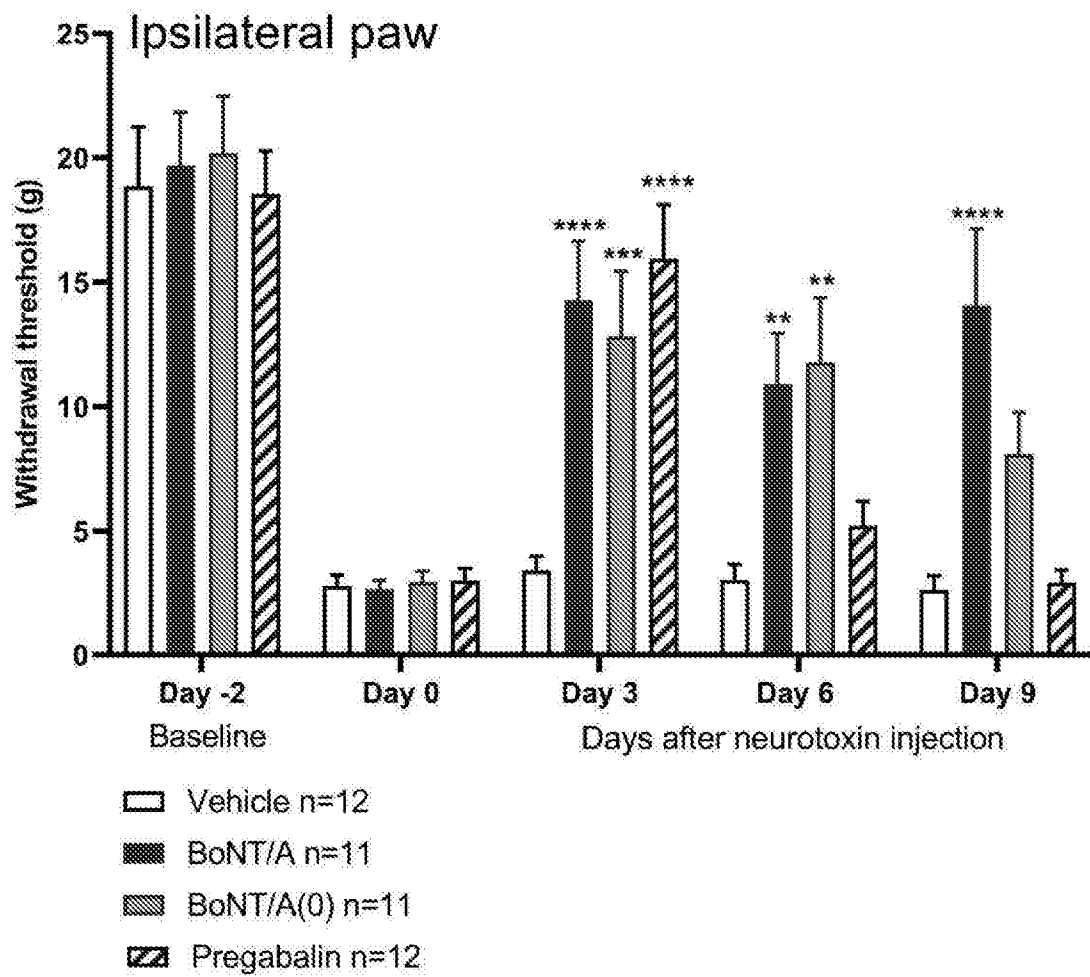
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FIGURE 6 CONTINUED

C

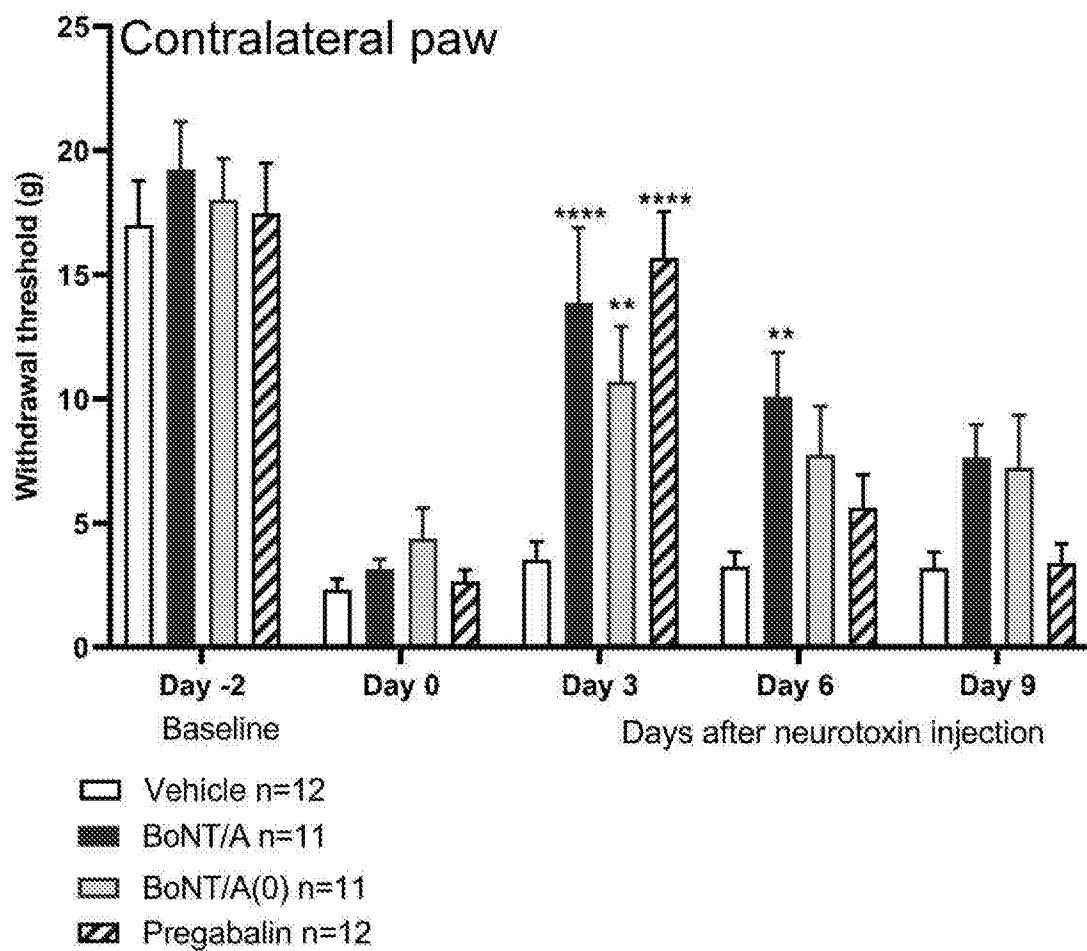


FIGURE 6 CONTINUED

D

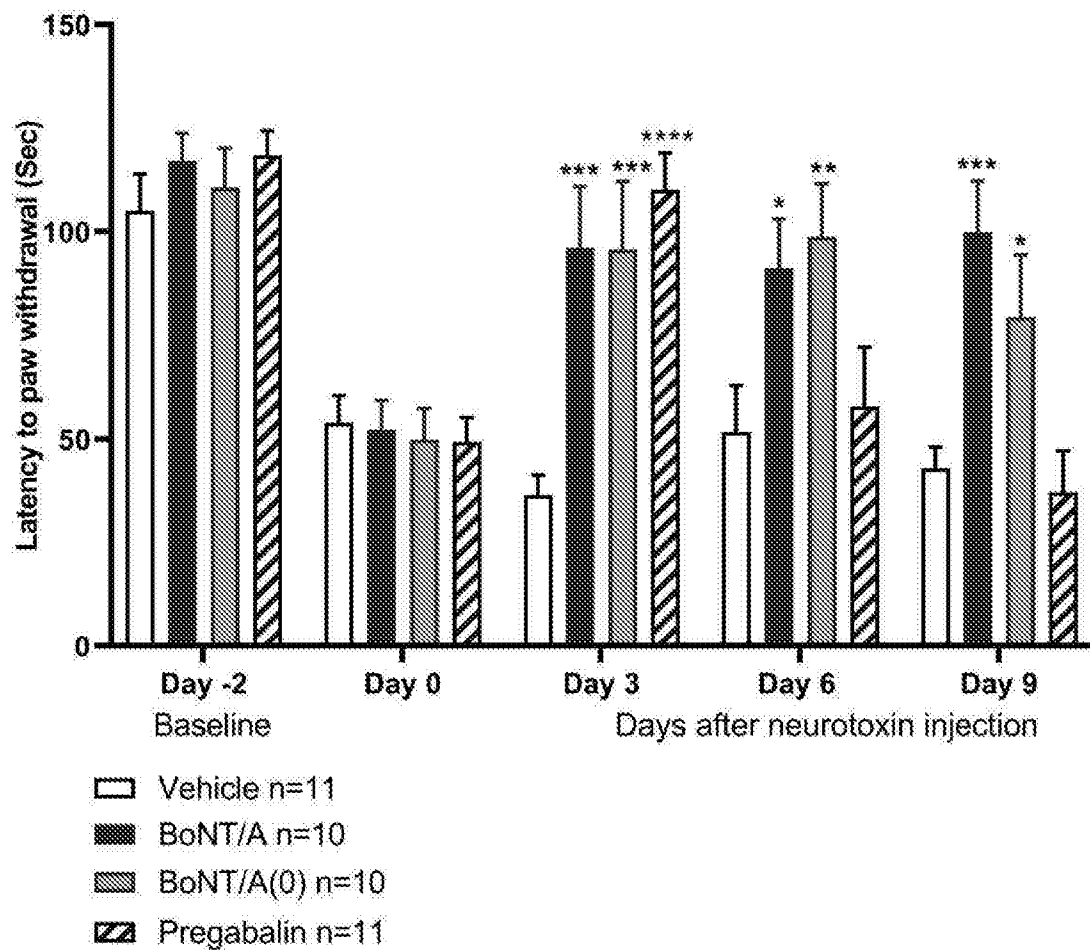


FIGURE 7

A

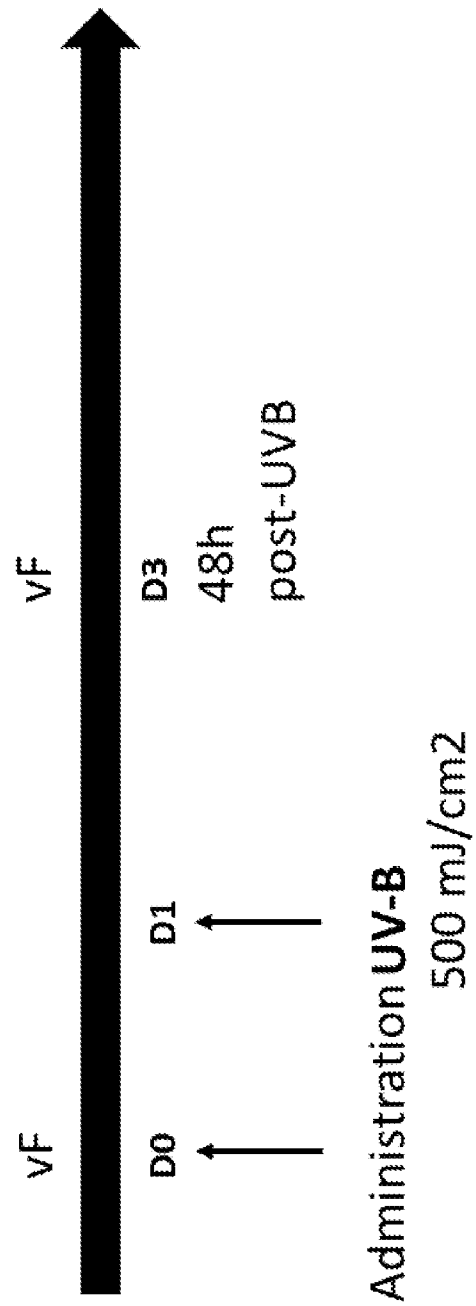


FIGURE 7 CONTINUED

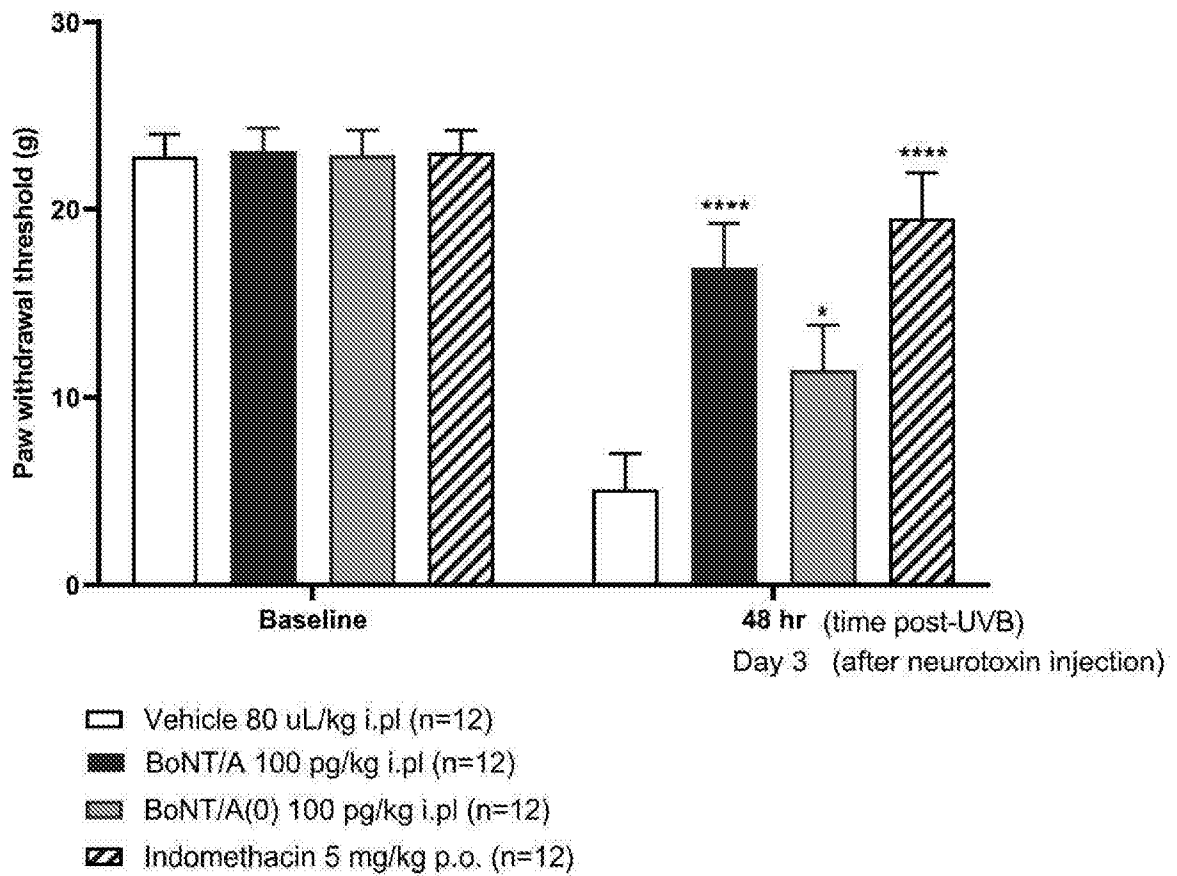
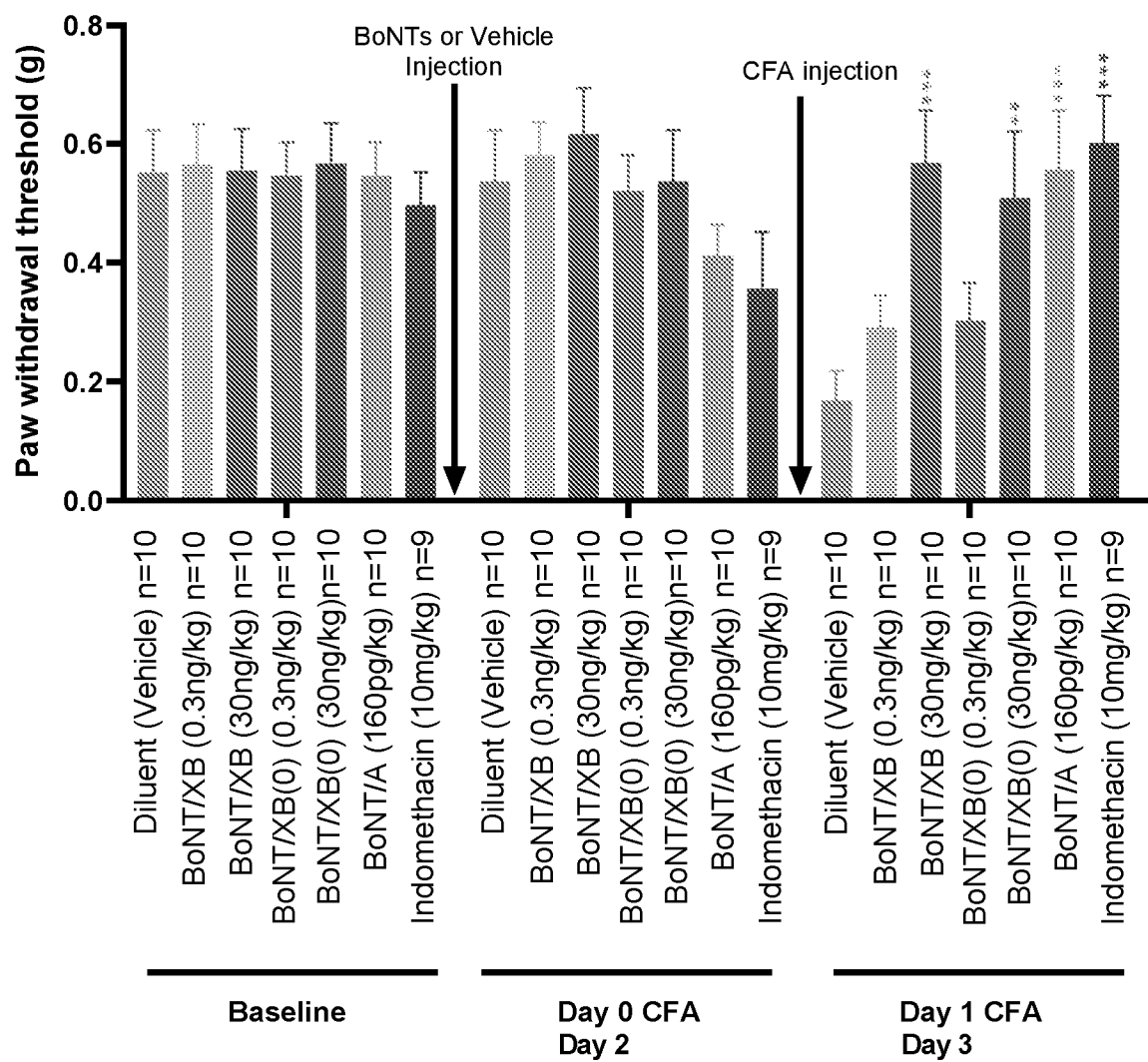
B

FIGURE 8



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2021/050783

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K38/48 A61P29/00 C07K14/33 C12N9/52 ADD.																				
According to International Patent Classification (IPC) or to both national classification and IPC																				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K A61P C12R C12N C07K																				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched																				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, BIOSIS, EMBASE, WPI Data																				
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>E</td> <td> WO 2021/064369 A1 (IPSEN BIOPHARM LTD [GB]) 8 April 2021 (2021-04-08) the whole document </td> <td> 1-4, 9-21,26, 27, 49-56, 58-67 </td> </tr> <tr> <td>X</td> <td> WO 2020/219358 A2 (CHILDRENS MEDICAL CENTER [US]) 29 October 2020 (2020-10-29) </td> <td> 1-4,9, 12-21, 32,48, 49,59, 65-67 </td> </tr> <tr> <td>Y</td> <td> see claims and page 85 </td> <td> 17,20,65 </td> </tr> <tr> <td>Y</td> <td> WO 2005/048949 A2 (ALLERGAN INC [US]; LI SHENGWEN [US]; AOKI KEI ROGER [US]) 2 June 2005 (2005-06-02) see claims and example 8 </td> <td> 17,20,65 </td> </tr> <tr> <td colspan="2" style="text-align: center;">-/-</td> <td></td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	E	WO 2021/064369 A1 (IPSEN BIOPHARM LTD [GB]) 8 April 2021 (2021-04-08) the whole document	1-4, 9-21,26, 27, 49-56, 58-67	X	WO 2020/219358 A2 (CHILDRENS MEDICAL CENTER [US]) 29 October 2020 (2020-10-29)	1-4,9, 12-21, 32,48, 49,59, 65-67	Y	see claims and page 85	17,20,65	Y	WO 2005/048949 A2 (ALLERGAN INC [US]; LI SHENGWEN [US]; AOKI KEI ROGER [US]) 2 June 2005 (2005-06-02) see claims and example 8	17,20,65	-/-		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
E	WO 2021/064369 A1 (IPSEN BIOPHARM LTD [GB]) 8 April 2021 (2021-04-08) the whole document	1-4, 9-21,26, 27, 49-56, 58-67																		
X	WO 2020/219358 A2 (CHILDRENS MEDICAL CENTER [US]) 29 October 2020 (2020-10-29)	1-4,9, 12-21, 32,48, 49,59, 65-67																		
Y	see claims and page 85	17,20,65																		
Y	WO 2005/048949 A2 (ALLERGAN INC [US]; LI SHENGWEN [US]; AOKI KEI ROGER [US]) 2 June 2005 (2005-06-02) see claims and example 8	17,20,65																		
-/-																				
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.																				
* Special categories of cited documents : <table border="0"> <tr> <td style="vertical-align: top;"> "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td style="vertical-align: top;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family </td> </tr> </table>			"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family																
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family																			
Date of the actual completion of the international search 19 November 2021		Date of mailing of the international search report 29/11/2021																		
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Merckling-Ruiz, V																		

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2021/050783

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RASETTI-ESCARGUEIL CHRISTINE ET AL: "Engineering Botulinum Neurotoxins for Enhanced Therapeutic Applications and Vaccine Development", TOXINS, vol. 13, no. 1, 1 January 2021 (2021-01-01), page 1, XP055860858, DOI: 10.3390/toxins13010001 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/artic les/PMC7821915/pdf/toxins-13-00001.pdf> see abstract, Fig.1, points 3.4 and 5 -----	17,20,65
Y	MATAK ET AL: "Mechanisms of Botulinum Toxin Type A Action on Pain", TOXINS, vol. 11, no. 8, 5 August 2019 (2019-08-05) , page 459, XP055860861, DOI: 10.3390/toxins11080459 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/artic les/PMC6723487/pdf/toxins-11-00459.pdf> see abstract, Tables 1-2 -----	17,20,65
A	WO 2020/065336 A1 (IPSEN BIOPHARM LTD [GB]) 2 April 2020 (2020-04-02) cited in the application see claims -----	1-69

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2021/050783

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2021/050783

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2021064369 A1	08-04-2021	TW 202120530 A WO 2021064369 A1	01-06-2021 08-04-2021
WO 2020219358 A2	29-10-2020	NONE	
WO 2005048949 A2	02-06-2005	AU 2004291152 A1 CA 2546383 A1 EP 1684799 A2 JP 2007511609 A US 2005106182 A1 WO 2005048949 A2	02-06-2005 02-06-2005 02-08-2006 10-05-2007 19-05-2005 02-06-2005
WO 2020065336 A1	02-04-2020	AU 2019348732 A1 BR 112021005017 A2 CA 3111962 A1 CN 113164565 A EP 3856232 A1 KR 20210068476 A SG 11202102323T A TW 202027782 A WO 2020065336 A1	15-04-2021 08-06-2021 02-04-2020 23-07-2021 04-08-2021 09-06-2021 29-04-2021 01-08-2020 02-04-2020