CLAIMS

1. A botulinum neurotoxin variant of SEQ ID NO:1, wherein the botulinum neurotoxin variant comprises a first thrombin cleavage site engineered immediately carboxy-terminal to amino acid N880 and a second thrombin cleavage site engineered immediately carboxy-terminal to amino acid D890, wherein the variant has botulinum neurotoxin activity.

2. The botulinum neurotoxin variant of claim 1, wherein the first or second thrombin cleavage site consists of SEQ ID NO:114.

3. The botulinum neurotoxin variant of claim 2, wherein both the first and second thrombin cleavage sites consist of SEQ ID NO:114.

4. The botulinum neurotoxin variant of claim 1,
wherein the first thrombin cleavage site is engineered by insertion of amino acid residues VP between L881 and R882 and insertion of amino acid residues GS between R882 and Y883, and,
wherein the second thrombin cleavage sequence is engineered by insertion of amino acid residues VPRG between L891 and S892.

5. The botulinum neurotoxin variant of claim 1 comprising SEQ ID NO: 531.

CLAIMS

1) A Clostridial toxin comprising a least one inactivation cleavage site located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain, wherein the at least one inactivation cleavage site comprises a dual Thrombin-Thrombin site, a Factor Xa site, a dual Factor Xa-Thrombin site, and/or a MMP-9 site.

2) A Clostridial toxin comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a Clostridial toxin binding domain, a di-chain loop region, an exogenous protease cleavage site, and at least two inactivation cleavage sites located within an inactivation cleavage site region; wherein the exogenous protease cleavage site located within the di-chain loop region.

3) The Clostridial toxin of 2, wherein the inactivation cleavage sites comprise a dual Thrombin-Thrombin site and/or a dual Factor Xa-Thrombin site.

4) A Clostridial toxin chimeric comprising a Clostridial toxin enzymatic domain, a Clostridial toxin translocation domain, a non-Clostridial toxin binding domain, and an inactivation cleavage located within an inactivation cleavage site region, wherein the inactivation cleavage site region is located in the translocation domain and/or the H_{CN} binding subdomain.

5) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids amino acids 462-496 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 458-492 of SEQ ID NO: 3; amino acids 464-487 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 463-496 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 458-491 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 434-467 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 453-486 of SEQ ID NO: 18, SEQ ID NO: 19, and/or SEQ ID NO: 20; amino acids 458-491 of SEQ ID NO: 21; amino acids 443-476 of SEQ ID NO: 23; and/or amino acids 434-467 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

6) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids 618-634 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 614-630 of SEQ ID NO: 3; amino acids 605-621 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 613-629 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 609-625 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 587-603 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 604-620 of SEQ ID NO: 18; amino acids 605-621 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 610-626 of SEQ ID NO: 21; amino acids 596-612 of SEQ ID NO: 23; and/or amino acids 587-603 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

7) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids 638-651 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 634-647 of SEQ ID NO: 3; amino acids 625-638 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 633-646 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 629-642 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 607-620 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 624-637 of SEQ ID NO: 18; amino acids 625-638 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 630-643 of SEQ ID NO: 21; amino acids 616-629 of SEQ ID NO: 23; and/or amino acids 607-620 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

8) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids 665-687 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 661-683 of SEQ ID NO: 3; amino acids 652-674 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 660-682 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 656-678 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 634-659 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 651-676 of SEQ ID NO: 18; amino acids 652-677 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 657-679 of SEQ ID NO: 21; amino acids 643-668 of SEQ ID NO: 23; and/or amino acids 634-659 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

9) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids 752-765 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 748-761 of SEQ ID NO: 3; amino acids 739-752 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 747-760 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 743-756 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 724-739 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 741-756 of SEQ ID NO: 18; amino acids 742-757 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 744-757 of SEQ ID NO: 21; amino acids 733-748 of SEQ ID NO: 23; and/or amino acids 724-739 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

10) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids 826-835 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 824-831 of SEQ ID NO: 3; amino acids 813-824 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 821-830 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 817-826 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 800-809 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 817-826 of SEQ ID NO: 18; amino acids 818-827 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 818-827 of SEQ ID NO: 21; amino acids 809-819 of SEQ ID NO: 23; and/or amino acids 800-809 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

11) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids 844-863 of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 840-859 of SEQ ID NO: 3;; amino acids 831-850 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 839-858 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 835-854 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 818-837 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 835-854 of SEQ ID NO: 18; amino acids 836-855 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 836-855 of SEQ ID NO: 21; amino acids 828-847 of SEQ ID NO: 23; and/or amino acids 818-837 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

12) The Clostridial toxin and/or Clostridial toxin chimeric of 1-4, wherein the inactivation cleavage site region comprises amino acids 871-895 of SEQ ID NO: 1,, SEQ ID NO: 2, SEQ ID NO: 4 and/or SEQ ID NO: 5; amino acids 867-891 of SEQ ID NO: 3; amino acids 858-882 of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and/or SEQ ID NO: 10; amino acids 866-890 of SEQ ID NO: 11 and/or SEQ ID NO: 12; amino acids 862-886 of SEQ ID NO: 13 and/or SEQ ID NO: 14; amino acids 845-869 of SEQ ID NO: 15, SEQ ID NO: 16, and/or SEQ ID NO: 17; amino acids 862-886 of SEQ ID NO: 18; amino acids 863-887 of SEQ ID NO: 19 and/or SEQ ID NO: 20; amino acids 863-887 of SEQ ID NO: 21; amino acids 855-879 of SEQ ID NO: 23; and/or amino acids 845-869 of SEQ ID NO: 24 and/or SEQ ID NO: 25.

13) The Clostridial toxin and/or Clostridial toxin chimeric of 1-12, wherein the Clostridial toxin enzymatic domain comprises a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a BaNT enzymatic domain, and/or a BuNT enzymatic domain.

14) The Clostridial toxin and/or Clostridial toxin chimeric of 1-13, wherein the inactivation cleavage site comprises Thrombin cleavage sites, Plasmin cleavage sites, Coagulation Factor VIIa cleavage sites, Coagulation Factor IXa cleavage sites, Coagulation Factor Xa cleavage sites, Coagulation Factor XIa cleavage sites, Coagulation Factor XIIa cleavage sites, plasma kallikrein cleavage sites, protease-activated G protein-coupled receptor-1 (PAR1) cleavage sites, PAR 2 cleavage sites, PAR3 cleavage sites, PAR4 cleavage sites, Matrix Metalloproteinase-2 (MMP-2) cleavage sites, Matrix Metalloproteinase-9 (MMP-9) cleavage sites, Furin cleavage sites, urokinase-type Plasminogen activator (uPA) cleavage sites, tissue-type Plasminogen activator (tPA) cleavage sites, Tryptase-ε cleavage sites, Mouse mast cell protease-7 (mMCP-7) cleavage sites, endothelin-converting enzyme-1 (ECE-1) cleavage sites, Kell blood group cleavage sites, DPPIV cleavage sites, ADAM metallopeptidase with thrombospondin type 1 motif-13 (ADAMTS13) cleavage sites, and/or Cathepsin L cleavage sites.

15) A Clostridial toxin comprising SEQ ID NO: 531, SEQ ID NO: 533, SEQ ID NO: 535, and/or SEQ ID NO: 537.