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A61K 48/00 (2006.01)(74) Agent: COHEN, Mark, S.; Pearl Cohen Zedek Latzer,
LLP, 1500 Broadway, 12th Floor, New York, NY 10036
(US).

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(71) Applicant (for all designated States except US): THE
TRUSTEES OF THE UNIVERSITY OF PENNSYL-
VANIA [US/US]; 3160 Chestnut Street-suite 200,
Philadelphia, PA 19104-6283 (US).

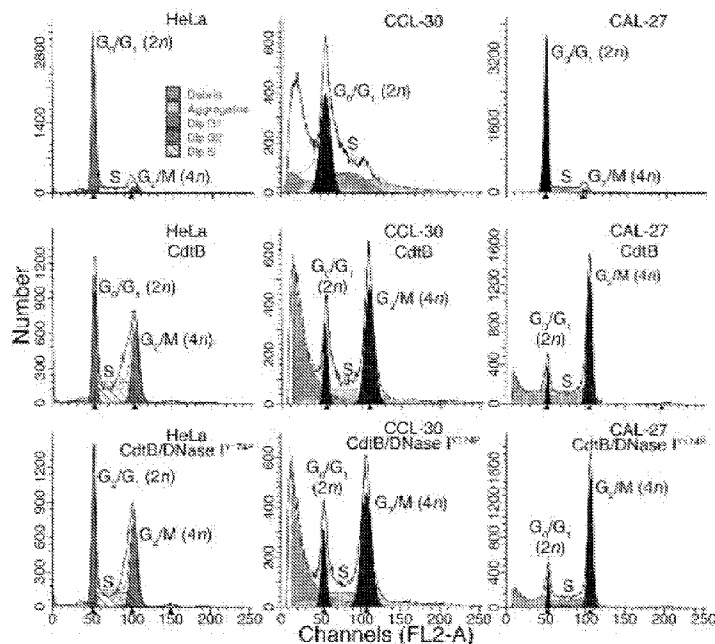
(72) Inventor; and

(75) Inventor/Applicant (for US only): DIRIENZO, Joseph,
M. [US/US]; 515 Shadeland Avenue, Drexel Hill, PA
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[Continued on next page]

(54) Title: CHIMERA COMPRISING BACTERIAL CYTOTOXIN AND METHODS OF USING THE SAME

Figure 13

(57) Abstract: This invention provides, a recom-
binant polypeptide encoding a chimera. The
chimera includes a DNase I fragment or a homo-
logue thereof and a Cdt fragment or a homologue
thereof. Further, the invention provides methods,
utilizing the recombinant polypeptide encoding the
chimera, such as a method for inhibiting the
proliferation of a neoplastic cell, a method for
treating a neoplastic disease in a human subject, a
method for inhibiting or suppressing a neoplastic
disease in a human subject, and a method for re-
ducing the symptoms associated with a neoplastic
disease in a human subject.



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FIELD OF INVENTION

5 [1] This invention provides recombinant polypeptides comprising a chimera that inhibit cell proliferation and a method of using the same.

BACKGROUND OF THE INVENTION

[2] Over the last few decades there has been developing interest in non-traditional treatments for certain types of cancer. Recombinant toxins, hybrid proteins composed of a bacterial toxin and either a growth factor
10 or a portion of a recombinant monoclonal antibody, have received significant attention in cancer therapeutics.

[3] Human DNase I has been used as a therapeutic agent for the treatment of wounds and ulcers, bronchitis, inflammatory conditions, herpes infection and most notably, cystic fibrosis. The cytolethal distending toxin (Cdt) is a genotoxin, produced by several species of bacteria including the periodontal pathogen *Actinobacillus actinomycetemcomitans*.

15 [4] Mammalian DNase I is secreted primarily by the pancreas and parotid gland and functions as a digestive enzyme. Bovine DNase I (EC 3.1.21.1) is the best-studied member of this nuclease family. It is a 30.4 kDa monomeric glycoprotein containing 260 amino acids and a carbohydrate side chain of 7-10 residues. The protein is an endonuclease that cleaves double-strand DNA at neutral pH by hydrolyzing the 3'-phosphodiester bond. The protein contains two disulphide bridges but only one is required to maintain
20 structure. The enzyme requires divalent metal cations and exhibits optimum activity when both Ca^{++} and Mg^{++} or Ca^{++} and Mn^{++} are included in the reaction buffer. DNA is nonspecifically degraded by a single-strand nicking mechanism when only Mg^{++} is present. The catalytic, metal ion binding and DNA contact residues in DNase I have been determined by mutational analysis and are conserved between the bovine and human enzymes. Bovine and human DNase I share 78% amino acid identity. Bovine DNase I is approximately 2.4-
25 fold more active than the human homolog. Both enzymes are inhibited by Zn^{++} and bind to and are inhibited by globular (G) actin. Six G-actin binding sites have been identified in human DNase I and have been altered to obtain an actin-resistant mutant for use in the treatment of cystic fibrosis.

[5] Cytolethal distending toxin (Cdt) is a family of secreted bacterial protein holotoxins that classically
30 arrest the growth of specific types of eukaryotic cells or cell lines at either the G0/G1 or G2/M phase of the cell cycle. The holotoxin is the product of three genes expressed by a handful of facultative or microaerophilic gram-negative pathogenic bacterial species. The species identified to date that express a biologically active CDT include select strains of enteropathogenic *Escherichia coli*, *Campylobacter jejuni*, *Campylobacter upsaliensis*, *Campylobacter coli*, *Shigella dysenteriae*, *Haemophilus ducreyi*, *Helicobacter hepaticus*,

Helicobacter flexispira, *Helicobacter bilis*, *Helicobacter canis* and, the periodontal pathogen, *Actinobacillus actinomycetemcomitans*. Organization of the genetic locus as well as the structure and biological activity of the holotoxin are fairly well conserved among the bacterial genera that express the Cdt. Biologically active toxin is a heterotrimer composed of approximately 18-25 kDa (CdtA), 31 kDa (CdtB) and 21 kDa (CdtC) protein subunits expressed from a polycistronic operon. An adjunct property of the *cdt* genes is that they appear to have a eukaryotic rather than prokaryotic heritage. The *cdt* gene products exhibit deduced amino acid sequence and structure/function similarities (albeit weak) to those of eukaryotic proteins.

SUMMARY OF THE INVENTION

[6] This invention provides, in one embodiment, a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.

[7] In another embodiment, the present invention provides a recombinant polynucleotide encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.

[8] In another embodiment, the present invention provides a DNA vector comprising a recombinant polynucleotide encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.

[9] In another embodiment, the present invention provides a plasmid comprising a recombinant polynucleotide encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.

[10] In another embodiment, the present invention provides a method for inhibiting the proliferation of a neoplastic cell comprising the step of contacting said cell with a recombinant polypeptide comprising a chimera or a nucleic acid molecule encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting the proliferation of a neoplastic cell.

[11] In another embodiment, the present invention provides a method for treating a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby treating a neoplastic disease in a subject.

[12] In another embodiment, the present invention provides a method for inhibiting or suppressing a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I

fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting or suppressing a neoplastic disease in a subject.

[13] In another embodiment, the present invention provides a method for reducing the symptoms associated with a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby reducing the symptoms associated with a neoplastic disease in a subject.

BRIEF DESCRIPTION OF THE FIGURES

[14] Figure 1. Characterization of recombinant CdtB-His₆. (A) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of affinity purified recombinant *A. actinomycetemcomitans* CdtB-His₆. The gel is stained with Coomassie Brilliant Blue. (B) Dose response kinetics of the conversion of supercoiled (S) plasmid DNA to relaxed (R) and linear (L) forms by CdtB-His₆. Agarose gel stained with ethidium bromide. The activity of CdtB labeled with a histidine tag at the amino terminal end of the protein is compared at the highest concentration (1 µg/1 µg of DNA) of CdtB-His₆ tested. (C) Dose response kinetics of the activity of bovine DNase I.

[15] Figure 2. DNA nicking assay containing increasing concentrations of either MgCl₂ (top panel), CaCl₂ (middle panel) or MnCl₂ (bottom panel) in the standard reaction buffer. Supercoiled (S), relaxed (R) and linear (L) forms of DNA were quantified by densitometry of ethidium bromide stained agarose gels (insets). The data shown is representative of several repeat experiments

[16] Figure 3. Heat liability of recombinant CdtB-His₆ and DNase I. (A) CdtB-His₆ heated for various periods in boiling water and then assayed for supercoiled DNA nicking activity. (B) bovine DNase I treated as in panel A. (C) effects of heating on the nicking activity of CdtB-DNase I and DNase I-CdtB. Numbers above gels lanes represent minutes. Abbreviations are the same as in Figure 1.

[17] Figure 4. The effect of globular (G) actin on the supercoiled DNAs nicking activity of CdtB-His₆, bovine DNase I and the two hybrid proteins. Abbreviations are the same as in Figure 1.

[18] Figure 5. (A) Computer models of the *A. actinomycetemcomitans* CdtB (as part of the Cdt heterotrimer) and bovine DNase I. (B) Theoretical model of the CdtB/DNase I hybrid protein construct. The CdtB (amino-terminal half) and DNase I (carboxy-terminal half) portions of the hybrid protein are shown in orange and magenta, respectively. The amino- (N) and carboxy (C)-termini are labeled. (C) Sequence alignment of the four His-tagged constructs (CdtB-H (SEQ ID NO: 16); CdtB/DNaseI (SEQ ID NO: 26); DNaseI/CdtB (SEQ ID NO: 27); human DNase I (SEQ ID NO: 7)). Conserved and identical residues are marked by dots and asterisks, respectively. The blue and orange arrows mark the gene fusion position and predicted signal sequence cleavage sites, respectively. Amino acid substitutions are marked in green and

predicted functional domains are in red. Location of the nuclear localization signal (NLS) and information about the location of functional residues in DNase I was from Nishikubo et al. (2003) and Pan et al. (1998), respectively. Disulfides in DNase I are designated by brackets.

[19] Figure 6. The biological and binding activities of the hybrid proteins. (A) heterotoxins were reconstituted with wild-type CdtA and CdtC and each of the hybrid proteins. CHO cells were treated with the preparations. Colonies were fixed, stained and counted after growth for six days and expressed as colony-forming units (CFU). (B) Saturation binding kinetics of the two hybrid proteins were compared to that of CdtB-His₆. The same proteins were incubated with wild-type CdtA and CdtC in refolding buffer and subjected to differential dialysis. The reconstituted samples were examined before (BD) and after (AD) dialysis on a western blot (inset). (C) The stoichiometric binding of the two hybrid proteins to wild-type CdtA and CdtC immobilized on thyroglobulin was examined by ELISA. Numbers above the columns are absorbance ratios calculated as described in Methods. The hybrid proteins, and CdtB-His₆ as a control, were mixed with wild-type CdtA and CdtC, as in the differential dialysis experiment in the inset in panel A, and the reconstituted samples assayed for DNA nicking activity (inset). The reaction examined in the first lane contained non-complexed CdtB-His₆. Abbreviations are the same as in the legend for Fig. 1. Experiments were performed a minimum of three times. Mean values and standard deviations were plotted where appropriate.

[20] Figure 7. A computer models of CdtB (as part of the Cdt heterotrimer) and the two hybrid proteins. The α -helix H1 and large loop domains are labeled. Note that the orientation of the DNase I/CdtB structure is flipped 180° relative to that of CdtB/DNase I. The *A. actinomycetemcomitans* Cdt was modeled using the program UCSF Chimera version 1.2197 and PDB coordinate file 2F2F. The theoretical hybrid protein structures were generated with Modeller 9.1 and visualized with UCSF Chimera.

[21] Figure 8. Nuclease activity of CdtB/DNase I^{mut5} was compared to that of CdtB-His₆ and the original CdtB/DNase I construct. The data is expressed as a percentage of the supercoiled (S) form DNA remaining after the reaction. These values were obtained by densitometry of ethidium bromide stained agarose gels (inset A). Semilogarithmic plot of the log of the substrate concentration [S] versus time of the reaction (expressed in minutes) for CdtB/DNase I^{mut5} (inset B). [S] represents the amount of S-form DNA obtained by densitometry of the agarose gel in the inset). The control lacked enzyme. The data shown is representative of several repeat experiments.

[22] Figure 9 depicts a flow cytometry of untreated and Cdt-treated HeLa cells, the human cancer cell line CAL-27 and primary HGEC (AL-1). Cultures treated with Cdt received 10 μ g of reconstituted recombinant protein/ml. Cell nuclei were prepared 36 h post-intoxication and were stained with propidium iodide. Quantitative values for the diploid G1, diploid G2 and S peaks are provided in Table 4.

[23] Figure 10 shows computer models of CdtB-His₆ and CdtB/DNase I^{mut5} containing the large loop substitution. The amino- and carboxy-terminal halves of the proteins are in red and magenta, respectively. The

α -helix H1 and large loop domains (yellow) are labeled. Distances are labeled in angstroms. The theoretical computer models were generated as described in the legend to Figure 6.

[24] Figure 11 is a bar graph showing wild-type and hybrid proteins that were added sequentially to CdtA pre-bound to thyroglobulin-coated 96-well plates. Bound protein was detected as described in the legend to Figure 6C.

[25] Figure 12 is a bar graph showing heterotoxins that were reconstituted with wild-type CdtA, wild-type CdtC and either wild-type CdtB or CdtB/DNase I^{Y174P} (SEQ. ID NO: 14 and 15). CHO cells were treated with the preparations. Colonies were fixed, stained and counted after growth for six days and expressed as colony-forming units (CFU).

[26] Figure 13 depicts flow cytometry of HeLa, CCL-30 and CAL-27 cancer cell lines with heterotoxin containing CdtB or CdtB/DNase I^{Y174P}. Cultures treated with heterotoxin received 10 μ g of reconstituted recombinant protein/ml. Cell nuclei were prepared 36 h post-intoxication and were stained with propidium iodide. Quantitative values for the diploid G1, diploid G2 and S peaks are provided in Table 5.

[27] Figure 14 is a phylogenetic tree showing the relationship of bacterial CdtB and mammalian DNase I deduced amino acid sequences. GenBank accession numbers are given in parentheses.

DETAILED DESCRIPTION OF THE INVENTION

[28] This invention provides, in one embodiment, a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment and a Cdt fragment. In another embodiment, the chimera comprises a DNase I fragment and a homologue of a Cdt fragment. In another embodiment, the chimera comprises a homologue of a DNase I fragment and a Cdt fragment. In another embodiment, the chimera comprises a homologue of a DNase I fragment and a homologue of a Cdt fragment. In another embodiment, the present invention provides a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof. In another embodiment, said chimera comprises a Cdt subunit.

[29] In one embodiment, DNase I is an endonuclease that nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5'-phosphorylated and 3'-hydroxylated ends. In one embodiment, DNase I acts on single- and double-stranded DNA, chromatin and RNA:DNA hybrids. In one embodiment, DNase I for use in the compositions and methods of the present invention is derived from humans. In one embodiment, DNase I for use in the compositions and methods of the present invention is derived from a murine species, which in one embodiment, is rat or mouse. In one embodiment, DNase I for use in the compositions and methods of the present invention is derived from *Mus musculus*, *Homo sapiens*, *Bos taurus*, *Bacillus subtilis*, *Rattus norvegicus*, *Oryctolagus cuniculus*, *Salmo salar*, *Gallus gallus*, *Canis lupus familiaris*, *Rhodopirellula baltica* SH 1, *Sus scrofa*, *Equus caballus*, *Dictyostelium discoideum*, *Pseudomonas putida* GB-1,

Pseudomonas putida W619, *Azoarcus* sp. BH72, *Synechococcus* sp., *Bacteroides caccae* ATCC 43185, *Xenopus laevis*, or *Aspergillus niger*.

[30] In one embodiment, a DNase I fragment of the present invention is an N-terminal fragment. In another embodiment, a DNase I fragment of the present invention is a C-terminal fragment.

5 [31] In one embodiment, the nucleic acid sequence encoding the DNase I fragment is:
TTGTACAAAAAGCAGGCTTGAAGGAGTTCGAACCATGAGGGGCATGAAGCTGCTGGGGGCG
CTGCTGGCACTGGCGGCCCTACTGCAGGGGGCCGTGTCCCTGAAGATCGCAGCCTTCAACATCC
AGACATTTGGGGAGACCAAGATGTCCAATGCCACCCTCGTCAGCTACATTGTGCAGATCCTGAG
CCGCTATGACATCGCCCTGGTCCAGGAGGTCAGAGACAGCCACCTGACTGCCGTGGGGAAGCTG
10 CTGGACAACCTCAATCAGGATGCACCAGACACCTATCACTACGTGGTCAGTGAGCCACTGGGAC
GGAACAGCTATAAGGAGCGCTACCTGTTCGTGTACAGGCCTGACCAGGTGTCTGCGGTGGACAG
CTACTACTACGATGATGGCTGCGAGCCCTGCGGGAACGACACCTTCAACCGAGAGCCAGCCATT
GTCAGGTTCTTCTCCCGGTTACAGAGGTCAGGGAGTTTGCCATTGTTCCCCTGCATGCGGCCCC
GGGGGACGCAGTATCCGAGATCGACGCTCTCTATGACGTCTACCTGGATGTCCAAGAGAAATGG
15 GGCTTGGAGGACGTCATGTTGATGGGCGACTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCC
AGTGGTCATCCATCCGCCTGTGGACAAGCCCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGA
CACCACAGCTACACCCACGCACTGTGCCTATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGA
GGCGCCGTTGTTCCCGACTCGGCTCTTCCCTTTAACTTCCAGGCTGCCAATGGCCTGAGTGACCA
ACTGGCCCAAGCCATCAGTGACCACTATCCAGTGGAGGTGATGCTGAAGTAGCTCGAGTGCGGC
20 CGCAACCCAGCTTTCTTGTAC (SEQ ID NO: 1). In one embodiment, the coding sequence of the nucleic acid sequence is underlined. In another embodiment, the DNase I fragment is a homologue of SEQ ID NO: 1. In another embodiment, the DNase I fragment is a variant of SEQ ID NO: 1. In another embodiment, the DNase I fragment is an isoform of SEQ ID NO: 1. In another embodiment, the DNase I fragment is a fragment of SEQ ID NO: 1. Each possibility represents a separate embodiment of the present invention.

25 [32] In another embodiment, the nucleic acid sequence encoding the DNase I fragment is:
ATGAGGGGCATGAAGCTGCTGGGGGCGCTGCTGGCACTGGCGGCCCTACTGCAGGGGGCCGTGT
CCCTGAAGATCGCAGCCTTCAACATCCAGACATTTGGGGAGACCAAGATGTCCAATGCCACCCT
CGTCAGCTACATTGTGCAGATCCTGAGCCGCTATGACATCGCCCTGGTCCAGGAGGTCAGAGAC
AGCCACCTGACTGCCGTGGGGAAGCTGCTGGACAACCTCAATCAGGATGCACCAGACACCTATC
30 ACTACGTGGTCAGTGAGCCACTGGGACGGAACAGCTATAAGGAGCGCTACCTGTTTCGTGTACAG
GCCTGACCAGGTGTCTGCGGTGGACAGCTACTACTACGATGATGGCTGCGAGCCCTGCGGGAAC
GACACCTTCAACCGAGAGCCAGCCATTGTCAGGTTCTTCTCCCGGTTACAGAGGTCAGGGAGT
TTGCCATTGTTCCCCTGCATGCGGCCCCGGGGGACGCAGTATCCGAGATCGACGCTCTCTATGAC
GTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCGACTTCAATG
35 CGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAGCCCCACCTT

CCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCCTATGACAGG
ATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCCCTTTAACTT
CCAGGCTGCCAATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTATCCAGTGGAG
GTGATGCTGAAGTAG (SEQ ID NO: 2). In another embodiment, the DNase I fragment is a homologue of
5 SEQ ID NO: 2. In another embodiment, the DNase I fragment is a variant of SEQ ID NO: 2. In another
embodiment, the DNase I fragment is an isoform of SEQ ID NO: 2. In another embodiment, the DNase I
fragment is a fragment of SEQ ID NO: 2. Each possibility represents a separate embodiment of the present
invention.

[33] In another embodiment, the nucleic acid sequence encoding the DNase I fragment is:
10 ATGGTGAGGGGAATGAAGCTGCTGGGGGCGCTGCTGGCACTGGCGGCCCTACTGCAGGGGGGCC
GTGTCCCTGAAGATCGCAGCCTTCAACATCCAGACATTTGGGGAGACCAAGATGTCCAATGCCA
CCCTCGTCAGCTACATTGTGCAGATCCTGAGCCGCTATGACATCGCCCTGGTCCAGGAGGTCAG
AGACAGCCACCTGACTGCCGTGGGGAAGCTGCTGGACAACCTCAATCAGGATGCACCAGACAC
CTATCACTACGTGGTCAGTGAGCCACTGGGACGGAACAGCTATAAGGAGCGCTACCTGTTTCGTG
15 TACAGGCCTGACCAGGTGTCTGCGGTGGACAGCTACTACTACGATGATGGCTGCGAGCCCTGCG
GGAACGACACCTTCAACCGAGAGCCAGCCATTGTCAGGTTCTTCTCCCGGTTACAGAGGTCAG
GGAGTTTGCCATTGTTCCCTGCATGCGGCCCGGGGGACGCAGTAGCCGAGATCGACGCTCTC
TATGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCGACT
TCAATGCGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAGCCC
20 CACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCCTAT
GACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCCCTT
TAACTTCCAGGCTGCCAATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTATCCA
GTGGAGGTGATGCTGAAGCTCGAGCACCACCACCACCACCACCATATGCTCGAGGATCCGGCTG
CTAACAAAGCC (SEQ ID NO: 3). In another embodiment, the DNase I fragment is a homologue of SEQ
25 ID NO: 3. In another embodiment, the DNase I fragment is a variant of SEQ ID NO: 3. In another
embodiment, the DNase I fragment is an isoform of SEQ ID NO: 3. In another embodiment, the DNase I
fragment is a fragment of SEQ ID NO: 3. Each possibility represents a separate embodiment of the present
invention.

[34] In another embodiment, the amino acid sequence encoding the DNase I fragment is:
30 MRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETKMSNATLVSYIVQILSRVDIALVQEVDRSHLT
AVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFNR
EPAIVRFFSRFTEVREFAIVPLHAAPGDAVSEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRP
SQWSSIRLWTSPTFQWLIPDSADTTATPTHCAVDRIVVAGMLLRGAVVPDSALPFNFQAANGLSDQL
AQAI SDHYPVEV MLK (SEQ ID NO: 4). In another embodiment, the DNase I fragment is a homologue of
35 SEQ ID NO: 4. In another embodiment, the DNase I fragment is a variant of SEQ ID NO: 4. In another
embodiment, the DNase I fragment is an isoform of SEQ ID NO: 4. In another embodiment, the DNase I

fragment is a fragment of SEQ ID NO: 4. Each possibility represents a separate embodiment of the present invention.

[35] In another embodiment, the amino acid sequence encoding the DNase I fragment is:
 MVRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETKMSNATLVSYIVQILSRDYDIALVQEVDRSHL
 5 TAVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFN
 REPAIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYV
 RPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAANGLSD
 QLAQAISDHYPVEVMLKLEHHHHHHHMLEDPAAANKA (SEQ ID NO: 5). In another embodiment, the
 DNase I fragment is a homologue of SEQ ID NO: 5. In another embodiment, the DNase I fragment is a
 10 variant of SEQ ID NO: 5. In another embodiment, the DNase I fragment is an isoform of SEQ ID NO: 5. In
 another embodiment, the DNase I fragment is a fragment of SEQ ID NO: 5. Each possibility represents a
 separate embodiment of the present invention.

[36] In another embodiment, the amino acid sequence encoding the DNase I fragment is:
 MVRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETKMSNATLVSYIVQIMSRDYDIALVQEVDRSH
 15 LTAVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTF
 NREPAIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSY
 VRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAANGLS
 DQLAQAISDHYPVEVMLKLEHHHHHHHMLEDPAAANKA (SEQ ID NO: 6). In another embodiment, the
 DNase I fragment is a homologue of SEQ ID NO: 6. In another embodiment, the DNase I fragment is a
 20 variant of SEQ ID NO: 6. In another embodiment, the DNase I fragment is an isoform of SEQ ID NO: 6. In
 another embodiment, the DNase I fragment is a fragment of SEQ ID NO: 6. Each possibility represents a
 separate embodiment of the present invention.

[37] In another embodiment, the amino acid sequence encoding the DNase I fragment is:
 MVRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETKMSNATLVSYIVQIMSRDYDIALVQEVDRSH
 25 LTAVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTF
 NREPAIVRFFSRFTEVREFAIVPLHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSY
 VRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAANGLS
 DQLAQAISDHYPVEVMLKLEHHHHHHHMLEDPAAANKA (SEQ ID NO: 7). In another embodiment, the
 DNase I fragment is a homologue of SEQ ID NO: 7. In another embodiment, the DNase I fragment is a
 30 variant of SEQ ID NO: 7. In another embodiment, the DNase I fragment is an isoform of SEQ ID NO: 7. In
 another embodiment, the DNase I fragment is a fragment of SEQ ID NO: 7. Each possibility represents a
 separate embodiment of the present invention.

[38] In another embodiment, a DNase I, or its fragment or homologue comprises a mutated form of a
 DNase I. In another embodiment, a DNase I, or its fragment or homologue comprises a mutated form of a
 35 DNase I fragment. In another embodiment, a mutated DNase I, a mutated DNase I fragment comprises a

deletion mutation. In another embodiment, a mutated DNase I, a mutated CdtB toxin fragment comprises an insertion mutation. In another embodiment, a mutated DNase I, a mutated DNase I fragment comprises a substitution mutation.

[39] In one embodiment, a chimera of the present invention comprises a DNase fragment. In another embodiment, a chimera of the present invention comprises a nuclease fragment. In another embodiment, a chimera of the present invention comprises an RNase fragment.

[40] In one embodiment, a DNase for use in the compositions and methods of the present invention is an exodeoxyribonuclease, and, in one embodiment, cleaves only residues at the ends of DNA molecules. In another embodiment, a DNase for use in the compositions and methods of the present invention is an endodeoxyribonuclease, and, in one embodiment, cleaves anywhere along the polynucleotide chain. In one embodiment, a DNase for use in the compositions and methods of the present invention is deoxyribonuclease I or, in another embodiment, deoxyribonuclease II.

[41] In another embodiment, the chimera provided herein is a Cdt-DNase I chimera. In another embodiment, the chimera provided herein is a multimeric chimera protein comprising DNase I or its fragment or homologue; and a Cdt toxin, or its fragment or homologue. In another embodiment, the chimera provided herein comprises DNase I or its fragment or homologue; and a Cdt toxin. In another embodiment, the chimera provided herein is a multimeric chimera protein comprising DNase I and a Cdt toxin, or its fragment or homologue. In another embodiment, the chimera provided herein consists DNase I or its fragment or homologue; and a Cdt toxin.

[42] In one embodiment, a Cdt fragment of the present invention is an N-terminal fragment. In another embodiment, a Cdt fragment of the present invention is a C-terminal fragment.

[43] In one embodiment, a CdtA, CdtB, CdtC, Cdt holotoxin, or fragment thereof used in the compositions and methods of the present invention is derived from *Escherichia coli*, *Campylobacter jejuni*, *Campylobacter upsaliensis*, *Campylobacter coli*, *Shigella dysenteriae*, *Haemophilus ducreyi*, *Helicobacter hepaticus*, *Helicobacter flexispira*, *Helicobacter bilis*, *Helicobacter canis*, *Aggregatibacter actinomycetemcomitans*, or *Actinobacillus actinomycetemcomitans*.

[44] In one embodiment, the Cdts are a family of heat-labile protein cytotoxins produced by several different bacterial species including diarrheal disease-causing enteropathogens such as some *Escherichia coli* isolates, *Campylobacter jejuni*, *Shigella species*, *Haemophilus ducreyi* and *Actinobacillus actinomycetemcomitans*. Thus, in one embodiment, a Cdt subunit of the present invention is derived from *Actinobacillus actinomycetemcomitans*. In another embodiment, a Cdt subunit of the present invention is derived from *Escherichia coli* isolates, *Campylobacter jejuni*, *Shigella species*, or *Haemophilus ducreyi*.

[45] Cdts are encoded by three genes, designated *cdtA*, *cdtB*, and *cdtC* which are arranged as an apparent operon. These three genes specify three polypeptides designated CdtA, CdtB and CdtC with apparent molecular masses of 28, 32 and 20 kDa, respectively, that form a heterotrimeric holotoxin. Several cell lines and cell types have been shown to be sensitive to Cdt including human lymphoid cells, fibroblasts, human embryonic intestinal epithelial cells, a human colon carcinoma cell line, and human keratinocytes, among others. In one embodiment, lymphocytes are the most sensitive (4-5 fold) to Cdt, suggesting that Cdt most likely functions as an immunotoxin. In response to Cdt, proliferating cells exhibit G2 arrest and eventually cell death resulting from activation of the apoptotic cascade. In another embodiment, the cell cycle arrest results in a cessation of cell division. In one embodiment, the Cdts produce other effects, including, in another embodiment, progressive cellular distention.

[46] The heterotrimeric Cdt holotoxin functions as an AB₂ toxin where CdtB is the active (A) unit, and the complex of CdtA and CdtC comprise the binding (B) unit. In one embodiment, the CdtA and CdtC subunits are involved in the adhesion to target cells. In one embodiment, CdtA and CdtC are required for the toxin to associate with lipid microdomains within lymphocyte membranes. In one embodiment, the CdtC subunit interacts specifically with cholesterol. In one embodiment, the CdtB must be internalized and associate with cellugyrin, a microvessicle associated protein, in order to induce cell cycle arrest. In one embodiment, CdtB acts as a PIP₃ phosphatase depleting cells of PIP₃ and thereby blocking the Akt survival/proliferation pathway. In one embodiment, CdtB does not act on phosphatides or proteins. In one embodiment, the Cdt holotoxin has structural homology with inositol polyphosphate 5-phosphatase. In another embodiment, the CdtB subunit has type I deoxyribonuclease-like activity. In another embodiment, the B subunit has endonuclease activity that results in double strand breaks and blunt ends. In one embodiment, the Cdt holotoxin has structural homology with DNase I.

[47] In another embodiment, *cdtA* is encoded by the following nucleic acid sequence:
 ATGGCTCCGAGGAGAGGTACAATGAAAAAGTTTTTACCTGGTCTTTTATTGATGGGTTTAGTGGC
 TTGTTCGTCAAATCAACGAATGAGTGACTATTCTCAGCCTGAATCTCAATCTGATTTAGCACCTA
 AATCTTCAACAACACAATTCCAACCCCAACCCCTATTATCAAAAGCATCTTCAATGCCATTGAAT
 TTGCTCTCTTCATCCAAGAATGGACAGGTATCGCCGTCTGAACCATCAAACCTTTATGACTTTGAT
 GGGACAAAATGGGGCACTGTTGACTGTCTGGGCGCTAGCAAAACGCAATTGGTTATGGGCTTAT
 CCCAATATATATTCGCAGGACTTTGGAAATATTCGTAATTGGAAGATAGAACCTGGTAAACACC
 GTGAATATTTTCGTTTTGTTAATCAATCTTTAGGTACATGTATTGAAGCTTACGGTAATGGTTTAA
 TTCATGATACTTGTAGTCTGGACAAATTAGCACAAGAGTTTGAGTTATTACCTACTGATAGTGGT
 GCGGTTGTCATTAAAAGTGTGTCACAAGGACGTTGTGTCACCTATAATCCTGTAAGTCCAACATA
 TTATTCAACAGTTACATTATCAACTTGTGATGGCGCAACAGAACCATTACGTGATCAAACATGGT
 ATCTCGCTCCTCCTGTATTAGAAGCAACAGCGGTTAATCACCACCACCACCACCGGATCCGG
 GCTGCTAACAAAGCCCCGAAAGGAAGC (SEQ ID NO: 8). In another embodiment, the CdtA subunit is
 a homologue of SEQ ID NO: 8. In another embodiment, the CdtA subunit is a variant of SEQ ID NO: 8. In

another embodiment, the CdtA subunit is an isoform of SEQ ID NO: 8. In another embodiment, the CdtA subunit is a fragment of SEQ ID NO: 8. Each possibility represents a separate embodiment of the present invention.

[48] In another embodiment, CdtA is encoded by the following amino acid sequence:
 5 MKKFLPGLLLMGLVACSSNQRMDSYQPSQSDLPKSSSTQFQPQLLSKASSMPLNLLSSSKNGQ
 VSPSEPSNFM TLMGQNGALLTVWALAKRNWLWAYPNIYSQDFGNIRNWKIEPGKHREYFRFVNQSL
 GTCIEAYGNGLIHDTCSLDKLAQEFELLPTDSGAVVIKSVSQGRCVTYNPVSPTYYSTVTLSLCDGAT
 EPLRDQTWYLAPPVLEATAVNHHHHHHHGSGLLTKPRKE (SEQ ID NO: 9). In another embodiment, the
 CdtA subunit is a homologue of SEQ ID NO: 9. In another embodiment, the CdtA subunit is a variant of SEQ
 10 ID NO: 9. In another embodiment, the CdtA subunit is an isoform of SEQ ID NO: 9. In another embodiment,
 the CdtA subunit is a fragment of SEQ ID NO: 9. Each possibility represents a separate embodiment of the
 present invention.

[49] In one embodiment, the amino acid sequence of a CdtA subunit of the present invention is:
 LLSSSKNGQVSPSEPSNFM TLMGQNGALLTVWALAKRNWLWAYPNIYSQDFGNIRNWKIEPGKHRE
 15 YFRFVNQSLGTCIEAYGNGLIHDTCSLDKLAQEFELLPTDSGAVVIKSVSQGRCVTYNPVSPTYYSTV
 TLSTCDGATEPLRDQTWYLAPPVLEATAV (SEQ ID NO: 10). In another embodiment, the CdtA subunit
 is a homologue of SEQ ID NO: 10. In another embodiment, the CdtA subunit is a variant of SEQ ID NO: 10.
 In another embodiment, the CdtA subunit is an isoform of SEQ ID NO: 10. In another embodiment, the CdtA
 subunit is a fragment of SEQ ID NO: 10. Each possibility represents a separate embodiment of the present
 20 invention.

[50] In another embodiment, the CdtA subunit has an amino acid sequence set forth in one of the following
 GenBank entries: AAF81760; AAF19157; AAD10621; AAB06707; AAA18785; NP_860977; AAP78043;
 YP_002343541; CAL34252; Q46668; AAT92047; AAC70897; AAZ16246; ABV51672.1; YP_001272540.1;
 BAF63360.1; YP_999805.1; ZP_02270538.1; AAB06707.1; EAQ71960.1; ABJ00842.1; YP_178099.1;
 25 AAW34670.1; NP_873397.1; or AAP95786.1. In another embodiment, the CdtA subunit has any CdtA
 subunit amino acid sequence known in the art. In another embodiment, the CdtA subunit is a homologue of a
 sequence from one of the above GenBank entries. In another embodiment, the CdtA subunit is a variant of a
 sequence from one of the above GenBank entries. In another embodiment, the CdtA subunit is an isoform of a
 sequence from one of the above GenBank entries. In another embodiment, the CdtA subunit is a fragment of a
 30 sequence from one of the above GenBank entries. Each possibility represents a separate embodiment of the
 present invention.

[51] In one embodiment, the nucleotide sequence of a CdtA subunit of the present invention is:
 ttgtctcttcatccaagaatggacaggtatcgccgtctgaacctcaaaactttatgactttgatgggacaaaatggggcactgttgactgtctgggcgctagcaaa
 acgcaattgggtatgggcttatcccaatatattcgacaggtttggaatattcgtaattggaagatagaacctggtaaacaccgtgaatatttcgtttgttaatca
 35 atcttttaggtacatgtattgaagcttacggtaatggttaattcatgatactgtagtctggacaaattagcacaagagtttgagttattacactactgatagtggtgcggtt

gtcattaaaagtgtgtcacaaggacgttggtgtcacttataatcctgtaagtccaacatattattcaacagttacattatcaactgtgatggcgcaacagaaccattacg
 tgatcaaacatgggtatctcgtctcctctgtattagaagcaacagcggtt (SEQ ID NO: 11). In another embodiment, the nucleotide
 sequence of the CdtA subunit is a homologue of SEQ ID NO: 11. In another embodiment, the nucleotide
 sequence of the CdtA subunit is a variant of SEQ ID NO: 11. In another embodiment, the nucleotide sequence
 of the CdtA subunit is an isoform of SEQ ID NO: 11. In another embodiment, the nucleotide sequence of the
 CdtA subunit is a fragment of SEQ ID NO: 11. Each possibility represents a separate embodiment of the
 present invention.

[52] In another embodiment, the CdtA subunit has a nucleic acid sequence set forth in one of the following
 GenBank entries: AL111168.1; AE017125.1; CP000814.1; AB285204.1; NZ_AASL01000001.1; U51121.1;
 CP000538.1; CP000468.1; CP000025.1; or AE017143.1. In another embodiment, the CdtA subunit has any
 CdtA subunit nucleic acid sequence known in the art. In another embodiment, the CdtA subunit is a
 homologue of a sequence from one of the above GenBank entries. In another embodiment, the CdtA subunit
 is a variant of a sequence from one of the above GenBank entries. In another embodiment, the CdtA subunit
 is an isoform of a sequence from one of the above GenBank entries. In another embodiment, the CdtA subunit
 is a fragment of a sequence from one of the above GenBank entries. Each possibility represents a separate
 embodiment of the present invention.

[53] In one embodiment, CdtA binds to a specific cell receptor, while in another embodiment, CdtA
 stabilizes the holotoxin. In one embodiment, analysis of the crystal structure of the toxin suggest that CdtA
 contains ricin-like domains, leading investigators to propose that it associates with carbohydrate.

[54] In one embodiment, the amino acid sequence of a CdtB subunit of the present invention is:
 NLSDFKVVATWNLQGSSAVNESKWNINVRQLLSGEQGADILMVQEAGSLPSSAVRTSRVIQHGGTPIE
 EYTWNLGTRSRPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFIVHSDSSVLQSRPAVGIRIGTDVFF
 TVHALATGGSDAVSLRNIFTTFTSSPSPERRGYSWMVVGDFNRAPVNLEAALRQEPVASENTIIIAP
 TEPTHRSGNILDYAILHDAHLPRREQARERIGASLMLNQLRSQITSDHFPVSFVRDR (SEQ ID NO: 12).
 In another embodiment, the CdtB subunit is a homologue of SEQ ID NO: 12. In another embodiment, the
 CdtB subunit is a variant of SEQ ID NO: 12. In another embodiment, the CdtB subunit is an isoform of SEQ
 ID NO: 12. In another embodiment, the CdtB subunit is a fragment of SEQ ID NO: 12. Each possibility
 represents a separate embodiment of the present invention.

[55] In another embodiment, the CdtB subunit has an amino acid sequence set forth in one of the following
 GenBank entries: ZP_01072217; NP_860978; YP_002343540; YP_002308521; ZP_03223221; NP_873398;
 YP_001481648; YP_852557; YP_999804; YP_434821; YP_178098; YP_001272541; ZP_01100899;
 ZP_01067880; ZP_00370497; or ZP_00369375. In another embodiment, the CdtB subunit has any CdtB
 subunit amino acid sequence known in the art. In another embodiment, the CdtB subunit is a homologue of a
 sequence from one of the above GenBank entries. In another embodiment, the CdtB subunit is a variant of a
 sequence from one of the above GenBank entries. In another embodiment, the CdtB subunit is an isoform of a

sequence from one of the above GenBank entries. In another embodiment, the CdtB subunit is a fragment of a sequence from one of the above GenBank entries. Each possibility represents a separate embodiment of the present invention.

[56] In one embodiment, the nucleotide sequence of a CdtB subunit of the present invention is:

5 aacttgagtgattcaagtagcaactggaatctgcaaggtcttcagctgtaaataaaagtaaataatgtgcgccaattattatcgggagaacaaggt
gcagatattttgatggtacaagaagcgggttcattaccaagttcggcagtaagaacctcacgagtaattcaacatgggggaacgccaattgaggaatatacctgg
aatttaggtactcgctcccgccaatatggtctatatttattcccgtttagatgtggggcaaacaggagtgaaacttagctatcgtgtcacgtcgtcaagccgatga
agcttttatcgtacattctgattcttctgtgttcaatctcgcccgagtaggtatccgcattggtactgatgtatttttacagtgcattgttggccacaggtggttct
gatgcggtaagtttaattcgaatatcttactacttttacctcatcaccatcatcaccggaaagacgaggatagctggatggtgtgtggtgatttcaatcgtgcgcc
10 ggtaaatctggaagctgcattaagacaggaacccgccgtgagtgaatacaattattattgcgccaacagaacggactcatcggtccggtaatattttagattatg
cgattttacatgacgcacattaccacgtcgagagcaagcacgtgaacgtatcggcgaagttaattgtaaatcagttacgtcacaaattacatccgatcttttc
ctgttagttttgttcgtgatc (SEQ ID NO: 13). In another embodiment, the nucleotide sequence of the CdtB subunit is
a homologue of SEQ ID NO: 13. In another embodiment, the nucleotide sequence of the CdtB subunit is a
variant of SEQ ID NO: 13. In another embodiment, the nucleotide sequence of the CdtB subunit is an isoform
15 of SEQ ID NO: 13. In another embodiment, the nucleotide sequence of the CdtB subunit is a fragment of SEQ
ID NO: 13. Each possibility represents a separate embodiment of the present invention.

[57] In another embodiment, the CdtB subunit has a nucleic acid sequence set forth in one of the following
GenBank entries: AL111168.1; AL627271.1; AE017125.1; CP000814.1; AB285204.1; EU794049.1;
DQ092613.1; CP000468.1; CP000026.1; CP000155.1; CP000025.1; AE017143.1; CP000538.1; U51121.1;
20 NZ_AASL01000001.1; or AE014613.1. In another embodiment, the CdtB subunit has any CdtB subunit
nucleic acid sequence known in the art. In another embodiment, the CdtB subunit is a homologue of a
sequence from one of the above GenBank entries. In another embodiment, the CdtB subunit is a variant of a
sequence from one of the above GenBank entries. In another embodiment, the CdtB subunit is an isoform of a
sequence from one of the above GenBank entries. In another embodiment, the CdtB subunit is a fragment of a
25 sequence from one of the above GenBank entries. Each possibility represents a separate embodiment of the
present invention.

[58] In one embodiment, compositions and methods of the present invention comprise or use a Cdt
fragment. In one embodiment, the Cdt fragment is a CdtB fragment or a homologue thereof.

[59] In another embodiment, the amino acid sequence encoding the CdtB fragment is:

30 MQWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSGEQGADILMVQ
EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTSRPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
VHSDSSVLQSRPAVGIRIGTDVFFTVHALATGGSDAVSLIRNIFTTFTSSPSPERRGYSWMVVGDFNR
APVNLEAALRQEPAVSENTIIIAPTEPTHQSGNILDYAILHDAHLPRREQVRERIGASMLNQLRSQITS
DHFP (SEQ ID NO: 14). In another embodiment, the CdtB fragment is a homologue of SEQ ID NO: 14. In
35 another embodiment, the CdtB fragment is a variant of SEQ ID NO: 14. In another embodiment, the CdtB

fragment is an isoform of SEQ ID NO: 14. In another embodiment, the CdtB fragment is a fragment of SEQ ID NO: 14. Each possibility represents a separate embodiment of the present invention.

[60] In another embodiment, the nucleic acid sequence encoding the CdtB fragment is:
 ATGCAATGGGTAAAGCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATGC
 5 TAACTTGAGTGATTTCAAAGTAGCAACTTGGAATCTGCAAGGTTCTTCAGCTGTAAATGAAAGT
 AAATGGAATATTAATGTGCGCCAATTATTATCGGGAGAACAAGGTGCAGATATTTTGATGGTAC
 AAGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATGGGGGAA
 CGCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTCCCGTCCAAATATGGTCTATATTTAT
 TATCCCGTTTAGATGTTGGGGCAAACCGAGTGAACCTTAGCTATCGTGTACGTCGTCAGCCGA
 10 TGAAGCTTTTATCGTACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCAGTAGGTATCCGCAT
 TGGTACTGATGTATTTTTTACAGTGCATGCTTTGGCCACAGGTGGTTCTGATGCGGTAAGTTTAA
 TTCGTAATATCTTCACTACTTTTACCTCATCACCATCATCACCGGAAAGACGAGGATATAGCTGG
 ATGGTTGTTGGTGATTTCAATCGTGCGCCGGTTAATCTGGAAGCTGCATTAAGACAGGAACCCG
 CCGTGAGTGAAAATACAATTATTATTGCGCCAACAGAACCGACTCATCAGTCCGGTAATATTTT
 15 AGATTATGCGATTTTACATGACGCACATTTACCACGTCGAGAGCAAGTACGTGAACGTATCGGC
 GCAAGTTTAATGTTAAATCAGTTACGCTCACAAATTACATCCGATCATTTTCCT (SEQ ID NO: 15).

In another embodiment, the CdtB fragment is encoded by a homologue of SEQ ID NO: 15. In another embodiment, the CdtB fragment is encoded by a variant of SEQ ID NO: 15. In another embodiment, the CdtB fragment is encoded by an isoform of SEQ ID NO: 15. In another embodiment, the CdtB fragment is encoded
 20 by a fragment of SEQ ID NO: 15. Each possibility represents a separate embodiment of the present invention.

[61] In another embodiment, the amino acid sequence encoding the CdtB fragment is:
 MQWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSGEQGADILMVQ
 EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRSRPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
 VHSDSSVLQSRPAVGIRIGTDVFFTVHALATGGSDAVSLIRNIFTTFTSSPSSPERRGYSWMVVGDFNR
 25 APVNLEAALRQEPVSENTIIAPTEPTHQSGNILDYAILHDAHLPRREQVRERIGASLMLNQLRSQITS
 DHFPVSFVHDRHHHHHHGSGC (SEQ ID NO: 16). In another embodiment, the CdtB fragment is a
 homologue of SEQ ID NO: 16. In another embodiment, the CdtB fragment is a variant of SEQ ID NO: 16. In
 another embodiment, the CdtB fragment is an isoform of SEQ ID NO: 16. In another embodiment, the CdtB
 fragment is a fragment of SEQ ID NO: 16. Each possibility represents a separate embodiment of the present
 30 invention.

[62] In another embodiment, a CdtB toxin, or its fragment or homologue comprises a mutated form of a CdtB toxin. In another embodiment, a CdtB toxin, or its fragment or homologue comprises a mutated form of a CdtB toxin fragment. In another embodiment, a mutated CdtB toxin or a mutated CdtB toxin fragment comprises a deletion mutation. In another embodiment, a mutated CdtB toxin or a mutated CdtB toxin

fragment comprises an insertion mutation. In another embodiment, a mutated CdtB toxin or a mutated CdtB toxin fragment comprises a substitution mutation.

[63] In one embodiment, a CdtB fragment of the present invention comprises a mutation. In one embodiment, the mutation adds a DNA contact residue. In one embodiment, the mutation is G55R, S99Y, V134R, or a combination thereof. In another embodiment, the mutation adds a metal ion binding residue. In one embodiment, the mutation is L62E. In another embodiment, the mutation adds a residue that hydrogen bonds to a catalytic histidine, which in one embodiment, is H160. In one embodiment, the mutation is R100E. In another embodiment, the mutation is Y174P. In one embodiment, the mutation is a combination of two or more of the mutations described hereinabove.

[64] In another embodiment, *cdtC* is encoded by the following nucleic acid sequence:
 ATGGTCGCTAAGGAGAATACTATGAAAAAATATTTATTGAGCTTCTTATTAAGCATGATATTGAC
 TTTGACGAGTCATGCAGAATCAAATCCTGATCCGACTACTTATCCTGATGTAGAGTTATCGCCTC
 CTCCACGTATTAGCTTGCGTAGTTTGCTTACGGCTCAACCAATTAAAAATGACCATTATGATTCA
 CATAATTATTTAAGTACACATTGGGAATTAATTGATTACAAGGGAAAAGAATATGAAAAATTAC
 GTGACGGTGGTACGTTGGTTCAATTTAAAGTGGTCGGTGCAGCAAAATGTTTTGCTTTCCAGGC
 GAAGGCACAACCTGATTGTAAAGATATTGATCATACTGTGTTTAACCTTATTCCAATAATACAGG
 TGCGTTTTTAATCAAAGATGCCCTATTAGGATTTTGTATGACAAGCCATGACTTTGATGATTTGA
 GGCTTGAACCTTGTGGAATTTCAAGTGGTTCGAACCTTTTCGTTGGCGTATCAATGGGGAATA
 TTACCTCCTTTTGGGCCAAGTAAAATTTTAAGACCACCGGTGGGGAGAAATCAGGGTAGCCACC
 ACCACCACCACCACGGATCCGGCTGCTAA (SEQ ID NO: 17). In another embodiment, the nucleic acid
 encoding the CdtC subunit is a homologue of SEQ ID NO: 17. In another embodiment, the nucleic acid
 encoding the CdtC subunit is a variant of SEQ ID NO: 17. In another embodiment, the nucleic acid encoding
 the CdtC subunit is an isoform of SEQ ID NO: 17. In another embodiment, the nucleic acid encoding the
 CdtC subunit is a fragment of SEQ ID NO: 17. Each possibility represents a separate embodiment of the
 present invention.

[65] In another embodiment, CdtC is encoded by the following amino acid sequence:
 MVAKENTMKKYLLSFLLSMILTLTSHAESNPDPPTYPDVELSPPPRISLRSLTAQPIKNDHYDSHNYL
 STHWELIDYKGKEYEKL RDGGTLVQFKVVGA AKCFAPGEGTTDCKDIDHTVFNLIPTNTGAFLIKD
 ALLGFCMTSHDFDDLRLPCGISVSGRTFSLAYQWGILPPFGPSKILRPPVGRNQGSHHHHHHGSGC
 (SEQ ID NO: 18). In another embodiment, the CdtC subunit is a homologue of SEQ ID NO: 18. In another
 embodiment, the CdtC subunit is a variant of SEQ ID NO: 18. In another embodiment, the CdtC subunit is an
 isoform of SEQ ID NO: 18. In another embodiment, the CdtC subunit is a fragment of SEQ ID NO: 18. Each
 possibility represents a separate embodiment of the present invention.

[66] In another embodiment, CdtC is encoded by the following amino acid sequence:
 MKKYLLSFLLSMILTLTSHAESNPDPPTYPDVELSPPPRISLRSLTAQPIKNDHYDSHNYLSTHWELID

YKGKEYEKL RDGGTLVQFKVVGA AKCFAPGEGTTDCKDIDHTVFNLIPTNTGAFLIKDALLGFCMT
SHDFDDLRL EPCGISVSGRTFSLAYQWGILPPFGPSKILRPPVGRNQGS HHHHHH HSGC (SEQ ID NO:
19). In another embodiment, the CdtC subunit is a homologue of SEQ ID NO: 19. In another embodiment, the
CdtC subunit is a variant of SEQ ID NO: 19. In another embodiment, the CdtC subunit is an isoform of SEQ
ID NO: 19. In another embodiment, the CdtC subunit is a fragment of SEQ ID NO: 19. Each possibility
represents a separate embodiment of the present invention.

[67] In one embodiment, the amino acid sequence of a CdtC subunit of the present invention is:
ESNPDP TTPDVELSPPPRISLRSL LTAQPIKNDHYDSHNYLSTHWELIDYKGKEYEKL RDGGTLVQF
KVVGA AKCFAPGEGTTDCKDIDHTVFNLIPTNTGAFLIKDALLGFCMTSHDFDDLRL EPCGISVSGR
TFSLAYQWGILPPFGPSKILRPPVGRNQGS (SEQ ID NO: 20). In another embodiment, the CdtC subunit
is a homologue of SEQ ID NO: 20. In another embodiment, the CdtC subunit is a variant of SEQ ID NO: 20.
In another embodiment, the CdtC subunit is an isoform of SEQ ID NO: 20. In another embodiment, the CdtC
subunit is a fragment of SEQ ID NO: 20. Each possibility represents a separate embodiment of the present
invention.

[68] In another embodiment, the CdtC subunit has an amino acid sequence set forth in one of the following
GenBank entries: YP_002343539.1; NP_860979.1; YP_001481647.1; YP_001272542.1; YP_852558.1;
YP_999803.1; YP_178097.1; NP_873399.1; CAL34250.1; AAP78045.1; ABV51670.1; BAF63362.1;
ABJ00844.1; EAQ72030.1; AAB06709.1; ZP_02270536.1; or AAW34668.1. In another embodiment, the
CdtC subunit has any CdtC subunit amino acid sequence known in the art. In another embodiment, the CdtC
subunit is a homologue of a sequence from one of the above GenBank entries. In another embodiment, the
CdtC subunit is a variant of a sequence from one of the above GenBank entries. In another embodiment, the
CdtC subunit is an isoform of a sequence from one of the above GenBank entries. In another embodiment, the
CdtC subunit is a fragment of a sequence from one of the above GenBank entries. Each possibility represents
a separate embodiment of the present invention.

[69] In one embodiment, the nucleotide sequence of a CdtC subunit of the present invention is:
gaatcaaatcctgatccgactactatcctgatgtagagtatcgctcctccacgtattagcttgcgtagtttgcttacggctcaaccaattaaaaatgaccattatga
ttcacataattatttaagtacacattgggaattaattgattacaagggaaaagaatatgaaaaattacgtgacgggtgtacgttggtcaatttaaagtggtcggtgca
gcaaaatgttttgcttcccaggcgaaggcacaactgattgtaaagatattgatcactgtgttaaccttattccaactaacaggtgcgttttaatacaaatgccc
ctattaggattttgatgacaagccatgactttgatgattgaggctgaacctgtggaatttcagtgagtggtcgaaccttttcgttgccgtatcaatggggaatatta
cctccttttgggccaagtaaaatttaagaccaccgggtggggagaaatcagggtagc (SEQ ID NO: 21). In another embodiment, the
nucleotide sequence of the CdtC subunit is a homologue of SEQ ID NO: 21. In another embodiment, the
nucleotide sequence of the CdtC subunit is a variant of SEQ ID NO: 21. In another embodiment, the
nucleotide sequence of the CdtC subunit is an isoform of SEQ ID NO: 21. In another embodiment, the
nucleotide sequence of the CdtC subunit is a fragment of SEQ ID NO: 21. Each possibility represents a
separate embodiment of the present invention.

[70] In another embodiment, the CdtC subunit has a nucleic acid sequence set forth in one of the following GenBank entries: AL111168.1; AE017125.1; CP000814.1; AB285204.1; CP000468.1; CP000538.1; U51121.1; NZ_AASL01000001.1; or CP000025.1. In another embodiment, the CdtC subunit has any CdtC subunit nucleic acid sequence known in the art. In another embodiment, the CdtC subunit is a homologue of a sequence from one of the above GenBank entries. In another embodiment, the CdtC subunit is a variant of a sequence from one of the above GenBank entries. In another embodiment, the CdtC subunit is an isoform of a sequence from one of the above GenBank entries. In another embodiment, the CdtC subunit is a fragment of a sequence from one of the above GenBank entries. Each possibility represents a separate embodiment of the present invention.

[71] In one embodiment, CdtC comprises a cholesterol recognition site and, in one embodiment, CdtC binds to both cell and model membranes in a cholesterol dependent manner.

[72] In another embodiment, the chimera provided herein is a CdtB-DNase I chimera. In another embodiment, the chimera provided herein is a multimeric chimera protein comprising DNase I or its fragment or homologue; and a CdtB toxin, or its fragment or homologue. In another embodiment, the chimera provided herein comprises DNase I or its fragment or homologue; and a CdtB toxin. In another embodiment, the chimera provided herein is a multimeric chimera protein comprising DNase I and a CdtB toxin, or its fragment or homologue. In another embodiment, the chimera provided herein consists of DNase I or its fragment or homologue; and a CdtB toxin.

[73] In one embodiment, a chimera of the present invention comprises (a) said CdtB fragment in the amino terminus of said chimera and said DNase I fragment in the carboxy terminus of said chimera or (b) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a Cdt fragment in the amino terminus of said chimera or (c) a DNase I in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera or (d) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera.

[74] In another embodiment, the DNase I is a clone of the DNase I gene from *Homo sapiens* chromosome 16 (GenBank accession number AC005203). In another embodiment, the *cdtB* gene is from the human periodontal bacterium *Actinobacillus actinomycetemcomitans* Y4 (GenBank accession number AF006830), which in another embodiment is called *Aggregatibacter actinomycetemcomitans*. In another embodiment, a unique *Sph*I restriction endonuclease cleavage site at about the midpoint of each sequence is used to construct two chimeric genes each containing opposite halves of the *cdtB* and DNase I genes.

[75] In another embodiment, the nucleic acid sequence encoding the chimera is:
TGCTAAATTCCCCTCTAGAATAATTTTGTTTAACTTTAAGAAGGAGATATACCATGGTGAGGGGA
ATGAAGCTGCTGGGGGCGCTGCTGGCACTGGCGGCCCTACTGCAGGGGGCCGTGTCCCTGAAGA
TGCGAGCCTTCAACATCCAGACATTTGGGGAGACCAAGATGTCCAATGCCACCCTCGTCAGCTA

CATTGTGCAGATCCTGAGCCGCTATGACATCGCCCTGGTCCAGGAGGTCAGAGACAGCCACCTG
ACTGCCGTGGGGAAGCTGCTGGACAACCTCAATCAGGATGCACCAGACACCTATCACTACGTGG
TCAGTGAGCCACTGGGACGGAACAGCTATAAGGAGCGCTACCTGTTCGTGTACAGGCCTGACCA
GGTGTCTGCGGTGGACAGCTACTACTACGATGATGGCTGCGAGCCCTGCGGGAACGACACCTTC
5 AACCGAGAGCCAGCCATTGTTCAGGTTCTTCTCCCGGTTACAGAGGTCAGGGAGTTTGCCATTGT
TCCCCTGCATGCTTTGGCCACAGGTGGTTCTGATGCGGTAAGTTTAATTCGTAATATCTTCACTA
CTTTTACCTCGTCACCATCATCACCGGAAAGACGAGGATATAGCTGGATGGTTGTTGGTGATTTC
AATCGTGCGCCGGTTAATCTGGAAGCTGCATTAAGACAGGAACCCGCCGTGAGTGAAAATACAA
TTATTATTGCGCCAACAGAACCGACTCATCAGTCCGGTAATATTTTAGATTATGCGATTTTACAT
10 GACGCACATTTACCACGTCGAGAGCAAGTACGTGAACGTATCGGCGCAAGTTTAATGTTAAATC
AGTTACGCTCACAAATTACATCCGATCATTTTCTCTGTTAGTTTTGTTTCATGATCGCCACCACCAC
CACCACCACGGATCCGGCTGCTAA (SEQ ID NO: 22, coding sequence underlined). In one embodiment,

the coding sequence of the nucleic acid sequence is underlined. In another embodiment, the nucleic acid encoding the chimera is a homologue of SEQ ID NO: 22. In another embodiment, the nucleic acid encoding the chimera is a variant of SEQ ID NO: 22. In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID NO: 22. In another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 22. Each possibility represents a separate embodiment of the present invention.

[76] In another embodiment, the nucleic acid sequence encoding the chimera is:
ATGCAATGGGTAAAGCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATGC
20 TAACTTGAGTGATTTCAAAGTAGCAACTTGGAATCTGCAAGGTTCTTCAGCTGTAAATGAAAGT
AAATGGAATATTAATGTGCGCCAATTATTATCGGGAGAACAAGGTGCAGATATTTTGATGGTAC
AAGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATGGGGGAA
CGCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTCCCGTCCAAATATGGTCTATATTTAT
TATTCCCGTTTTAGATGTTGGGGCAAACCGAGTGAACCTTAGCTATCGTGTACGTCGTCAGCCGA
25 TGAAGCTTTTATCGTACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCAGTAGGTATCCGCAT
TGGTACTGATGTATTTTTTACAGTGCATGCGGCCCCGGGGGACGCAGTAGCCGAGATCGACGCT
CTCTATGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCG
ACTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAG
CCCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCC
30 TATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCC
CTTTAACTTCCAGGCTGCCAATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTAT
CCAGTGGAGGTGATGCTGAAGGGATCCCGGGAGCTCGTGGATCCGAATTCTGTACAGGCGCGCC
TGCAGGACGTCGACGGTACCATCGATACGCGTTCGAAGCTTGCGGCCGCACAGCTGTATACACG
TGCAAGCCAGCCAGAACTCGCTCCTGAAGACCCAGAGGATCTCGAGCACCACCACCACCACCAC
35 CATATGCTCGAGGATCCGGCTGCTAACAAAGCCCGAAAAGAAGG (SEQ ID NO: 23). In another
embodiment, the nucleic acid encoding the chimera is a homologue of SEQ ID NO: 23. In another

embodiment, the nucleic acid encoding the chimera is a variant of SEQ ID NO: 23. In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID NO: 23. In another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 23. Each possibility represents a separate embodiment of the present invention.

5 [77] In another embodiment, the amino acid sequence encoding the chimera is:
 MVRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETKMSNATLVSYIVQILSRVDIALVQEVDRDHL
 TAVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFN
 REPAIVRFFSRFTEVREFAIVPLHALATGGSDAVSLIRNIFTTFTSSPSPERRGGYSWMVVGDFNRAPV
 NLEAALRQEPASSENTIIAPTEPTHQSGNILDYAILHDAHLPRREQVRERIGASLMLNQLRSQITSDFH
 10 PVSFVHDRHHHHHHGSGC (SEQ ID NO: 24). In another embodiment, the chimera is a homologue of SEQ
 ID NO: 24. In another embodiment, the chimera is a variant of SEQ ID NO: 24. In another embodiment, the
 chimera is an isoform of SEQ ID NO: 24. In another embodiment, the chimera is a fragment of SEQ ID NO:
 24. Each possibility represents a separate embodiment of the present invention.

[78] In another embodiment, the amino acid sequence encoding the chimera is:
 15 MQWVKQLNVVFCTMLFSFSSYANLSDFKVVATWNLQGSSAVNESKWNINVRQLLSGEQGADILMVQ
 EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRSRPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
 VHSDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGC
 SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAVDRIVVAGMLLRGAVVPDSALPFNFQAANG
 LSDQLAQAI SDHYPVEV MLKGSREL VDPNSVQARLQDVGTDIDTRSKLAAAQLYTRASQPELAPEDP
 20 EDLEHHHHHHHMLLEDPAANKARKEG (SEQ ID NO: 25). In another embodiment, the chimera is a
 homologue of SEQ ID NO: 25. In another embodiment, the chimera is a variant of SEQ ID NO: 25. In
 another embodiment, the chimera is an isoform of SEQ ID NO: 25. In another embodiment, the chimera is a
 fragment of SEQ ID NO: 25. Each possibility represents a separate embodiment of the present invention.

[79] In another embodiment, the amino acid sequence encoding the chimera is:
 25 MQWVKQLNVVFCTMLFSFSSYANLSDFKVVATWNLQGSSAVNESKWNINVRQLLSGEQGADILMVQ
 EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRSRPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
 VHSDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGC
 SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAVDRIVVAGMLLRGAVVPDSALPFNFQAANG
 LSDQLAQAI SDHYPVEV MLKHHHHHHHMLLEDPAANKAQQK (SEQ ID NO: 26). In another
 30 embodiment, the chimera is a homologue of SEQ ID NO: 26. In another embodiment, the chimera is a variant
 of SEQ ID NO: 26. In another embodiment, the chimera is an isoform of SEQ ID NO: 26. In another
 embodiment, the chimera is a fragment of SEQ ID NO: 26. Each possibility represents a separate embodiment
 of the present invention.

[80] In another embodiment, the amino acid sequence encoding the chimera is:
 35 MVRGMKLLGALLALAALLQGAVSLKIAAFNIQTFGETKMSNATLVSYIVQILSRVDIALVQEVDRDHL

TAVGKLLDNLNQDAPDTYHYVVSEPLGRNSYKERYLFVYRPDQVSAVDSYYYDDGCEPCGNDTFN
 REPAIVRFFSRFTEVREFAIVPLHALATGGSDAVSLIRNIFTTFTSSPSPERRRGYSWMVVGDFNRAPV
 NLEAALRQEPAVSENTIIAPTEPTHQSGNILDYAILHDAHLPRREQVRERIGASLMLNQLRSQITSDFH
 PVSFVHDRHHHHHHHSGGC (SEQ ID NO: 27). In another embodiment, the chimera is a homologue of SEQ
 ID NO: 27. In another embodiment, the chimera is a variant of SEQ ID NO: 27. In another embodiment, the
 chimera is an isoform of SEQ ID NO: 27. In another embodiment, the chimera is a fragment of SEQ ID NO:
 27. Each possibility represents a separate embodiment of the present invention.

[81] In one embodiment a chimera of the present invention binds to both CdtA and CdtC. In another
 embodiment, a chimera of the present invention binds to CdtC only.

In another embodiment, the chimera is encoded by the following nucleic acid sequence:
 ATGGAATGGGTAaagCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATG
 CTAACCTTGAGTGATTTCAAAGTAGCAACTTGGAATCTGCAAGGTTCTTCAGCTGTAAATGAA
 AGTAAATGGAATATTAATGTGCGCCAATTATTATCGAGGGAACAAGGTGCAGATATTGAGAT
 GGTACAAGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATG
 GGGGAACGCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTATGAGCCAAATATGGTC
 TATATTTATTATCCCGTTTTAGATGTTGGGGCAAACCGAGTGAACCTAGCTATCGTGTACAGT
 CGTCAAGCCGATGAAGCTTTTATCCGACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCA
 GTAGGTATCCGCATTGGTACTGATGTATTTTTTACAGTGCATGCGGCCCCGGGGGACGCAGT
 AGCCGAGATCGACGCTCTCCCTGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGG
 ACGTCATGTTGATGGGCGACTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCAGTGGTCA
 TCCATCCGCCTGTGGACAAGCCCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCAC
 AGCTACACCCACGCACTGTGCCTATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCG
 CCGTTGTTCCCGACTCGGCTCTTCCCTTTAACTTCCAGGCTGCCTTAATGTTAAATCAGTTAC
 GCTCACAATTACAAGTGACCACTATCCAGTGGAGGTGATGCTGAAGCACCACCACCACCAC
 CACCATATGCTCGAGGATCCGGCTGCTAACAAGCTGAAAGAAGC (SEQ ID NO: 28;
 CdtB/DNase^{Y174}). In another embodiment, the nucleic acid encoding the chimera is a homologue of SEQ
 ID NO: 28. In another embodiment, the nucleic acid encoding the chimera is a variant of SEQ ID NO: 28.
 In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID NO: 28. In
 another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 28. Each
 possibility represents a separate embodiment of the present invention.

[82] In another embodiment, the chimera is encoded by the following amino acid sequence:
 MEWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSREQGADIEMVQ
 EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRYEPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
 RHSDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALPDVYLDVQEKWGLEDVMLMGDFNAGC
 SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCA YDRIVVAGMLLRGAVVPDSALPFNFQAALM

LNQLRSQITSDHYPVEVMLKHHHHHHHMLEDPAAANKLKEA (SEQ ID NO: 29; CdtB/DNase^{Y174}). In another embodiment, the chimera is a homologue of SEQ ID NO: 29. In another embodiment, the chimera is a variant of SEQ ID NO: 29. In another embodiment, the chimera is an isoform of SEQ ID NO: 29. In another embodiment, the chimera is a fragment of SEQ ID NO: 29. Each possibility represents a separate embodiment of the present invention. Each possibility represents a separate embodiment of the present invention.

[83] In another embodiment, the chimera is encoded by the following nucleic acid sequence:
 ATGGAATGGGTAaagCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATGCT
 AACTTGAGTGATTTCAAAGTAGCAACTTGAATCTGCAAGGTTCTTCAGCTGTAAATGAAAGTA
 AATGGAATATTAATGTGCGCCAATTATTATCGAGGGAACAAGGTGCAGATATTGAGATGGTACA
 AGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATGGGGGAAC
 GCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTATGAGCCAAATATGGTCTATATTTATT
 ATTCCCGTTTAGATGTTGGGGCAAACCGAGTGAACCTAGCTATCGTGTACGTCGTCAAGCCGAT
 GAAGCTTTTATCCGACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCAGTAGGTATCCGCAT
 TGGTACTGATGTATTTTTTACAGTGCATGCGGCCCGGGGGACGCAGTAGCCGAGATCGACGCT
 CTCTATGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCG
 ACTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAG
 CCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCC
 TATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCC
 CTTTAACTTCCAGGCTGCCAATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTAT
 CCAGTGGAGGTGATGCTGAAGCACCACCACCACCACCACCATATGCTCGAGGATCCGGCTGCTA
 ACAAGCTGAAAGAAGC (SEQ ID NO: 30). In another embodiment, the nucleic acid encoding the chimera is a homologue of SEQ ID NO: 30. In another embodiment, the nucleic acid encoding the chimera is a variant of SEQ ID NO: 30. In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID NO: 30. In another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 30. Each possibility represents a separate embodiment of the present invention.

[84] In another embodiment, the chimera is encoded by the following amino acid sequence:
 MEWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSREQGADIEMVQ
 EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRYEPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
 RHDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGC
 SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCA YDRIVVAGMLLRGAVVPDSALPFNFQAANG
 LSDQLAQAI SDHYPVEVMLKHHHHHHHMLEDPAAANKLKEA (SEQ ID NO: 31). In another embodiment, the chimera is a homologue of SEQ ID NO: 31. In another embodiment, the chimera is a variant of SEQ ID NO: 31. In another embodiment, the chimera is an isoform of SEQ ID NO: 31. In another embodiment, the chimera is a fragment of SEQ ID NO: 31. Each possibility represents a separate embodiment of the present invention. Each possibility represents a separate embodiment of the present invention.

[85] In another embodiment, the chimera is encoded by the following nucleic acid sequence:

ATGGAATGGGTAAAGCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATGC
 TAACTTGAGTGATTTCAAAGTAGCAACTTGGAATCTGCAAGGTTCTTCAGCTGTAAATGAAAGT
 AAATGGAATATTAATGTGCGCCAATTATTATCGGGAGAACAAGGTGCAGATATTTTGATGGTAC
 5 AAGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATGGGGGAA
 CGCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTATGAGCCAAATATGGTCTATATTTAT
 TATTCCCGTTTATAGATGTTGGGGCAAACCGAGTGAACCTTAGCTATCGTGTACGTCGTCAAGCCGA
 TGAAGCTTTTATCGTACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCAGTAGGTATCCGCAT
 TGGTACTGATGTATTTTTTACAGTGCATGCGGCCCGGGGGACGCAGTAGCCGAGATCGACGCT
 10 CTCTATGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCG
 ACTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAG
 CCCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCC
 TATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCC
 CTTTAACTTCCAGGCTGCCAATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTAT
 15 CCAGTGGAGGTGATGCTGAAGCACCACCACCACCACCACCATATGCTCGAGGATCCGGCTGCTA
 ACAAGCTGAAAGAAGC (SEQ ID NO: 32). In another embodiment, the nucleic acid encoding the chimera

is a homologue of SEQ ID NO: 32. In another embodiment, the nucleic acid encoding the chimera is a variant of SEQ ID NO: 32. In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID NO: 32. In another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 32. Each possibility represents a separate embodiment of the present invention.

[86] In another embodiment, the chimera is encoded by the following amino acid sequence:

MEWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSGEQGADILMVQ
 EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRYEPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
 VHSDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGC
 25 SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCA YDRIVVAGMLLRGAVVPDSALPFNFQAANG
 LSDQLAQAI SDHYPVEV MLKHHHHHHH MLEDPAANKLKEA (SEQ ID NO: 33). In another

embodiment, the chimera is a homologue of SEQ ID NO: 33. In another embodiment, the chimera is a variant of SEQ ID NO: 33. In another embodiment, the chimera is an isoform of SEQ ID NO: 33. In another embodiment, the chimera is a fragment of SEQ ID NO: 33. Each possibility represents a separate embodiment of the present invention. Each possibility represents a separate embodiment of the present invention.

[87] In another embodiment, the chimera is encoded by the following nucleic acid sequence:

ATGGAATGGGTAAagCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATGCT
 AACTTGAGTGATTTCAAAGTAGCAACTTGGAATCTGCAAGGTTCTTCAGCTGTAAATGAAAGTA
 AATGGAATATTAATGTGCGCCAATTATTATCGAGGGAACAAGGTGCAGATATTGAGATGGTACA
 35 AGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATGGGGGAAC
 GCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTATGAGCCAAATATGGTCTATATTTATT

ATTCCCGTTTAGATGTTGGGGCAAACCGAGTGAACCTAGCTATCGTGTACGTCGTCAAGCCGAT
 GAAGCTTTTATCCGACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCAGTAGGTATCCGCAT
 TGGTACTGATGTATTTTTTACAGTGCATGCGGCCCCGGGGGACGCAGTAGCCGAGATCGACGCT
 CTCTATGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCG
 5 ACTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAG
 CCCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCC
 TATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCC
 CTTTAACTTCCAGGCTGCCTTAATGTAAATCAGTTACGCTCACAAATTACAAGTGACCACTATC
 CAGTGGAGGTGATGCTGAAGCACCACCACCACCACCACCATATGCTCGAGGATCCGGCTGCTAA

10 CAAGCTGAAAGAAGC (SEQ ID NO: 34). In another embodiment, the nucleic acid encoding the chimera is a homologue of SEQ ID NO: 34. In another embodiment, the nucleic acid encoding the chimera is a variant of SEQ ID NO: 34. In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID NO: 34. In another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 34. Each possibility represents a separate embodiment of the present invention.

15 [88] In another embodiment, the chimera is encoded by the following amino acid sequence:
 MEWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSREQGADIEMVQ
 EAGSLPSSAVRTSRVIQHGGTPIEETWNLGTRYEPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
 RHSDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGC
 SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCA YDRIVVAGMLLRGAVVPDSALPFNFQAALM
 20 LNQLRSQITSDHYPVEVMLKHHHHHHHMLEDPAANKLKEA (SEQ ID NO: 35). In another
 embodiment, the chimera is a homologue of SEQ ID NO: 35. In another embodiment, the chimera is a variant of SEQ ID NO: 35. In another embodiment, the chimera is an isoform of SEQ ID NO: 35. In another embodiment, the chimera is a fragment of SEQ ID NO: 35. Each possibility represents a separate embodiment of the present invention. Each possibility represents a separate embodiment of the present invention.

25 [89] In another embodiment, the chimera is encoded by the following nucleic acid sequence:
 ATGGAATGGGTAaagCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATGCT
 AACTTGAGTGATTTCAAAGTAGCAACTTGGAACTCTGCAAGGTTCTTCAGCTGTAAATGAAAGTA
 AATGGAATATTAATGTGCGCCAATTATTATCGGGAGAACAAGGTGCAGATATTGAGATGGTACA
 AGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATGGGGGAAC
 30 GCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTATGAGCCAAATATGGTCTATATTTATT
 ATTCCCGTTTAGATGTTGGGGCAAACCGAGTGAACCTAGCTATCGTGTACGTCGTCAAGCCGAT
 GAAGCTTTTATCGTACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCAGTAGGTATCCGCATT
 GGTACTGATGTATTTTTTACAGTGCATGCGGCCCCGGGGGACGCAGTAGCCGAGATCGACGCTC
 TCTATGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCGA
 35 CTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAGC
 CCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCCT

ATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCCC
TTTAACTTCCAGGCTGCCAATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTATC
CAGTGGAGGTGATGCTGAAGCACCACCACCACCACCACCATATGCTCGAGGATCCGGCTGCTAA
CAAGCTGAAAGAAGC (SEQ ID NO: 36). In another embodiment, the nucleic acid encoding the chimera
5 is a homologue of SEQ ID NO: 36. In another embodiment, the nucleic acid encoding the chimera is a variant
of SEQ ID NO: 36. In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID
NO: 36. In another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 36. Each
possibility represents a separate embodiment of the present invention.

[90] In another embodiment, the chimera is encoded by the following amino acid sequence:
10 MEWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSGEQGADIEMVQ
EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRYEPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
VHSDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGC
SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAANG
LSDQLAQAI SDHYPVEV MLKHHHHHHH MLEDPAANKLKEA (SEQ ID NO: 37). In another
15 embodiment, the chimera is a homologue of SEQ ID NO: 37. In another embodiment, the chimera is a variant
of SEQ ID NO: 37. In another embodiment, the chimera is an isoform of SEQ ID NO: 37. In another
embodiment, the chimera is a fragment of SEQ ID NO: 37. Each possibility represents a separate embodiment
of the present invention. Each possibility represents a separate embodiment of the present invention.

[91] In another embodiment, the chimera is encoded by the following nucleic acid sequence:
20 ATGGAATGGGTAaagCAATTAAATGTGGTTTTCTGTACGATGTTATTTAGCTTTTCAAGTTATGCT
AACTTGAGTGATTTCAAAGTAGCAACTTGAATCTGCAAGGTTCTTCAGCTGTAAATGAAAGTA
AATGGAATATTAATGTGCGCCAATTATTATCGAGGGAACAAGGTGCAGATATTGAGATGGTACA
AGAAGCGGGTTCATTACCAAGTTCGGCAGTAAGAACCTCACGAGTAATTCAACATGGGGGAAC
GCCAATTGAGGAATATACCTGGAATTTAGGTACTCGCTATGAGCCAAATATGGTCTATATTTATT
25 ATTCCCGTTTAGATGTTGGGGCAAACCGAGTGAACCTAGCTATCGTGTACGTCGTCAGCCGAT
GAAGCTTTTATCGTACATTCTGATTCTTCTGTGCTTCAATCTCGCCCGGCAGTAGGTATCCGCATT
GGTACTGATGTATTTTTTACAGTGCATGCGGCCCCGGGGGACGCAGTAGCCGAGATCGACGCTC
TCTATGACGTCTACCTGGATGTCCAAGAGAAATGGGGCTTGGAGGACGTCATGTTGATGGGCGA
CTTCAATGCGGGCTGCAGCTATGTGAGACCCTCCCAGTGGTCATCCATCCGCCTGTGGACAAGC
30 CCCACCTTCCAGTGGCTGATCCCCGACAGCGCTGACACCACAGCTACACCCACGCACTGTGCCT
ATGACAGGATCGTGGTTGCAGGGATGCTGCTCCGAGGCGCCGTTGTTCCCGACTCGGCTCTTCCC
TTTAACTTCCAGGCTGCCAATGGCCTGAGTGACCAACTGGCCCAAGCCATCAGTGACCACTATC
CAGTGGAGGTGATGCTGAAGCACCACCACCACCACCACCATATGCTCGAGGATCCGGCTGCTAA
CAAGCTGAAAGAAGC (SEQ ID NO: 38). In another embodiment, the nucleic acid encoding the chimera
35 is a homologue of SEQ ID NO: 38. In another embodiment, the nucleic acid encoding the chimera is a variant
of SEQ ID NO: 38. In another embodiment, the nucleic acid encoding the chimera is an isoform of SEQ ID

NO: 38. In another embodiment, the nucleic acid encoding the chimera is a fragment of SEQ ID NO: 38. Each possibility represents a separate embodiment of the present invention.

[92] In another embodiment, the chimera is encoded by the following amino acid sequence:
MEWVKQLNVVFCTMLFSFSSYANLSDFKVATWNLQGSSAVNESKWNINVRQLLSREQGADIEMVQ
5 EAGSLPSSAVRTSRVIQHGGTPIEEYTWNLGTRYEPNMVYIYYSRLDVGANRVNLAIVSRRQADEAFI
VHSDSSVLQSRPAVGIRIGTDVFFTVHAAPGDAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGC
SYVRPSQWSSIRLWTSPTFQWLIPDSADTTATPTHCA YDRIVVAGMLLRGAVVPDSALPFNFQAANG
LSDQLAQAI SDHYPVEV MLKHHHHHHHMLEDP AANKLKEA (SEQ ID NO: 39). In another
embodiment, the chimera is a homologue of SEQ ID NO: 39. In another embodiment, the chimera is a variant
10 of SEQ ID NO: 39. In another embodiment, the chimera is an isoform of SEQ ID NO: 39. In another
embodiment, the chimera is a fragment of SEQ ID NO: 39. Each possibility represents a separate embodiment
of the present invention. Each possibility represents a separate embodiment of the present invention.

[93] In another embodiment, a deletion mutant comprises a single deletion. In another embodiment, a
deletion mutant comprises a deletion of at least a single nucleotide. In another embodiment, a deletion mutant
15 comprises a deletion of at least a single amino acid. In another embodiment, a deletion mutant comprises a
deletion of at least 2 amino acids. In another embodiment, a deletion mutant comprises a deletion of at least 3
amino acids. In another embodiment, a deletion mutant comprises a deletion of at least 4 amino acids. In
another embodiment, a deletion mutant comprises a deletion of at least 5 amino acids. In another
embodiment, a deletion mutant comprises a deletion of at least 6 amino acids. In another embodiment, a
20 deletion mutant comprises a deletion of at least 8 amino acids. In another embodiment, a deletion mutant
comprises a deletion of at least 10 amino acids.

[94] In another embodiment, an insertion mutant comprises a single insertion. In another embodiment, an
insertion mutant comprises an insertion of at least a single nucleotide. In another embodiment, an insertion
mutant comprises an insertion of at least a single amino acid. In another embodiment, an insertion mutant
25 comprises an insertion of at least 2 amino acids. In another embodiment, an insertion mutant comprises an
insertion of at least 3 amino acids. In another embodiment, an insertion mutant comprises an insertion of at
least 4 amino acids. In another embodiment, an insertion mutant comprises an insertion of at least 5 amino
acids. In another embodiment, an insertion mutant comprises an insertion of at least 6 amino acids. In another
embodiment, an insertion mutant comprises an insertion of at least 8 amino acids. In another embodiment, an
30 insertion mutant comprises an insertion of at least 10 amino acids.

[95] In another embodiment, a substitution mutant comprises a single substitution. In another
embodiment, a substitution mutant comprises a substitution of at least a single amino acid. In another
embodiment, a substitution mutant comprises a substitution of at least 2 amino acids. In another embodiment,
a substitution mutant comprises a substitution of at least 3 amino acids. In another embodiment, a substitution
35 mutant comprises a substitution of at least 4 amino acids. In another embodiment, a substitution mutant

comprises a substitution of at least 5 amino acids. In another embodiment, a substitution mutant comprises a substitution of at least 6 amino acids. In another embodiment, a substitution mutant comprises a substitution of at least 8 amino acids. In another embodiment, a substitution mutant comprises a substitution of at least 10 amino acids.

5 [96] In one embodiment, a mutation of the present invention comprises a substitution of a sequence of DNase I with the large loop in the CdtB sequence, which in one embodiment, is present in residues L261-S272, which is predicted to bind to CdtA, in one embodiment. In one embodiment, the DNase I fragment comprises a substitution of SEQ ID NO: 58 for SEQ ID NO: 59.

10 [97] In another embodiment, a mutated Cdt toxin or a mutated Cdt toxin fragment comprises an insertion mutation, a deletion mutation, a substitution mutation, or any combination thereof. In another embodiment, a mutated CdtB toxin or a mutated CdtB toxin fragment comprises an insertion mutation, a deletion mutation, a substitution mutation, or any combination thereof. In another embodiment, a mutated DNase I or a mutated DNase I fragment comprises an insertion mutation, a deletion mutation, a substitution mutation, or any combination thereof.

15 [98] In another embodiment, the sequence of a mutated chimera as provided herein may comprise the DNA sequence as set forth in SEQ ID NOS: 28, 30, 32, 34, 36, or 38. In another embodiment, the sequence of a mutated chimera as provided herein may comprise the amino acid sequence as set forth in SEQ ID NOS: 29, 31, 33, 35, 37, or 39.

20 [99] In another embodiment, the chimera as provided herein is His-tagged. In another embodiment, the chimera as provided herein is His₆-tagged. In another embodiment, methods and compositions of the present invention utilize a chimeric molecule, comprising a fusion of a recombinant chimeric polypeptide with a tag polypeptide that provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is placed, in other embodiments, at the amino- or carboxyl-terminus of the protein or in an internal location therein. The presence of such epitope-tagged forms of the chimeric polypeptide is detected, in another
25 embodiment, using an antibody against the tag polypeptide. In another embodiment, inclusion of the epitope tag enables the recombinant chimeric polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 (Field *et al.*, Mol. Cell. Biol., 8: 2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al.*, Molecular and Cellular Biology, 5: 3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky *et al.*, Protein Engineering, 3(6): 547-553 (1990)). Other tag polypeptides include the Flag-peptide (Hopp *et al.*, BioTechnology, 6: 1204-1210 (1988)); the KT3 epitope peptide (Martin *et al.*, Science, 255: 192-194 (1992)); a tubulin epitope peptide (Skinner *et al.*, J. Biol. Chem., 266:15163-
30 15166 (1991)); and the T7 gene 10 protein peptide tag (Lutz-Freyermuth *et al.*, Proc. Natl. Acad. Sci. USA,
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87: 6393-6397 (1990)). Methods for constructing fusion proteins are well known in the art, and are described, for example, in LaRoche *et al.*, J. Cell Biol., 139(2): 357-66 (1995); Heidaran *et al.*, FASEB J., 9(1): 140-5 (1995); Ashkenazi *et al.*, Int. Rev. Immunol., 10(2-3): 219-27 (1993) and Cheon *et al.*, PNAS USA, 91(3): 989-93 (1994).

5 [100] In another embodiment, provided herein is a chimera comprising a Cdt fragment in the amino terminus of the chimera and a DNase I fragment in the carboxy terminus of the chimera. In another embodiment, provided herein is a chimera comprising a Cdt fragment in the amino terminus of the chimera and a homologue of a DNase I fragment in the carboxy terminus of the chimera. In another embodiment, provided herein is a chimera comprising a homologue of a Cdt fragment in the amino terminus of the chimera and a DNase I in the carboxy terminus of the chimera. In another embodiment, provided herein is a chimera comprising a homologue of a Cdt fragment in the amino terminus of the chimera and a homologue of a DNase I fragment in the carboxy terminus of the chimera.

[101] In another embodiment, provided herein is a chimera comprising a CdtB fragment in the amino terminus of the chimera and a DNase I fragment in the carboxy terminus of the chimera. In another embodiment, provided herein is a chimera comprising a CdtB fragment in the amino terminus of the chimera and a homologue of a DNase I fragment in the carboxy terminus of the chimera. In another embodiment, provided herein is a chimera comprising a homologue of a CdtB fragment in the amino terminus of the chimera and a DNase I in the carboxy terminus of the chimera. In another embodiment, provided herein is a chimera comprising a homologue of a CdtB fragment in the amino terminus of the chimera and a homologue of a DNase I fragment in the carboxy terminus of the chimera.

[102] In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds CdtC. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds CdtA. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds both CdtA and CdtC. In another embodiment, provided herein is a recombinant polypeptide consisting of a chimera, wherein the recombinant polypeptide binds both CdtA and CdtC. In another embodiment, provided herein is a recombinant polypeptide consisting of a chimera and a leader peptide, wherein the recombinant polypeptide binds both CdtA and CdtC. In another embodiment, provided herein is a recombinant polypeptide consisting of a chimera and a signal peptide, wherein the recombinant polypeptide binds both CdtA and CdtC.

[103] In another embodiment, provided herein is a recombinant polypeptide that inhibits proliferation of a cell. In another embodiment, provided herein is a recombinant polypeptide that inhibits proliferation of a eukaryotic cell. In another embodiment, provided herein is a recombinant polypeptide that inhibits proliferation of a neoplastic cell. In another embodiment, provided herein is a recombinant polypeptide that induces cell cycle arrest. In another embodiment, provided herein is a recombinant polypeptide comprising a

chimera, wherein the recombinant polypeptide binds both CdtA and CdtC and induces cell cycle arrest. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds CdtC and induces cell cycle arrest. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds CdtA and induces cell cycle arrest. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds both CdtA and CdtC and inhibits proliferation of a neoplastic cell. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds CdtC and inhibits proliferation of a neoplastic cell. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera, wherein the recombinant polypeptide binds CdtA and inhibits proliferation of a neoplastic cell.

[104] In another embodiment, provided herein is a recombinant polypeptide comprising a chimera encoded by the DNA sequence of SEQ ID NO: 28, wherein the recombinant polypeptide binds both CdtA and CdtC and inhibits proliferation of a neoplastic cell. In another embodiment, provided herein is a recombinant polypeptide comprising a chimera encoded by the amino acid sequence of SEQ ID NO: 29, wherein the recombinant polypeptide binds both CdtA and CdtC and inhibits proliferation of a neoplastic cell.

[105] In another embodiment, a chimera as described herein comprises a human DNase I/CdtB protein comprising potent supercoiled DNA nicking activity and cell delivery and nuclear localization mechanisms.

[106] In another embodiment, provided herein is a method for inhibiting the proliferation of a neoplastic cell comprising the step of contacting a cell with a recombinant polypeptide comprising a chimera as described herein or a nucleic acid molecule encoding the same, thereby inhibiting the proliferation of a neoplastic cell. In another embodiment, provided herein is a method for inhibiting the proliferation of a neoplastic cell comprising the step of contacting a cell with a nucleic acid molecule encoding a recombinant polypeptide comprising a chimera as described herein comprising the step of administering a DNA vector comprising said nucleic acid molecule. In one embodiment, methods of contacting a cell with a nucleic acid molecule by administering a DNA vector are known to one of skill in the art.

[107] In another embodiment, the vector consists of a DNA sequence encoding a recombinant polypeptide comprising a chimera and a larger sequence that serves of the "backbone" of the vector. In another embodiment, the vector consists of a DNA sequence encoding a recombinant polypeptide consisting of a chimera and a larger sequence that serves of the "backbone" of the vector. In another embodiment, the vector multiplies in the target cell. In another embodiment, the vector is expressed in the target cell, thus expressing a recombinant polypeptide such as those described herein. In another embodiment, the vector is an expression vector.

[108] In one embodiment, the formulations and methods of the instant invention comprise a nucleic acid sequence operably linked to one or more regulatory sequences. In one embodiment, a nucleic acid molecule

introduced into a cell is in a form suitable for expression in the cell of the gene product encoded by the nucleic acid. Accordingly, in one embodiment, the nucleic acid molecule includes coding and regulatory sequences required for transcription of a gene (or portion thereof). When the gene product is a protein or peptide, the nucleic acid molecule includes coding and regulatory sequences required for translation of the nucleic acid molecule include promoters, enhancers, polyadenylation signals, sequences necessary for transport of an encoded protein or peptide.

[109] In one embodiment, nucleotide sequences which regulate expression of a gene product (e.g., promoter and enhancer sequences) are selected based upon the type of cell in which the gene product is to be expressed and the desired level of expression of the gene product. For example, a promoter known to confer cell-type specific expression of a gene linked to the promoter can be used. A promoter specific for myoblast gene expression can be linked to a gene of interest to confer muscle-specific expression of that gene product. Muscle-specific regulatory elements which are known in the art include upstream regions from the dystrophin gene (Klamut et al., (1989) *Mol. Cell Biol.* 9:2396), the creatine kinase gene (Buskin and Hauschka, (1989) *Mol. Cell Biol.* 9:2627) and the troponin gene (Mar and Ordahl, (1988) *Proc. Natl. Acad. Sci. USA.* 85:6404). Negative response elements in keratin genes mediate transcriptional repression (Jho Sh et al, (2001). *J. Biol Chem.*). Regulatory elements specific for other cell types are known in the art (e.g., the albumin enhancer for liver-specific expression; insulin regulatory elements for pancreatic islet cell-specific expression; various neural cell-specific regulatory elements, including neural dystrophin, neural enolase and A4 amyloid promoters). Alternatively, a regulatory element which can direct constitutive expression of a gene in a variety of different cell types, such as a viral regulatory element, can be used. Examples of viral promoters commonly used to drive gene expression include those derived from polyoma virus, Adenovirus 2, cytomegalovirus (CMV) and Simian Virus 40, and retroviral LTRs. Alternatively, a regulatory element which provides inducible expression of a gene linked thereto can be used. The use of an inducible regulatory element (e.g., an inducible promoter) allows for modulation of the production of the gene product in the cell. Examples of potentially useful inducible regulatory systems for use in eukaryotic cells include hormone-regulated elements (e.g., see Mader, S. and White, J. H. (1993) *Proc. Natl. Acad. Sci. USA* 90:5603-5607), synthetic ligand-regulated elements (see, e.g., Spencer, D. M. et al (1993) *Science* 262:1019-1024) and ionizing radiation-regulated elements (e.g., see Manome, Y. Et al. (1993) *Biochemistry* 32:10607-10613; Datta, R. et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:1014-10153). Additional tissue-specific or inducible regulatory systems which may be developed can also be used in accordance with the invention.

[110] In one embodiment, a regulatory sequence of the instant invention may comprise a CMV promoter, while in another embodiment, the regulatory sequence may comprise a CAG promoter. In one embodiment, a CAG promoter is a composite promoter that combines the human cytomegalovirus immediate-early enhancer and a modified chicken beta-actin promoter and first intron. In one embodiment, a regulatory sequence may comprise a simian virus (SV)-40 polyadenylation sequence, which in one embodiment, is the mechanism by which most messenger RNA molecules are terminated at their 3' ends in eukaryotes. In one embodiment, the

polyadenosine (poly-A) tail protects the mRNA molecule from exonucleases and is important for transcription termination, for export of the mRNA from the nucleus, and for translation. In another embodiment, a formulation of the present invention may comprise one or more regulatory sequences.

5 [111] In another embodiment, the vector comprises a promoter sequence that drives expression of the gene encoding a recombinant polypeptide comprising a chimera, as described herein. In another embodiment, the vector is a transcription vector.

[112] In another embodiment, the promoter is a constitutively active promoter. In another embodiment, the promoter is an inducible promoter.

10 [113] In another embodiment, the vector is a plasmid. In another embodiment, the vector is a viral vector. In another embodiment, the vector comprises an origin of replication. In another embodiment, the vector comprises a "multiple cloning site". In another embodiment, the vector is a bacterial vector. In another embodiment, a bacterial vector comprising a transgene encoding a recombinant polypeptide as described herein induces the expression of the recombinant polypeptide

15 [114] In another embodiment, the vector is a genetically-engineered virus. In another embodiment, the vector further comprises a helper virus or packaging lines for large-scale transfection. In another embodiment, the vector is designed for permanent incorporation of the insert into a host genome.

[115] In another embodiment, methods for contacting a cell with a nucleic acid provided herein include "naked DNA" technology. In another embodiment, methods including "naked DNA" technology result in transiently expressed recombinant polypeptide.

20 [116] This invention provides, in one embodiment, a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.

25 [117] In one embodiment, "protein" or "polypeptide" refers to an amino acid chain comprising multiple peptide subunits, and may, in one embodiment, include a full-length protein, oligopeptides, and fragments thereof, wherein the amino acid residues are linked by covalent peptide bonds. In one embodiment, a protein described in the present invention may comprise a polypeptide of the present invention. In one embodiment, a protein is a multimeric structure. In one embodiment, a protein of the present invention is a holotoxin.

30 [118] The term "native" or "native sequence" refers to a polypeptide having the same amino acid sequence as a polypeptide that occurs in nature. A polypeptide is considered to be "native" in accordance with the present invention regardless of its mode of preparation. Thus, such native sequence polypeptide can be isolated from nature or can be produced by recombinant and/or synthetic means. The terms "native" and "native sequence" specifically encompass naturally-occurring truncated or secreted forms (e.g., an

extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of a polypeptide.

[119] As used herein in the specification and in the examples section which follows the term "peptide" includes native peptides (either degradation products, synthetically synthesized peptides or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides), such as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body or more capable of penetrating into bacterial cells. Such modifications include, but are not limited to N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH₂-NH, CH₂-S, CH₂-S=O, O=C-NH, CH₂-O, CH₂-CH₂, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

[120] Peptide bonds (-CO-NH-) within the peptide may be substituted, for example, by N-methylated bonds (-N(CH₃)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylen bonds (-CO-CH₂-), α -aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH₂-NH-), hydroxyethylene bonds (-CH(OH)-CH₂-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), peptide derivatives (-N(R)-CH₂-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom.

[121] These modifications can occur at any of the bonds along the peptide chain and even at several (2-3) at the same time.

[122] Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

[123] In addition to the above, the peptides of the present invention may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

[124] As used herein in the specification and in the claims section below the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

[125] Either naturally occurring amino acids or non-conventional or modified amino acids may be used in the compositions and methods of the present invention.

[126] As used herein, the term "amino acid" refers to either the D or L stereoisomer form of the amino acid, unless otherwise specifically designated. Also encompassed within the scope of this invention are equivalent proteins or equivalent peptides, e.g., having the biological activity of purified wild type tumor suppressor protein. "Equivalent proteins" and "equivalent polypeptides" refer to compounds that depart from the linear sequence of the naturally occurring proteins or polypeptides, but which have amino acid substitutions that do not change its biologically activity. These equivalents can differ from the native sequences by the replacement of one or more amino acids with related amino acids, for example, similarly charged amino acids, or the substitution or modification of side chains or functional groups.

[127] In another embodiment, the present invention provides a recombinant polynucleotide encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.

[128] In one embodiment, the term "nucleic acid" or "polynucleotide" refers to oligonucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA) or mimetic thereof. The term should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotide. This term includes oligonucleotides composed of naturally occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

[129] In one embodiment, the term "nucleic acid" or "oligonucleotide" refers to a molecule, which may include, but is not limited to, prokaryotic sequences, eukaryotic mRNA, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also refers to sequences that include any of the known base analogs of DNA and RNA.

[130] The nucleic acids can be produced by any synthetic or recombinant process such as is well known in the art. Nucleic acids can further be modified to alter biophysical or biological properties by means of techniques known in the art. For example, the nucleic acid can be modified to increase its stability against nucleases (e.g., "end-capping"), or to modify its solubility, or binding affinity to complementary sequences. These nucleic acids may comprise the vector, the expression cassette, the promoter sequence, the gene of interest, or any combination thereof. In another embodiment, its lipophilicity may be modified, which, in turn, will reflect changes in the systems employed for its delivery, and in one embodiment, may further be influenced by whether such sequences are desired for retention within, or permeation through the skin, or any

of its layers. Such considerations may influence any compound used in this invention, in the methods and systems described.

[131] The term "promoter" means a nucleotide sequence that, when operably linked to a DNA sequence of interest, promotes transcription of that DNA sequence.

5 [132] DNA according to the invention can also be chemically synthesized by methods known in the art. For example, the DNA can be synthesized chemically from the four nucleotides in whole or in part by methods known in the art. Such methods include those described in Caruthers (1985). DNA can also be synthesized by preparing overlapping double-stranded oligonucleotides, filling in the gaps, and ligating the ends together (see, generally, Sambrook et al. (1989) and Glover et al. (1995)). DNA expressing functional homologues of
10 the protein can be prepared from wild-type DNA by site-directed mutagenesis (see, for example, Zoller et al. (1982); Zoller (1983); and Zoller (1984); McPherson (1991)). The DNA obtained can be amplified by methods known in the art. One suitable method is the polymerase chain reaction (PCR) method described in Saiki et al. (1988), Mullis et al., U.S. Pat. No.4,683,195, and Sambrook et al. (1989).

[133] Methods for modifying nucleic acids to achieve specific purposes are disclosed in the art, for example,
15 in Sambrook et al. (1989). Moreover, the nucleic acid sequences of the invention can include one or more portions of nucleotide sequence that are non-coding for the protein of interest. Variations in DNA sequences, which are caused by point mutations or by induced modifications (including insertion, deletion, and substitution) to enhance the activity, half-life or production of the polypeptides encoded thereby, are also encompassed in the invention.

20 [134] The formulations of this invention may comprise nucleic acids, in one embodiment, or in another embodiment, the methods of this invention may include delivery of the same, wherein, in another embodiment, the nucleic acid is a part of a vector.

[135] The efficacy of a particular expression vector system and method of introducing nucleic acid into a cell can be assessed by standard approaches routinely used in the art as described hereinbelow.

25 [136] As will be appreciated by one skilled in the art, a fragment or derivative of a nucleic acid sequence or gene that encodes for a protein or peptide can still function in the same manner as the entire wild type gene or sequence. Likewise, forms of nucleic acid sequences can have variations as compared to wild type sequences, nevertheless encoding the protein or peptide of interest, or fragments thereof, retaining wild type function exhibiting the same biological effect, despite these variations. Each of these represents a separate embodiment
30 of this present invention.

[137] In another embodiment, the present invention provides a DNA vector comprising a recombinant polynucleotide encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.

[138] In one embodiment, the term "vector" or "expression vector" refers to a carrier molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. In one embodiment, the vector is a DNA vector. In one embodiment, the vector is a plasmid. In one embodiment, the nucleic acid molecules are transcribed into RNA, which in some cases are then translated into a protein, polypeptide, or peptide. In one embodiment, expression vectors can contain a variety of "control sequences" which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host cell. In another embodiment, a vector further includes an origin of replication. In one embodiment the vector may be a shuttle vector, which in one embodiment can propagate both in prokaryotic and eukaryotic cells, or in another embodiment, the vector may be constructed to facilitate its integration within the genome of an organism of choice. The vector, in other embodiments may be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a virus or an artificial chromosome. In one embodiment, the vector is a viral vector, which in one embodiment may be a bacteriophage, mammalian virus, or plant virus. In one embodiment, a vector is a plasmid.

[139] In another embodiment, provided herein is a method for treating a neoplastic disease in a subject comprising the step of administering to a subject a recombinant polypeptide as described herein or a nucleic acid encoding the same, thereby treating a neoplastic disease in a subject.

[140] In another embodiment, provided herein is a method for inhibiting or suppressing a neoplastic disease in a subject comprising the step of administering to a subject a recombinant polypeptide as described herein or a nucleic acid encoding the same, thereby inhibiting or suppressing a neoplastic disease in a subject.

[141] In another embodiment, provided herein is a method for reducing the symptoms associated with a neoplastic disease in a subject comprising the step of administering to a subject the recombinant polypeptide as described herein or a nucleic acid encoding the same, thereby reducing the symptoms associated with a neoplastic disease in a subject.

[142] In one embodiment, the present invention provides a method for inhibiting the proliferation of a neoplastic cell comprising the step of contacting said cell with a recombinant polypeptide comprising a chimera or a nucleic acid molecule encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting the proliferation of a neoplastic cell.

[143] In one embodiment, the present invention provides a method for treating a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby treating a neoplastic disease in a subject.

[144] In one embodiment, the present invention provides a method for inhibiting or suppressing a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting or suppressing a neoplastic disease in a subject.

[145] In one embodiment, the present invention provides a method for reducing the symptoms associated with a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby reducing the symptoms associated with a neoplastic disease in a subject.

[146] In one embodiment, the present invention provides a method for inhibiting the proliferation of a neoplastic cell comprising the step of contacting said cell with a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting the proliferation of a neoplastic cell.

[147] In one embodiment, the present invention provides a method for treating a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby treating a neoplastic disease in a subject.

[148] In one embodiment, the present invention provides a method for inhibiting or suppressing a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting or suppressing a neoplastic disease in a subject.

[149] In one embodiment, the present invention provides a method for reducing the symptoms associated with a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby reducing the symptoms associated with a neoplastic disease in a subject.

[150] In one embodiment, the present invention provides a method for inhibiting the proliferation of a neoplastic cell comprising the step of contacting said cell with a nucleic acid molecule encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting the proliferation of a neoplastic cell.

[151] In one embodiment, the present invention provides a method for treating a neoplastic disease in a subject comprising the step of administering to said subject a nucleic acid encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby treating a neoplastic disease in a subject.

5 [152] In one embodiment, the present invention provides a method for inhibiting or suppressing a neoplastic disease in a subject comprising the step of administering to said subject a nucleic acid encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting or suppressing a neoplastic disease in a subject.

10 [153] In one embodiment, the present invention provides a method for reducing the symptoms associated with a neoplastic disease in a subject comprising the step of administering to said subject a nucleic acid encoding a recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby reducing the symptoms associated with a neoplastic disease in a subject.

15 [154] In one embodiment, the present invention provides a use of a recombinant polypeptide comprising a chimera or a nucleic acid molecule encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof in the preparation of a pharmaceutical composition for inhibiting the proliferation of a neoplastic cell.

[155] In one embodiment, the present invention provides a use of a recombinant polypeptide comprising a chimera or a nucleic acid molecule encoding the same, wherein said chimera comprises a DNase I fragment or
20 a homologue thereof and a Cdt fragment or a homologue thereof in the preparation of a pharmaceutical composition for treating a neoplastic disease in a subject.

[156] In one embodiment, the present invention provides a use of a recombinant polypeptide comprising a chimera or a nucleic acid molecule encoding the same, wherein said chimera comprises a DNase I fragment or
25 a homologue thereof and a Cdt fragment or a homologue thereof in the preparation of a pharmaceutical composition for inhibiting or suppressing a neoplastic disease in a subject.

[157] In one embodiment, the present invention provides a use of a recombinant polypeptide comprising a chimera or a nucleic acid molecule encoding the same, wherein said chimera comprises a DNase I fragment or
30 a homologue thereof and a Cdt fragment or a homologue thereof in the preparation of a pharmaceutical composition for reducing the symptoms associated with a neoplastic disease in a subject.

[158] In one embodiment, a composition of the present invention is targeted to a rapidly proliferating cell, which in one embodiment, is an undifferentiated epithelial cell, and, in another embodiment, is a lymphocyte. In one embodiment, immortalized epithelial-like cell lines such as HeLa, KB, HEP-2 and GSM-K (SV40

transformed) are particularly sensitive to Cdt, or in another embodiment, to Cdt-containing chimera of the present invention, or, in another embodiment, a CdtB-containing chimera of the present invention.

[159] In one embodiment, a composition of the present invention may be used to treat, suppress, or inhibit a cancer of epitheloid origin, which in one embodiment, is a carcinoma, which in one embodiment, is a squamous cell carcinoma, adenocarcinoma, or transitional cell carcinoma, which in one embodiment, is a lung, breast, ovarian, or colon cancer. In one embodiment, the squamous cell carcinoma is oral squamous cell carcinoma. In one embodiment, breast cancer, prostate cancer, bladder cancer, brain cancer and hepatic cancer are of epitheloid origin.

[160] In another embodiment, basal cell carcinoma, gastrointestinal cancer, lip cancer, mouth cancer, esophageal cancer, small bowel cancer and stomach cancer, colon cancer, liver cancer, bladder cancer, pancreas cancer, ovary cancer, cervical cancer, lung cancer, and skin cancer, such as squamous cell and basal cell cancers, renal cell carcinoma are of epitheloid origin. Other known cancers that effect epithelial cells throughout the body are known in the art.

[161] In one embodiment, a neoplastic disease is a disease that involves the formation of a tumor or other abnormal tissue growth. In another embodiment, a neoplastic disease is a disease in which a neoplasm is present. In one embodiment, a neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues, and persists in the same excessive manner after cessation of the stimulus which evoked the change. In one embodiment, a neoplasm does not form a tumor. In one embodiment, the neoplastic disease is malignant. In another embodiment, the neoplastic disease is pre-malignant. In another embodiment, the neoplastic disease is benign. In one embodiment, the neoplastic disease is a sarcoma, lymphoma, leukemia, carcinoma, or multiple myeloma. In one embodiment, the neoplastic disease is diagnosed in soft tissue tumors of the head and neck, skin, salivary glands, thyroid gland, breast, skeletal system, maxilla, orbit or, temporal fossa, nervous system, lungs, pleura or mediastinum, esophagus or stomach, small intestine, large intestine, liver or gallbladder, pancreas, kidneys, adrenal, glands, or, ureters-carcinoma, urinary bladder-carcinoma, cancers of the female genital tract, prostate gland, testicles, or penis.

[162] In another embodiment, the compositions and methods of the present invention may be used for reducing the symptoms associated with neoplastic disease. Such symptoms are dependent on the location of the neoplastic disease, and are known to a skilled artisan and may, in one embodiment, include fevers, chills, night sweats, weight loss, loss of appetite, fatigue, malaise, or a combination thereof. In one embodiment, lung tumors can cause coughing, shortness of breath, or chest pain while, in one embodiment, tumors of the colon can cause weight loss, diarrhea, constipation and blood in the stool.

[163] In one embodiment, the compositions and methods of the present invention may be used for treating, suppressing, or inhibiting tumor metastasis. In one embodiment, "metastasis" refers to the condition of spread of cancer from the organ of origin to additional distal sites in the patient.

[164] In one embodiment, the compositions and methods of the present invention may be used for treating, suppressing, or inhibiting a "primary tumor", which, in one embodiment, is a tumor appearing at a first site within the subject and can be distinguished from a "metastatic tumor" which appears in the body of the subject at a remote site from the primary tumor. In one embodiment, a primary tumor is a solid tumor.

[165] In one embodiment, the compositions and methods of the present invention may be used for treating, suppressing, or inhibiting wounds, ulcers, bronchitis, inflammatory conditions, herpes infection, or cystic fibrosis. In one embodiment, the compositions and methods of the present invention may be used for reducing the viscoelasticity of pulmonary secretions (mucus). In one embodiment, the compositions and methods of the present invention may be used for treating, suppressing, or inhibiting pneumonia and cystic fibrosis (CF). See e.g., Lourenco, et al., Arch. Intern. Med. 142:2299-2308 (1982); Shak, et al., Proc. Nat. Acad. Sci. 87:9188-9192 (1990); Hubbard, et al., New Engl. J. Med. 326:812-815 (1992); Fuchs, et al., New Engl. J. Med. 331:637-642 (1994); Bryson, et al., Drugs 48:894-906 (1994). In one embodiment, the compositions and methods of the present invention may be used for treating, suppressing, or inhibiting chronic bronchitis, asthmatic bronchitis, bronchiectasis, emphysema, acute and chronic sinusitis, the common cold, in which, in one embodiment, mucus also contributes to its morbidity.

[166] The examples provided herein demonstrate that functional chimeras of the cdtB and human DNase I genes may be obtained and strongly supports the close relationship between the two gene products. The chimeric cdtB-DNase I genes were expressed in E. coli and the gene products exhibited nuclease activity, in vitro, comparable to that of CdtB. The fact that these artificial prokaryotic/eukaryotic gene constructs were expressed by E. coli and satisfied very stringent requirements to maintain a specific enzymatic activity provides compelling and novel evidence that the cdtB gene has evolved as an atypical divalent cation-dependent nuclease with remarkable similarities to mammalian DNase I.

[167] In one embodiment, the successful construction of the chimeric genes indicates that there is significant potential to exploit the functional relationship between CdtB and human DNase I to genetically engineer novel therapeutic reagents that take advantage of the most desirable features of each native protein. In one embodiment, DNase I has potent DNA-cutting/nicking activity. In one embodiment, CdtB can enter cells and translocate to the cell nucleus. Thus, in one embodiment, the CdtB portion or portions of the chimera allows cell entry and translocation of the chimera and the DNase I portion or portions of the chimera provide potent DNA-cutting/nicking activity. In one embodiment, the CdtB portion or portions of the chimera confers cell type specificity to the chimera, which in one embodiment, is specificity for epitheloid cells.

[168] In another embodiment, the compositions of the present invention further comprise a targeting molecule used to target the chimera to a particular cell type of interest. In one embodiment, the targeting molecule is an antibody to a specific polypeptide expressed by a tumor or to a polypeptide expressed by a particular cell type. In one embodiment, somatostatin, neurotensin, bombesin receptor binding molecules, monoclonal antibodies, penetratinesTM, or glycoproteins, may be used as targeting molecules. Other targeting molecules are known in the art.

[169] In one embodiment, “fragment” refers to a portion of a larger polypeptide or polynucleotide. In one embodiment, a fragment retains one or more particular functions as the larger molecule from which it was derived. In one embodiment, a fragment may maintain a functional domain, which in one embodiment, is a nuclear localization signal (NLS), a glycosylation site, a cleavage site, a binding site, a DNA contact site or residues, a G-actin binding site or residues, a metal binding site or residues, a Sph chimera fusion site, a catalytic His, a Hi hydrogen-bond pair, or a combination thereof. In one embodiment, a fragment may be approximately 50% of the length of the source polypeptide or polynucleotide.

[170] In another embodiment, a fragment may be approximately 90% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 75% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 70% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 60% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 40% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 30% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 25% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 10% of the length of the source polypeptide or polynucleotide. In another embodiment, a fragment may be approximately 5% of the length of the source polypeptide or polynucleotide.

[171] In another embodiment, a fragment is approximately 150 amino acids. In another embodiment, a fragment is approximately 180 amino acids. In another embodiment, a fragment is approximately 200 amino acids. In another embodiment, a fragment is 100-200 amino acids. In another embodiment, a fragment is 125-175 amino acids. In another embodiment, a fragment is 140-160 amino acids. In another embodiment, a fragment is 50-150 amino acids. In another embodiment, a fragment is the equivalent number of nucleic acids required to encode an amino acid as described, as would be understood by a skilled artisan.

[172] In one embodiment, “isoform” refers to a version of a molecule, for example, a protein, with only slight differences to another isoform of the same protein. In one embodiment, isoforms may be produced from different but related genes, or in another embodiment, may arise from the same gene by alternative splicing. In another embodiment, isoforms are caused by single nucleotide polymorphisms.

[173] In one embodiment, “variant” refers to an amino acid or nucleic acid sequence (or in other embodiments, an organism or tissue) that is different from the majority of the population but is still sufficiently similar to the common mode to be considered to be one of them, for example splice variants. In one embodiment, the variant may be a sequence conservative variant, while in another embodiment, the variant may be a functional conservative variant. In one embodiment, a variant may comprise an addition, deletion or substitution of 1 amino acid. In one embodiment, a variant may comprise an addition, deletion, substitution, or combination thereof of 2 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 3 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 4 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 5 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 7 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 10 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 2-15 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 3-20 amino acids. In one embodiment, a variant may comprise an addition, deletion or substitution, or combination thereof of 4-25 amino acids.

[174] In another embodiment, a chimeric polypeptide or isolated nucleic acid of the present invention is homologous to a sequence set forth hereinabove, either expressly or by reference to a GenBank entry. The terms “homology,” “homologous,” etc, when in reference to any protein or peptide, or any sequence, whether amino acid or nucleotide sequence, refer, in one embodiment, to a percentage of amino acid residues or nucleic acid residues, as appropriate, in the candidate sequence that are identical with the residues of a corresponding native polypeptide or nucleic acid, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. Methods and computer programs for the alignment are well known in the art.

[175] Homology is, in another embodiment, determined by computer algorithm for sequence alignment, by methods well described in the art. For example, computer algorithm analysis of nucleic acid sequence homology can include the utilization of any number of software packages available, such as, for example, the BLAST, DOMAIN, BEAUTY (BLAST Enhanced Alignment Utility), GENPEPT and TREMBL packages.

[176] In another embodiment, “homology” refers to the identity between two sequences being greater than 70%. In another embodiment, “homology” refers to the identity between two sequences being greater than 72%. In another embodiment, “homology” refers to the identity between two sequences being greater than 75%. In another embodiment, “homology” refers to the identity between two sequences being greater than 78%. In another embodiment, “homology” refers to the identity between two sequences being greater than 80%. In another embodiment, “homology” refers to the identity between two sequences being greater than 82%. In another embodiment, “homology” refers to the identity between two sequences being greater than

83%. In another embodiment, "homology" refers to the identity between two sequences being greater than 85%. In another embodiment, "homology" refers to the identity between two sequences being greater than 87%. In another embodiment, "homology" refers to the identity between two sequences being greater than 88%. In another embodiment, "homology" refers to the identity between two sequences being greater than 90%. In another embodiment, "homology" refers to the identity between two sequences being greater than 92%. In another embodiment, "homology" refers to the identity between two sequences being greater than 93%. In another embodiment, "homology" refers to the identity between two sequences being greater than 95%. In another embodiment, "homology" refers to the identity between two sequences being greater than 96%. In another embodiment, "homology" refers to the identity between two sequences being greater than 97%. In another embodiment, "homology" refers to the identity between two sequences being greater than 98%. In another embodiment, "homology" refers to the identity between two sequences being greater than 99%. In another embodiment, "homology" refers to the identity between two sequences being 100%.

[177] In another embodiment, homology is determined via determination of candidate sequence hybridization, methods of which are well described in the art (See, for example, "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., Eds. (1985); Sambrook *et al.*, 2001, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel *et al.*, 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y). In other embodiments, methods of hybridization are carried out under moderate to stringent conditions, to the complement of a DNA encoding a native caspase peptide. Hybridization conditions being, for example, overnight incubation at 42°C in a solution comprising: 10-20 % formamide, 5 X SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7. 6), 5 X Denhardt's solution, 10 % dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA.

[178] Homology for any amino acid or nucleic acid sequence listed herein is determined, in another embodiment, by methods well described in the art, including immunoblot analysis, or via computer algorithm analysis of amino acid sequences, utilizing any of a number of software packages available, via established methods. Some of these packages include the FASTA, BLAST, MPsrch or Scanps packages, and, in another embodiment, employ the use of the Smith and Waterman algorithms, and/or global/local or BLOCKS alignments for analysis, for example. Each method of determining homology represents a separate embodiment of the present invention.

[179] In some embodiments, any of the chimeric polypeptides or nucleic acids of and for use in the methods of the present invention will comprise a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, or an isolated nucleic acid encoded said components, in any form or embodiment as described herein. In some embodiments, any of the chimeric polypeptides or nucleic acids of and for use in the methods of the present invention will consist of a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, or an isolated nucleic acid encoded said components of the present

invention, in any form or embodiment as described herein. In some embodiments, the chimeric polypeptides or nucleic acids of this invention will consist essentially of a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, or an isolated nucleic acid encoded said components of the present invention, in any form or embodiment as described herein. In some embodiments, the term “comprise” refers to the inclusion of other fragments of DNase I or Cdt, additional polypeptides, as well as inclusion of other proteins that may be known in the art. In some embodiments, the term “consisting essentially of” refers to a vaccine, which has a DNase I polypeptide or fragment thereof and a Cdt fragment or a homologue thereof. However, other peptides may be included that are not involved directly in the utility of the chimeric polypeptide. In some embodiments, the term “consisting” refers to a chimeric polypeptide having the specific DNase I polypeptide or fragment thereof and a Cdt fragment or a homologue thereof of the present invention, in any form or embodiment as described herein.

[180] In one embodiment, a “chimeric” polypeptide or toxin is a protein or toxin created through the joining of two or more nucleotides or genes which originally coded for separate proteins. In one embodiment, translation of this fusion polynucleotide results in a single polypeptide with functional properties derived from each of the original proteins. In one embodiment, a chimeric polypeptide of the present invention comprises an IgE Fc or fragment thereof and a Cdt subunit.

Pharmaceutical Compositions

[181] In another embodiment, the use of a recombinant polypeptide as described hereinabove and/or its analog, derivative, isomer, metabolite, pharmaceutically acceptable salt, pharmaceutical product, hydrate, N-oxide, or combinations thereof for treating, preventing, suppressing, inhibiting or reducing the incidence of cancer.

[182] Thus, in one embodiment, the methods of the present invention comprise administering a recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering a derivative of the recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering an isomer of the recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering a metabolite of the recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering a pharmaceutically acceptable salt of the recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering a pharmaceutical product of the recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering a hydrate of the recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering an N-oxide of the recombinant polypeptide as described hereinabove. In another embodiment, the methods of the present invention comprise administering any of a combination of an analog, derivative,

isomer, metabolite, pharmaceutically acceptable salt, pharmaceutical product, hydrate or N-oxide of the recombinant polypeptide as described hereinabove.

[183] As used herein, "pharmaceutical composition" means a "therapeutically effective amount" of the active ingredient, i.e. the recombinant polypeptide as described hereinabove, together with a pharmaceutically acceptable carrier or diluent. A "therapeutically effective amount" refers, in one embodiment, to that amount which provides a therapeutic effect for a given condition and administration regimen.

[184] The pharmaceutical compositions containing the recombinant polypeptide as described hereinabove can be administered to a subject by any method known to a person skilled in the art, such as parenterally, paracancerally, trans-mucosally, trans-dermally, intramuscularly, intravenously, intra-dermally, subcutaneously, intra-peritoneally, intra-ventricularly, intra-cranially, intra-vaginally or intra-tumorally.

[185] In one embodiment, the pharmaceutical compositions are administered orally, and are thus formulated in a form suitable for oral administration, i.e. as a solid or a liquid preparation. Suitable solid oral formulations include tablets, capsules, pills, granules, pellets and the like. Suitable liquid oral formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment of the present invention, the recombinant polypeptide as described hereinabove is formulated in a capsule. In accordance with this embodiment, the compositions of the present invention comprise, in addition to the recombinant polypeptide as described hereinabove and the inert carrier or diluent, a hard gelating capsule.

[186] Further, in another embodiment, the pharmaceutical compositions are administered by intravenous, intraarterial, or intramuscular injection of a liquid preparation. Suitable liquid formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment, the pharmaceutical compositions are administered intravenously and are thus formulated in a form suitable for intravenous administration. In another embodiment, the pharmaceutical compositions are administered intraarterially and are thus formulated in a form suitable for intraarterial administration. In another embodiment, the pharmaceutical compositions are administered intramuscularly and are thus formulated in a form suitable for intramuscular administration.

[187] Further, in another embodiment, the pharmaceutical compositions are administered topically to body surfaces and are thus formulated in a form suitable for topical administration. Suitable topical formulations include gels, ointments, creams, lotions, drops and the like. For topical administration, the recombinant polypeptides as described hereinabove or their physiologically tolerated derivatives such as salts, esters, N-oxides, and the like are prepared and applied as solutions, suspensions, or emulsions in a physiologically acceptable diluent with or without a pharmaceutical carrier.

[188] Further, in another embodiment, the pharmaceutical compositions are administered as a suppository, for example a rectal suppository or a urethral suppository. Further, in another embodiment, the

pharmaceutical compositions are administered by subcutaneous implantation of a pellet. In a further embodiment, the pellet provides for controlled release of the recombinant polypeptides as described hereinabove over a period of time.

[189] In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez- Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

[190] As used herein "pharmaceutically acceptable carriers or diluents" are well known to those skilled in the art. The carrier or diluent may be a solid carrier or diluent for solid formulations, a liquid carrier or diluent for liquid formulations, or mixtures thereof.

[191] Solid carriers/diluents include, but are not limited to, a gum, a starch (e.g. corn starch, pregeletanized starch), a sugar (e.g., lactose, mannitol, sucrose, dextrose), a cellulosic material (e.g. microcrystalline cellulose), an acrylate (e.g. polymethylacrylate), calcium carbonate, magnesium oxide, talc, or mixtures thereof.

[192] For liquid formulations, pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, emulsions or oils. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, and fish-liver oil.

[193] Parenteral vehicles (for subcutaneous, intravenous, intraarterial, or intramuscular injection) include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions. Examples of oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, mineral oil, olive oil, sunflower oil, and fish-liver oil.

[194] In addition, the compositions may further comprise binders (e.g. acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g. cornstarch, potato starch, alginic acid, silicon dioxide, croscarmellose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-HCl, acetate, phosphate) of various pH and ionic strength,

additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g. sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g. hydroxypropyl cellulose, hydroxypropylmethyl cellulose), viscosity increasing agents (e.g. carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum),
5 sweeteners (e.g. aspartame, citric acid), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), lubricants (e.g. stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g. colloidal silicon dioxide), plasticizers (e.g. diethyl phthalate, triethyl citrate), emulsifiers (e.g. carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film
10 forming agents (e.g. ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

[195] In one embodiment, the pharmaceutical compositions provided herein are controlled-release compositions, i.e. compositions in which the recombinant polypeptide as described hereinabove is released over a period of time after administration. Controlled- or sustained-release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). In another embodiment, the composition is an immediate-
15 release composition, i.e. a composition in which all of the recombinant polypeptide as described hereinabove is released immediately after administration.

[196] In yet another embodiment, the pharmaceutical composition can be delivered in a controlled release system. For example, the agent may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump may be
20 used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984). Other controlled-release
25 systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

[197] The compositions may also include incorporation of the active material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts.) Such compositions influence the physical state, solubility, stability, rate of in vivo release, and
30 rate of in vivo clearance.

[198] Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors.

[199] Also comprehended by the invention are compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline. The modified compounds are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuchowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired in vivo biological activity may be achieved by the administration of such polymer-compound adducts less frequently or in lower doses than with the unmodified compound.

[200] The preparation of pharmaceutical compositions that contain an active component, for example by mixing, granulating, or tablet-forming processes, is well understood in the art. The active therapeutic ingredient is often mixed with excipients that are pharmaceutically acceptable and compatible with the active ingredient. For oral administration, the recombinant polypeptide as described hereinabove or their physiologically tolerated derivatives such as salts, esters, N-oxides, and the like are mixed with additives customary for this purpose, such as vehicles, stabilizers, or inert diluents, and converted by customary methods into suitable forms for administration, such as tablets, coated tablets, hard or soft gelatin capsules, aqueous, alcoholic or oily solutions. For parenteral administration, the recombinant polypeptide as described hereinabove or their physiologically tolerated derivatives such as salts, esters, N-oxides, and the like are converted into a solution, suspension, or emulsion, if desired with the substances customary and suitable for this purpose, for example, solubilizers or other substances.

[201] An active component can be formulated into the composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule), which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

[202] In one embodiment, the term "treating" includes preventative as well as disorder remittative treatment. As used herein, the terms "reducing", "suppressing" and "inhibiting" have their commonly understood meaning of lessening or decreasing. As used herein, the term "progression" means increasing in scope or severity, advancing, growing or becoming worse. As used herein, the term "recurrence" means the return of a disease after a remission.

[203] In one embodiment, "treating" refers to either therapeutic treatment or prophylactic or preventative measures, wherein the object is to prevent or lessen the targeted condition or disorder as described

hereinabove. Thus, in one embodiment, treating may include directly affecting or curing, suppressing, inhibiting, preventing, reducing the severity of, delaying the onset of, reducing symptoms associated with the disease, disorder or condition, or a combination thereof. Thus, in one embodiment, "treating" refers inter alia to delaying progression, expediting remission, inducing remission, augmenting remission, speeding recovery, increasing efficacy of or decreasing resistance to alternative therapeutics, or a combination thereof. In one embodiment, "preventing" refers, inter alia, to delaying the onset of symptoms, preventing relapse to a disease, decreasing the number or frequency of relapse episodes, increasing latency between symptomatic episodes, or a combination thereof. In one embodiment, "suppressing" or "inhibiting", refers inter alia to reducing the severity of symptoms, reducing the severity of an acute episode, reducing the number of symptoms, reducing the incidence of disease-related symptoms, reducing the latency of symptoms, ameliorating symptoms, reducing secondary symptoms, reducing secondary infections, prolonging patient survival, or a combination thereof.

[204] "Contacting," in one embodiment, refers to directly contacting the target cell with a chimeric polypeptide of the present invention. In another embodiment, "contacting" refers to indirectly contacting the target cell with a chimeric polypeptide of the present invention. Thus, in one embodiment, methods of the present invention include methods in which the subject is contacted with a chimeric polypeptide which is brought in contact with the target cell by diffusion or any other active transport or passive transport process known in the art by which compounds circulate within the body.

[205] In one embodiment, the methods of the present invention comprising the step of administering a composition of the present invention to a subject in need. In one embodiment, "administering" refers to directly introducing into a subject by injection or other means a composition of the present invention. In another embodiment, "administering" refers to contacting a cell with a composition of the present invention. In one embodiment, the term "administering" refers to bringing a subject in contact with the recombinant polypeptide as described hereinabove. As used herein, administration can be accomplished in vitro, i.e. in a test tube, or in vivo, i.e. in cells or tissues of living organisms, for example humans. In one embodiment, the present invention encompasses administering the compounds of the present invention to a subject. In one embodiment, the compositions of the present invention are administered chronically, while in another embodiment, they are administered intermittently.

[206] In one embodiment, "chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain a desired effect or level of agent(s) for an extended period of time.

[207] In one embodiment, "intermittent" administration is treatment that is not consecutively done without interruption, but rather is periodic in nature.

[208] Administration "in combination with" or "in conjunction with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[209] An "effective amount" is an amount sufficient to effect beneficial or desired therapeutic (including preventative) results. An effective amount can be administered in one or more administrations.

5 [210] In one embodiment, the methods of the present invention comprise administering a chimera as the sole active ingredient. In another embodiment, the present invention provides methods that comprise administering a chimera in combination with one or more therapeutic agents.

EXPERIMENTAL DETAILS SECTION

MATERIALS AND METHODS

10 **Construction of chimeric cdtB genes**

[211] Plasmids and primers used in this study are listed in Tables 1 and 2, respectively. All PCR and restriction digestion reactions were performed using standard techniques. Restriction endonucleases were purchased from New England Biolabs (Beverly, MA) and PCR oligonucleotide primers were obtained from Integrated DNA Technologies (IDT, Coralville, IA). Constructs were first selected in *E. coli* DH5 α [supE44
15 Δ lacU169 (ϕ 80lacZ Δ M15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1] and transformed into *E. coli* BL-21(DE3) for gene expression analysis and isolation of the gene product. Bacteria were grown in LB broth containing 75 μ g/ml ampicillin at 37°C with vigorous shaking. Plasmid DNA was isolated using either a QIAprep Miniprep (QIAGEN, Valencia, CA) or the Wizard Miniprep Kit (Promega Corporation, Fitchburg, WI). All constructs and mutations were confirmed by DNA sequencing. Automated cycle sequencing reactions
20 were conducted by the DNA Sequencing Facility at the University of Pennsylvania Abramson Cancer Center using an Applied Biosystems 96-capillary 3730XL sequencer with BigDye Taq FS Terminator V 3.1.

[212] The cdtB gene sequence used throughout these experiments was from construct pJDB7 originally made in Cao et al., (2005) and based on the sequence deposited in GenBank (accession no. AF006830). The full length DNase I sequence (GenBank accession no. AC005203) was amplified from plasmid GC-C0001
25 (GeneCopoeia, Inc., Germantown, MD) using the PCR primers N-DNase-F and X-DNase-R. This amplicon was cloned, in frame, to the His-tag in pETBlue2 (EMD Chemicals-Novagen, San Diego, CA) using NcoI and XhoI restriction endonuclease cleavage sites. Portions of the DNA sequence from the resulting clone, pJD1, were used to construct the chimeras. To construct the DNase I/cdtB gene chimera the 5'-half of the DNase I gene sequence was obtained by digestion of pJD1 with NcoI and SphI, the 3'-half of the cdtB gene was
30 obtained by digesting pJDB7 with SphI and BamHI and pET15b was cut with NcoI and BamHI. The three DNA fragments were ligated to obtain pETDB1 (Table 1). To construct the cdtB/DNase I gene chimera the PCR primers N-cdtB-F and S-cdtB-R were used to amplify the 5'-half of cdtB using pJDB7 as the template DNA. pJD1 was digested with NcoI and SphI and the large DNA fragment was ligated to the pJDB7 amplicon after digestion with the same enzymes (clone pBDN1). The insert DNA fragment from pBDN1 was amplified

using the PCR primers N-cdtB-F and NdeI-His and the amplicon was cloned into the NcoI and NdeI sites of pET15b. The resulting plasmid, pETBDN2, contained the cdtB/DNase I chimeric gene, with codons for a His-tag at the 3'-end, in the same vector background as the DNase I/cdtB gene chimera.

5 Table 1. Plasmids used in this study

Plasmid	Features	Source or reference
GC-C0001	human DNase I gene in pReceiver-B02	GeneCopoeia
pJD1	DNase I gene containing 6 His codons at 3'-end in pETBlue2	This study
pJDB7	cdtB gene containing 6 His codons at 3'-end in pET15b	Cao et al., 2005
pMUT160cdtB	pJDB7 with substitution H160A	This study
pDN1	human DNase I gene in pETBlue2 with 3'-His ₆ -tag	This study
pETDN1	human DNase I gene containing 7 His codons at 3'-end in pET15b	This study
pBDN1	cdtB/DNase I chimeric gene containing 6 His codons at 3'-end in pETBlue2	This study
pETBDN1	cdtB/DNase I hybrid gene in pET15b with 3'-His ₆ -tag	This study
pETBDN2	cdtB/DNase I chimeric gene containing 7 His codons at 3'-end in pET15b	This study
pETDNB1	DNase I/cdtB chimeric gene containing 6 His codons at 3'-end in pET15b	This study
pETBDNmut5	pETBDN1 containing 5 codon changes in the hybrid gene (Table 4)	This study
pETBDN2-1	pETBDN2 with substitutions S99Y and R100E	This study
pETBDN2-2	pETBDN2-1 with substitution L62E	This study
pETBDN2-4	pETBDN2-3 with substitution G55R	This study
pETBDN2-5	pETBDN2-4 with substitution V134R	This study
pETBDN2-5LL	pETBDN2-5 with substitution N265-I275 to L265-T275	This study
pETBlue2	Cloning vector	Novagen
pET15b	Cloning vector	Novagen

Amino acid substitutions mutants

[213] Amino acid substitutions were made in the CdtB/DNase I hybrid protein by site-directed mutagenesis as described previously (Cao et al., 2006). Synthetic oligonucleotide primer pairs were used to change S99Y and R100E (both in same primer), L62E, G55R and V134R. Briefly, mutant DNA strands were made using PfuUltra DNA polymerase (Stratagene, La Jolla, CA) in PCR. Plasmid DNA from pETBDN2 was used as the first PCR template. Additional mutations were then made sequentially using plasmid DNA from pETBDN2-1 to pETBDN2-5. Methylated parental DNA strands were digested with DpnI and the intact mutated DNA strand was transformed into *E. coli* TOP10 [F^- mcrA Δ (mrr-hsdRMS-mcrBC) ϕ 80lacZ Δ M15 Δ lacX74 recA1 araD139 Δ (ara-leu)7697 galU galK rpsL(str^R) endA1 nupG] chemically competent cells (Invitrogen, Carlsbad, CA). The mutations were confirmed by sequencing of the plasmid insert DNA from a single transformant.

[214] The same strategy was used to change the active site H160 in CdtB-His₆ to Ala. DNA from pJDB7 was used as the PCR template for primers H160for and H160rev. The mutated protein from this clone was used as a negative activity control in the bioassays.

[215] The large loop domain was added to CdtB/DNase I using an inverse PCR method (Cao et al., 2004) with the Loop-F and Loop-R primers (Table 2) and the insert fragment from pETBDN2-5 (Table 1), containing the cdtB/DNase I nucleotide sequence, as the template DNA. Codons for LMLNQLRSQIT (SEQ ID NO: 58) replaced NGLSDQLAQAI (SEQ ID NO: 59) in pETBDN2-5LL.

20

Table 2. Primers used in this study

[illegible]

CdtB-DN-L62E-F	5'- AATTATTATCGCGAGAACAAGGTGCAGATATT GAG AT GGTACAAGAAGC-3'	50
CdtB-DN-L62E-R	5'- GCTTCTTGTACCAT CTCA ATATCTGCACCTTGTTCCTCG CGATAATAATT-3'	51
CdtB-DN-G55R-F	5'-AATGTGCGCCAATTATTATCG AGG GAACAAGGTGC- 3'	52
CdtB-DN-G55R-R	5'-GCACCTTGTTC CCT CGATAATAATTGGCGCACATT-3'	53
CdtB-DN-V134R-F	5'- CAAGCCGATGAAGCTTTTAT CCG ACATTCTGATTCTTC TGTGCT-3'	54
CdtB-DN-V134R-R	5'- AGCACAGAAGAATCAGAATGT TCG GATAAAAGCTTCA TCGGCTTG-3'	55
Loop-F	5' - CGCTC ACAAATTACAAGTGACCACTATCCAG-3'	56
Loop-R	5' - TA ACTGATT TAA CATTAAGGCAGCCTGGAAG-3'	57

* Underlined bases show the NcoI (CCATGG), XhoI (CTCGAG), SphI (GCATGC), NdeI (CATATG) and BamHI (GGATCC) restriction endonuclease cleavage sites. Bold type marks the changed codon(s).

5 Isolation of gene products and heterotoxin reconstitution

[216] *Escherichia coli* BL-21(DE3) [*F⁻ omp^T hsd_SB (r_B⁻ m_B⁻) gal dcm* (DE3)] (Novagen) containing pJDA9 (wild-type CdtA-His₆), pJDC2 (wild-type CdtC-His₆), pJDB7 (wild-type CdtB-His₆), pMUT160cdtB (active site mutant), pETBDN2 (cdB-DNase I chimera), pETDNB1 (DNase I-cdB chimera) and pETBDN2-1 to pETBDN2-5 (cdB-DNase I mutated chimera) were used to isolate gene products by affinity chromatography on nickel-iminodiacetic acid columns (Novagen) as described previously (Cao et al., 2005). All of the gene products have a His₆₋₇ tag at the carboxy-terminal end. The final protein preparations were dialyzed to remove urea, passed through 45 micron filters and quantified with the Micro BCA protein assay kit (Pierce, Rockville, IL). Purity was assessed by analysis on 10-20% polyacrylamide gels. The presence of the His-tag on all protein constructs was verified by western blotting using Anti-His•Tag Monoclonal Antibody (Novagen). Aliquots of the quantified protein samples were stored at -70°C in a buffer containing 10 mM Tris-HCl (pH 7), 100 mM NaCl, 5 mM MgCl₂ and 5 mM imidazole for use in the various bioassays. Wild-type heterotoxin and heterotoxin containing hybrid and mutated hybrid proteins were reconstituted as described previously (Mao & DiRienzo, 2002). Attempts to purify recombinant human DNase I from either *E. coli* BL-21(DE3) (pJD1) or *E. coli* BL-21(DE3) (pETDN1) were unsuccessful most likely due to the poor expression of the human DNase I gene in *E. coli* as noted by Linardou et al. (2000).

In vitro DNase activity

[217] Supercoiled DNA nicking activity was determined, with minor modifications, as described previously (Elwell & Dreyfus, 2000; Mao & DiRienzo, 2000). In a typical assay 1 µg of supercoiled pBluescript II SK(+) DNA (Sigma-Aldrich, St. Louis, MO) was incubated with 1 µg of wild-type, mutant or hybrid CdtB protein in 25 mM HEPES (Sigma-Aldrich) (pH 7.0) containing 50 mM MgCl₂ at 37°C for 1 h. DNase I (0.1 ng), from

bovine pancreas (Sigma-Aldrich), was used in place of human DNase I. Bovine DNase I is approximately 2.4-fold more active than the human homolog (Pan et al., 1998). Each form of DNA (supercoiled, relaxed, linear) was quantified and compared using ImageJ version 1.34 (<http://rsb.info.nih.gov/ij/>) and digitized images of ethidium bromide-stained gels. Protein and divalent cation concentrations were varied in some kinetic and biochemical analyses.

[218] Heat lability was compared by pretreating proteins at 100°C for up to 30 min prior to determining DNA nicking activity. Actin inhibition of DNA nicking activity was performed essentially as described by Ulmer et al, (1996). Rabbit skeletal muscle G-actin (Sigma-Aldrich) was de-polymerized at room temperature for 15 min in a buffer containing 25 mM HEPES (pH 7.5), 5 mM CaCl₂, 5 mM MgCl₂, 0.1% BSA, 0.05% Tween 20 and 0.5 mM 2-mercaptoethanol. Five micrograms of the depolymerized G-actin were preincubated with 1 µg of CdtB-His₆ or 0.1 ng of bovine DNase I for 60 min at 37°C. The treated proteins were then examined in the nicking assay. Subunit protection of DNA nicking activity was assessed as described previously (Nešić et al., 2004) except that incubation was performed at room temperature for 1 hour.

In vivo CdtB activity

[219] Cell proliferation was measured with a colony-forming assay employing CHO cells [300 cells in 3 ml of medium per well (6-well plate) in triplicate] as described in Mao & DiRienzo (2002). The number of colonies per well was expressed as colony-forming units (CFU). A dose response curve for wild-type reconstituted heterotoxin has been published (Cao et al., 2005). Cell cycle arrest was determined by flow cytometry of propidium iodide stained nuclei as described previously (Cao et al., 2005).

Binding kinetics

[220] Saturation kinetics were used to assess binding of the hybrid and mutated hybrid proteins to wild-type CdtA-His₆ and CdtC-His₆ in a thyroglobulin ELISA (Cao et al., 2005). Wild-type CdtA-His₆ (4.0 µg) and CdtC-His₆ (3.5 µg) were added to thyroglobulin-coated wells. Hybrid or mutated hybrid protein (0-4 µg) was then added to triplicate wells. Bound protein was detected with a 1 x 10⁻⁶ dilution of anti-CdtB IgG rabbit antiserum and a 1 x 10⁻³ dilution of donkey anti-rabbit IgG-horseradish peroxidase conjugate (Amersham Pharmacia Biotech, Piscataway, NJ) (Cao et al., 2008). The ability of the hybrid and mutated hybrid proteins to bind the other subunits was also determined by stoichiometric binding in the thyroglobulin ELISA as described previously (Cao et al., 2005). Wild-type CdtA-His₆ was prebound to thyroglobulin-coated 96-well microtiter plates as described above. Hybrid and mutated hybrid proteins (4.5 µg) and wild-type CdtC-His₆ (3.5 µg) were added to triplicate wells. Total bound protein was detected with anti-His₆ Tag monoclonal antibody and anti-mouse IgG horseradish peroxidase conjugate both at a 1 x 10⁻³ dilution. An absorbance ratio of 3.0 is indicative of the binding of the hybrid or mutated hybrid protein to the other two subunits. CdtB-His₆ and DNase I do not bind to thyroglobulin. ELISA plates were washed in a BioTek Model EL405 HT microplate washer (BioTek Instruments, Inc., Winooski, VT). Absorbance values were obtained with a BioTek Synergy 2 Multi-Detection Microplate Reader.

[221] Differential dialysis was performed as described previously using dialysis membrane with a molecular weight exclusion limit of 100 kDa (Spectrum Laboratories, Inc., Rancho Dominguez, CA) (Cao et al., 2008). Immunopositive bands on western blots were quantified with ImageJ.

5 Computer modeling

[222] The European Molecular Biology Open Software Suite (EMBOSS release 3.0; <http://emboss.sourceforge.net>) (Rice et al., 2000) was used to obtain the deduced amino acid sequences for cdtB-His₆ (from pJDB7), cdtB/DNase I, DNase I/cdtB and human DNase I (from pETDN1) and to create the alignment using ClustalX 1.83. The crystal structures of the *A. actinomycetemcomitans* Y4 Cdt (Yamada et al., 2006) and bovine DNase I were modeled with UCSF Chimera 1.2197 (<http://www.cgl.ucsf.edu/chimera/>) (Pettersen et al., 2004). Coordinates were obtained from the Protein Data Bank (accession nos. 2F2F and 3DNI, respectively). Human DNase I has not been crystalized. CdtB/DNase I and DNase I/CdtB structures were predicted using Modeller version 9v1 (<http://salilab.org/modeller/>) (Marti-Renom et al., 2000).

15 Polyclonal CdtB and CdtC antisera

[223] Subunit protein specific antisera for CdtB-His₆ and CdtCHis₆, used in some experiments, were made in rabbits (Cocalico Biologicals, Inc., Reamstown, PA). IgG fractions were purified using a Montage Antibody Purification Kit (Millipore, Billerica, MA). IgG titers were obtained by ELISA using purified CdtB-His₆ and CdtC-His₆ as antigens. Cross-reactivity with all three Cdt proteins was assessed by western blotting. Bound IgG was detected with a 1:3000 dilution of donkey anti-rabbit IgG-horseradish peroxidase conjugate (Amersham Pharmacia Biotech).

EXAMPLE 1:

USING A CDTB/DNASE I HYBRID PROTEIN AS A THERAPEUTIC AGENT

[224] In spite of the phylogenetic relationship (Figure 14) in CdtB/DNase, there are key biological, compositional and structural differences between the CdtB and DNase I (Table 3). However, the similarities between these proteins are strong enough to suggest that genetic modification is possible to adapt them for potential use as growth inhibitors of cancer cells.

Table 3. Comparison of physical and biochemical properties of CdtB and DNase I

Property	CdtB	DNase I	
	<i>A. actinomycetemcomitans</i>	human	bovine
Number of amino acids	283	282	261
Molecular weight (Da) ^a	31,494	31,434	29,183
pI ^b	7.8	3.5-4.3	4.7-5.2

WO 2009/123950		PCT/US2009/038740	
Disulfide	no	yes (C124-C127, C196-C232)	yes (C102-C105, C174-C210)
Carbohydrate modification ^c	no	yes (N18 and N106)	yes (N18)
<i>in vitro</i> DNA nicking activity	yes	Yes	yes
Actin inhibition	no	Yes	yes
Cations required for activity	Mg ⁺⁺ or Ca ⁺⁺ or Mn ⁺⁺	Mg ⁺⁺ or Ca ⁺⁺ or Mn ⁺⁺	Mg ⁺⁺ or Ca ⁺⁺ or Mn ⁺⁺
Specific activity for nicking dsDNA ^d	100-1000 fold less	2.4 fold less	-

^aCalculated molecular weight without carbohydrate moiety [additional 1.3 kDa per carbohydrate chain (NAG₂-MAN₅); 2 chains bovine, 1 chain human]

^bRange due to heterogeneity in glycosylation

5 ^cAmino acid location from processed proteins (minus signal sequence)

^dRelative to bovine DNase

[225] DNase I cannot enter cells unaided. Even with some type of physical-, immuno- or ligand-based transport system the protein would not migrate to the cell nucleus. In contrast, CdtB appears to be taken up by cells (possibly with CdtC) via the Golgi complex and transported to the endoplasmic reticulum. However, this uptake occurs only after the Cdt binds to the susceptible cell. Initial binding may be to cholesterol-rich membrane patches (possibly lipid rafts). The CdtB travels to the nucleus by an unknown mechanism and gains access to the chromatin via a nuclear localization sequence. The CdtB sends the cells on a destructive pathway due to activation of DNA damage checkpoint responses leading to growth arrest or apoptosis. These effects are most pronounced in rapidly proliferating cells such as undifferentiated epithelial cells and lymphocytes. The experiments performed revealed that immortalized epithelial-like cell lines such as HeLa, KB, HEp-2 and GSMK-K (SV40 transformed) are particularly sensitive to the toxin. In contrast, oral primary fibroblast-like cell types including human periodontal ligament fibroblasts (HPLF), human gingival fibroblasts (HGF), cementoblasts, and osteoblasts are resistant to the DNA-damaging and cytotoxic effects of the Cdt.

20 [226] The fact that CdtB is phylogenically related to human DNase I provides an additional advantage over typical prokaryotic-based recombinant toxins. Furthermore, the DNA-damaging strategy (double-strand DNA breaks) of the CdtB subunit of Cdt is likened to the effects of ionizing radiation. Targeting a potent DNA-damaging protein to the nucleus of cancer cells is analogous to a form of localized radiation therapy.

EXAMPLE 2:

25 **KINETIC ANALYSIS OF RECOMBINANT CDTB-HIS₆ FROM A. ACTINOMYCETEMCOMITANS AND BOVINE DNASE I**

[227] The difference in specific activities between recombinant CdtB-His₆ and bovine DNase I is exemplified in Figure 1. Purified recombinant A. actinomycetemcomitans Y4 CdtB from pJDB7 (Fig. 1a),

converted supercoiled [superhelical (S) circular, Form I] plasmid DNA to relaxed [(R) nicked circular, Form II] and linear [(L) Form III] forms with dose dependent kinetics in the standard DNA nicking assay (Fig. 1b). This conversion was not affected by the location (amino- or carboxy-terminus) of the His₆-tag. One µg of recombinant CdtB-His₆ converted 95% of 1 µg of supercoiled plasmid DNA to relaxed or linear forms in 1
5 hour at 37°C. The substitution mutant CdtBH160A (from pMUT160cdtB in Table 1) had no effect on supercoiled plasmid DNA confirming that recombinant CdtB-His₆ preparations were not contaminated with E. coli nucleases (data not shown). In comparison, 0.1 ng bovine DNase I converted 100% of 1 µg of supercoiled plasmid DNA to relaxed form in 1 hour at 37°C (Fig. 1c). A ten-fold higher concentration of DNase I converted 98% of 1 µg of supercoiled DNA to linear form and a 100-fold higher concentration of the
10 nuclease completely digested the DNA into small fragments. Based on these results, the specific activity of recombinant CdtB-His₆ was estimated to be as much as 10³-fold lower than that of bovine DNase I.

[228] Maximum conversion of supercoiled DNA to relaxed and linear forms was obtained with either 50 mM MgCl₂, CaCl₂ or MnCl₂ (Figure 2). Reactions containing CaCl₂ consistently failed to go to completion. CdtB-His₆ nuclease activity was inhibited by MgCl₂, CaCl₂ and MnCl₂ when the cation concentrations were
15 greater than 150-200 mM. No conversion of supercoiled DNA was observed at any divalent cation concentration in the absence of CdtB and various combinations of the cations (50 mM of each) did not affect digestion patterns relative to those obtained with the individual cations. A time course of digestion with 50 mM MgCl₂ in the reaction established that 1 µg of CdtB-His₆ completely converted 1 µg of supercoiled DNA to relaxed and linear forms in 1 hour at 37°C (data not shown). Incubation times of 2 hours or greater
20 generated more linear form DNA. Thus, CdtB-His₆ requires either Mg⁺⁺, Ca⁺⁺ or Mn⁺⁺ at an individual or combined concentration of 50 mM for optimum activity. This is a 10-fold higher concentration than that required for optimum DNase I activity (Price, 1975). The standard in vitro DNase activity assay used in all subsequent experiments contained 50 mM MgCl₂.

[229] Cdt-B-His₆ retained greater than 90% of its DNA nicking activity following incubation for 5 min in a
25 boiling water bath (Figure 3 A). There was a loss of virtually all enzymatic activity after heating for 10 min. In contrast, bovine DNase I DNA nicking activity was less than 50% of the control after 5 min of heating (Figure 3B). The DNA nicking activity of both enzymes was significantly more heat stable than the double-strand cleavage activity.

[230] CdtB-His₆ is not inhibited by G-actin (Figure 4). These results confirmed that both CdtB-His₆ and
30 bovine DNase I have supercoiled DNA-nicking activity and that the two enzymes can be distinguished by several key biochemical properties (Table 3).

EXAMPLE 3:

CHARACTERIZATION OF CDTB/DNASE I AND DNASE I/CDTB CHIMERIC GENES AND GENE PRODUCTS

[231] Notable differences between the biochemical properties of CdtB and DNase I suggested that a genetic strategy based on the analysis of the products of chimeric genes could be used to further examine functional similarities and differences between the two proteins. This approach was facilitated by the presence of a unique SphI restriction endonuclease cleavage site at approximately the mid-point of both wild-type gene sequences. Using each half of the CdtB-His₆ and human DNase I gene sequences two genetic constructs, pETBDN2 and pETDNB1 containing the chimeric ORFs cdtB/DNase I and DNase I/cdtB, respectively (Table 1) were made. Both ORFs were placed immediately downstream from a highly efficient, inducible promoter and contained six or seven histidine codons in frame at the 3'-end of each sequence.

[232] Two active site catalytic histidines are conserved in CdtB from *A. actinomycetemcomitans* and bovine DNase I (Figure 5A). A deduced amino acid sequence alignment showed that both hybrid gene products contain the two histidines [residues H160/157 and H278/271 in CdtB/DNase I and DNase I/CdtB, respectively] (Figure 5C). Computer modeling indicated that only the CdtB/DNase I hybrid protein maintained a folded structure similar to those of native CdtB and DNase I (Figure 5B). However, both hybrid proteins exhibited optimum *in vitro* supercoiled DNA nicking activity in the presence of 50 mM MgCl₂. The DNA nicking activity of CdtB/DNase I was not affected after incubation in a boiling water bath for 5 min but there was a significant reduction in the DNA nicking activity of DNase I/CdtB after the same treatment (Figure 3). In contrast to bovine DNase I, G-actin had no effect on the ability of CdtB-His₆ and CdtB/DNase I to convert supercoiled DNA to relaxed and linear forms. Conversely, actin inhibited the DNA nicking activity of DNase I/CdtB (Figure 4). The results of these assays demonstrated that the amino terminal portions of CdtB and DNase I carry the biochemical properties of thermostability and actin binding, respectively.

EXAMPLE 4:

SUBUNIT ASSEMBLY OF CDTB/DNASE I AND DNASE I/CDTB HYBRID PROTEINS

[233] The ability of the hybrid proteins to form biologically active reconstituted heterotoxin was tested. Each hybrid protein was mixed with wild-type CdtA and CdtC in refolding buffer as described previously. CHO cell cultures were then treated with the reconstituted preparations. Neither hybrid protein yielded a biologically active heterotoxin as measured in the cell proliferation assay (Figure 6A). A property specific to CdtB is the ability to bind the CdtA and CdtC subunits to form a biologically active heterotrimer. CdtB/DNase I but not DNase I/CdtB bound to CdtA-CdtC heterodimer at concentrations comparable to CdtB-His₆ (Figure 6b). Both the CdtB-His₆ control and CdtB/DNase I hybrid proteins reached saturation binding at approximately 3-4 µg per 4.0 µg of CdtA-His₆ and 3.5 µg of CdtC-His₆. Consistent with these results, CdtB/DNase I but not DNase I/CdtB, formed a heterotrimer complex in the differential dialysis assay (Figure 6b; inset). In addition, CdtB/DNase I but not DNase I/CdtB bound stoichiometrically to CdtA and CdtC (Figure 6c) and reconstituted heterotrimer preparations containing the CdtB/DNase I hybrid protein failed to convert supercoiled DNA to linear and relaxed forms (Figure 6c; inset). Although these four independent

assays established that the CdtB/DNase I hybrid protein formed a heterotrimer, this complex failed to inhibit the proliferation and cell cycle progression of CHO cells.

[234] Computer modeling of the two hybrid proteins provided supporting evidence for reconciling the results of the proliferation inhibition (Figure 6A) and thyroglobulin ELISA (Figure 6B) assays. There are two structural domains in CdtB, an α -helix labeled H1 (residues N41-S54) and a large loop (residues L261-S272), that are predicted to be important for the binding of this subunit to CdtC and CdtA, respectively (Figures 5 and 7). A modeled ribbon structure of the CdtB/DNase I hybrid protein shows that α -helix H1, located in the amino terminal half of CdtB, is still present but the large loop, located in the carboxy-terminal half of CdtB is missing. The presence of only the α -helix H1 suggests that CdtB/DNase I is capable of binding only to CdtC. Addition of the large loop CdtA-binding domain to CdtB/DNase I is discussed below. In contrast, the ribbon model of the DNase I/CdtB hybrid protein contains only the large loop structure and displays a significant change in overall protein conformational relative to that of the native CdtB.

EXAMPLE 5:

EFFECT OF TARGETED AMINO ACID SUBSTITUTIONS ON THE DNA NICKING AND SUBUNIT BINDING ACTIVITIES OF CDTB/DNASE I

[235] Since the DNase I/CdtB hybrid protein failed to bind CdtA and CdtC (Figure 6), lacked a key domain for nuclear localization (Figure 5C) and appeared to have an unfolded conformation in computer models (data not shown), it was not studied further. Single amino acid substitutions were sequentially made in the CdtB/DNase I hybrid protein based on the results from mutagenesis studies of the bovine and human forms of the DNase I gene (Pan et al., 1998). Substitutions G55R, S99Y and V134R added DNA contact residues, L62E added a metal ion binding residue and R100E added a residue that hydrogen bonds to the catalytic H160 in DNase I (Figure 5C and Tables 1 and 4). Five hundred ng of the final mutated hybrid gene product, CdtB/DNase I^{mut5} (clone pETBDN2-5), converted 100% of 1 μ g of supercoiled DNA to relaxed and linear forms under standard assay conditions resulting in an approximately 1.4-fold increase in specific activity relative to CdtB-His₆ and CdtB/DNase I (Figure 8 and inset B). CdtB/DNase I^{mut5} exhibited classical first-order kinetics (inset A).

Table 4. Successive amino acid substitutions made in the CdtB/DNase I hybrid protein.

Amino acid substitution	Position (residue number)^a	Description of mutation
R→E	100	Add acidic residue that forms a hydrogen-bond pair with catalytic His residue 160
G→R	55	Add DNA contact residue
S→Y	99	Add DNA contact residue

V→R	134	Add DNA contact residue
E→E	62	Add metal ion binding residue

^aBased on the deduced amino acid sequence alignment of CdtB/DNase I and human DNase I (see Figure 5).

EXAMPLE 6:

CDT-INDUCED CELL CYCLE ARREST OF HUMAN CANCER CELL LINES

5 [236] As a prelude to determining the therapeutic efficacy of heterotoxin reconstituted with CdtB/DNase I^{mut5}, experiments were initiated to detail the response of established human cancer cell lines to the native Cdt. Four cancer cell lines derived from carcinomas of the tongue, nasal septum and pharynx were purchased from the American Type Culture Collection (ATCC) (Table 5). HeLa cells were used as a transformed human cell control. The response of the cancer cell lines to that of normal primary human gingival epithelial cells (HGEC strain AL-1) which have been recently isolated and cultured in our laboratory was performed. Each of the cell lines were treated with reconstituted recombinant *A. actinomycetemcomitans* Cdt for 24 hours and effects on the cell cycle were assessed by flow cytometry as described previously. All four Cdt-treated cancer cell cultures exhibited a significant accumulation of cells having a 4n DNA content indicative of a G₂ block in cell cycle progression (Table 5). The portion of the cancer cell populations having a 4n DNA content ranged from 10 49-70 percent compared to 44 and 45 percent for HeLa and AL-1 cells, respectively. The accumulation of Cdt-treated CAL-27 cells at the G₂/M interphase of the cell cycle is shown, compared to controls, as an example in 15 Figure 9.

[237] Thus, all of the cancer cell lines examined exhibited cell cycle arrest and on average appeared to be more sensitive to the Cdt than the HeLa cells and normal primary HGEC. These cancer cell lines were used in 20 experiments described below to test the cytotoxic effects of heterotrimer made with the final genetically modified hybrid protein. These cells are used to generate solid tumors in mice to test the efficacy of the hybrid protein.

Table 5. Cell cycle analysis of cancer cell lines treated with reconstituted Cdt

ATCC number	Cell line ^{a,b}	Source	Disease	Treatment	Diploid G1 (2n) (%)	Diploid G2 (4n) (%)	Diploid S (%)	Coefficient of variance (%)
CRL-1624	SCC-4	tongue	squamous cell carcinoma	none Cdt	73.98 17.90	7.42 63.52	18.59 18.58	5.33 4.61
CRL-2095	CAL-27	tongue	squamous cell carcinoma	none Cdt	74.35 39.23	2.04 49.01	23.61 11.76	5.57 3.74
CCL-30	RPMI 2850	nasal septum	squamous cell carcinoma	none Cdt	73.87 11.38	8.71 69.90	17.43 19.02	4.25 4.37
HTB-43	FaDu	pharynx	squamous cell carcinoma	none Cdt	62.00 22.01	3.91 56.06	34.09 21.93	3.96 4.46
CCL-2	HeLa	cervix	adenocarcinoma	none Cdt	65.16 39.19	1.22 43.58	33.62 17.23	5.86 4.16
-	AL-1	human gingiva	none	none Cdt	43.57 26.89	24.42 45.40	32.01 27.61	8.43 5.02

^aAll are adherent cells with epithelial cell morphology.

^bAll cancer cell lines will form solid tumors in mice except HeLa cells.

EXAMPLE 7:

MODIFICATION OF CDTB/DNASE I^{mut5} TO IMPROVE BINDING TO CDTA

[238] The genetically modified hybrid protein CdtB/DNase I^{mut5} (see Table 1) had an increased specific activity, relative to CdtB and CdtB/DNase I (see Figure 8), and formed a heterotrimer with wild-type CdtA and CdtC. However, this heterotrimer failed to inhibit the proliferation of cells or cause cell cycle arrest. As seen in the Cdt structure depicted in Figure 7, two regions in CdtB appear to be in contact with CdtA (large loop; L261-S272 in Figure 5) and CdtC (α -helix H1; N41-S54 in Figure 5). The large loop is not present in CdtB/DNase I^{mut5}. The CdtB/DNase I hybrid protein does not inhibit cell proliferation (see Figure 6A), yet appears to bind to the other subunits when CdtA is immobilized on thyroglobulin or subjected to differential dialysis (Figure 6B). This result could be obtained if CdtB/DNase I^{mut5} binds in the heterotrimer only to CdtC instead of to both CdtA and CdtC thus forming an aberrant heterotrimer. To entice the CdtB/DNase I hybrid to form a correctly assembled heterotoxin, the large loop region was added to the CdtB/DNase I^{mut5} hybrid protein by genetically substituting the sequence LMLNQLRSQIT (SEQ ID NO: 58, positions 261-271) for NGLSDQLAQAI (SEQ ID NO: 59, positions 265-275).

[239] A computer model of CdtB/DNase I^{mut5} containing the restored large loop sequence shows an increased distance (32.48 Å) between this putative CdtA binding domain and the CdtC binding domain (α -helix H1) relative to that in CdtB (10.03 Å) (Figure 10). It was hypothesized that this difference in conformation between CdtB and the hybrid protein may affect assembly of the heterotrimer. In a series of modeling exercises a proline residue was added at key theoretical folding regions in the CdtB/DNase I^{mut5} structure. One theoretical substitution, Y174P (Figure 5) reduced the estimated distance between the putative subunit binding domains to 15.38 Å. This proline was genetically added to the CdtB/DNase I^{mut5} hybrid protein that contained the large loop modification.

[240] The new mutated hybrid protein designated CdtB/DNase I^{Y174P} (SEQ ID NO: 29, 28), containing both the large loop and Y174P substitutions, was used to make a heterotrimer with wild-type CdtA and CdtC. Like the previous variations of the CdtB/DNase I hybrid proteins, CdtB/DNase I^{Y174P} bound to CdtA and CdtC as measured in the thyroglobulin ELISA (Figure 11). The stoichiometric increase in binding suggested that a heterotrimer was formed. Unlike previous versions of the hybrid protein, the heterotrimer formed with CdtB/DNase I^{Y174P} inhibited proliferation of CHO cells (Figure 12). To confirm that the new mutated hybrid protein formed an active heterotoxin culture, HeLa cells and two of the oral squamous cell carcinoma lines were treated with the reconstituted heterotrimer preparation. All three cell lines treated with heterotoxin made with CdtB/DNase I^{Y174P} exhibited a significant accumulation of cells having a 4n DNA content indicative of a G₂ block in cell cycle progression (Table 6). The portion of the cancer cell populations having a 4n DNA content ranged from 57-75 percent compared to 39 percent for HeLa cells. The accumulation of CdtB/DNase I^{Y174P}-treated cells at the G₂/M interphase of the cell cycle is shown, compared to controls, in Figure 13.

Table 6. Cell cycle analysis of cancer cells treated with reconstituted mutant heterotoxin

Cell line	Wild-type or mutant protein in heterotrimer	Diploid G1 (2n)	Diploid G2 (4n)	Diploid S	Coefficient of variance
		(%)	(%)	(%)	(%)
HeLa	none	71.54	5.97	22.49	4.60
	CdtB ^a	25.42	32.79	41.80	4.50
	CdtB/DNase I ^{Y174P}	30.06	38.75	31.19	4.47
CCL-30	none	58.83	0.00	41.17	12.05
	CdtB ^a	18.47	54.09	27.44	5.32
	CdtB/DNase I ^{Y174P}	19.91	56.73	23.37	6.10
CAL-27	none	75.38	2.63	21.99	4.68
	CdtB ^a	12.37	78.85	8.79	3.23
	CdtB/DNase I ^{Y174P}	11.51	74.58	13.92	3.07

^aWild-type recombinant protein from *E. coli* BL-21(DE3) (pJDB7).

What is claimed is:

1. A recombinant polypeptide comprising a chimera, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof.
2. The recombinant polypeptide of claim 1, wherein said Cdt fragment is a CdtB fragment.
3. The recombinant polypeptide of claim 2, wherein said CdtB fragment is as set forth in SEQ ID NO: 14 or a fragment thereof.
4. The recombinant polypeptide of claim 2, wherein said CdtB fragment comprises a mutation.
5. The recombinant polypeptide of claim 4, wherein said mutation is G55R, L62E, S99Y, R100E, V134R, Y174P or their combination.
6. The recombinant polypeptide of claim 4, wherein said DNase I fragment comprises a substitution of SEQ ID NO: 58 for SEQ ID NO: 59.
7. The recombinant polypeptide of claim 1, wherein said DNase I fragment is encoded by a sequence as set forth in SEQ ID NO: 1-7 or a fragment thereof.
8. The recombinant polypeptide of claim 1, wherein said chimera is His₆-tagged.
9. The recombinant polypeptide of claim 1, wherein said chimera comprises (a) said CdtB fragment in the amino terminus of said chimera and said DNase I fragment in the carboxy terminus of said chimera or (b) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a Cdt fragment in the amino terminus of said chimera or (c) a DNase I in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera or (d) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera.
10. The recombinant polypeptide of claim 1, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 24-27.
11. The recombinant polypeptide of claim 1, wherein said recombinant polypeptide binds both CdtA and CdtC.
12. The recombinant polypeptide of claim 1, wherein said recombinant polypeptide inhibits proliferation of a neoplastic cell.
13. The recombinant polypeptide of claim 1, wherein said recombinant polypeptide inhibits proliferation of a neoplastic cell and binds both CdtA and CdtC.
14. The recombinant polypeptide of claim 1, wherein the amino acid sequence of said recombinant polypeptide is as set forth in SEQ ID NO: 29.
15. A recombinant polynucleotide encoding the chimera of claim 1.

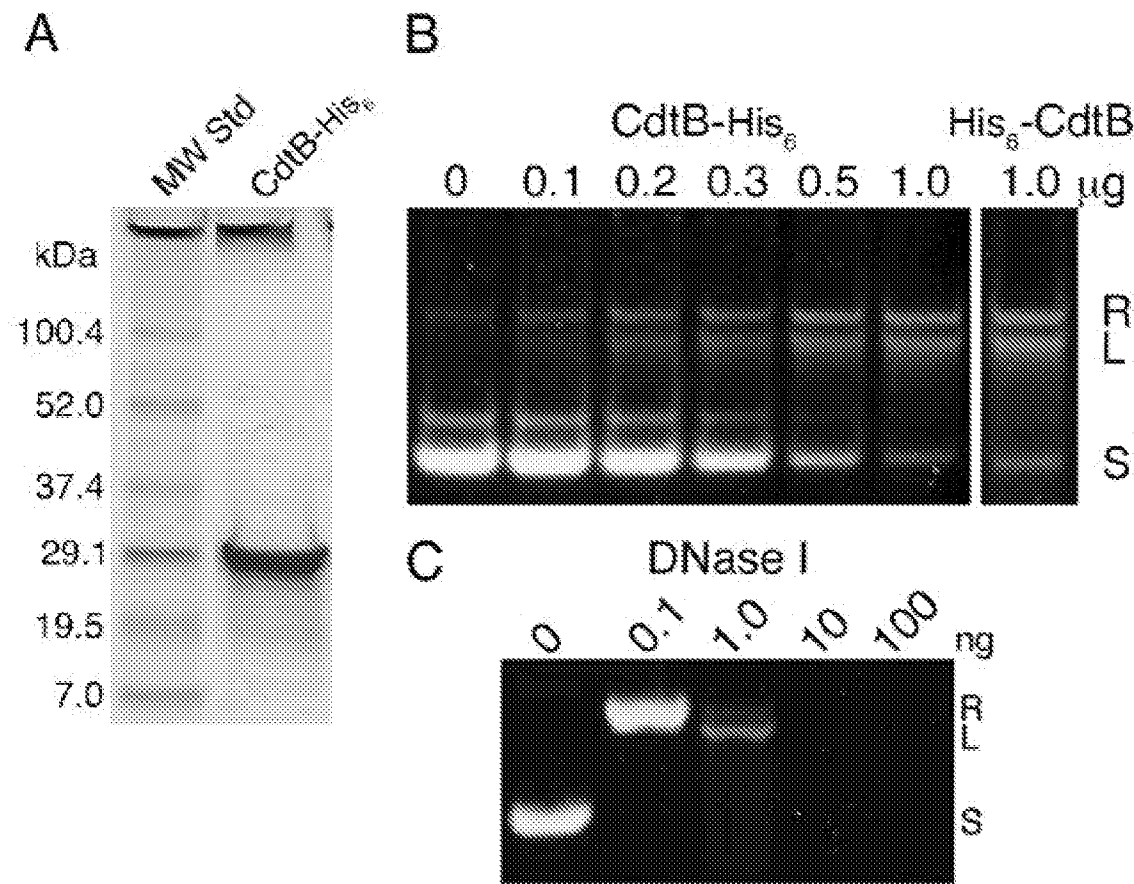
16. The recombinant polynucleotide of claim 15, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 22-23.
17. The recombinant polynucleotide of claim 15, wherein the nucleic acid sequence of said chimera is as set forth in SEQ ID NO: 28.
18. A DNA vector comprising the recombinant polynucleotide of claim 15.
19. A plasmid comprising the recombinant polynucleotide of claim 15.
20. The DNA vector claim 17, wherein said vector further comprises a promoter.
21. The DNA vector claim 19, wherein said promoter is a constitutively active promoter.
22. A method for inhibiting the proliferation of a neoplastic cell comprising the step of contacting said cell with a recombinant polypeptide comprising a chimera or a nucleic acid molecule encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting the proliferation of a neoplastic cell.
23. The method of claim 22, wherein said Cdt fragment is a CdtB fragment.
24. The method of claim 23, wherein said CdtB fragment is as set forth in SEQ ID NO: 14 or a fragment thereof.
25. The method of claim 23, wherein said CdtB fragment comprises a mutation.
26. The method of claim 25, wherein said mutation is G55R, L62E, S99Y, R100E, V134R, Y174P or their combination.
27. The method of claim 25, wherein said DNase I fragment comprises a substitution of SEQ ID NO: 58 for SEQ ID NO: 59.
28. The method of claim 22, wherein said DNase I fragment is encoded by a sequence as set forth in SEQ ID NO: 1-7 or a fragment thereof.
29. The method of claim 22, wherein said chimera is His₆-tagged.
30. The method of claim 22, wherein said chimera comprises (a) said CdtB fragment in the amino terminus of said chimera and said DNase I fragment in the carboxy terminus of said chimera or (b) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a Cdt fragment in the amino terminus of said chimera or (c) a DNase I in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera or (d) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera.
31. The method of claim 22, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 24-27.

32. The method of claim 22, wherein said chimera further binds both CdtA and CdtC.
33. The method of claim 22, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 29.
34. The method of claim 22, wherein said contacting comprises administering a DNA vector comprising said nucleic acid molecule.
35. The method of claim 34, wherein said DNA vector is a plasmid.
36. The method of claim 34, wherein said vector further comprises a promoter.
37. The method of claim 36, wherein said promoter is a constitutively active promoter.
38. The method of claim 22, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 22-23.
39. The method of claim 22, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 28.
40. A method for treating a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby treating a neoplastic disease in a subject.
41. The method of claim 40, wherein said Cdt fragment is a CdtB fragment.
42. The method of claim 41, wherein said CdtB fragment is as set forth in SEQ ID NO: 14 or a fragment thereof.
43. The method of claim 41, wherein said CdtB fragment comprises a mutation.
44. The method of claim 43, wherein said mutation is G55R, L62E, S99Y, R100E, V134R, Y174P or their combination.
45. The method of claim 41, wherein said DNase I fragment comprises a substitution of SEQ ID NO: 58 for SEQ ID NO: 59.
46. The method of claim 40, wherein said DNase I fragment is encoded by a sequence as set forth in SEQ ID NO: 1-7 or a fragment thereof.
47. The method of claim 40, wherein said chimera is His₆-tagged.
48. The method of claim 40, wherein said chimera comprises (a) said CdtB fragment in the amino terminus of said chimera and said DNase I fragment in the carboxy terminus of said chimera or (b) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a Cdt fragment in the amino terminus of said chimera or (c) a DNase I in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said

- chimera or (d) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera.
49. The method of claim 40, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 24-27.
 50. The method of claim 40, wherein said chimera further binds both CdtA and CdtC.
 51. The method of claim 40, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 29.
 52. The method of claim 40, wherein said contacting comprises administering a DNA vector comprising said nucleic acid molecule.
 53. The method of claim 52, wherein said DNA vector is a plasmid.
 54. The method of claim 52, wherein said vector further comprises a promoter.
 55. The method of claim 54, wherein said promoter is a constitutively active promoter.
 56. The method of claim 40, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 22-23.
 57. The method of claim 40, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 28.
 58. A method for inhibiting or suppressing a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby inhibiting or suppressing a neoplastic disease in a subject.
 59. The method of claim 58, wherein said Cdt fragment is a CdtB fragment.
 60. The method of claim 59, wherein said CdtB fragment is as set forth in SEQ ID NO: 14 or a fragment thereof.
 61. The method of claim 59, wherein said CdtB fragment comprises a mutation.
 62. The method of claim 61, wherein said mutation is G55R, L62E, S99Y, R100E, V134R, Y174P or their combination.
 63. The method of claim 59, wherein said DNase I fragment comprises a substitution of SEQ ID NO: 58 for SEQ ID NO: 59.
 64. The method of claim 58, wherein said DNase I fragment is encoded by a sequence as set forth in SEQ ID NO: 1-7 or a fragment thereof.
 65. The method of claim 58, wherein said chimera is His₆-tagged.

66. The method of claim 58, wherein said chimera comprises (a) said CdtB fragment in the amino terminus of said chimera and said DNase I fragment in the carboxy terminus of said chimera or (b) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a Cdt fragment in the amino terminus of said chimera or (c) a DNase I in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera or (d) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera.
67. The method of claim 58, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 24-27.
68. The method of claim 58, wherein said chimera further binds both CdtA and CdtC.
69. The method of claim 58, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 29.
70. The method of claim 58, wherein said contacting comprises administering a DNA vector comprising said nucleic acid molecule.
71. The method of claim 70, wherein said DNA vector is a plasmid.
72. The method of claim 70, wherein said vector further comprises a promoter.
73. The method of claim 72, wherein said promoter is a constitutively active promoter.
74. The method of claim 58, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 22-23.
75. The method of claim 58, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 28.
76. A method for reducing the symptoms associated with a neoplastic disease in a subject comprising the step of administering to said subject a recombinant polypeptide comprising a chimera or a nucleic acid encoding the same, wherein said chimera comprises a DNase I fragment or a homologue thereof and a Cdt fragment or a homologue thereof, thereby reducing the symptoms associated with a neoplastic disease in a subject.
77. The method of claim 76, wherein said Cdt fragment is a CdtB fragment.
78. The method of claim 77, wherein said CdtB fragment is as set forth in SEQ ID NO: 14 or a fragment thereof.
79. The method of claim 77, wherein said CdtB fragment comprises a mutation.
80. The method of claim 79, wherein said mutation is G55R, L62E, S99Y, R100E, V134R, Y174P or their combination.

81. The method of claim 77, wherein said DNase I fragment comprises a substitution of SEQ ID NO: 58 for SEQ ID NO: 59.
82. The method of claim 76, wherein said DNase I fragment is encoded by a sequence as set forth in SEQ ID NO: 1-7 or a fragment thereof.
83. The method of claim 76, wherein said chimera is His₆-tagged.
84. The method of claim 76, wherein said chimera comprises (a) said CdtB fragment in the amino terminus of said chimera and said DNase I fragment in the carboxy terminus of said chimera or (b) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a Cdt fragment in the amino terminus of said chimera or (c) a DNase I in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera or (d) a homologue of a DNase I fragment in the carboxy terminus of said chimera and a homologue of a Cdt fragment in the amino terminus of said chimera.
85. The method of claim 76, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 24-27.
86. The method of claim 76, wherein said recombinant polypeptide further binds both CdtA and CdtC.
87. The method of claim 76, wherein the amino acid sequence of said chimera is as set forth in SEQ ID NO: 29.
88. The method of claim 76, wherein said contacting comprises administering a DNA vector comprising said nucleic acid molecule.
89. The method of claim 88, wherein said DNA vector is a plasmid.
90. The method of claim 88, wherein said vector further comprises a promoter.
91. The method of claim 90, wherein said promoter is a constitutively active promoter.
92. The method of claim 76, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 22-23.
93. The method of claim 76, wherein the nucleic acid sequence encoding said chimera is as set forth in SEQ ID NO: 28.



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Figure 2

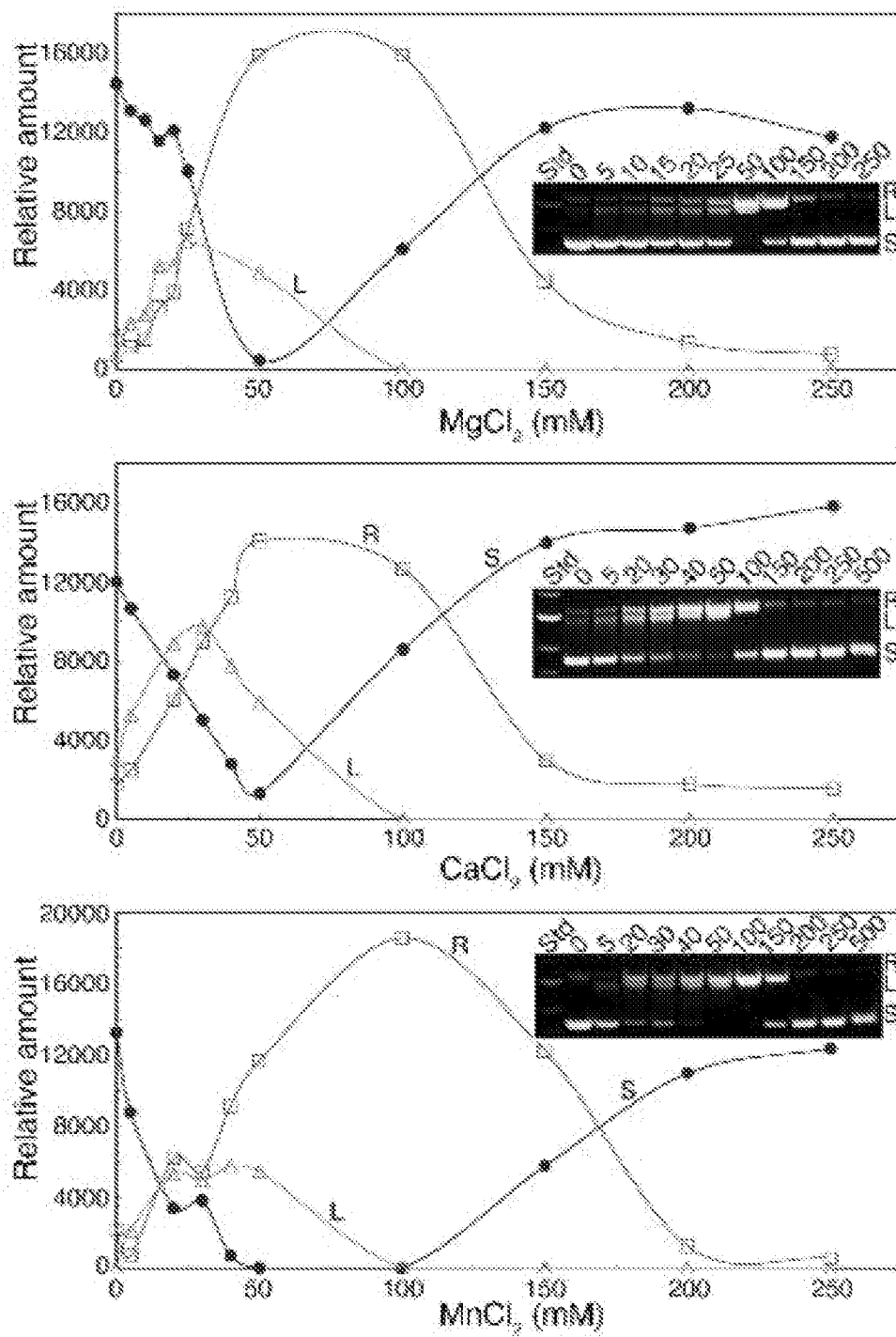


Figure 3

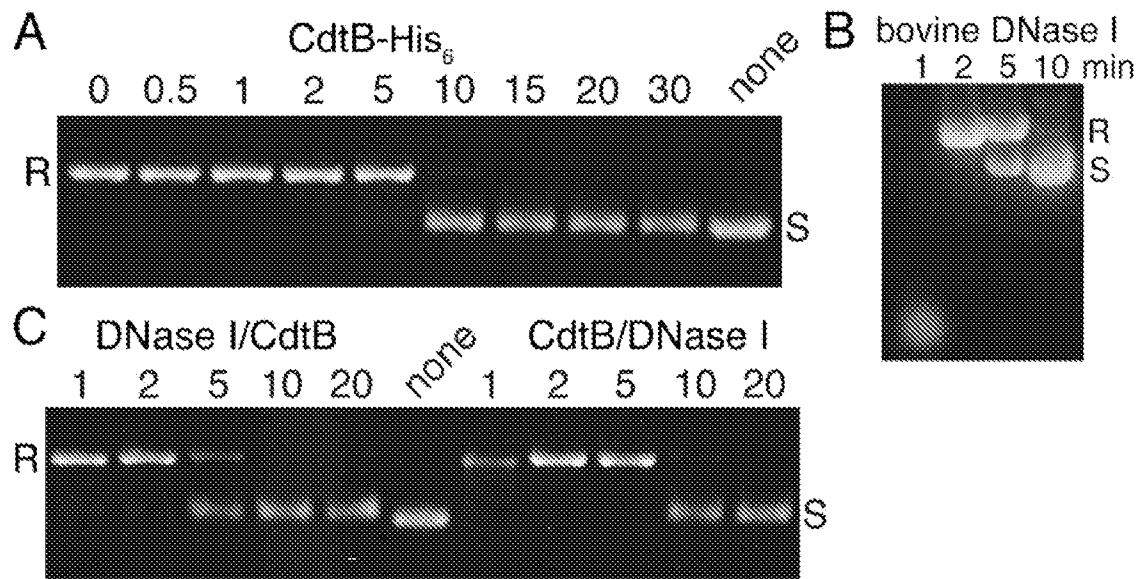
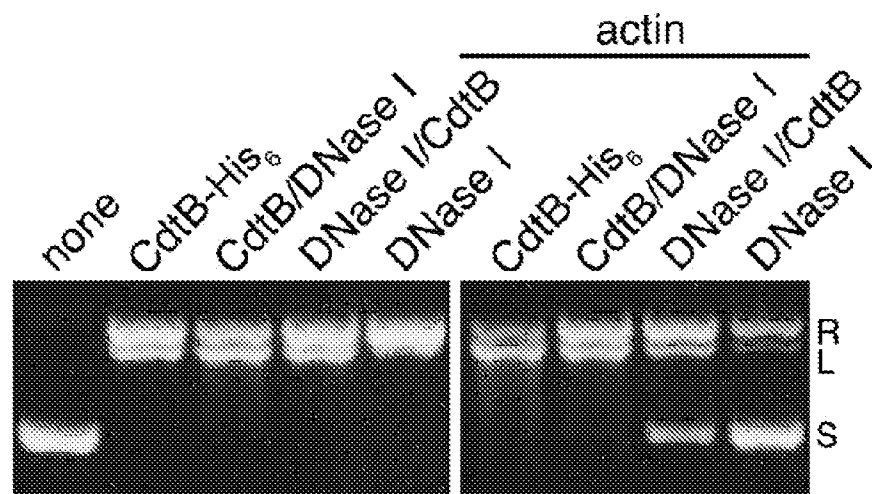
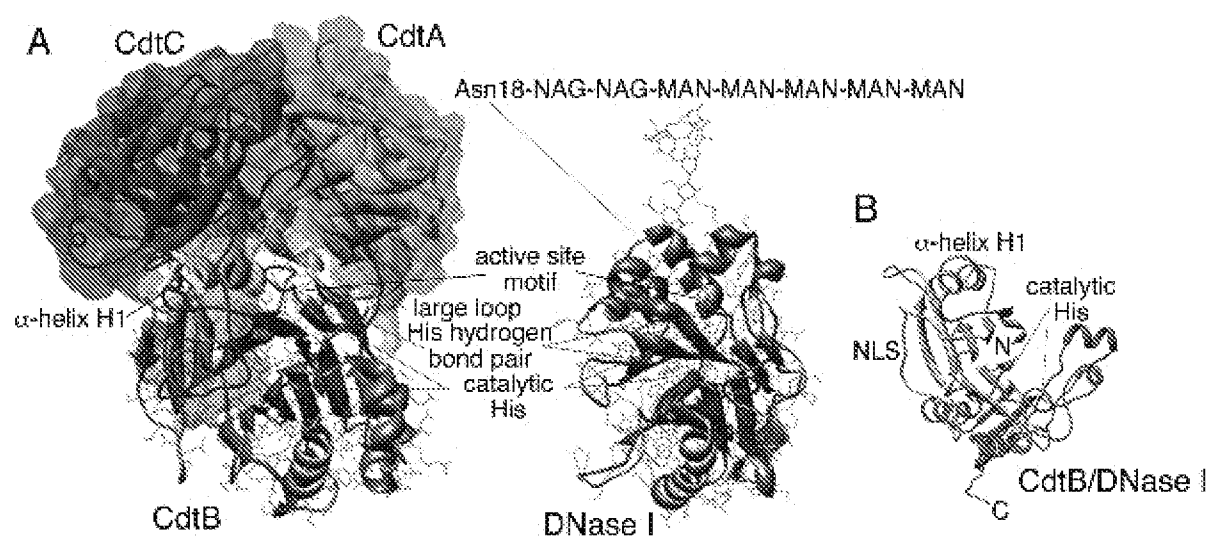


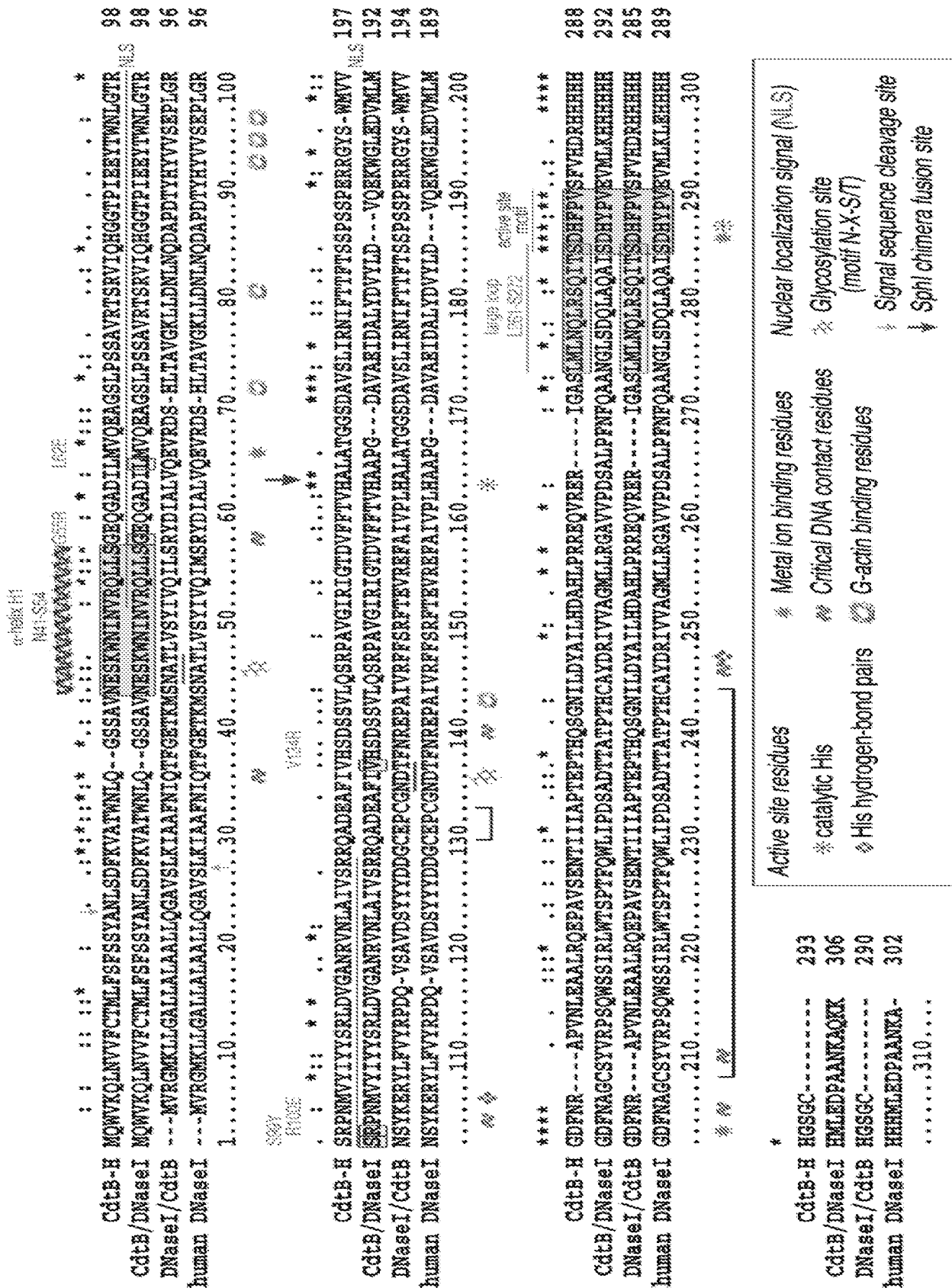
Figure 4



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Figure 5





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Figure 6A

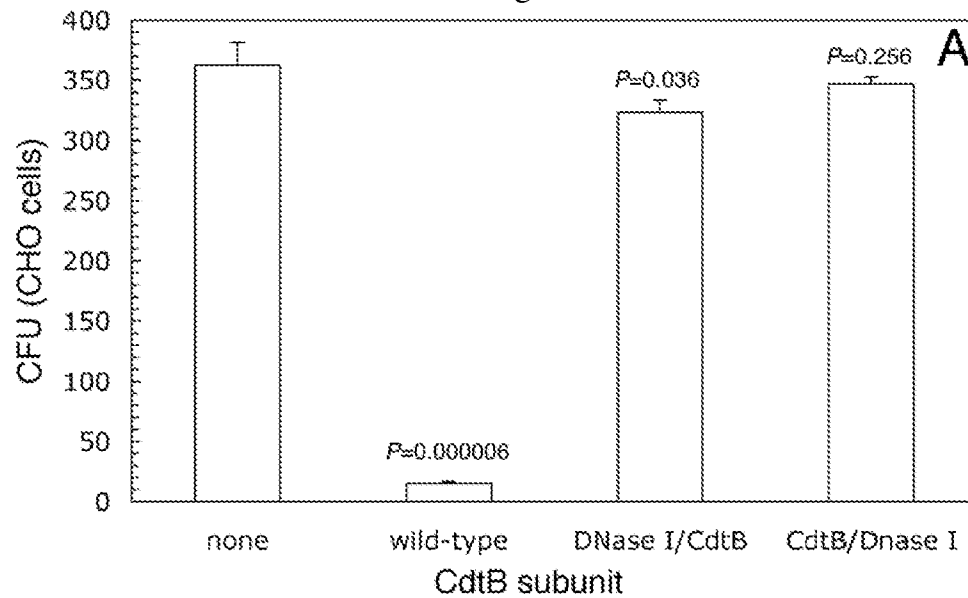
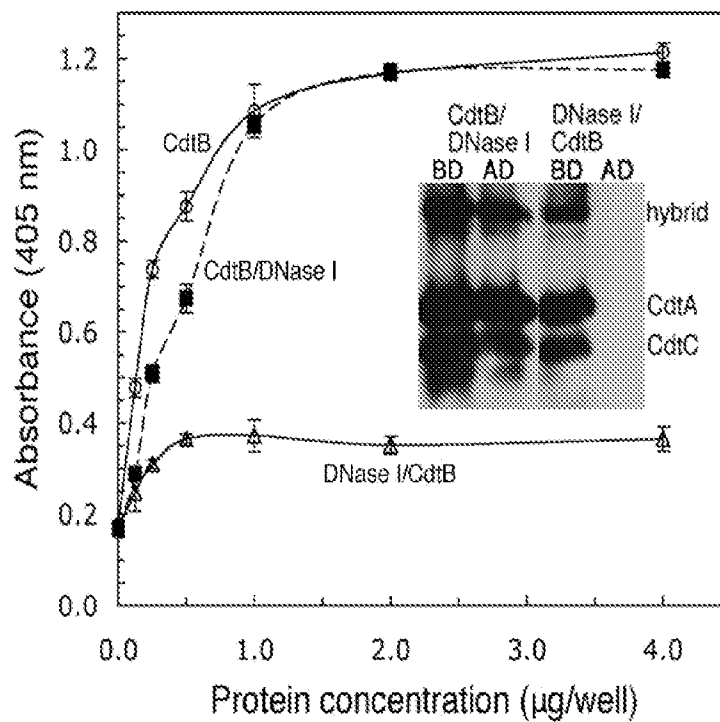
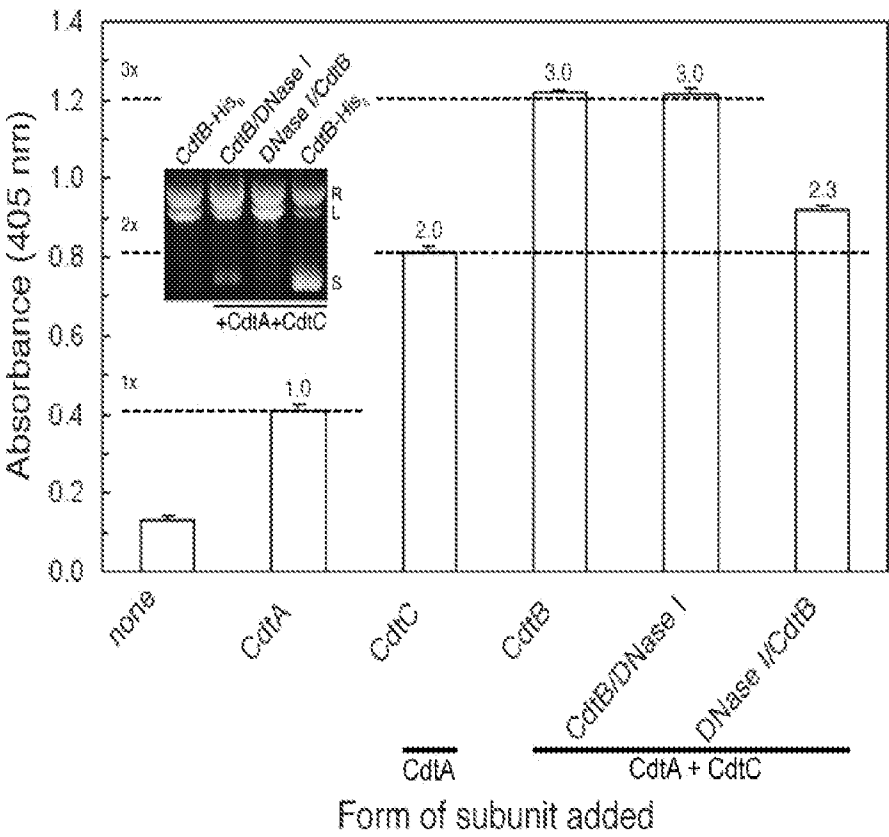


Figure 6B

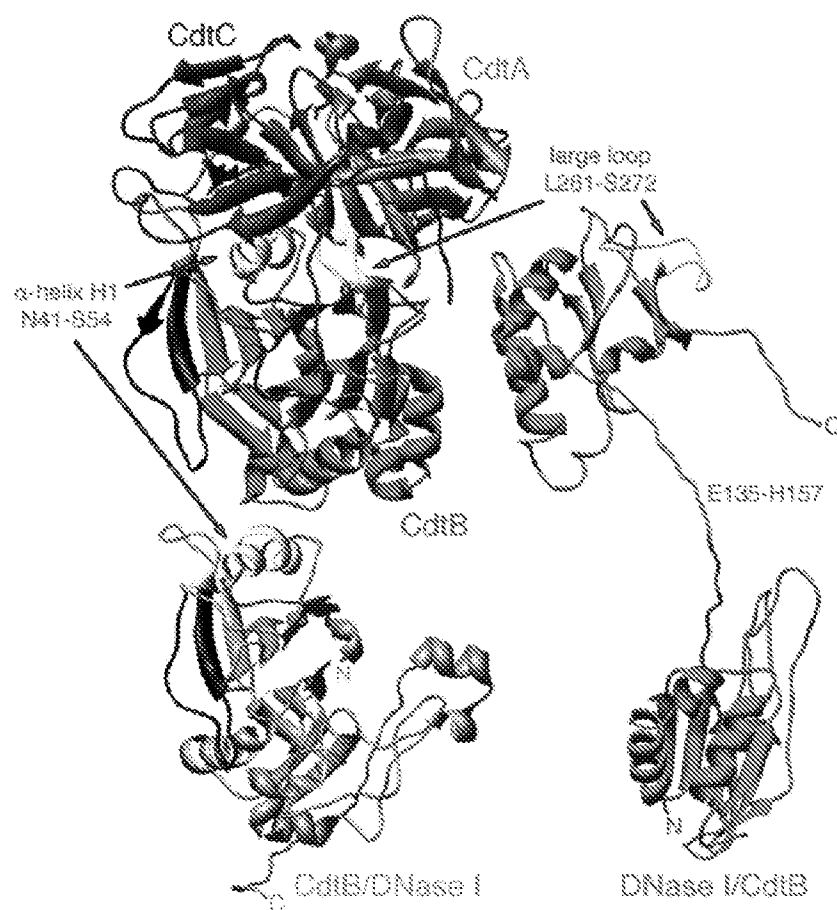


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Figure 6C



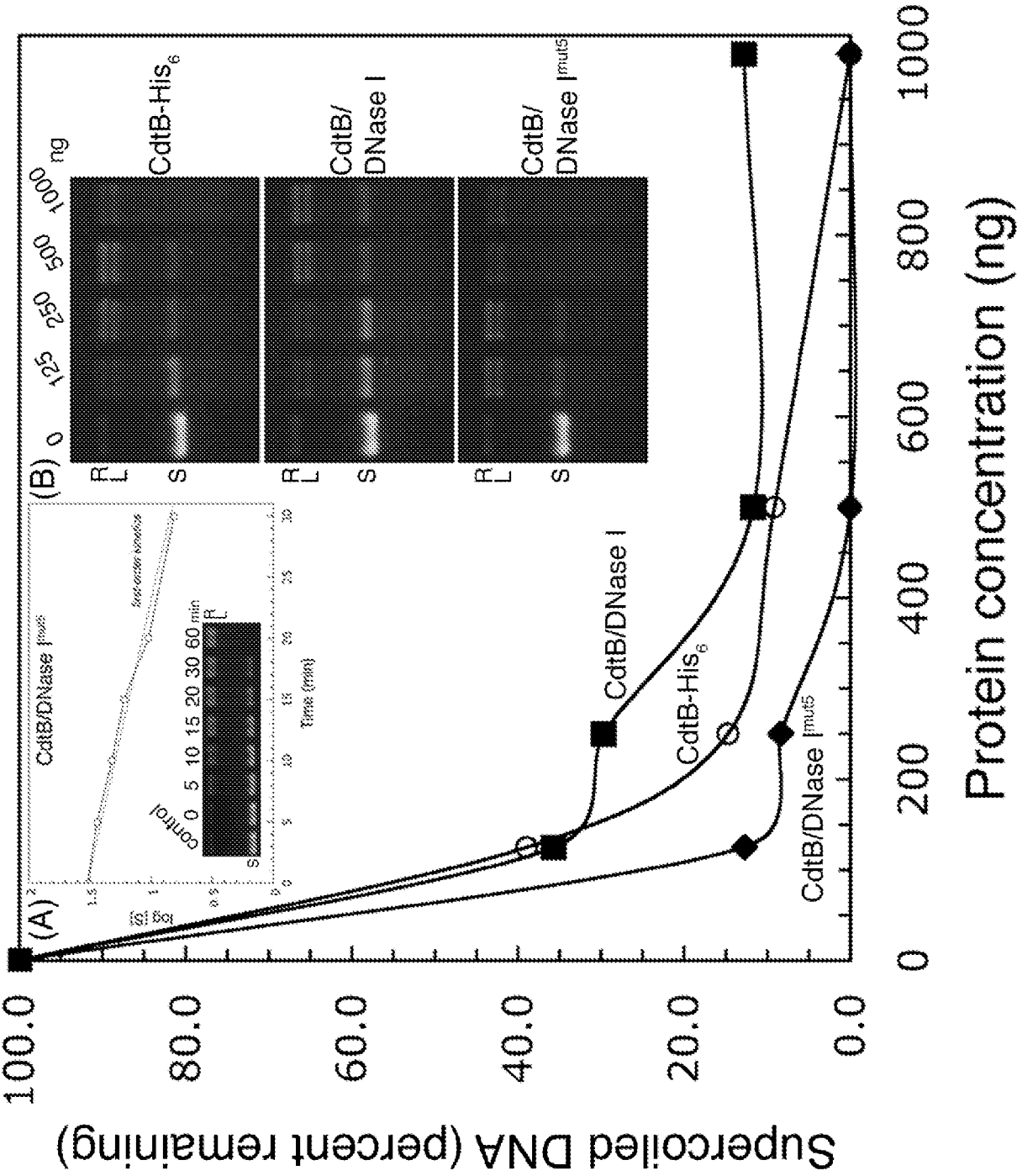
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Figure 7



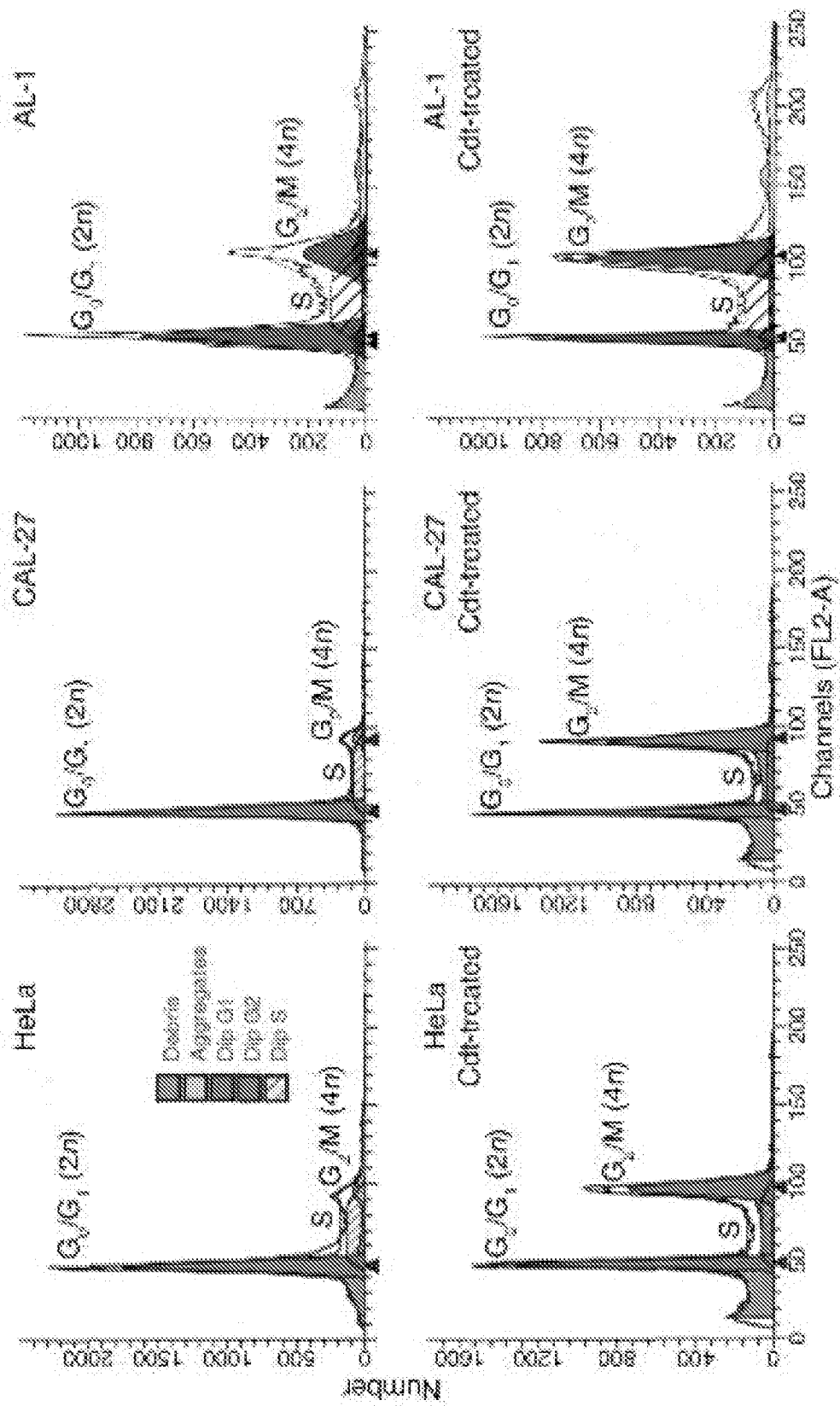
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Figure 8

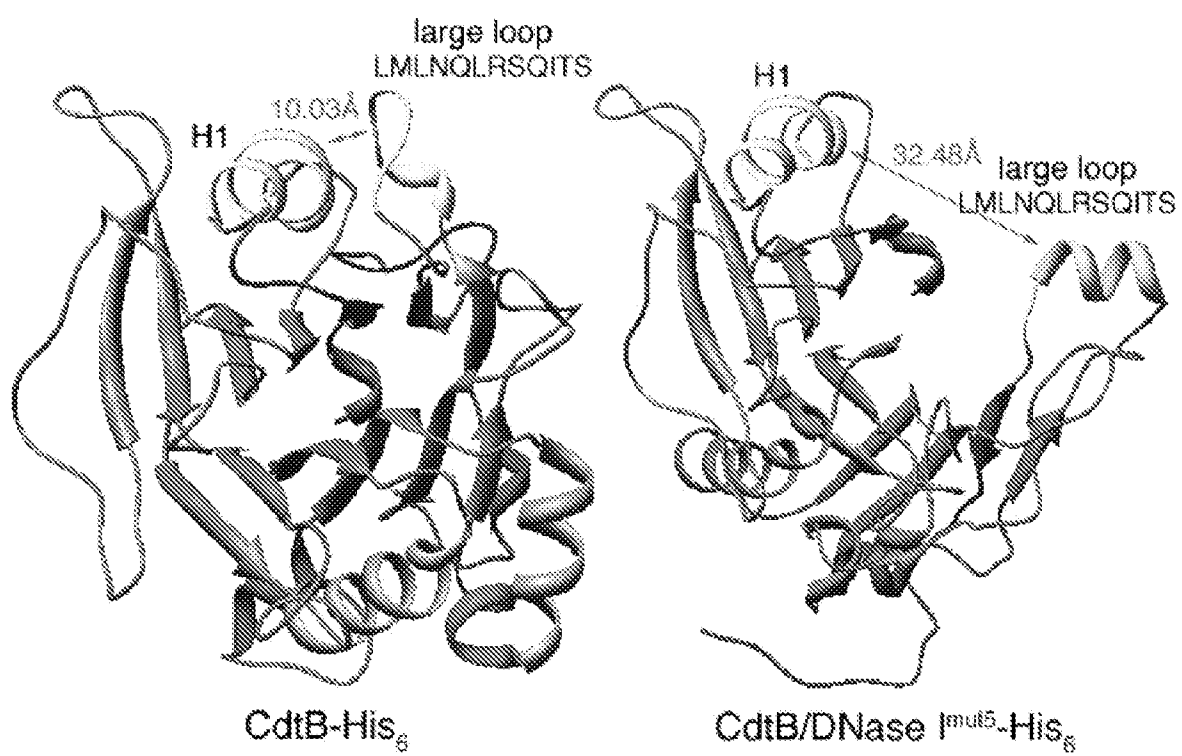


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Figure 9



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Figure 10



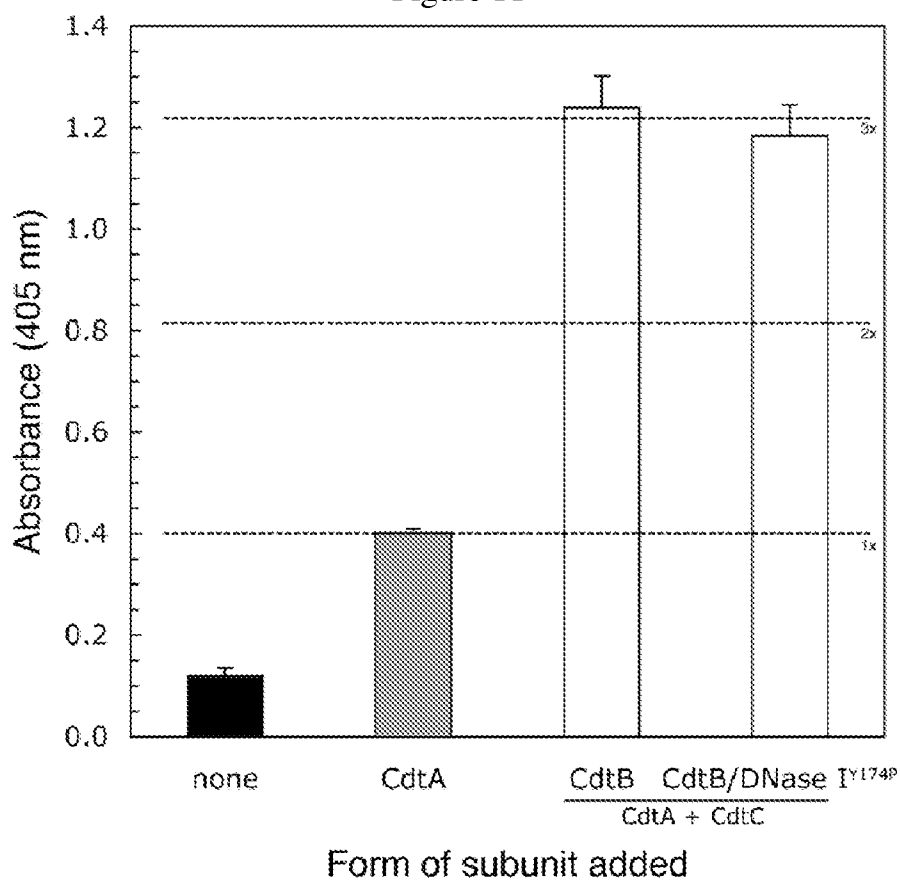
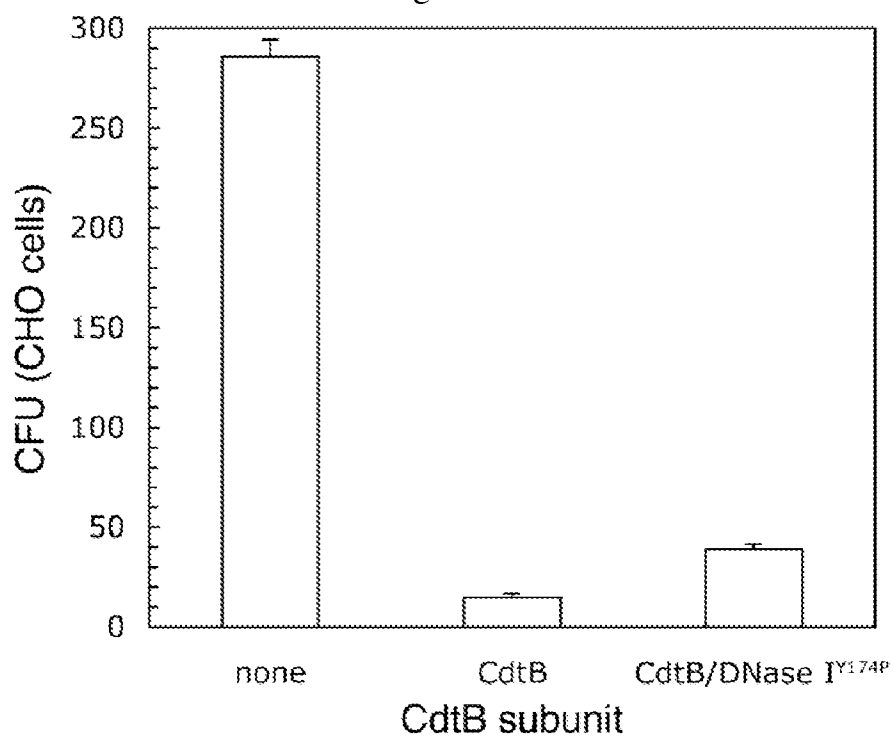
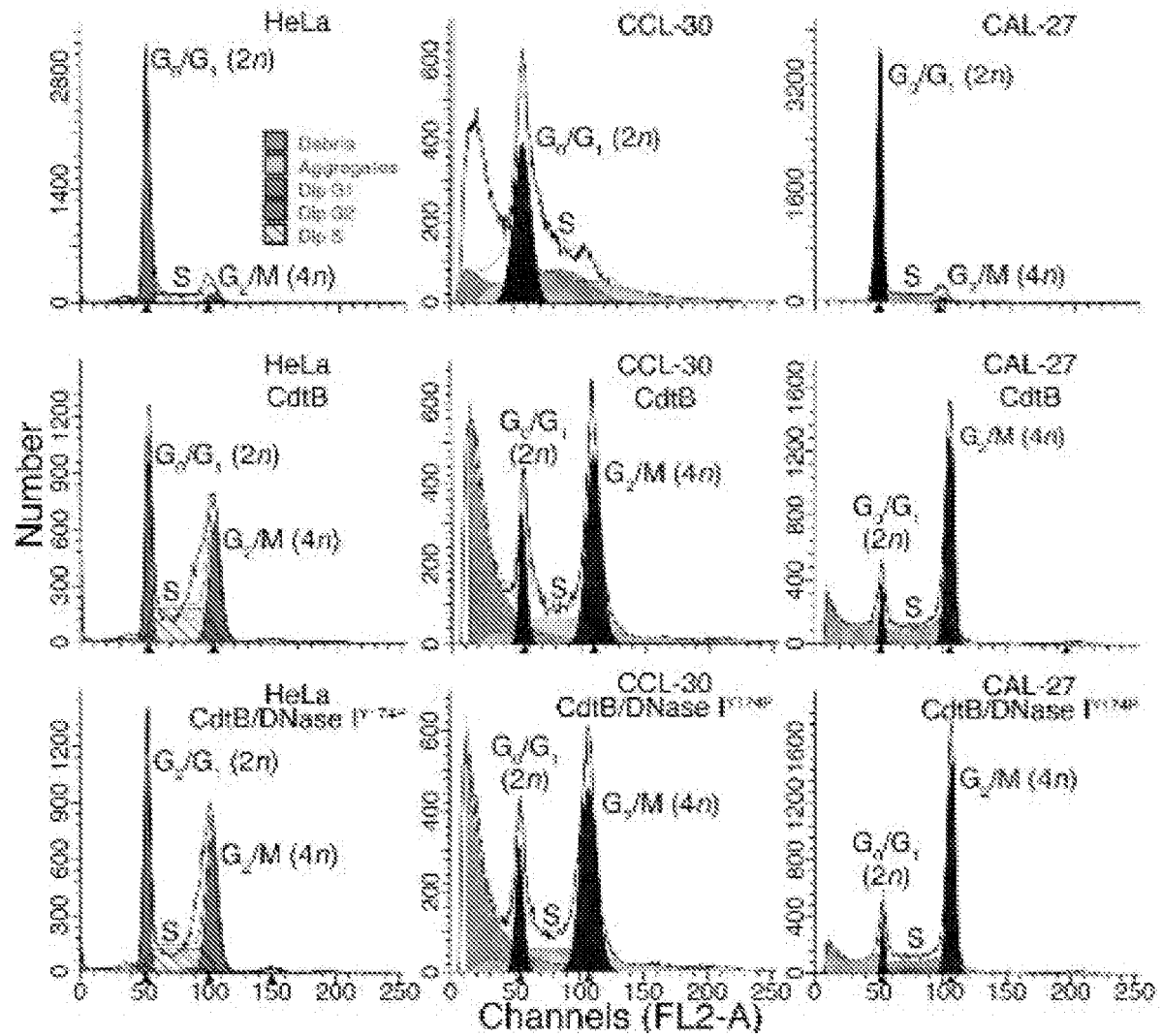
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Figure 11

Figure 12



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Figure 13



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Figure 14

