(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
03 April 2025 (03.04.2025)

WIPO | PCT

(10) International Publication Number
WO 2025/072772 A2

(51) International Patent Classification:
C07K 14/31 (2006.01) *A61K 38/16* (2006.01)

(21) International Application Number:
PCT/US2024/048990

(22) International Filing Date:
27 September 2024 (27.09.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/541,166 28 September 2023 (28.09.2023) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

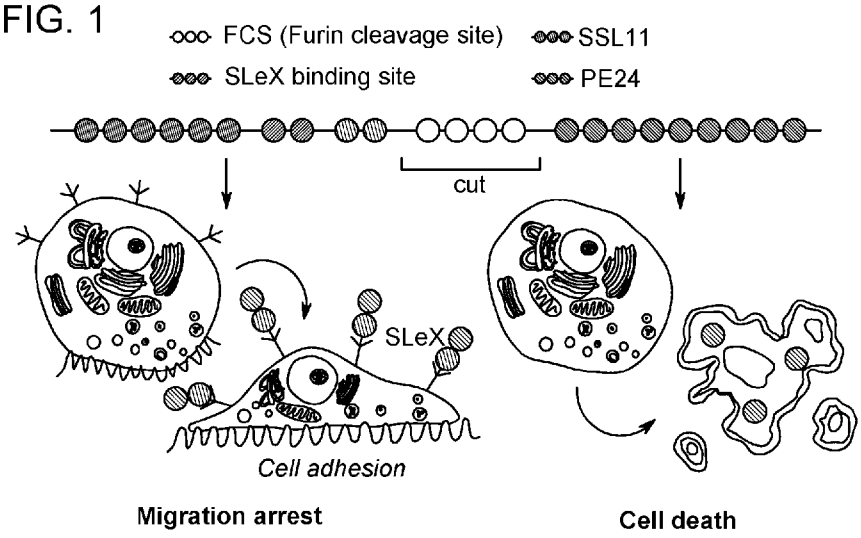
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: RECOMBINANT FUSION PROTEIN FOR TREATING CANCER

FIG. 1



(57) Abstract: Disclosed is a recombinant fusion protein comprising a fragment derived from a *Staphylococcus aureus* superantigen-like (SSL) protein and a fragment derived from a killer protein such as a Pseudomonas exotoxin (PE). The SSL protein fragment binds to a Sialyl Lewis X (SLeX) fragment of a cancer, thereby inhibiting cell migration. Also disclosed is a method for treating a cancer by administering the recombinant fusion protein.

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RECOMBINANT FUSION PROTEIN FOR TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit from U.S. provisional patent application no. 63/541,166, filed on September 28, 2023, the entire content of which is incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted herewith and is hereby incorporated by reference in its entirety. Said .xml copy, created on September 27, 2024, is named 144240598858, and is 55,850 bytes in size.

FIELD OF THE DISCLOSURE

[0003] This disclosure relates to a recombinant fusion protein and a method for treating a cancer in a subject in need thereof administering the recombinant fusion protein.

BACKGROUND

[0004] Lung cancer is the most frequent cause of cancer-related deaths worldwide. It has been transformed from a rare disease into a global problem and public health issue. Every year, 1.8 million people are diagnosed with lung cancer, and 1.6 million people die due to the disease. 5-year survival rates vary from 4-17% depending on the stage and regional differences. In the United States, lung and bronchus cancer (collectively referred to as lung cancer) are the most common causes of cancer-related deaths, with only 20.5% of the 5-year overall survival rate.

Low lung cancer survival rates reflect the large proportion of patients (57%) diagnosed with distant metastases, for which the 5-year relative survival rate is 5%. Cancer imposes an economic burden on the affected person and society. The National Institutes of Health (NIH) estimated the 2009 overall annual costs of cancer were $216.6 billion, including $86.6 billion in direct medical costs (total of all health expenditures) and $130 billion in indirect mortality costs (cost of lost productivity due to premature death). Despite these encouraging advances, cancer is a significant public health problem worldwide, requiring new strategies and treatment modalities to optimize patient outcomes.

[0005] Late-stage cancers are often associated with late diagnosis and high rates of metastases. Treatments for late-stage cancers are challenging due to intensive metastasis and poor drug penetration for solid tumors. A hallmark of cancer is uncontrolled cell growth, which leads to hypoxia. Hypoxia alters cancer cell metabolism and promotes metastasis and therapy resistance. Although there are different cell markers for different types of cancers, hypoxia leads to common cell changes across a broad spectrum of cancers. These changes include significantly increased expression of tumor-associated carbohydrate antigen Sialyl Lewis X (SLeX). Expression of SLeX is significantly increased due to tumor hypoxia, which correlates with recurrence, poor prognosis, and survival rate in a broad spectrum of cancers, e.g., in the lung, breast, kidney, prostate, ovary, melanoma, pancreas, liver, cervix, and stomach. Accordingly, there is a need to develop therapies against cancers prone to metastasis.

SUMMARY

[0006] The present disclosure provides a recombinant fusion protein comprising at least one fragment derived from a *Staphylococcus aureus* superantigen-like (SSL) protein; and at least one fragment derived from a Pseudomonas exotoxin (PE).

[0007] In an embodiment, the SSL is SSL11. In an embodiment, the at least one fragment derived from the SSL protein binds to a Sialyl Lewis X (SLeX) fragment of a cancer, thereby inhibiting cell migration. In an embodiment, the at least one fragment derived from the SSL protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 2. In an embodiment, the at least one fragment derived from the SSL protein comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO. 2. In an embodiment, the at least one fragment derived from the SSL protein comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO. 2.

[0008] In an embodiment, the PE is in a 24 kDa truncated form (PE24). In an embodiment, the at least one fragment derived from the PE inactivates eukaryotic elongation factor-2 (eEF-2), thereby inducing cell death. In an embodiment, the at least one fragment derived from the PE comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 3. In an embodiment, the at least one fragment derived from the PE comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO. 3. In an embodiment, the at least one fragment derived from the PE comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO. 3.

[0009] In an embodiment, the at least one fragment derived from the SSL protein and the at least one fragment derived from the PE are linked through a furin cleavage site. In an

embodiment, the furin cleavage site comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 4. In an embodiment, the furin cleavage site comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO. 4. In an embodiment, the furin cleavage site comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO. 4.

[0010] In an embodiment, the recombinant fusion protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 1. In an embodiment, the recombinant fusion protein comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO. 1. In an embodiment, the recombinant fusion protein comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO. 1.

[0011] The present disclosure also provides a polynucleotide encoding the recombinant fusion protein.

[0012] The present disclosure also provides a recombinant vector encoding the recombinant fusion protein.

[0013] The present disclosure also provides a pharmaceutical composition comprising the recombinant fusion protein and a pharmaceutically acceptable carrier. In an embodiment, the pharmaceutical composition comprises an additional anti-cancer agent.

[0014] The present disclosure also provides a method for treating a cancer in a subject in need thereof, comprising contacting the SLeX fragment of the cancer with the recombinant fusion protein to inhibit cell migration.

[0015] The present disclosure also provides a method for treating a cancer in a subject in need thereof, comprising contacting the eEF-2 with the recombinant fusion protein to induce cell death.

[0016] The present disclosure also provides a method for treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the recombinant fusion protein. In an embodiment, the method further comprises administering an additional active agent to the subject.

[0017] In an embodiment, the subject is human.

[0018] In an embodiment, the therapeutically effective amount is administered orally, subcutaneously, or intravenously.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0020] Figure 1 is a schematic representation showing the design of an exemplary recombinant fusion protein, and its mechanism for migration arrest and cell death. The exemplary recombinant fusion protein comprises SSL11, which inhibits cell migration by binding to SLeX, and PE24, a truncated form of PE, which inactivates eEF-2 to induce cell death.

[0021] Figure 2A is an electropherogram image showing Coomassie staining of SSL11-$PE24^{M10}$ and $PE24^{M10}$. Figure 2B is an electropherogram image showing Coomassie staining of SSL11-$PE24^{M10}$ after being cleaved by furin and run on SDS-PAGE under reducing and non-reducing conditions.

[0022] Figure 3A shows micrographic images of Hela cells treated with various concentrations of SSL11-PE24^{M10} and PE24^{M10}. Figure 3B is a graphical representation of percentages of live cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10}.

[0023] Figure 4A is a graphical representation of percentages of live cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 24 and 48 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in Hela cells. Figure 4B is a graphical representation of percentages of live cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 24 and 48 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in HCI-H1299 cells. Figure 4C is a graphical representation of percentages of live cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 24 and 48 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in A549 cells. Figure 4D is a graphical representation of percentages of live NCI-H1299 cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 72 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in NCI-H1299 cells. Figure 4E is a graphical representation of percentages of live A549 cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 72 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in A549 cells. Figure 4F is a graphical representation of percentages of live Hela cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 72 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in Hela cells. Figure 4G is a graphical representation of percentages of live Lovo cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 72 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in Lovo cells. Figure 4H is a graphical representation of percentages of live ZR 75-1 cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 72 hours showing that

SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in ZR 75-1 cells. Figure 4I is a graphical representation of percentages of live BT 20 cells after treatment with different amounts of SSL11-PE24^{M10} and PE24^{M10} for 72 hours showing that SSL11-PE24^{M10}, but not PE24^{M10}, induces cell death in BT 20 cells

[0024] Figure 5A is a graphical representation of percentages of live BEAS-2B cells after treatment with fusion proteins at 37°C for 72 hours. Cells were washed to remove the dead cells and stained using crystal violet. Percentage of live cells after treatment with different amounts of platform proteins. Figure 5B is a graphical representation of percentages of live cells after treatment with 160 nM of platform proteins at 37°C for 72 hours.

[0025] Figure 6 is a Western Blot image showing SSL11-PE24^{M10} and PE24^{M10} ADP ribosylated e-EF2 in NCI-H1299 cell lysate.

[0026] Figure 7A shows micrograph images of Alexa Fluor 647 labeled phalloidin for F-actin staining and DAPI for nuclei staining for NCI-H1299 cells incubated with various concentrations of Alex Fluor 568 labeled SSL11-PE24^{M10} and PE24^{M10}. Figure 7B is a graphical representation showing cell association being quantified as the fluorescence intensity ratio between platform proteins and DAPI staining by Image J. Figure 7C is a graphical representation showing flow cytometry analysis of NCI-H1299 cells incubated with 160 nM of Alex Fluor 568 labeled SSL11-PE24^{M10} and PE24^{M10} on ice for 30 mins.

[0027] Figure 8 shows micrograph images of ^{3XFLAG}SSL11-^{IIA}PE24^{M10} incubated with NCI-H1299 cells at 37 °C for 60 mins. Cells were washed, fixed and stained by immunofluorescence. SSL11 (stained with anti-FLAG), PE24^{M10} (stained with anti-HA), and Golgi (stained with anti-Golgi97).

[0028] Figure 9A shows micrograph images of cell wounds created on a monolayer of NCI-H1299 cells. The cells were incubated with 80-640 nM of SSL11-PE24^{M10} and PE24^{M10} at 37 °C for 24 or 48 hours. Figure 9B shows graphical representations of quantification of the percentages of wound area per image. Figure 9C shows micrograph images of cell wounds created on a monolayer of A549 cells. The cells were incubated with 80-640 nM of SSL11-PE24^{M10} and PE24^{M10} at 37 °C for 24 or 48 hours. Figure 9D shows graphical representations of quantification of the percentages of wound area per image.

[0029] Figure 10A shows micrograph images of cell wounds created on a monolayer of NCI-H1299 cells with a 96-well woundmaker tool. Cells were incubated with 160-640 nM of SSL11-PE24^{M10} and PE24^{M10} at 37 °C for 24 hours, and plates were scanned every two hours by Incucyte® S3. Figure 10B shows graphical representations of quantification of the percentages of wound area per image.

[0030] Figure 11A shows micrograph images of cell wounds created on a monolayer of A549 cells with a 96-well woundmaker tool. Cells were incubated with 160-640 nM of SSL11-PE24^{M10} and PE24^{M10} at 37 °C for 24 hours, and plates were scanned every two hours. Figure 11B shows graphical representations of quantification of the percentages of wound area per image.

[0031] Figure 12A shows micrograph images of cell wounds created on a monolayer of BEAS-2B cells with a 96-well woundmaker tool. Cells were incubated with 160-640 nM of SSL11-PE24^{M10} and PE24^{M10} at 37 °C for 24 hours, and plates were scanned every two hours. Figure 12B shows graphical representations of quantification of the percentages of wound area per image.

[0032] Figure 13 (SEQ ID Nos: 46-59) shows SSL1-14 sequences aligned using Clustal Omega. The SLeX binding sites are indicated by a box.

[0033] Figure 14 shows a graphical representation of quantification of the levels of cell adhesion levels induced by SSL4 and SSL11 in the differentiated HL60 cells (**** $p < 0.0001$, *** $p < 0.001$, unpaired two-tailed *t* test by GraphPad Prism 10.3.1).

DETAILED DESCRIPTION

[0034] Aberrant glycosylation of tumor cells is recognized as a universal hallmark of cancer pathogenesis. Tumor hypoxia accelerates the expression of abnormal glycans in cancer cells such as Sialyl Lewis X (SLeX [Neu5Aca2-3Galb1-4(Fuc1-3)GlcNAc]) and Sialyl Lewis A (SLeA [Neu5Aca2-3Galb1-3(Fuc1-4)GlcNAc]). Expressions of SLeX and SLeA are increased in cancer tissues compared to normal epithelial cells. SLeX and SLeA are useful tumor markers for cancers, e.g., in the lung, breast, kidney, prostate, stomach, colon, pancreas, and ovary. The SLeX and SLeA glycan serve as ligands for E-selectin expressed on vascular endothelial cells. Enhanced interaction of cancer cells with endothelial cells facilitates tumor angiogenesis and hematogenous metastasis.

[0035] Metastasis is one of the main causes of death among cancer patients and correlates with poor prognosis. Histologically, lung cancer is classified into two major types: small cell lung cancer (SCLC), which represents 10% of lung cancer cases, and non-small cell lung cancer (NSCLCs), which is the most common type and represents 85% of lung cancer cases. Stages I or II NSCLC patients with MUC5AC (a major carrying protein of SLeX and SLeA) and/or SLeX expression have a higher rate of postoperative metastasis and shorter overall survival. The higher expression of the SLeX, the worse the survival rate for lung cancer patients.

NSCLC is one of the most common primary tumors to metastasize to the brain in adults, which is associated with the high expression of SLeX. Twenty to forty percent of NSCLC patients develop brain metastasis at or within a short period of primary tumor diagnosis. Clinical studies have shown that patients with tumors expressing high levels of SLeX have a significantly higher risk of developing invasion and metastasis than patients with tumors expressing low levels of this antigen. In one study, out of 92 lung cancer samples examined, SLeX was detected in 52 cases (52%), and higher expression of SLeX was reported in 72% of lung adenocarcinoma patients. Surprisingly, the present disclosure describes a treatment for cancer, especially cancers prone to metastasis, that targets SLeX, inhibits cancer cell migration, and stimulates cell death.

[0036] Staphylococcal superantigen-like protein 11 (SSL11) binds to SLeX and inhibits cell migration. SSL11 is a promising platform for delivering a "killing" protein to cancers that overexpress SLeX. As used herein, a "killing protein" refers to a full length or truncated polypeptide of viral, bacterial, fungi, plant, or animal origin that are toxic to mammalian cells, particularly to mammalian cancer cells and/or induce death of mammalian cancer cells. In contrast to conventional carbohydrate-specific antibodies that typically have low affinity and specificity, SSL11 is better suited because of its binding specificity and cell migration inhibition.

[0037] Toxins derived from bacteria or plants have evolved with human beings for their efficiency and potency and have been engineered for therapeutic treatments against different diseases. A good example is Pseudomonas exotoxin A (PE)-based immunotoxins against cancers, which is a potent bacterial toxin to induce cell death. PE belongs to the AB toxin family, which includes an enzymatic domain (A subunit) and a receptor-binding domain (B subunit). The A subunit of PE is an ADP-ribosyltransferase that inactivates eEF-2. This halts protein synthesis and eventually leads to cell death. PE has been used to construct immunotoxins

targeting cancers with antibodies replacement of the receptor-binding domain of PE. This effect was observed when a 38 kDa truncated form of PE (PE38) was fused to a variety of immunotoxins to inactivate eEF-2 in cancer cells. Due to higher immunogenicity, in lieu of PE38, the present disclosure describes use of PE24, a 24 kDa truncated form of PE, and more specifically $PE24^{M10}$ (SEQ. ID. NO: 3), which has shown promising results against multiple cancers.

[0038] In this context, the inventors have surprisingly found that the ability of SSL11 inhibiting cell migration via binding to SLeX is a great platform to deliver therapeutic reagents to cancer cells overexpressing SLeX, which is an excellent target for cancer biology against a spectrum of cancers, especially end-stage cancers prone to metastasis. The combination of SSL11 and PE24 not only kills cancer cells via PE24 but also inhibits cancer metastasis via SSL11.

[0039] One of the major challenges for immunotoxins is the penetration into the solid tumor, as antigens on the solid tumor are not as accessible as those on, e.g., leukemia. Solid tumors are often associated with tumor hypoxia, which accelerates the expression of SLeX. Advantageously, recombinant fusion proteins containing a suitable SSL and a killing protein, as exemplified by SSL11-$PE24^{M10}$ in the present disclosure, targeting SLeX serves as a great candidate against solid tumors that overexpress SLeX. In addition, the recombinant fusion protein provides a novel therapy against cancers overexpressing SLeX, which are often prone to metastasis. SSL11 enters cells via binding to SLeX, inhibits cell migration quickly (within 15 minutes), and delivers the "killing" protein, e.g., PE24, to the cytosol to induce cell death. In an embodiment, PE24 is further engineered as $PE24^{M10}$ with ten mutations to reduce immunogenicity.

[0040] As used herein, the term "about" refers to plus or minus 10% of the indicated value. Unless otherwise stated, weight percentages are provided based on the total amount of the composition in which they are described.

[0041] As used herein, the singular forms "a," "an," and "the" include plural referents unless stated otherwise.

[0042] As used herein, the term "cancer" refers to a disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, ocular cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like.

[0043] As used herein, the term "recombinant protein" refers to a protein encoded by recombinant DNA that has been cloned in an expression vector that supports expression of the gene and translation of messenger RNA. *Escherichia coli* (bacteria) is one of the organisms of choice for the production of recombinant proteins. Its use as a cell factory is well-established and it has become the most popular expression platform. High-level expression of many recombinant proteins in *Escherichia coli* leads to the formation of highly aggregated protein commonly referred to as inclusion bodies. Inclusion bodies are normally formed in the cytoplasm. Bacterial inclusion bodies are mesoscale protein aggregates commonly observed in recombinant bacteria, primarily formed by recombinant protein.

[0044] As used herein, the term "fusion protein" refers to a protein comprising a plurality of heterologous proteins, protein domains, or peptides, e.g., a binding protein and a functional

effector protein, associated with each other via a peptide linkage, thus forming a single amino acid sequence. In certain embodiments, a fusion protein is encoded by a gene.

[0045] As used herein, the term “peptide” refers to a chain-type polymer formed by amino acid residues which are linked to each other via peptide bonds, and used interchangeably with "polypeptide." Further, a "polypeptide" includes a peptide and a protein.

[0046] Further, the term “peptide” includes amino acid sequences that are conservative variations of those peptides specifically exemplified herein. The term “conservative variation,” as used herein, denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include substitution of one hydrophobic residue, such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine, or methionine for another, or substitution of one polar residue for another, for example, substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which may be substituted for one another include asparagine, glutamine, serine, and threonine.

[0047] A person having ordinary skill in the art may make similar substitutions to obtain peptides having higher cell permeability and a broader host range. For example, one aspect disclosed in the present application provides peptides corresponding to amino acid sequences (e.g., SEQ ID NOS: 1-6 and 14-18) provided herein, as well as analogues, homologs, isomers, derivatives, amidated variations, and conservative variations thereof, as long as the cell permeability of the peptide remains.

[0048] Minor modifications to primary amino acid sequences disclosed in the present application may result in peptides which have substantially equivalent or enhanced cell

permeability, as compared to the specific peptides described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous.

[0049] All peptides may be synthesized using L-amino acids, but D forms of all of the peptides may be synthetically produced. In addition, C-terminal derivatives, such as C-terminal methyl esters and C-terminal amidates, may be produced in order to increase the cell permeability of the peptide according to one embodiment disclosed in the present application.

[0050] All of the peptides produced by these modifications are included herein, as long as, in the case of amidated versions of the peptide, the cell permeability of the original peptide is altered or enhanced such that the amidated peptide is therapeutically useful. It is envisioned that such modifications are useful for altering or enhancing cell permeability of a particular peptide.

[0051] Furthermore, deletion of one or more amino acids may also result in a modification to the structure of the resultant molecule without any significant change in its cell permeability. This may lead to the development of a smaller active molecule which may also have utility. For example, amino- or carboxyl-terminal amino acids which may not be required for the cell permeability of a particular peptide may be removed.

[0052] As used herein, the term "gene" refers to an arbitrary nucleic acid sequence or a part thereof having a functional role in protein coding or transcription, or regulation of other gene expression. The gene may be composed of all nucleic acids encoding a functional protein or a part of the nucleic acid encoding or expressing the protein. The nucleic acid sequence may include a gene mutation in exon, intron, initiation or termination region, promoter sequence, other regulatory sequence, or a unique sequence adjacent to the gene.

[0053] As used herein, the terms "therapeutically effective amount" is intended to mean that the non-aqueous solutions of lamotrigine will elicit the biological or medical response of a

tissue, a system, animal, or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. In a preferred embodiment, the term "therapeutically effective amount" means an amount that alleviates at least one clinical symptom in a human patient. The terms "prophylactically effective (or efficacious) amount" and similar descriptions such as "an amount efficacious for prevention" are intended to mean that amount that will prevent or reduce the risk of occurrence of the biological or medical event that is sought to be prevented in a tissue, a system, animal or human by a researcher, veterinarian, medical doctor or other clinician.

[0054] As used herein, the disclosure of numerical ranges within this specification is considered to be a disclosure of all numerical values and ranges within that range. For example, if a range is from about 1 to about 50, it is deemed to include, for example, 1, 7, 34, 46.1, 23.7, 50 or any other value or range within the range. Moreover, as used herein, the term "at least" includes the stated number, e.g., "at least 50" includes 50.

[0055] As used herein, the term "administration" and variants thereof (e.g., "administering") in reference to an active agent of the disclosure means introducing or delivering the active agent to a subject in need of treatment. Administering of an active agent of the disclosure to the subject includes both self-administration and administration to the subject by another, including a medical professional. Administration may be via any common route, and in forms suitable for each administration route. Such routes include, but are not limited to, parenteral (e.g., subcutaneous, intramuscular, intraperitoneal or intravenous) and oral.

[0056] As used herein, "treat", "treating", or "treatment" includes treating for the purpose of curing or ameliorating a neurological disorder or a mental disorder, or for the purpose of suppressing the progression, occurrence, or recurrence of the neurological disorder or the mental disorder or alleviating one or more of the associated symptoms.

[0057] As used herein, "administered every day" or "daily" or "once a day" or "once a day on each day" includes an administration schedule based on a regimen in which dosing is performed on every day of a treatment cycle (i.e., treatment period). For example, an active ingredient may be administered every day of a treatment cycle. In some embodiments, a "drug holiday" may be provided as each treatment cycle ends. In some embodiments, an active agent is administered once during a treatment cycle.

[0058] As used herein, the "treatment cycle" refers to the period of time during which the administration according to the methods of the disclosure takes place. During a treatment cycle, the active agent of the method is administered sequentially or simultaneously as described herein.

[0059] As used herein, "administered intermittently" is not particularly limited as long as the conditions of at least twice during the treatment cycle and an administration interval of at least one day between dosing (the number of days between a certain day of administration and the next day of administration) are satisfied.

[0060] One aspect of the disclosure provides a recombinant fusion protein comprising at least one fragment derived from an SSL protein and at least one fragment derived from a PE.

[0061] In an embodiment, the recombinant fusion protein of the disclosure can be in a form of an inclusion body or bodies. The fusion may be formed via a peptide bond or a chemical bond. The chemical bond can be disulfide bonds, diamine bonds, sulfide-amine bonds, carboxyl-amine bonds, ester bonds, and covalent bonds.

[0062] In an embodiment, the recombinant fusion protein of the disclosure has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least

95%, at least 98%, at least 99%, or at least 99.5% amino acid sequence identity to SEQ ID NO: 1.

[0063] In an embodiment, the SSL is SSL11. In an embodiment, the at least one fragment derived from the SSL protein binds to an SLeX fragment of a cancer, thereby inhibiting cell migration. In other embodiments, different SSLs may be utilized, for example any one of SSL2 (SEQ ID NO: 14), SSL3 (SEQ ID NO: 15), SSL4 (SEQ ID NO: 16), SSL5 (SEQ ID NO: 17), or SSL6 (SEQ ID NO: 18), which can also bind to an SLeX fragment of a cancer.

[0064] In an embodiment, the SSL is a chimeric protein comprising an SLeX binding site from any one of SSL2 (SEQ ID NO: 8), SSL3 (SEQ ID NO: 9), SSL4 (SEQ ID NO: 10), SSL5 (SEQ ID NO: 11), SSL6 (SEQ ID NO: 12), or SSL11 (SEQ ID NO: 13) inserted into any one of heterologous SSL proteins in a way that replaces its native binding site. In an embodiment, the chimeric SSL is an SSL2 (SEQ ID NO: 14) protein wherein the native SSL2 SLeX binding site (SEQ ID NO: 8) has been replaced by an SLeX binding site of any one of SSL3 (SEQ ID NO: 9), SSL4 (SEQ ID NO: 10), SSL5 (SEQ ID NO: 11), SSL6 (SEQ ID NO: 12), or SSL11 (SEQ ID NO: 13) protein. In an embodiment, SSL is an SSL3 (SEQ ID NO: 15) protein wherein the native SSL3 SLeX binding site (SEQ ID NO: 9) has been replaced by the SLeX binding site of any one of SSL2 (SEQ ID NO: 8), SSL4 (SEQ ID NO: 10), SSL5 (SEQ ID NO: 11), SSL6 (SEQ ID NO: 12), or SSL11 (SEQ ID NO: 13) protein. In an embodiment, SSL is an SSL4 (SEQ ID NO: 16) protein wherein the native SSL4 SLeX binding site (SEQ ID NO: 10) has been replaced by the SLeX binding site of any one of SSL2 (SEQ ID NO: 8), SSL3 (SEQ ID NO: 9), SSL5 (SEQ ID NO: 11), SSL6 (SEQ ID NO: 12), or SSL11 (SEQ ID NO: 13) protein. In an embodiment, SSL is an SSL5 (SEQ ID NO: 17) protein wherein the native SSL5 SLeX binding site (SEQ ID NO: 11) has been replaced by the SLeX binding site of any one of SSL2 (SEQ ID

NO: 8), SSL3 (SEQ ID NO: 9), SSL4 (SEQ ID NO: 10), SSL6 (SEQ ID NO: 12), or SSL11 (SEQ ID NO: 13) protein. In an embodiment, SSL is an SSL6 (SEQ ID NO: 18) protein wherein the native SSL6 SLeX binding site (SEQ ID NO: 12) has been replaced by the SLeX binding site of any one of SSL2 (SEQ ID NO: 8), SSL3 (SEQ ID NO: 9), SSL4 (SEQ ID NO: 10), SSL5 (SEQ ID NO: 11), or SSL11 (SEQ ID NO: 13) protein. In an embodiment, SSL is an SSL11 (SEQ ID NO: 2) protein wherein the native SSL11 SLeX binding site 9SEQ ID NO; 13) has been replaced by the SLeX binding site of any one of SSL2 (SEQ ID NO: 8), SSL3 (SEQ ID NO: 9), SSL4 (SEQ ID NO: 10), SSL5 (SEQ ID NO: 11), or SSL6 (SEQ ID NO: 12) protein.

[0065] In an embodiment, the at least one fragment derived from the SSL protein of the recombinant fusion protein of the disclosure has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% amino acid sequence identity to SEQ ID NO: 2 or SEQ ID NOs: 14-18. In an embodiment, the at least one fragment derived from the SSL protein of the recombinant fusion protein of the disclosure has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% amino acid sequence identity to SEQ ID NO: 2.

[0066] In an embodiment, the PE is in a 24 kDa truncated form. In an embodiment, the at least one fragment derived from the PE inactivates eEF-2, thereby inducing cell death. In other embodiments, alternative full length or truncated killing proteins can be used. Non-limiting examples of alternative full length or truncated killing proteins include diphtheria toxin (DT), ricin toxin, and lethal factor (LF) and edema factor (EF) obtained from anthrax toxin. DT can be used to induce cell death by ADP ribosylation of eEF-2. Ricin toxin can be used to induce cell death by eliminating adenine at residue 4324 from a conserved 28S rRNA loop to inhibit the

translation process. LF can be used to induce cell death by cleaving MAPKKs. EF can be used to induce cell death by catalyzing cAMP formation.

[0067] In an embodiment, the at least one fragment derived from the PE of the recombinant fusion protein of the disclosure has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% amino acid sequence identity to SEQ ID NO: 3.

[0068] In an embodiment, the at least one fragment derived from the SSL protein and the at least one fragment derived from the PE are linked through a cleavage site recognized by proteases present in the cell environment, especially in the tumor cell environment. In an embodiment, a cleavage site is a sequence recognized by the metalloprotease MMP, for example sequences PLGLAG (SEQ ID NO: 20), PLGIAGE (SEQ ID NO: 21), or PLGLAGQ (SEQ ID NO: 22). In an embodiment, a cleavage site is a sequence recognized by urokinase uPA, for example RVVR (SEQ ID NO: 23) sequence. In an embodiment, a cleavage site is a sequence recognized by furin, for example sequences RKKR (SEQ ID NO: 24) or RKKRVKR (SEQ ID NO: 25) . In a preferred embodiment, a cleavage site is a furin cleavage site.

[0069] In an embodiment, the furin cleavage site of the recombinant fusion protein of the disclosure has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% amino acid sequence identity to SEQ ID NO: 4.

[0070] In an embodiment, the cleavage site may be linked to the SSL protein and the killer protein through a linker. Exemplary sequences of the furin cleavage site of SEQ ID NO: 6 including linkers are shown in SEQ ID NO: 5 and SEQ ID NO: 6. The linkers in SEQ ID NO: 5 and SEQ ID NO: 6 can be replaced by other linkers known in the art.

[0071] In an embodiment, the furin cleavage site of the recombinant fusion protein of the disclosure has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% amino acid sequence identity to SEQ ID NO: 5.

[0072] In an embodiment, the furin cleavage site of the recombinant fusion protein of the disclosure has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% amino acid sequence identity to SEQ ID NO: 6.

[0073] Apart from the main functional elements of the fusion protein, binding, killing, and cleavage site domain(s), the fusion proteins of the invention may contain a one or more neutral sequences of a flexible steric linker (spacer) comprised of alanine, glycine, glutamine, cysteine, histidine and serine residues. Such linkers/spacers are well known and described in the literature. Their incorporation into the sequence of the fusion protein is intended to promote the correct folding of proteins produced by the process of its overexpression in the host cells. In an embodiment, the linker/spacer sequences are positioned between functional domains of the proteins, e.g., between a PE or a PE-derived domain and a cleavage site, or between a cleavage and an SSL domain or an SSL-derived domain.

[0074] Additionally, the fusion proteins of the invention may further contain a transporting domain attached to the killing domain selected from the group consisting of: a sequence directing to the endoplasmic reticulum, a polyarginine sequence transporting through the cell membrane, comprised of 6, 7, 8 or 9 Arg residues, a translocation domain of Pseudomonas aeruginosa, a membrane transporting domain, a nuclear localization domain, and a mitochondrial targeting domain, and combinations thereof

[0075] Furthermore, the combination of transporting domains may include domains located next to each other and connected to one end of the killing domain and/or domains linked to different ends of the killing domain.

[0076] It should be understood that in the case when the fusion protein has both the transporting domain attached to the killing domain and the cleavage site between the transporting and killing domains, then the cleavage site is located in such a manner that after cleavage of the construct the transporting domain remains attached to the killing domain. In other words, if the fusion protein contains both the transporting domain and the cleavage site, then the transporting domain is located between the killing domain and the cleavage site.

[0077] The transporting sequence may be attached at the N-terminus or at the C-terminus of the killing domain. In some embodiments, the transporting sequence may be also terminal part of the whole construct, such as C-terminal part or N-terminal part, depending on the manner of attachment of the killing domain and binding domain.

[0078] Translocation domain of Pseudomonas aeruginosa is capable of translocation through the lysosomal membrane into the cytoplasm and can be used to introduce the effector peptide to the tumor cell compartments. The translocation domain sequence of Pseudomonas aeruginosa is well known in the art. The sequence directing to endoplasmic reticulum may be any signal sequence directing to endoplasmic reticulum known in the art, such as for example, but not limited to, KDEL (SEQ ID NO: 38), HDEL (SEQ ID NO: 39), RDEL (SEQ ID NO: 40), DDEL (SEQ ID NO: 41), ADEL (SEQ ID NO: 42), SDEL (SEQ ID NO: 43), KEDL (SEQ ID No: 44). The membrane transporting domain may be any signal sequence transporting through the plasma membrane known in the art, such as for example and not limited to, KPRRPY (SEQ

ID NO: 26) or KPRRPYR (SEQ ID NO: 27). The nuclear localization sequence may be any signal sequence directing into nucleus known in the art, such as for example and not limited to, EEEAAGRKRKKRT (SEQ. No. 19), FFFAAGRKRKKRT (SEQ ID NO: 28), NNNAAGRKRKKRT (SEQ ID NO: 29), YYYAAGRKRKKRT (SEQ ID NO: 30), AAKKK (SEQ ID NO: 31), or GRKRKKRT (SEQ ID NO: 32). The mitochondrial targeting domain (d6) may be any signal sequence directing to mitochondrium known in the art, such as for example and not limited to RVSFCRPGWSAMARSRLTATSVSQVQENGFVK (SEQ ID NO: 33), fragment MLATRVFSLVGKRAISTSVCVR (SEQ ID NO: 34) of human cytochrome oxidase subunit IV (hCOXIV1), or the ornithine transcarbamylase leader peptide.

[0079] Apart from the main functional elements of the fusion protein, transporting domains and the cleavage site domains, the fusion proteins of the invention may contain domain (e), i.e. a polycysteine motif facilitating trimer stabilisation, as, for example, and not limited to, CAACAAAC (SEQ ID NO: 35) sequence or CAAECAAAC (SEQ ID NO: 36).

[0080] Furthermore, for selection and purification purposes the fusion proteins of the invention may contain an affinity tag. Any affinity tag may be used according to the invention as long as it is small enough and will not interfere with the overall structure and function of the macromolecular complex. Examples of affinity tags are a His-tag, a FLAG-tag, Arg-tag, T7-tag, Strep-tag, S-tag, aptamer-tag, or any combination of these tags. Preferably, the affinity tag is a His_6-tag (SEQ ID NO: 37).

[0081] As shown in Figure 1, an embodiment of the recombinant fusion protein includes an SSL11 sequence, a PE24 sequence and a furin cleavage site positioned between the SSL11

sequence and the PE24 sequence. SSL11, via its SLeX binding site, promotes cell adhesion (and prevents cell migration) while PE24 inactivates eEF-2 to induce cell death.

[0082] Another aspect of this disclosure provides a polynucleotide sequence encoding the recombinant fusion protein.

[0083] In an embodiment, the polynucleotide sequence has at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% nucleotide sequence identity to SEQ ID NO: 7. Due to the degenerate nature of the genetic code a polynucleotide sequence having at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% nucleotide sequence identity to SEQ ID NO: 7 can still encode fusion proteins of the present invention. In an embodiment, the polynucleotide sequence encoding fusion proteins of the invention comprises codons optimized for expression in an *e.g.*, bacterial host, such as *Escherichia coli*. Use of codon optimized sequences allows to increase the efficiency of a further step of target protein synthesis in *e.g., Escherichia coli*.

[0084] Still another aspect of this disclosure provides a recombinant expression vector including the polynucleotide sequence.

[0085] In an embodiment, the vector may be inserted in a host cell and recombined with the host cell genome, or refers to any nucleic acid including a nucleotide sequence competent to replicate spontaneously as an episome. Such a vector may include a linear nucleic acid, a plasmid, a phagemid, a cosmid, an RNA vector, a viral vector, etc.

[0086] In an embodiment, the vector may be genetically engineered to incorporate the nucleic acid sequence encoding the recombinant protein in an orientation either N-terminal and/or C-terminal to a nucleic acid sequence encoding a peptide, a polypeptide,

a protein domain, or a full-length protein of interest, and in the correct reading frame so that the recombinant fusion protein including SSL11 and PE may be expressed. Expression vectors may be selected from those readily available for use in prokaryotic or eukaryotic expression systems.

[0087] Standard recombinant nucleic acid methods may be used to express a genetically engineered recombinant protein. The nucleic acid sequence encoding the recombinant protein according to one embodiment disclosed in the present application may be cloned into a nucleic acid expression vector, e.g., with appropriate signal and processing sequences and regulatory sequences for transcription and translation, and the protein may be synthesized using automated organic synthetic methods.

[0088] In order to obtain high level expression of a cloned gene or nucleic acid, for example, a cDNA encoding the recombinant protein according to one embodiment disclosed in the present application, the recombinant protein sequence may be typically subcloned into an expression vector that includes a strong promoter for directing transcription, a transcription/translation terminator, and in the case of a nucleic acid encoding a protein, a ribosome binding site for translational initiation. Suitable bacterial promoters are well known in the art. Bacterial expression systems for expression of the recombinant protein are available in, e.g., *E. coli*, *Bacillus sp.*, and *Salmonella*. Kits for such expression systems are commercially available. Eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available. The eukaryotic expression vector may be preferably an adenoviral vector, an adeno-associated vector, or a retroviral vector.

[0089] The polynucleotide sequence according to one embodiment disclosed in the present application may be present in a vector in which the polynucleotide sequence is operably

linked to regulatory sequences capable of providing for the expression of the polynucleotide sequence by a suitable host cell.

[0090] Within an expression vector, the term "operably linked" is intended to mean that the polynucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the polynucleotide sequence. The term "regulatory sequence" is intended to include promoters, enhancers, and other expression control elements. Such operable linkage with the expression vector can be achieved by conventional gene recombination techniques known in the art, while site-directed DNA cleavage and linkage are carried out by using conventional enzymes known in the art.

[0091] The expression vectors may contain a signal sequence or a leader sequence for membrane targeting or secretion, as well as regulatory sequences such as a promoter, an operator, an initiation codon, a termination codon, a polyadenylation signal, an enhancer and the like. The promoter may be a constitutive or an inducible promoter. Further, the expression vector may include one or more selectable marker genes for selecting the host cell containing the expression vector, and may further include a polynucleotide sequence that enables the vector to replicate in the host cell in question.

[0092] The expression vector constructed according to an embodiment of this disclosure may be the vector where the polynucleotide encoding the recombinant protein is inserted within the multiple cloning sites (MCS) of a pT7 vector.

[0093] The recombinant protein may be introduced into an appropriate host cell, e.g., a bacterial cell, a yeast cell, an insect cell, or a tissue culture cell. The recombinant protein may also be introduced into embryonic stem cells in order to generate a transgenic organism. Large

numbers of suitable vectors and promoters are known to those skilled in the art and are commercially available for generating the recombinant protein.

[0094] Known methods may be used to construct vectors including the polynucleotide sequence according to one embodiment disclosed in the present application and appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* recombination/genetic recombination.

[0095] Still another aspect of this disclosure provides a transformant transformed with the recombinant expression vector.

[0096] The transformation includes transfection, and refers to a process whereby a foreign (extracellular) DNA, with or without an accompanying material, enters into a host cell. The "transfected cell" refers to a cell into which the foreign DNA is introduced into the cell, and thus the cell harbors the foreign DNA. The DNA may be introduced into the cell so that a nucleic acid thereof may be integrated into the chromosome or replicable as an extrachromosomal element. The cell with the replicable foreign DNA is called a transformant.

[0097] As used herein, “introducing” of a protein, a peptide, an organic compound into a cell may be used interchangeably with the expression of “carrying,” “penetrating,” “transporting,” “delivering,” “permeating” or “passing.”

[0098] It is understood that the host cell refers to a eukaryotic or prokaryotic cell into which one or more DNAs or vectors are introduced, and refers not only to the particular subject cell but also to the progeny or potential progeny thereof. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[0099] The host cells may be preferably bacterial cells, and as the bacterial cells, there are, in principle, no limitations. They may be eubacteria (gram-positive or gram-negative) or archaebacteria, as long as they allow genetic manipulation for insertion of a gene of interest, preferably for site-specific integration, and they may be cultured on a manufacturing scale. Preferably, the host cells may have the property to allow cultivation to high cell densities.

[0100] Examples of bacterial host cells that may be used in the preparation of the recombinant protein are *E. coli*, *Bacillus subtilis*, *Pseudomonas fluorescens* as well as various *Corynebacterium* and *Lactococcus lactis* strains. Preferably, the host cells are *Escherichia coli* cells.

[0101] In an embodiment, the host cell may include an RNA polymerase capable of binding to a promoter regulating the gene of interest. The RNA polymerase may be endogenous or exogenous to the host cell.

[0102] In an embodiment, host cells with a foreign strong RNA polymerase may be used. For example, *Escherichia coli* strains engineered to carry a foreign RNA polymerase (e.g. like in the case of using a T7 promoter a T7-like RNA polymerase in the so-called "T7 strains") integrated in their genome may be used. Examples of T7 strains, e.g. BL21(DE3), HMS174(DE3), and their derivatives or relatives (see Novagen, pET System manual, 11th edition), may be widely used and commercially available. Preferably, BL21-CodonPlus (DE3)-RIL or BL21-CodonPlus (DE3)-RIPL may be used. These strains are DE3 lysogens containing the T7 RNA polymerase gene under control of the lacUV5 promoter. Induction with IPTG allows production of T7 RNA polymerase which then directs the expression of the gene of interest under the control of the T7 promoter.

[0103] The host cell strains, *E. coli* BL21(DE3) or HMS174(DE3), which have received their genome-based T7 RNA polymerase via the phage DE3, are lysogenic. It is preferred that the T7 RNA polymerase contained in the host cell has been integrated by a method which avoids, or preferably excludes, the insertion of residual phage sequences in the host cell genome since lysogenic strains have the disadvantage to potentially exhibit lytic properties, leading to undesirable phage release and cell lysis.

[0104] Still another aspect of this disclosure provides a preparing method of the recombinant protein including preparing the recombinant expression vector; preparing the transformant using the recombinant expression vector; culturing the transformant; and recovering the recombinant protein expressed by culturing.

[0105] Culturing may be preferably in a mode that employs the addition of a feed medium, this mode being selected from the fed-batch mode, semi-continuous mode, or continuous mode, and the bacterial expression host cells may include a DNA construct, integrated in their genome, carrying the DNA sequence encoding the protein of interest under the control of a promoter that enables expression of said protein.

[0106] There are no limitations in the type of the culture medium. The culture medium may be semi-defined, i.e., containing complex media compounds (e.g. yeast extract, soy peptone, casamino acids), or it may be chemically defined, without any complex compounds. Preferably, a defined medium may be used. The defined media (also called minimal or synthetic media) are exclusively composed of chemically defined substances, i.e., carbon sources such as glucose or glycerol, salts, vitamins, and, in view of a possible strain auxotrophy, specific amino acids or other substances such as thiamine. Most preferably, glucose may be used as a carbon source.

Usually, the carbon source of the feed medium serves as the growth-limiting component which controls the specific growth rate.

[0107] Host cells may be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or the use of cell lysing agents. There are a number of general methods known in the art for purifying recombinant (and non-recombinant) proteins. The methods may include, e.g., ion-exchange chromatography, size-exclusion chromatography, affinity chromatography, selective precipitation, dialysis, and hydrophobic interaction chromatography. These methods may be adapted to devise a purification strategy for the cell permeable recombinant protein. If the cell permeable recombinant protein includes a purification handle, such as an epitope tag or a metal chelating sequence, affinity chromatography may be used to easily purify the protein.

[0108] The amount of the protein produced may be evaluated by detecting the advanced macromolecule transduction domain directly (e.g., using Western analysis) or indirectly (e.g., by assaying materials derived from the cells for specific DNA binding activity, such as by electrophoretic mobility shift assay). Proteins may be detected prior to purification, during any stage of purification, or after purification. In some implementations, purification or complete purification may not be necessary.

[0109] The recombinant proteins according to an embodiment of the disclosure are cell permeable proteins, and may be used as protein-based vaccines, particularly in the case where killed or attenuated whole organism vaccines are impractical.

[0110] The recombinant proteins according to an embodiment of the disclosure can be preferably used for treating a cancer in a subject. The cell permeable recombinant proteins can be delivered to the interior of the cell, eliminating the need to transfect or transform the cell with

a recombinant vector. The cell permeable recombinant proteins can be used *in vitro* to investigate protein function or may be used to maintain cells in a desired state.

[0111] Dosages of the Recombinant Fusion Protein of the Disclosure

[0112] The dosage regimen utilizing the recombinant fusion protein of the disclosure is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the potency of the compound chosen to be administered; the route of administration; and the renal and hepatic function of the patient. A consideration of these factors is well within the purview of the ordinarily skilled clinician for the purpose of determining the therapeutically effective or prophylactically effective dosage amount needed to prevent, counter, or arrest the progress of the condition. It is understood that a specific daily dosage amount can simultaneously be both a therapeutically effective amount, *e.g.*, for treatment of an oncological condition, and a prophylactically effective amount, *e.g.*, for prevention of an oncological condition.

[0113] While individual needs vary, determination of optimal ranges of effective amounts of the recombinant fusion protein of the disclosure is within the skill of the art. For administration to a human in, for example, the curative or prophylactic treatment of the conditions and disorders identified herein, the typical dosages of the recombinant fusion protein of the disclosure can be about 0.05 mg/kg/day to about 50 mg/kg/day, or at least 0.05 mg/kg, or at least 0.08 mg/kg, or at least 0.1 mg/kg, or at least 0.2 mg/kg, or at least 0.3 mg/kg, or at least 0.4 mg/kg, or at least 0.5 mg/kg, and any amount therebetween, to about 50 mg/kg or less, or about 40 mg/kg or less, or about 30 mg/kg or less, or about 20 mg/kg or less, or about 10 mg/kg or less and any amount therebetween, which can be, for example, about 2.5 mg/day (0.5 mg/kg x 5 kg) to about 5000 mg/day (50 mg/kg x 100 kg). For example, dosages of the compounds can be

about 0.1 mg/kg/day to about 50 mg/kg/day, or about 0.05 mg/kg/day to about 10 mg/kg/day, or about 0.05 mg/kg/day to about 5 mg/kg/day, or about 0.05 mg/kg/day to about 3 mg/kg/day, or about 0.07 mg/kg/day to about 3 mg/kg/day, or about 0.09 mg/kg/day to about 3 mg/kg/day, or about 0.05 mg/kg/day to about 0.1 mg/kg/day, or about 0.1 mg/kg/day to about 1 mg/kg/day, or about 1 mg/kg/day to about 10 mg/kg/day, or about 1 mg/kg/day to about 5 mg/kg/day, or about 1 mg/kg/day to about 3 mg/kg/day, or about 3 mg/day to about 500 mg/day, or about 5 mg/day to about 250 mg/day, or about 10 mg/day to about 100 mg/day, or about 3 mg/day to about 10 mg/day, or about 100 mg/day to about 250 mg/day. Such doses may be administered in a single dose or may be divided into multiple doses.

[0114] Pharmaceutical Compositions

[0115] The recombinant fusion protein of the disclosure can be administered to animals, preferably to mammals, and in particular to humans, as pharmaceuticals by themselves, in mixtures with one another or in the form of pharmaceutical compositions. The term "subject" or "patient" includes animals, preferably mammals and especially humans, who use the instant active agents for the prevention or treatment of a medical condition. Administering of the drug to the subject includes both self-administration and administration to the patient by another person. The subject may be in need of, or desire, treatment for an existing disease or medical condition, or may be in need of or desire prophylactic treatment to prevent or reduce the risk of occurrence of said disease or medical condition. As used herein, a subject "in need" of treatment of an existing condition or of prophylactic treatment encompasses both a determination of need by a medical professional as well as the desire of a patient for such treatment.

[0116] The present disclosure therefore also provides the recombinant fusion protein of the disclosure for use as pharmaceuticals, their use for inhibiting cell migration by binding to

SLeX and modulating the activity eEF-2 and in particular their use in the therapy and prophylaxis of the below-mentioned diseases or disorders as well as their use for preparing medicaments for these purposes. In certain embodiments, the recombinant fusion protein of the disclosure inhibit the activity of eEF-2.

[0117] Furthermore, the present disclosure provides pharmaceutical compositions which comprise as active component an effective dose of the recombinant fusion protein of the disclosure and a customary pharmaceutically acceptable carrier, *i.e.*, one or more pharmaceutically acceptable carrier substances and/or additives.

[0118] Thus, the present disclosure provides, for example, said recombinant fusion protein of the disclosure for use as pharmaceutical compositions which comprise as active component an effective dose of the recombinant fusion protein of the disclosure and a customary pharmaceutically acceptable carrier, and the uses of said recombinant fusion protein of the disclosure in the therapy or prophylaxis of the below-mentioned diseases or disorders, *e.g.*, cancer, as well as their use for preparing medicaments for these purposes.

[0119] The pharmaceutical compositions according to the disclosure can be administered orally, for example, in the form of pills, tablets, lacquered tablets, sugar-coated tablets, granules, hard and soft gelatin capsules, aqueous, alcoholic or oily solutions, syrups, emulsions or suspensions, or rectally, for example, in the form of suppositories. Administration can also be carried out parenterally, for example subcutaneously, intramuscularly or intravenously in the form of solutions for injection or infusion.

[0120] Other suitable administration forms are, for example, percutaneous or topical administration, for example, in the form of ointments, tinctures, sprays or transdermal

therapeutic systems, or, for example, microcapsules, implants or rods. The preferred administration form depends, for example, on the disease to be treated and on its severity.

[0121] The amount of the active recombinant fusion protein of the disclosure in the pharmaceutical composition normally is from 0.01 to 200 mg, or from 0.1 to 200 mg, or from 1 to 200 mg, per dose, but depending on the type of the pharmaceutical composition, it can also be higher. In some embodiments, the amount of active recombinant fusion protein of the disclosure in the pharmaceutical composition is from 0.01 to 10 mg per dose. The pharmaceutical compositions usually comprise 0.5 to 90 percent by weight of the recombinant fusion protein of the disclosure. The preparation of the pharmaceutical compositions can be carried out in a manner known *per se*. For this purpose, the recombinant fusion protein of the disclosure, together with one or more solid or liquid pharmaceutical carrier substances and/or additives (or auxiliary substances) and, if desired, in combination with other pharmaceutically active compounds having therapeutic or prophylactic action, are brought into a suitable administration form or dosage form which can then be used as a pharmaceutical in human or veterinary medicine.

[0122] For the production of pills, tablets, sugar-coated tablets and hard gelatin capsules, it is possible to use, for example, lactose, starch, for example, maize starch, or starch derivatives, talc, stearic acid or its salts, etc. Carriers for soft gelatin capsules and suppositories are, for example, fats, waxes, semisolid and liquid polyols, natural or hardened oils, etc. Suitable carriers for the preparation of solutions, for example, of solutions for injection, or of emulsions or syrups are, for example, water, physiologically acceptable sodium chloride solution, alcohols such as ethanol, glycerol, polyols, sucrose, invert sugar, glucose, mannitol, vegetable oils, etc. It is also possible to lyophilize the recombinant fusion protein of the disclosure and to use the

resulting lyophilisates, for example, for preparing preparations for injection or infusion. Suitable carriers for microcapsules, implants or rods are, for example, copolymers of glycolic acid and lactic acid.

[0123] Besides the active compounds and carriers, the pharmaceutical compositions can also contain customary additives, for example, fillers, disintegrants, binders, lubricants, wetting agents, stabilizers, emulsifiers, dispersants, preservatives, sweeteners, colorants, flavorings, aromatizers, thickeners, diluents, buffer substances, solvents, solubilizers, agents for achieving a depot effect, salts for altering the osmotic pressure, coating agents and/or antioxidants.

[0124] Methods of Using the Recombinant Fusion Protein of the Disclosure

[0125] The present application provides a method of inhibiting cell migration comprising contacting a cell with the recombinant fusion protein of the disclosure. Inhibition of cell migration can be assessed and demonstrated by a wide variety of ways known in the art. Kits and commercially available assays can be utilized for determining the degree of cell migration.

[0126] The present application also provides a method of inducing cell death comprising contacting a cell with the recombinant fusion protein of the disclosure. Induction of cell death can be assessed and demonstrated by a wide variety of ways known in the art. Kits and commercially available assays can be utilized for determining the degree of cell death.

[0127] In some embodiments, a method for treatment of cancer is provided, the method comprising administering a therapeutically effective amount of the recombinant fusion protein of the disclosure or any of the foregoing pharmaceutical compositions comprising such a recombinant fusion protein of the disclosure to a subject in need of such treatment.

[0128] The disclosed compounds inhibit anchorage-independent cell growth and therefore have the potential to inhibit tumor metastasis. Accordingly, another embodiment of the

present disclosure provides a method for inhibiting tumor metastasis, the method comprising administering an effective amount the recombinant fusion protein of the disclosure.

[0129] Certain embodiments are directed to administration of the recombinant fusion protein of the disclosure (*e.g.*, in the form of a pharmaceutical composition) to a subject in need of treatment of a hematological malignancy. Such malignancies include, but are not limited to leukemias and lymphomas. For example, the presently disclosed compounds can be used for treatment of diseases such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), chronic myelogenous leukemia (CML), acute monocytic leukemia (AMoL) and/ or other leukemias. In other embodiments, the compounds are useful for treatment of lymphomas such as Hodgkin's lymphoma or non-Hodgkin's lymphoma. In various embodiments, the compounds are useful for treatment of plasma cell malignancies such as multiple myeloma, mantle cell lymphoma, and Waldenstrom's macroglubunemia.

[0130] The present application also provides a method of treating a hyperproliferative disorder comprising administering a therapeutically effective amount of the recombinant fusion protein of the disclosure to a subject in need thereof. In some embodiments, said method relates to the treatment of a subject who suffers from a cancer such as acute myeloid leukemia, cancer in adolescents, adrenocortical carcinoma childhood, AIDS- related cancers (*e.g.*, lymphoma and Kaposi's Sarcoma), anal cancer, appendix cancer, astrocytomas, atypical teratoid, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain stem glioma, brain tumor, breast cancer, bronchial tumors, Burkitt lymphoma, carcinoid tumor, atypical teratoid, embryonal tumors, germ cell tumor, primary lymphoma, cervical cancer, childhood cancers, chordoma, cardiac tumors, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML),

chronic myleoproliferative disorders, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, extrahepatic ductal carcinoma in situ (DCIS), embryonal tumors, CNS cancer, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, Ewing sarcoma, extracranial germ cell tumor, extragonadal germ cell tumor, eye cancer, fibrous histiocytoma of bone, gall bladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumors (GIST), germ cell tumor, gestational trophoblastic tumor, hairy cell leukemia, head and neck cancer, heart cancer, liver cancer, Hodgkin's lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell tumors, pancreatic neuroendocrine tumors, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liver cancer, lobular carcinoma in situ (LCIS), lung cancer, lymphoma, metastatic squamous neck cancer with occult primary, midline tract carcinoma, mouth cancer; multiple endocrine neoplasia syndromes, multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplasia syndromes, myelodysplastic/myeloproliferative neoplasms, multiple myeloma, Merkel cell carcinoma, malignant mesothelioma, malignant fibrous histiocytoma of bone and osteosarcoma, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin's lymphoma, non-small cell lung cancer (NSCLC), oral cancer, lip and oral cavity cancer, oropharyngeal cancer, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pleuropulmonary blastoma, primary central nervous system (CNS) lymphoma, prostate cancer, rectal cancer, transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, skin cancer, stomach (gastric) cancer, small cell lung cancer; small intestine cancer, soft tissue sarcoma, T-Cell lymphoma, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor, unusual cancers of

childhood, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, or viral-induced cancer. In some embodiments, said method relates to the treatment of a non-cancerous hyperproliferative disorder such as benign hyperplasia of the skin (*e.g.*, psoriasis), restenosis, or prostate (*e.g.*, benign prostatic hypertrophy (BPH)).

[0131] In some embodiments, the methods for treatment are directed to treating lung cancers, and the methods comprise administering a therapeutically effective amount of the recombinant fusion protein of the disclosure (or pharmaceutical composition comprising such a recombinant fusion protein of the disclosure) to a subject in need thereof. In certain embodiments, the lung cancer is a non-small cell lung carcinoma (NSCLC), for example, adenocarcinoma, squamous-cell lung carcinoma or large-cell lung carcinoma. In some embodiments, the lung cancer is a small cell lung carcinoma. Other lung cancers which the recombinant fusion protein of the disclosure may provide therapeutic benefit for include, but are not limited to, glandular tumors, carcinoid tumors and undifferentiated carcinomas.

[0132] Combination Therapies

[0133] One or more additional pharmacologically active agents may be administered in combination with the recombinant fusion protein of the disclosure. An additional active agent (or agents) is intended to mean a pharmaceutically active agent (or agents) that is active in the body, including pro-drugs that convert to pharmaceutically active form after administration, which are different from the recombinant fusion protein of the disclosure. The additional active agents also include free-acid, free-base and pharmaceutically acceptable salts of said additional active agents. Generally, any suitable additional active agent or agents, including chemotherapeutic agents or therapeutic antibodies, may be used in any combination with the recombinant fusion protein of the disclosure in a single dosage formulation (e.g., a fixed dose

drug combination), or in one or more separate dosage formulations which allows for concurrent or sequential administration of the active agents (co-administration of the separate active agents) to subjects. In addition, the recombinant fusion protein of the disclosure can be administered in combination with radiation therapy, hormone therapy, surgery or immunotherapy.

[0134] The present application also provides methods for combination therapies in which the additional active agent is known to modulate other pathways, or other components of the same pathway, or even overlapping sets of target enzymes which are used in combination with the recombinant fusion protein of the disclosure. In one embodiment, such therapy includes, but is not limited to, the combination of the recombinant fusion protein of the disclosure with chemotherapeutic agents, immunotherapeutic agents, hormonal and anti-hormonal agents, targeted therapy agents, and anti-angiogenesis agents, to provide a synergistic or additive therapeutic effect. In another embodiment, such therapy includes radiation treatment to provide a synergistic or additive therapeutic effect.

[0135] Examples of additional active agents (i.e., additional anti-cancer agents) include chemotherapeutic agents (e.g., cytotoxic agents), immunotherapeutic agents, hormonal and anti-hormonal agents, targeted therapy agents, and anti-angiogenesis agents. Many anti-cancer agents can be classified within one or more of these groups. While certain anti-cancer agents have been categorized within a specific group(s) or subgroup(s) herein, many of these agents can also be listed within one or more other group(s) or subgroup(s), as would be presently understood in the art. It is to be understood that the classification herein of a particular agent into a particular group is not intended to be limiting. Many anti-cancer agents are presently known in the art and can be used in combination with the compounds of the present disclosure.

[0136] Further, an agent can be an agonist, antagonist, allosteric modulator, toxin or, more generally, may act to inhibit or stimulate its target (e.g., receptor or enzyme activation or inhibition). For example, suitable for use are one or more agents (e.g., antibodies, antigen binding regions, or soluble receptors) that specifically bind and inhibit the activity of growth factors, such as antagonists of hepatocyte growth factor (HGF, also known as Scatter Factor), and antibodies or antigen binding regions that specifically bind its receptor "c-met".

[0137] In an embodiment, the additional anti-cancer agent is a chemotherapeutic agent, an immunotherapeutic agent, a hormonal agent, an anti-hormonal agent, a targeted therapy agent, or an anti-angiogenesis agent (or angiogenesis inhibitor). In an embodiment, the additional anti-cancer agent is selected from the group consisting of a chemotherapeutic agent, a mitotic inhibitor, a plant alkaloid, an alkylating agent, an anti-metabolite, a platinum analog, an enzyme, a topoisomerase inhibitor, a retinoid, an aziridine, an antibiotic, a hormonal agent, an anti-hormonal agent, an anti-estrogen, an anti-androgen, an anti-adrenal, an androgen, a targeted therapy agent, an immunotherapeutic agent, a biological response modifier, a cytokine inhibitor, a tumor vaccine, a monoclonal antibody, an immune checkpoint inhibitor, an anti-PD-1 agent, an anti-PD-L1 agent, a colony-stimulating factor, an immunomodulator, an immunomodulatory imide (IMiD), an anti-CTLA4 agent, an anti-LAGl agent, an anti-LAG3 agent, an anti-ILT4 agent, an anti-OX40 agent, a GITR agonist, a CAR-T cell, a BiTE, a signal transduction inhibitor, a growth factor inhibitor, a tyrosine kinase inhibitor, an EGFR inhibitor, a histone deacetylase (HDAC) inhibitor, a proteasome inhibitor, a cell-cycle inhibitor, an anti-angiogenesis agent, a matrix-metalloproteinase (MMP) inhibitor, a hepatocyte growth factor inhibitor, a TOR inhibitor, a KDR inhibitor, a VEGF inhibitor, a HIF-1α inhibitor, a HIF-2α inhibitor, a fibroblast growth factor (FGF) inhibitor, a RAF inhibitor, a MEK inhibitor, an ERK

inhibitor, a PI3K inhibitor, an AKT inhibitor, an MCL-1 inhibitor, a BCL-2 inhibitor, an SHP2 inhibitor, a HER-2 inhibitor, a BRAF-inhibitor, a gene expression modulator, an autophagy inhibitor, an apoptosis inducer, an antiproliferative agent, and a glycolysis inhibitor.

[0138] In an embodiment, the additional anti-cancer agent(s) is a chemotherapeutic agent. Non-limiting examples of chemotherapeutic agents include mitotic inhibitors and plant alkaloids, alkylating agents, anti-metabolites, platinum analogs, enzymes, topoisomerase inhibitors, retinoids, aziridines, and antibiotics.

[0139] Non-limiting examples of mitotic inhibitors and plant alkaloids include taxanes such as cabazitaxel, docetaxel, larotaxel, ortataxel, paclitaxel, and tesetaxel; demecolcine; epothilone; eribulin; etoposide (VP- 16); etoposide phosphate; navelbine; noscapine; teniposide; thaliblastine; vinblastine; vincristine; vindesine; vinflunine; and vinorelbine.

[0140] Non-limiting examples of alkylating agents include nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, cytophosphane, estramustine, ifosfamide, mannomustine, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, tris(2-chloroethyl)amine, trofosfamide, and uracil mustard; alkyl sulfonates such as busulfan, improsulfan, and piposulfan; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine, streptozotocin, and TA-07; ethylenimines and methylamelamines such as altretamine, thiotepa, triethylenemelamine, triethylenethiophosphaoramide, trietylenephosphoramide, and trimethylolomelamine; ambamustine; bendamustine; dacarbazine; etoglucid; irofulven; mafosfamide; mitobronitol; mitolactol; pipobroman; procarbazine; temozolomide; treosulfan; and triaziquone.

[0141] Non-limiting examples of anti-metabolites include folic acid analogues such as aminopterin, denopterin, edatrexate, methotrexate, pteropterin, raltitrexed, and trimetrexate;

purine analogs such as 6-mercaptopurine, 6-thioguanine, fludarabine, forodesine, thiamiprine, and thioguanine; pyrimidine analogs such as 5-fluorouracil (5-FU), 6-azauridine, ancitabine, azacytidine, capecitabine, carmofur, cytarabine, decitabine, dideoxyuridine, doxifiuridine, doxifluridine, enocitabine, floxuridine, galocitabine, gemcitabine, and sapacitabine; 3-aminopyridine-2-carboxaldehyde thiosemicarbazone; broxuridine; cladribine; cyclophosphamide; cytarabine; emitefur; hydroxyurea; mercaptopurine; nelarabine; pemetrexed; pentostatin; tegafur; and troxacitabine.

[0142] Non-limiting examples of platinum analogs include carboplatin, cisplatin, dicycloplatin, heptaplatin, lobaplatin, nedaplatin, oxaliplatin, satraplatin, and triplatin tetranitrate.

[0143] Non-limiting examples of enzymes include asparaginase and pegaspargase.

[0144] Non-limiting examples of topoisomerase inhibitors include acridine carboxamide, amonafide, amsacrine, belotecan, elliptinium acetate, exatecan, indolocarbazole, irinotecan, lurtotecan, mitoxantrone, razoxane, rubitecan, SN-38, sobuzoxane, and topotecan.

[0145] Non-limiting examples of retinoids include alitretinoin, bexarotene, fenretinide, isotretinoin, liarozole, RII retinamide, and tretinoin.

[0146] Non-limiting examples of aziridines include benzodopa, carboquone, meturedopa, and uredopa.

[0147] Non-limiting examples of antibiotics include intercalating antibiotics; anthracenediones; anthracycline antibiotics such as aclarubicin, amrubicin, daunomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, menogaril, nogalamycin, pirarubicin, and valrubicin; 6-diazo-5-oxo- L-norleucine; aclacinomysins; actinomycin; authramycin; azaserine; bleomycins; cactinomycin; calicheamicin; carabicin; carminomycin; carzinophilin; chromomycins; dactinomycin; detorubicin; esorubicin; esperamicins; geldanamycin;

marcellomycin; mitomycins; mitomycin C; mycophenolic acid; olivomycins; novantrone; peplomycin; porfiromycin; potfiromycin; puromycin; quelamycin; rebeccamycin; rodorubicin; streptonigrin; streptozocin; *tanespimycin*; tubercidin; ubenimex; zinostatin; zinostatin stimalamer; and zorubicin.

[0148] In an embodiment, the additional anti-cancer agent(s) is a hormonal and/or anti-hormonal agent (i.e., hormone therapy). Non-limiting examples of hormonal and anti-hormonal agents include anti-androgens such as abiraterone, apalutamide, bicalutamide, darolutamide, enzalutamide, flutamide, goserelin, leuprolide, and nilutamide; anti-estrogens such as 4- hydroxy tamoxifen, aromatase inhibiting 4(5)-imidazoles, EM-800, fosfestrol, fulvestrant, keoxifene, LY 117018, onapristone, raloxifene, tamoxifen, toremifene, and trioxifene; anti-adrenals such as aminoglutethimide, dexaminoglutethimide, mitotane, and trilostane; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, and testolactone; abarelix; anastrozole; cetrorelix; deslorelin; exemestane; fadrozole; finasteride; formestane; histrelin (RL 0903); human chorionic gonadotropin; lanreotide; LDI 200 (Milkhaus); letrozole; leuprorelin; mifepristone; nafarelin; nafoxidine; osaterone; prednisone; thyrotropin alfa; and triptorelin.

[0149] In an embodiment, the additional anti-cancer agent(s) is an immunotherapeutic agent (i.e., immunotherapy). Non-limiting examples of immunotherapeutic agents include biological response modifiers, cytokine inhibitors, tumor vaccines, monoclonal antibodies, immune checkpoint inhibitors, colony-stimulating factors, and immunomodulators.

[0150] Non-limiting examples of biological response modifiers, including cytokine inhibitors (cytokines) such as interferons and interleukins, include interferon alfa/interferon alpha such as interferon alfa-2, interferon alfa-2a, interferon alfa-2b, interferon alfa-nl, interferon alfa-n3, interferon alfacon-1, peginterferon alfa-2a, peginterferon alfa-2b, and leukocyte alpha

interferon; interferon beta such as interferon beta-1a, and interferon beta-1b; interferon gamma such as natural interferon gamma-1a, and interferon gamma-1b; aldesleukin; interleukin-1 beta; interleukin-2; oprelvekin; sonermin; tasonermin; and virulizin.

[0151] Non-limiting examples of tumor vaccines include APC 8015, AVICINE, bladder cancer vaccine, cancer vaccine (Biomira), gastrin 17 immunogen, Maruyama vaccine, melanoma lysate vaccine, melanoma oncolysate vaccine (New York Medical College), melanoma vaccine (New York University), melanoma vaccine (Sloan Kettering Institute), TICE® BCG (Bacillus Calmette-Guerin), and viral melanoma cell lysates vaccine (Royal Newcastle Hospital).

[0152] Non-limiting examples of monoclonal antibodies include abagovomab, adecatumumab, aflibercept, alemtuzumab, blinatumomab, brentuximab vedotin, CA 125 MAb (Biomira), cancer MAb (Japan Pharmaceutical Development), daclizumab, daratumumab, denosumab, edrecolomab, gemtuzumab zogamicin, HER- 2 and Fc MAb (Medarex), ibritumomab tiuxetan, idiotypic 105AD7 MAb (CRC Technology), idiotypic CEA MAb (Trilex), ipilimumab, quavonlimab, vibostolimab, favezelimab, lintuzumab, LYM-1 -iodine 131 MAb (Techni clone), mitumomab, moxetumomab, ofatumumab, polymorphic epithelial mucin-yttrium 90 MAb (Antisoma), ranibizumab, rituximab, and trastuzumab.

[0153] Non-limiting examples of immune checkpoint inhibitors include anti-PD-1 agents or antibodies such as cemiplimab, nivolumab, and pembrolizumab; anti-PD-L1 agents or antibodies such as atezolizumab, avelumab, and durvalumab; anti-CTLA-4 agents or antibodies such as ipilumumab and quavonlimab; anti-LAG1 agents; anti-LAG3 agents such as favezelimab, and anti-OX40 agents.

[0154] Non-limiting examples of colony-stimulating factors include darbepoetin alfa, epoetin alfa, epoetin beta, filgrastim, granulocyte macrophage colony stimulating factor,

lenograstim, leridistim, mirimostim, molgramostim, nartograstim, pegfilgrastim, and sargramostim.

[0155] Non-limiting examples of additional immunotherapeutic agents include BiTEs, CAR-T cells, GITR agonists, imiquimod, immunomodulatory imides (IMiDs), mismatched double stranded RNA (Ampligen), resiquimod, SRL 172, and thymalfasin.

[0156] In an embodiment, the additional anti-cancer agent(s) is a targeted therapy agent (i.e., targeted therapy). Targeted therapy agents include, for example, monoclonal antibodies and small molecule drugs. Non-limiting examples of targeted therapy agents include signal transduction inhibitors, growth factor inhibitors, tyrosine kinase inhibitors, EGFR inhibitors, histone deacetylase (HDAC) inhibitors, proteasome inhibitors, cell-cycle inhibitors, angiogenesis inhibitors, matrix-metalloproteinase (MMP) inhibitors, hepatocyte growth factor inhibitors, TOR inhibitors, KDR inhibitors, VEGF inhibitors, fibroblast growth factors (FGF) inhibitors, MEK inhibitors, ERK inhibitors, PI3K inhibitors, AKT inhibitors, MCL-1 inhibitors, BCL-2 inhibitors, SHP2 inhibitors, HER-2 inhibitors, BRAF-inhibitors, BTK inhibitors (e.g., nemtabrutinib), gene expression modulators, autophagy inhibitors, apoptosis inducers, antiproliferative agents, and glycolysis inhibitors.

[0157] Non-limiting examples of signal transduction inhibitors include tyrosine kinase inhibitors, multiple-kinase inhibitors, anlotinib, avapritinib, axitinib, dasatinib, dovitinib, imatinib, lenvatinib, lonidamine, nilotinib, nintedanib, pazopanib, pegvisomant, ponatinib, vandetanib, and EGFR inhibitory agents.

[0158] Non-limiting examples of EGFR inhibitory agents include small molecule antagonists of EGFR such as afatinib, brigatinib, erlotinib, gefitinib, lapatinib, and osimertinib; and antibody-based EGFR inhibitors, including any anti-EGFR antibody or antibody fragment

that can partially or completely block EGFR activation by its natural ligand. Antibody-based EGFR inhibitory agents may include, for example, those described in Modjtahedi, H., et al., 1993, Br. J. Cancer 67:247-253; Teramoto, T., et al., 1996, Cancer 77:639-645; Goldstein et al, 1995, Clin. Cancer Res. 1 : 1311-1318; Huang, S. M., et al., 1999, Cancer Res. 15:59(8): 1935-40; and Yang, X., et al., 1999, Cancer Res. 59: 1236-1243; monoclonal antibody Mab E7.6.3 (Yang, 1999 supra); Mab C225 (ATCC Accession No. HB-8508), or an antibody or antibody fragment having the binding specificity thereof; specific antisense nucleotide or siRNA; afatinib, cetuximab; matuzumab; necitumumab; nimotuzumab; panitumumab; and zalutumumab.

[0159] Non-limiting examples of histone deacetylase (HDAC) inhibitors include belinostat, panobinostat, romidepsin, and vorinostat.

[0160] Non-limiting examples of proteasome inhibitors include bortezomib, carfilzomib, ixazomib, marizomib (salinosporamide a), and oprozomib.

[0161] Non-limiting examples of cell-cycle inhibitors, including CDK inhibitors, include abemaciclib, alvocidib, palbociclib, and ribociclib.

[0162] In an embodiment, the additional anti-cancer agent(s) is an anti-angiogenic agent (or angiogenesis inhibitor) including, but not limited to, matrix-metalloproteinase (MMP) inhibitors; VEGF inhibitors; EGFR inhibitors; TOR inhibitors such as everolimus and temsirolimus; PDGFR kinase inhibitory agents such as crenolanib; HIF-lα inhibitors such as PX 478; HIF-2α inhibitors such as belzutifan and the HIF-2α inhibitors described in WO 2015/035223; fibroblast growth factor (FGF) or FGFR inhibitory agents such as B-FGF and RG 13577; hepatocyte growth factor inhibitors; KDR inhibitors; anti-Ang1 and anti-Ang2 agents; anti-Tie2 kinase inhibitory agents; Tek antagonists (US 2003/0162712; US 6,413,932); anti-TWEAK agents (US 6,727,225); ADAM distintegrin domain to antagonize the binding of

integrin to its ligands (US 2002/0042368); anti-eph receptor and/or anti-ephrin antibodies or antigen binding regions (US 5,981,245; 5,728,813; 5,969,110; 6,596,852; 6,232,447; and 6,057,124); and anti-PDGF-BB antagonists as well as antibodies or antigen binding regions specifically binding to PDGF-BB ligands.

[0163] Non-limiting examples of matrix-metalloproteinase (MMP) inhibitors include MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloproteinase 9) inhibitors, prinomastat, RO 32-3555, and RS 13-0830. Examples of useful matrix metalloproteinase inhibitors are described, for example, in WO 96/33172, WO 96/27583, EP 1004578 , WO 98/07697, WO 98/03516, WO 98/34918, WO 98/34915, WO 98/33768, WO 98/30566, EP 0606046, EP 0931788, WO 90/05719, WO 99/52910, WO 99/52889, WO 99/29667, WO 1999/007675 , EP 1786785, EP 1181017, US 2009/0012085 , US 5,863,949, US 5,861,510, and EP 0780386. Preferred MMP-2 and MMP-9 inhibitors are those that have little or no activity inhibiting MMP-1. More preferred, are those that selectively inhibit MMP-2 and/or MMP-9 relative to the other matrix-metalloproteinases (i.e., MAP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP- 7, MMP- 8, MMP-10, MMP-11, MMP-12, and MMP-13).

[0164] Non-limiting examples of VEGF and VEGFR inhibitory agents include bevacizumab, cediranib, CEP 7055, CP 547632, KRN 633, orantinib, pazopanib, pegaptanib, pegaptanib octasodium, semaxanib, sorafenib, sunitinib, VEGF antagonist (Borean, Denmark), and VEGF-TRAP™.

[0165] The additional anti-cancer agent(s) may also be another anti-angiogenic agent including, but not limited to, 2-methoxyestradiol, AE 941, alemtuzumab, alpha-D148 Mab (Amgen, US), alphastatin, anecortave acetate, angiocidin, angiogenesis inhibitors, (SUGEN, US), angiostatin, anti-Vn Mab (Crucell, Netherlands), atiprimod, axitinib, AZD 9935, BAY RES

2690 (Bayer, Germany, BC 1 (Genoa Institute of Cancer Research, Italy), beloranib, benefin (Lane Labs, US), cabozantinib, CDP 791 (Celltech Group, UK), chondroitinase AC, cilengitide, combretastatin A4 prodrug, CP 564959 (OSI, US), CV247, CYC 381 (Harvard University, US), E 7820, EHT 0101, endostatin, enzastaurin hydrochloride, ER-68203-00 (IVAX, US), fibrinogen-E fragment, Flk-1 (ImClone Systems, US), forms of FLT 1 (VEGFR 1), FR-111142, GCS-100, GW 2286 (GlaxoSmithKline, UK), IL-8, ilomastat, IM-862, irsogladine, KM-2550 (Kyowa Hakko, Japan), lenalidomide, lenvatinib, MAb alpha5beta3 integrin, second generation (Applied Molecular Evolution, USA and Medlmmune, US), MAb VEGF (Xenova, UK), marimastat, maspin (Sosei, Japan), metastatin, motuporamine C, M-PGA, ombrabulin, OXI4503, PI 88, platelet factor 4, PPI 2458, ramucirumab, rBPI 21 and BPI-derived antiangiogenic (XOMA, US), regorafenib, SC-236, SD-7784 (Pfizer, US), SDX 103 (University of California at San Diego, US), SG 292 (Telios, US), SU-0879 (Pfizer, US), TAN-1120, TBC-1635, tesevatinib, tetrathiomolybdate, thalidomide, thrombospondin 1 inhibitor, Tie-2 ligands (Regeneron, US), tissue factor pathway inhibitors (EntreMed, US), tumor necrosis factor-alpha inhibitors, tumstatin, TZ 93, urokinase plasminogen activator inhibitors, vadimezan, vandetanib, vasostatin, vatalanib, VE-cadherin-2 antagonists, xanthorrhizol, XL 784 (Exelixis, US), ziv-aflibercept, and ZD 6126.

[0166] In embodiments, the additional anti-cancer agent(s) is an additional active agent that disrupts or inhibits RAS-RAF-ERK or PI3K-AKT-TOR signaling pathways or is a PD-1 and/or PD-L1 antagonist. In embodiments, the additional anti-cancer agent(s) is a RAF inhibitor, EGFR inhibitor, MEK inhibitor, ERK inhibitor, PI3K inhibitor, AKT inhibitor, TOR inhibitor, MCL-1 inhibitor, BCL-2 inhibitor, SHP2 inhibitor, proteasome inhibitor, or immune therapy, including monoclonal antibodies, immunomodulatory imides (IMiDs), anti-PD-1, anti-PDL-1,

anti-CTLA4, anti-LAGl, anti-LAG3, and anti-OX40 agents, GITR agonists, CAR-T cells, and BiTEs.

[0167] Non-limiting examples of RAF inhibitors include dabrafenib, encorafenib, regorafenib, sorafenib, and vemurafenib.

[0168] Non-limiting examples of MEK inhibitors include binimetinib, CI-1040, cobimetinib, PD318088, PD325901, PD334581, PD98059, refametinib, selumetinib, and trametinib.

[0169] Non-limiting examples of ERK inhibitors include LY3214996, LTT462, MK-8353, SCH772984, ravoxertinib, ulixertinib, and an ERKi as described in WO 2017/068412.

[0170] Non-limiting examples of PI3K inhibitors include 17-hydroxywortmannin analogs (*e.g.*, WO 06/044453); AEZS-136; alpelisib; AS-252424; buparlisib; CAL263; copanlisib; CUDC-907; dactolisib (WO 06/122806); demethoxyviridin; duvelisib; GNE-477; GSK1059615; IC87114; idelalisib; INK1117; LY294002; Palomid 529; paxalisib; perifosine; PI-103; PI-103 hydrochloride; pictilisib (*e.g.*, WO 09/036,082; WO 09/055,730); PIK 90; PWT33597; SF1126; sonolisib; TGI 00-115; TGX-221; XL147; XL-765; wortmannin; and ZSTK474.

[0171] Non-limiting examples of AKT inhibitors include Akt-1-1 (inhibits Aktl) (Barnett *et al.* (2005) *Biochem. J.*, 385 (Pt. 2), 399-408); Akt-1-1,2 (Barnett *et al.* (2005) *Biochem. J.* 385 (Pt. 2), 399-408); API-59CJ-Ome (*e.g.*, Jin et al. (2004) Br. J. Cancer 91, 1808-12); l-H-imidazo[4,5-c]pyridinyl compounds (*e.g.*, WO05011700); indole-3-carbinol and derivatives thereof (*e.g.*, U.S. Patent No. 6,656,963; Sarkar and Li (2004) *J Nutr.* 134(12 Suppl), 3493S-3498S); perifosine, Dasmahapatra *et al.* (2004) *Clin. Cancer Res.* 10(15), 5242-52, 2004); phosphatidylinositol ether lipid analogues (*e.g.*, Gills and Dennis (2004) *Expert. Opin. Investig.*

Drugs 13, 787-97); triciribine (Yang *et al.* (2004) Cancer Res. 64, 4394-9); imidazooxazone compounds including trans-3-amino-1-methyl-3-[4-(3-phenyl-5H-imidazo[1,2-c]pyrido[3,4-e][1,3]oxazin-2-yl)phenyl]-cyclobutanol hydrochloride (WO 2012/137870) ; afuresertib;; capivasertib; MK2206; patasertib, and those disclosed in WO 2011/082270 and WO 2012/177844.

[0172] Non-limiting examples of TOR inhibitors include deforolimus; ATP-competitive TORC1/TORC2 inhibitors, including PI-103, PP242, PP30, and Torin 1; TOR inhibitors in FKBP12 enhancer, rapamycins and derivatives thereof, including temsirolimus, everolimus, WO 9409010; rapalogs, *e.g.* as disclosed in WO 98/02441 and WO 01/14387, *e.g.* AP23573, AP23464, or AP23841; 40-(2-hydroxyethyl)rapamycin, 40-[3-hydroxy(hydroxymethyl)methylpropanoate]-rapamycin ; 40-epi-(tetrazolyl)-rapamycin (also called ABT578); 32-deoxorapamycin; 16-pentynyloxy-32(S)-dihydrorapanycin, and other derivatives disclosed in WO 05/005434; derivatives disclosed in US 5,258,389, WO 94/090101, WO 92/05179, US 5,118,677, US 5,118,678, US 5,100,883, US 5,151,413, US 5,120,842, WO 93/111130, WO 94/02136, WO 94/02485, WO 95/14023, WO 94/02136, WO 95/16691, WO 96/41807, WO 96/41807 and US 5,256,790; and phosphorus-containing rapamycin derivatives (*e.g.*, WO 05/016252).

[0173] Non-limiting examples of MCL-1 inhibitors include AMG-176, MIK665, and S63845.

[0174] Non-limiting examples of SHP2 inhibitors include SHP2 inhibitors described in WO 2019/167000 and WO 2020/022323.

[0175] Additional non-limiting examples of anti-cancer agents that are suitable for use include 2-ethylhydrazide, 2,2',2"-trichlorotriethylamine, ABVD, aceglatone, acemannan,

aldophosphamide glycoside, alpharadin, amifostine, aminolevulinic acid, anagrelide, ANCER, ancestim, anti-CD22 immunotoxins, antitumorigenic herbs, apaziquone, arglabin, arsenic trioxide, azathioprine, BAM 002 (Novelos), bcl-2 (Genta), bestrabucil, biricodar, bisantrene, bromocriptine, brostallicin, bryostatin, buthionine sulfoximine, calyculin, cell-cycle nonspecific antineoplastic agents, celmoleukin, clodronate, clotrimazole, cytarabine ocfosfate, DA 3030 (Dong-A), defofamine, denileukin diftitox, dexrazoxane, diaziquone, dichloroacetic acid, dilazep, discodermolide, docosanol, doxercalciferol, edelfosine, eflornithine, EL532 (Elan), elfomithine, elsamitrucin, eniluracil, etanidazole, exisulind, ferruginol, folic acid replenisher such as frolinic acid, gacytosine, gallium nitrate, gimeracil/oteracil/tegafur combination (S-1), glycopine, histamine dihydrochloride, HIT diclofenac, HLA-B7 gene therapy (Vical), human fetal alpha fetoprotein, ibandronate, ibandronic acid, ICE chemotherapy regimen, imexon, iobenguane, IT-101 (CRLX101), laniquidar, LC 9018 (Yakult), leflunomide, lentinan, levamisole + fluorouracil, lovastatin, lucanthone, masoprocol, melarsoprol, metoclopramide, miltefosine, miproxifene, mitoguazone, mitozolomide, mopidamol, motexafin gadolinium, MX6 (Galderma), naloxone + pentazocine, nitracrine, nolatrexed, NSC 631570 octreotide (Ukrain), olaparib, P-30 protein, PAC-1, palifermin, pamidronate, pamidronic acid, pentosan polysulfate sodium, phenamet, picibanil, pixantrone, platinum, podophyllinic acid, porfimer sodium, PSK (Polysaccharide-K), rabbit antithymocyte polyclonal antibody, rasburiembodiment, retinoic acid, rhenium Re 186 etidronate, romurtide, samarium (153 Sm) lexidronam, sizofiran, sodium phenylacetate, sparfosic acid, spirogermanium, strontium-89 chloride, suramin, swainsonine, talaporfin, tariquidar, tazarotene, tegafur-uracil, temoporfin, tenuazonic acid, tetrachlorodecaoxide, thrombopoietin, tin ethyl etiopurpurin, tirapazamine, TLC ELL-12,

tositumomab-iodine 131, trifluridine and tipiracil combination, troponin I (Harvard University, US), urethan, valspodar, verteporfin, zoledronic acid, and zosuquidar.

[0176] The present disclosure further provides a method for using the recombinant fusion protein of the disclosure provided herein, in combination with radiation therapy to treat cancer. Techniques for administering radiation therapy are known in the art, and these techniques can be used in the combination therapy described herein. The administration of the recombinant fusion protein of the disclosure in this combination therapy can be determined as described herein.

[0177] Radiation therapy can be administered through one of several methods, or a combination of methods, including, without limitation, external-beam therapy, internal radiation therapy, implant radiation, stereotactic radiosurgery, systemic radiation therapy, radiotherapy and permanent or temporary interstitial brachy therapy. The term "brachythisherapy," as used herein, refers to radiation therapy delivered by a spatially confined radioactive material inserted into the body at or near a tumor or other proliferative tissue disease site. The term is intended, without limitation, to include exposure to radioactive isotopes (*e.g.*, At-211, I-131, I -125, Y-90, Re-186, Re-188, Sm- 153, Bi-212, P-32, and radioactive isotopes of Lu). Suitable radiation sources for use as a cell conditioner of the present disclosure include both solids and liquids. By way of non-limiting example, the radiation source can be a radionuclide, such as I-125, I -131, Yb-169, Ir-192 as a solid source, I-125 as a solid source, or other radionuclides that emit photons, beta particles, gamma radiation, or other therapeutic rays. The radioactive material can also be a fluid made from any solution of radionuclide(s), e.g., a solution of I-125 or I-131, or a radioactive fluid can be produced using a slurry of a suitable fluid containing small particles of solid radionuclides, such as Au-198, Y-90. Moreover, the radionuclide(s) can be embodied in a gel or radioactive microspheres.

[0178] The present disclosure also provides methods for combination therapies in which the additional active agent is known to modulate other pathways, or other components of the same pathway, or even overlapping sets of target enzymes which are used in combination with the recombinant fusion protein of the disclosure. In one embodiment, such therapy includes, but is not limited to, the combination of the recombinant fusion protein of the disclosure with chemotherapeutic agents, immunotherapeutic agents, hormonal therapy agents, therapeutic antibodies, targeted therapy agents, and radiation treatment, to provide a synergistic or additive therapeutic effect.

[0179] The compounds of the disclosure can be used in combination with the agents disclosed herein or other suitable agents, depending on the condition being treated. Hence, in some embodiments the one or more compounds of the disclosure will be co-administered with other agents as described above. When used in combination therapy, the compounds described herein are administered with the second agent simultaneously or separately. This administration in combination can include simultaneous administration of the two agents in the same dosage form, simultaneous administration in separate dosage forms, and separate administration. That is, the recombinant fusion protein of the disclosure and any of the agents described above can be formulated together in the same dosage form and administered simultaneously. Alternatively, the recombinant fusion protein of the disclosure and any of the agents described above can be simultaneously administered, wherein both the agents are present in separate formulations. In another alternative, the recombinant fusion protein of the disclosure can be administered just followed by and any of the agents described above, or vice versa. In some embodiments of the separate administration protocol, the recombinant fusion protein of the disclosure and any of the agents described above are administered a few minutes apart, or a few hours apart, or a few days

apart. The recombinant fusion protein of the disclosure may be administered at different frequencies such as, for example, from 1 to 20 times per week (or any number therein), from 1 to 90 times per month (or any number therein). For example, the recombinant fusion protein of the disclosure may be administered once per day, twice per day, three times per day, or more than three times per day. The recombinant fusion protein of the disclosure is administered for various durations such as, for example, a week, a month, two months, a year, or more than a year. For example, in some embodiments, the recombinant fusion protein of the disclosure is administered every day for a week, every day for a month, every day for two months, every day for a year, or every day for more than a year.

[0180] In some embodiments, the recombinant fusion protein of the disclosure is administered every day or intermittently. In some embodiments, the administration according to the methods of the disclosure is for at least one treatment cycle. In some embodiments, the recombinant fusion protein of the disclosure is administered every day, intermittently, or once during a treatment cycle.

[0181] In some embodiments, a treatment cycle is 1 – 365 days. In an embodiment, a treatment cycle is 1 day, 2 days, one week (i.e., 7 days), 2 weeks (i.e., 14 days), 3 weeks (i.e., 21 days), 4 weeks (i.e., 28 days), 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks, 18 weeks, 19 weeks, or 20 weeks, one month, months , months, 6 months or about one year. As used herein, a "week" means seven consecutive days.

[0182] In some embodiments, the administration is for 1 treatment cycle. In some embodiments, the administration is for at least one treatment cycle and one additional treatment cycle (i.e., at least two treatment cycles). In some embodiments, the administration is for at least

2 treatment cycles, or at least 3 treatment cycles, or at least 4 treatment cycles, or at least 5 treatment cycles, or at least 6 treatment cycles, or at least 7 treatment cycles, or at least 8 treatment cycles, or at least 9 treatment cycles, or at least 10 treatment cycles, or at least 11 treatment cycles, or at least 12 treatment cycles, or at least 15 treatment cycles, or at least 20 treatment cycles, or at least 25 treatment cycles, or at least 30 treatment cycles, or at least 35 treatment cycles, or at least 40 treatment cycles. In some embodiments, the treatment is continuous until an endpoint which may be determined by a medical professional. In some embodiments, the administration is for less than or equal to 35 treatment cycles.

[0183] In some embodiments, the administered amount of the recombinant fusion protein of the disclosure is held constant during one treatment cycle. In some embodiments, the administered amount of the recombinant fusion protein of the disclosure is held constant for at least one treatment cycle and at least one additional treatment cycle (i.e., more than one treatment cycle). In some embodiments, the administered amount of the recombinant fusion protein of the disclosure can be increased in a second or subsequent treatment cycle (i.e., an additional treatment cycle). In some embodiments, the administered amount of the recombinant fusion protein of the disclosure can be held constant for at least two treatment cycles and decreased in a third or subsequent treatment cycle. In some embodiments, the administered amount of the recombinant fusion protein of the disclosure can be held constant for at least two treatment cycles and increased in a third or subsequent treatment cycle.

[0184] In some embodiments, the recombinant fusion protein of the disclosure is administered once a day on each day of a treatment cycle. In some embodiments, the recombinant fusion protein of the disclosure is administered intermittently during a treatment cycle.

[0185] As one aspect of the present disclosure contemplates the treatment of the disease/conditions with a combination of pharmaceutically active compounds that may be administered separately, the disclosure further relates to combining separate pharmaceutical compositions in kit form. The kit comprises two separate pharmaceutical compositions: the recombinant fusion protein of the disclosure, and a second pharmaceutical compound. The kit comprises a container for containing the separate compositions such as a divided bottle or a divided foil packet. Additional examples of containers include syringes, boxes, and bags. In some embodiments, the kit comprises directions for the use of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (*e.g.*, oral and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing health care professional.

[0186] The present disclosure also provides for the recombinant fusion protein of the disclosure for use in therapy, or use of the recombinant fusion protein of the disclosure in therapy. The present disclosure also provides for the recombinant fusion protein of the disclosure for use in treating cancer, or use of the recombinant fusion protein of the disclosure for treating cancer. The present disclosure also provides for the recombinant fusion protein of the disclosure for the preparation of a medicament for the treatment of cancer, or use of the recombinant fusion protein of the disclosure for the preparation of a medicament for the treatment of cancer. The present disclosure also provides for the recombinant fusion protein of the disclosure and an additional anti-cancer agent, for use in the treatment of cancer, or use of the recombinant fusion protein of the disclosure and the additional anti-cancer agent for treating cancer. The disclosure also provides the recombinant fusion protein of the disclosure and an

additional anti-cancer agent, for the preparation of a medicament for the treatment of cancer, or use of the recombinant fusion protein of the disclosure and the additional anti-cancer agent, for the preparation of a medicament for the treatment of cancer. The present disclosure also provides for a pharmaceutical composition comprising the recombinant fusion protein of the disclosure for use in the treatment of cancer, or use of the pharmaceutical composition comprising the recombinant fusion protein of the disclosure for treating cancer. The present disclosure also provides for a pharmaceutical composition comprising the recombinant fusion protein of the disclosure and an additional anti-cancer agent, for use in the treatment of cancer, or use of the pharmaceutical composition comprising the recombinant fusion protein of the disclosure and the additional anti-cancer agent, for treating cancer.

[0187] Further aspects of the present disclosure are provided by the subject matter of the following clauses.

[0188] A recombinant fusion protein comprising at least one fragment derived from a *Staphylococcus aureus* superantigen-like (SSL) protein and at least one fragment derived from a killing protein.

[0189] The recombinant fusion protein according to the preceding clause, wherein the SSL is selected from SSL2, SSL3, SSL4, SSL5, SSL6 and SSL11.

[0190] The recombinant fusion protein according to any preceding claus, wherein the at least one fragment derived from the SSL protein is selected from an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOs: 2 and 14-18.

[0191] The recombinant fusion protein according to any preceding clause, wherein the SSL is a chimeric protein comprising an SLeX binding site from any one of SSL2 (SEQ ID NO:

8), SSL3 (SEQ ID NO: 9), SSL4 (SEQ ID NO: 10), SSL5 (SEQ ID NO: 11), SSL6 (SEQ ID NO: 12), or SSL11 (SEQ ID NO: 13) protein inserted into any one of heterologous SSL proteins in a way that replaces its native binding site.

[0192] The recombinant fusion protein according to any preceding clause, wherein the killing protein is selected from Pseudomonas exotoxin (PE), diphtheria toxin (DT), ricin toxin, anthrax toxin lethal factor (LF), and anthrax toxin edema factor (EF).

[0193] The recombinant fusion protein according to any preceding clause wherein the killing protein is derived from a Pseudomonas exotoxin (PE).

[0194] The recombinant fusion protein according to any preceding clause, wherein the SSL is SSL11.

[0195] The recombinant fusion protein according to any preceding clause, wherein the at least one fragment derived from the SSL protein binds to a Sialyl Lewis X (SLeX) fragment of a cancer, thereby inhibiting cell migration.

[0196] The recombinant fusion protein according to any preceding clause, wherein the at least one fragment derived from the SSL protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 2.

[0197] The recombinant fusion protein according to any preceding clause, wherein the at least one fragment derived from the SSL protein comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO. 2.

[0198] The recombinant fusion protein according to any preceding clause, wherein the at least one fragment derived from the SSL protein comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO. 2.

[0199] The recombinant fusion protein according to any preceding clause, wherein the killing protein is PE in a 24 kDa truncated form (PE24).

[0200] The recombinant fusion protein according to any preceding clause, wherein the killing protein is PE in a modified 24 kDa truncated form ($PE24^{M10}$).

[0201] The recombinant fusion protein according to the preceding clause, wherein the at least one fragment derived from the PE inactivates eukaryotic elongation factor-2 (eEF-2), thereby inducing cell death.

[0202] The recombinant fusion protein according to any preceding clause, wherein the killing protein comprises at least one fragment derived from the killing protein is a PE comprising an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 3.

[0203] The recombinant fusion protein according to the preceding clause, wherein the at least one fragment derived from the killing protein is a PE comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO. 3.

[0204] The recombinant fusion protein according to the preceding clause, wherein the at least one fragment derived from the killing protein is a PE comprising an amino acid sequence having at least 99% sequence identity to SEQ ID NO. 3.

[0205] The recombinant fusion protein according to any preceding clause, wherein the at least one fragment derived from the SSL protein and the killing protein are linked through a furin cleavage site.

[0206] The recombinant fusion protein according to any preceding clause, wherein the at least one fragment derived from the SSL protein and the killing protein the at least one fragment derived from the PE are linked through a furin cleavage site.

[0207] The recombinant fusion protein according to the preceding clause, wherein the furin cleavage site comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 4.

[0208] The recombinant fusion protein according to the preceding clause, wherein the furin cleavage site comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 5 or SEQ ID NO. 6.

[0209] The recombinant fusion protein according to any preceding clause, wherein the recombinant fusion protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 1.

[0210] The recombinant fusion protein according to any preceding clause, wherein the recombinant fusion protein comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO. 1.

[0211] The recombinant fusion protein according to any preceding clause, wherein the recombinant fusion protein comprises an amino acid sequence having at least 99% sequence identity to SEQ ID NO. 1.

[0212] A method for treating a cancer in a subject in need thereof, comprising contacting the SLeX fragment of the cancer with the recombinant fusion protein as described in of any of the preceding clauses, to inhibit cell migration.

[0213] A method for treating a cancer in a subject in need thereof, comprising contacting eEF-2 with the recombinant fusion protein as described in of any of the preceding clauses, to induce cell death.

[0214] A method for treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the recombinant fusion protein as described in of any of the preceding clauses.

[0215] The method according to the preceding clause, further comprising administering an additional active agent to the subject.

[0216] The method according to any one of the preceding clauses, wherein the subject is a mammal.

[0217] The method according to any one of the preceding clauses, wherein the subject is human.

[0218] The method according to any preceding clause, wherein the therapeutically effective amount is administered orally, subcutaneously, or intravenously.

[0219] The recombinant fusion protein identified described in any preceding clause for use in therapy, or use of the recombinant fusion protein according to any preceding clause in therapy.

[0220] The recombinant fusion protein identified in any preceding clause for use in treating cancer, or use of the recombinant fusion protein identified in any preceding clause for treating cancer.

[0221] The for the preparation of a medicament for the treatment of cancer, or use of the recombinant fusion protein identified in any preceding clause for the preparation of a medicament for the treatment of cancer.

[0222] The recombinant fusion protein identified in any preceding clause and an additional anti-cancer agent for use in the treatment of cancer, or use of the recombinant fusion protein identified in any preceding clause and the additional anti-cancer agent for treating cancer.

[0223] The recombinant fusion protein identified in any preceding clause and an additional anti-cancer agent, for the preparation of a medicament for the treatment of cancer, or use of t recombinant fusion protein identified in any preceding clause and the additional anti-cancer agent for the preparation of a medicament for the treatment of cancer.

[0224] A pharmaceutical composition comprising the recombinant fusion protein of any one of the preceding clauses and a pharmaceutically acceptable carrier.

[0225] The pharmaceutical composition protein according to the preceding clause, further comprising an additional anti-cancer agent.

[0226] A pharmaceutical composition as identified in any preceding clause for use in the treatment of cancer, or use of the pharmaceutical composition identified in any preceding clause for treating cancer.

[0227] A polynucleotide encoding the recombinant fusion protein of any one of the preceding clauses.

[0228] A recombinant vector encoding the recombinant fusion protein of any one of the preceding clauses.

EXAMPLES

[0229] Specific embodiments will now be demonstrated by reference to the following examples. It should be understood that these examples are disclosed solely by way of illustrating the invention and should not be taken in any way to limit the scope of the present invention.

[0230] Example 1: SSL11 as an anticancer delivery platform

[0231] Fourteen SSLs were identified by bioinformatic screening of the staphylococcal genome using the two conserved motifs of Staphylococcal Superantigens (SAgs). SSLs did not activate T cells as SAgs without the critical residues of SAgs. Depending on the presence of a glycan SLeX-binding pocket, SSLs is divided into two subfamilies where SSL2-6 and SSL11 possess this carbohydrate-binding site. SSL11 mediated cell motility arrest by interacting with SLeX. Since SLeX is overexpressed in many types of cancers, and due to the properties of binding glycan SLeX and inhibiting cell motility of SSL11, SSL was chosen as the delivery platform against cancers that overexpress SLeX.

[0232] Example 2: Construction of SSL-PE24^{M10} and PE24^{M10}

[0233] SSL11-PE24^{M10} had a furin cleavage site (SEQ ID NO: 4) as a linker. To protect the protein from being cleaved and inactivated before internalization by cell surface furin or other proteases in the bloodstream or microenvironment, the furin cleavage site was flanked by cysteine-containing peptides (SEQ ID NOS: 5 and 6), which was used to optimize immunotoxin

SSL11-PE24. SSL11^{T168P}, which does not bind to SLeX, was engineered as a control construct SSL11^{T168P}-PE24^{M10} for SLeX-dependent cell binding and entry. PE24^{M10} and SSL11 was used as controls as well. SSL11^{T168P}-PE24^{M10} and PE24^{M10} were utilized to provide information on the off-target effects of the fusion protein SSL11-PE24^{M10}.

[0234] *E. coli* codon-optimized sequences of the proteins SSL11-PE24^{M10}, SSL11^{T168P}-PE24^{M10}, and PE24^{M10} were synthesized and subcloned into a pET28a vector for protein expression. All constructs had an N-terminal Hisx6 tag for protein purification (GenScript). Plasmids encoding these proteins were transformed into *E. coli* BL21 (DE3). Cells were cultured at 37 °C to an optical density at 600 nm (OD600) of 0.6, followed by T7 promoter expression with one mM IPTG induction cultured overnight at 16 °C. Cells were pelleted and lysed with a French press and clarified by centrifugation. HisX$_6$-tagged (SEQ ID NO: 37) proteins were purified using Ni^{2+}-nitrilotriacetic acid (NTA) resin (Qiagen). SSL11-PE24^{M10} and PE24^{M10} were successfully purified with great solubility (>95%) and yields (10-15 mg/2 L culture), as shown in Figure 2A. As SSL11 and PE24^{M10} are linked by a furin cleavage site with cysteine-containing peptides when SSL11-PE24^{M10} was treated with furin and analyzed by SDS-PAGE under reducing conditions, it was cleaved into SSL11 and PE24^{M10} fragments (same molecular weight). Figure 2B shows that SSL11-PE24^{M10} maintains full length without furin cleavage and under non-reducing conditions.

[0235] Example 3: Glycan specificity

[0236] Glycan specificity of the protein SSL11-PE24^{M10} was tested. SSL11^{T168P}-PE24^{M10} and PE24^{M10} served as negative controls. SSL11 served as a positive control. SSL11, SSL11-PE24^{M10}, and SSL11^{T168P}-PE24^{M10} were labeled with an Alexa Fluor Protein labeling kit (Invitrogen, A10238). Proteins were dialyzed in PBS at 4 °C overnight. 50 μL of 1 M

bicarbonate was added to 0.5 ml of 2 mg/mL proteins, followed by the transfer of the protein solutions to a vial of reactive dye. The reaction mixture was stirred for two hours at room temperature, followed by dialysis in PBS at 4 °C overnight to eliminate free dyes. Labeled proteins were stored at 4 °C. Fluorescence labeled SSL11, SSL11-PE24^{M10}, and SSL11^{T168P}-PE24^{M10} (2, 20, 200 nM) were tested for binding against 562 different glycans using a glycan microarray provided by NCFG (National Center for Functional Glycomics, Harvard University).

[0237] SSL11 preferably bound to the glucan structure sLacNac, a subcomponent of SLeX. As PE24^{M10} is a truncated fragment from PE without the receptor-binding domain. SSL11-PE24^{M10} and SSL11 were expected to bind preferably to glycans with the sLacNac structure, such as SLeX, while SSL11^{T168P}-PE24^{M10} and PE24^{M10} did not.

[0238] Example 4: Inducement of cell death

[0239] A Hela cell line, two lung cancer cell lines, two breast cancer cell lines, and a colon cancer cell line (Lovo) that overexpress SLeX were used as model systems to test the activities of SSL11-PE24^{M10}. The two lung cancer cell lines were the brain metastatic lung cancer cell line (NCI-H1299) and the primary lung cancer cell line (A549). The two lung cancer cell lines were ZR 75-1 and BT 20. The normal lung epithelial cell line BEAS-2B was used as a control. The SLeX expression in all the cell lines was determined by immunofluorescence using the antibody CSLEX1 (BD). All cell lines were purchased from the American Type Culture Collection (ATCC) and cultured as suggested. SSL11^{T168P}-PE24^{M10} and free PE24^{M10} were used as controls in all the cell-based assays.

[0240] Hela, NCI-H1299, A549, Lovo, ZR 75-1, BT 20, and BEAS-2B (control) cells (2,000 cells/well) were plated in 96-well plates, left overnight to adhere, and incubated with SSL11-PE24^{M10}, or SSL11^{T168P}-PE24^{M10}, or SSL11, or PE24^{M10} (80, 160, 320, 640, 1280 nM) at

37 °C up to 24, 48, or 72 hours at a final volume of 0.2 mL. 0.2% digitonin which induces cell death by damaging cell membranes, was used as a positive control. At the end of the incubation period, cytotoxicity was evaluated by two assays. i) LDH release from dead cells. A colorimetric LDH assay was used to quantify the release of the cytoplasmic enzyme lactate dehydrogenase with high sensitivity from culture supernatants. ii) Viable cells at the end of experiments. 96-well plates were washed three times with PBS to remove dead cells. Live cells adherent to the plates were incubated with 0.5% crystal violet at room temperature for 10 min, followed by four PBS washes. The plates were dried and followed by the addition of ethanol to solubilize cell-bound crystal violet. Absorbance at 595 nm was measured to quantify crystal violet and shown as live cell percentages.

[0241] As shown in Figures 3A and 3B, SSL11-PE24^{M10}, but not PE24^{M10}, induced cell death in a dose-dependent manner in the Hela cell line. Figures 4A-4I show that SSL11-PE24^{M10}, but not PE24^{M10}, induced cell death in a dose-dependent manner in all six cancer cell lines, strongly suggesting that SSL11 successfully delivered PE24^{M10} into cells to induce cell death.

[0242] Example 5: SSL11-PE24^{M10} displays lower toxicity in normal human cells.

[0243] To test whether SSL11-PE24^{M10} is toxic to normal human cells, the normal human lung epithelial cell line BEAS-2B was used. SSL11-PE24^{M10} is less toxic in BEAS-2B cells than in all cancer cell lines tested when incubated at 37°C for 72 hours. Although at the highest concentration of 640 nM, SSL11-PE24^{M10} displays some toxicity in BEAS-2B cells. It has much lower toxicity in normal lung cells at lower concentrations than cancer cells (Figure 5). IC_{50} of SSL11-PE24^{M10} in all the cell lines was determined by GraphPad Prism. IC_{50} in BEAS-2B cells is about fivefold of the IC_{50} in NCI-H1299, A549, Lovo, and Hela cells (Table 1).

Table 1. IC_{50} of SSL11-$PE24^{M10}$ in multiple cell lines.

Cell lines	**IC_{50} of SSL11-$PE24^{M10}$**
NCI-H1299	121 nM
A549	141 nM
Hela	168 nM
Lovo	240 nM
BEAS-2B	685 nM

[0244] Example 6: ADP ribosylation

[0245] Platform proteins' *in vitro* ADP ribosylation (ADPR) activities were tested using Biotin-NAD as the substrate. Cell lysate from NCI-H1299, A549, and BEAS-2B cells was collected using RIPA buffer. The ADP ribosylation reaction was set up with 40 µg of cell lysate, 5 mM Biotin-NAD (Trevigen, 250 mM stock), 100 ng of SSL11-$PE24^{M10}$ and $PE24^{M10}$ (positive control), and no Biotin-NAD, with Biotin-NAD but without ADPR proteins (negative control), and reaction buffer (10 mM Tris-HCl, 20 mM NaCl, PH7.6) to make the final reaction to 60 µL. The ADP ribosylation reaction was done at room temperature for one hour. Samples were subjected to SDS-PAGE followed by Western blotting with streptavidin HRP conjugate (Invitrogen) to detect ADP ribosylated EF2. Total cell lysate EF2 and GAPDH were used as loading controls by Western blotting. As shown in Figure 6 pointed by the arrow, SSL11-$PE24^{M10}$ displayed the same ADPR activity as $PE24^{M10}$, while no Biotin-NAD and no ADPR proteins did not, as shown by the nonspecific bands, confirming that platform protein SSL11-$PE24^{M10}$ has the same ADPR activity as the $PE24^{M10}$ and SSL11-$PE24^{M10}$ does not affect the ADPR of $PE24^{M10}$.

[0246] Example 7: Cell binding and entering

[0247] NCI-H1299 cells were seeded in 24 well plated coated with glass coverslips for 24 hours. SSL11-$PE24^{M10}$ and $PE24^{M10}$ were labeled with Alexa Fluor 568 and different

amounts of proteins (80, 160, 320, 640 nM) were incubated with NCI-H1299 cells at 37 °C for 30 minutes. Cells were washed and incubated with Alexa Fluor 647 labeled phalloidin for F-actin staining and DAPI for nuclei staining. Cell images with Z stacks (0.5 μm/slide for 5 μm) were captured with a Nikon TiE2 Microscope using an ORCA-Fusion Gen-III sCMOS Camera. Images were processed using Nikon NIS deconvolution software. Cell association was quantified as the fluorescence intensity ratio between platform proteins and DAPI staining by Image J. As shown in Figures 7A and 7B, SSL11-PE24^{M10} entered cells in a dose-dependent manner, while PE24^{M10} did not. Further, in the SSL11-PE24^{M10} treated cells, platform proteins were localized to peri-nuclear regions in a dose-dependent manner. The perinuclear region corresponded to the Golgi/ER region. PE24^{M10} trafficked to the Golgi/ER region by binding to the KDEL (SEQ ID NO: 38) receptor via the terminal REDL (SEQ ID NO: 45) motif, suggesting that SSL11 delivered PE24^{M10} inside cells and did not interfere with PE24^{M10} trafficking. In sum, SSL11 delivered PE24^{M10} inside cells by binding to SLeX on the cell surface, while PE24^{M10} failed to enter cells missing the receptor binding domain.

[0248] Example 8: SSL11-PE24^{M10} trafficking in NCI-H1299 cells

[0249] To better understand the intracellular trafficking of the SSL11-PE24^{M10}, a 3XFLAG tag in the N-terminal of SSL11 and a HA-tag in the N-terminal of PE24^{M10} were added to generate a ^{3XFLAG}SSL11-^{HA}PE24^{M10} construct. Recombinant ^{3XFLAG}SSL11-^{HA}PE24^{M10} (640 and 160 nM) was incubated with NCI-H1299 cells at 37 °C for 60 mins. Cells were washed and fixed, and immunofluorescence staining was performed using anti-FLAG (SSL11), anti-HA (PE24^{M10}), and anti-Golgi97 (Golgi marker). The results show that PE24^{M10} trafficked to the peri-nuclear region and colonized with Golgi, while SSL11 stayed on the plasma membrane and cytosol. Cells treated with 640 nM of SSL11-PE24^{M10} started to be round, suggesting that it

started to induce cell death (Figure 8). This shows that after SSL11-PE24^{M10} enters cells, intracellular furin cleaves the platform into SSL11 and PE24M^{10} via the FCS site. PE24^{M10} traffics to the Golgi/ER region and translocates to the cytosol later to cleave eEF2 to induce cell death

[0250] Example 9: Cell migration

[0251] A scratch wound healing assay was performed to determine cell migration. 24-well culture plates with small 2-well silicone inserts per well with a cell-free gap of 500 μm (Ibidi USA, Inc) were used. Briefly, NCI-H1299, A549, and BEAS-2B (control) cells were seeded in each culture insert and incubated in a 37°C, 5% CO_2 incubator until a confluent cell monolayer is achieved. Inserts were removed with sterile tweezers. Wounds were created by scratching the monolayer cells with a pipette tip to form a cross. Cells were treated with SSL11-PE24^{M10}, SSL11^{T168P}-PE24^{M10}, SSL11, or PE24^{M10} (0, 80, 160, 320, 640 nM) at 37 °C for up to 24 or 48 hours. Pictures were taken, and wound healing was quantified using the Image J Wound healing sizes tool and displayed as % wound area per image. Since NCI-H1299 is a brain metastatic lung cancer cell line and A549 is a primary lung cancer cell line, NCI-H1299 cells migrated much faster than A549 cells, as shown in Figures 9A-9D. Also shown is that SSL11-PE24^{M10} inhibited cell migration in a dose- and time-dependent manner in both NCI-H1299 an dA549 cells. For NCI-H1299, while SSL11-PE24^{M10} inhibited cell migration at 640 nM at 24 hours, it inhibited cell migration starting at 160 nM at 48 hours. Whereas PE24^{M10} did not show any inhibition for cell migration.

[0252] To better quantify the cell migration assay, an Incucyte Scratch Wound assay was performed in NCI-H1299, A549, and BEAS-2B cells. Briefly, all cells were grown in 96-well plates until confluency. A 96-well woundmaker tool was used to make scratches in all wells.

Cells were incubated with 160-640 nM of SSL11- $PE24^{M10}$, $PE24^{M10}$, and SSL11 at 37 °C for 24 hours. Plates were scanned every two hours. Cell migration was quantified by the Incucyte software and shown as Relative Wound Density (%).Consistent with the wound-healing assay, SSL11-$PE24^{M10}$ significantly inhibited NCI-H1299 cell migration in a dose- and time-dependent manner, while $PE24^{M10}$ and SSL11 did not affect cell migration (Figure 10). As A549 cells are primary lung cancer cells, it was found that wounds only closed 20% after 24 hours in A549 cells and that only 640 nM of SSL11-$PE24^{M10}$ inhibited cell migration (Figure 11), which is consistent with the wound healing assay (Figure 9). BEAS-2B cells migrated at a similar rate as NCI-H1299 cells as wounds closed around 90-100% after 24 hours. SSL11-$PE24^{M10}$ did not inhibit cell migration in BEAS-2B cells (Figure 12), which is consistent with the fact that SSL11-$PE24^{M10}$ displays lower toxicity in BEAS-2B cells (Figure 5 and Table 1). This supports the hypothesis that SSL11-$PE24^{M10}$ is an anti-cancer agent.

[0253] Example 10: SSL-$PE24^{M10}$ platform optimization

[0254] To test whether SSL4 induces cell adhesion in a way similar to SSL11, the cell adhesion assay with the differentiated HL60 cells as the neutrophil cell model, was performed as previously published. Briefly, dHL60 cells were incubated with 160 nM of SSL4 or SSL11 in fibronectin-coated 96-well plates at 37 °C for 30 min, followed by two PBS washes. Adherent cells were quantified by crystal violet staining and shown as adhesion arbitrary units (AU). It was observed that SSL4 induced increased cell adhesion as compared to SSL11 (Figure 14), suggesting that SSL4-$PE24^{M10}$ may provide better efficacy than SSL11-$PE24^{M10}$.

[0255] Figure 13 shows sequences of SSL1-SSL14 (SEQ ID Nos: 46-59) in the binding regions aligned using Clustal Omega. Polynucleotides encoding chimeric SSL proteins

containing an SLeX binding site, as indicated in Figure 13, from one of SSL2, SSL3, SSL4, SSL5, SSL6, or SSL11 (SEQ ID Nos: 8-13) protein inserted into a heterologous SSL protein will be generated and used to construct nucleic acids encoding chimeric SSL-PE24^{M10} fusion proteins. These will be subcloned into an *E. coli* expression vector and the chimeric SSL-PE24^{M10} fusion proteins will be expressed and purified in the same manner as described above. The chimeric SSL SSL-PE24^{M10} fusion proteins will be subsequently tested for glycan specificity, inducement of cell death, ADP ribosylation, cell binding and entering, cell migration, toxicity, and intracellular trafficking in the manner described above.

[0256] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

[0257] While the disclosure has been described above with reference to specific embodiments thereof, it is apparent that many changes, modification, and variations can be made without departing from the concept disclosed herein. Accordingly, it is intended to embrace all such changes, modifications, and variations that fall within the spirit and broad scope of the appended claims.

WHAT IS CLAIMED IS:

1. A recombinant fusion protein comprising:

at least one fragment derived from a *Staphylococcus aureus* superantigen-like (SSL) protein; and

at least one fragment derived from a Pseudomonas exotoxin (PE).

2. The recombinant fusion protein of claim 1, wherein the SSL is SSL11.

3. The recombinant fusion protein of claim 2, wherein the at least one fragment derived from the SSL protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 2.

4. The recombinant fusion protein of claim 1 or claim 2, wherein the PE is in a 24 kDa truncated form (PE24).

5. The recombinant fusion protein of claim 4, wherein the at least one fragment derived from the PE inactivates eukaryotic elongation factor-2 (eEF-2), thereby inducing cell death.

6. The recombinant fusion protein of claim 4, wherein the at least one fragment derived from the PE comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 3.

7. The recombinant fusion protein of claim 1, wherein the at least one fragment derived from the SSL protein and the at least one fragment derived from the PE are linked through a furin cleavage site.

8. The recombinant fusion protein of claim 7, wherein the furin cleavage site comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 4.

9. The recombinant fusion protein of claim 1, wherein the recombinant fusion protein comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO. 1.

10. A polynucleotide encoding the recombinant fusion protein of claim 1.

11. A recombinant vector encoding the recombinant fusion protein of claim 1.

12. A pharmaceutical composition comprising the recombinant fusion protein of claim 1and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition of claim 12, further comprising an additional anti-cancer agent.

14. A method for treating a cancer in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the recombinant fusion protein of claim 1.

15. The method of claim 14, wherein the therapeutically effective amount is administered orally, subcutaneously, or intravenously.

16. The method of claim 14, further comprising administering an additional anti-cancer agent.

17. A recombinant fusion protein comprising:

at least one fragment derived from a *Staphylococcus aureus* superantigen-like (SSL) protein; and

a killing protein.

18. The recombinant fusion protein of claim 17, wherein the SSL is selected from a group consisting of SSL2, SSL3, SSL4, SSL5, and SSL6.

19. The recombinant fusion protein of claim 18, wherein the at least one fragment derived from the SSL protein is selected from an amino acid sequence having at least 90% sequence identity to any one of SEQ ID NOs: 14-18.

20. The recombinant fusion protein of claim 17 , wherein the SSL is a chimeric protein comprising an SLeX binding site from any one of SSL2 (SEQ ID NO: 8), SSL3 (SEQ ID NO: 9), SSL4 (SEQ ID NO: 10), SSL5 (SEQ ID NO: 11), SSL6 (SEQ ID NO: 12), or SSL11 (SEQ ID NO: 13) protein inserted into any one of heterologous SSL proteins in a way that replaces its native binding site.

21. The recombinant fusion protein of claim 17, wherein the killing protein is selected from a group consisting of diphtheria toxin (DT), ricin toxin, anthrax toxin lethal factor (LF), and anthrax toxin edema factor (EF).

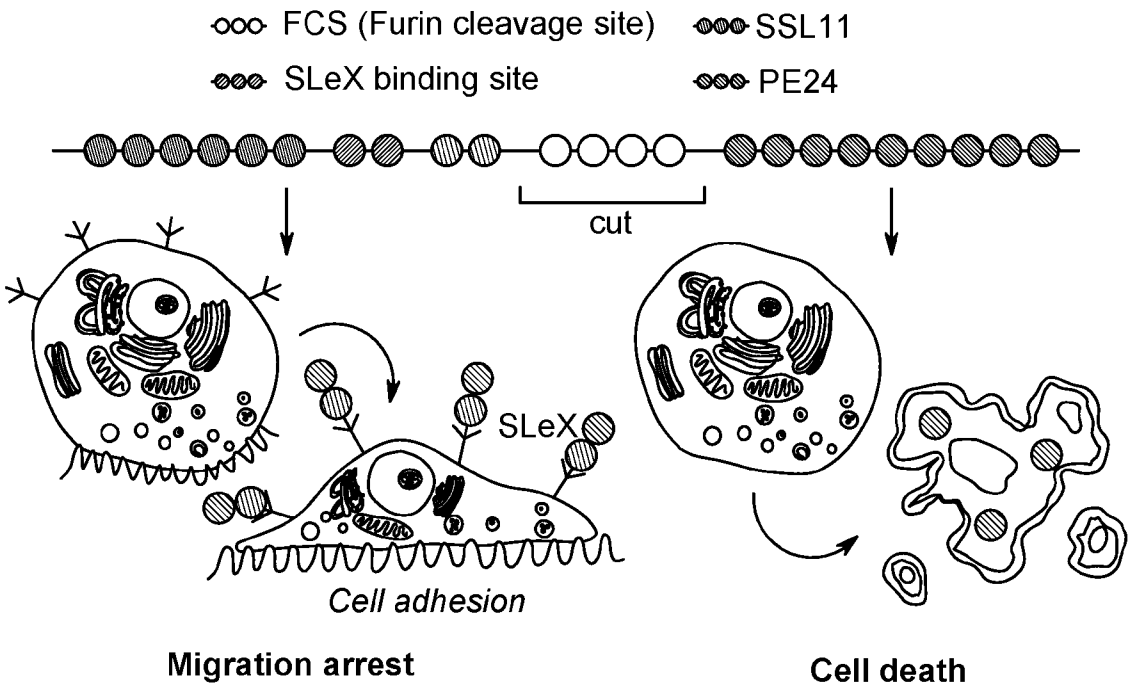
FCS (Furin cleavage site)
SSL11
SLeX binding site
PE24
cut
SLeX
Cell adhesion
Migration arrest
Cell death

FIG. 1

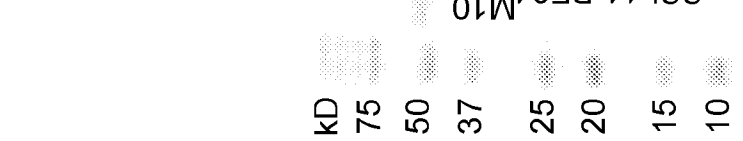
kD
75
50
37
25
20
15
10
SSL11-PE24M10
PE24M10

FIG. 2A

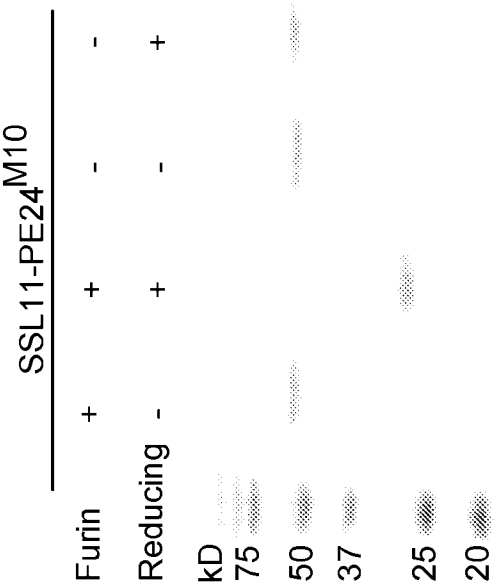
SSL11-PE24M10
Furin + + - -
Reducing - + - +
kD
75
50
37
25
20

FIG. 2B

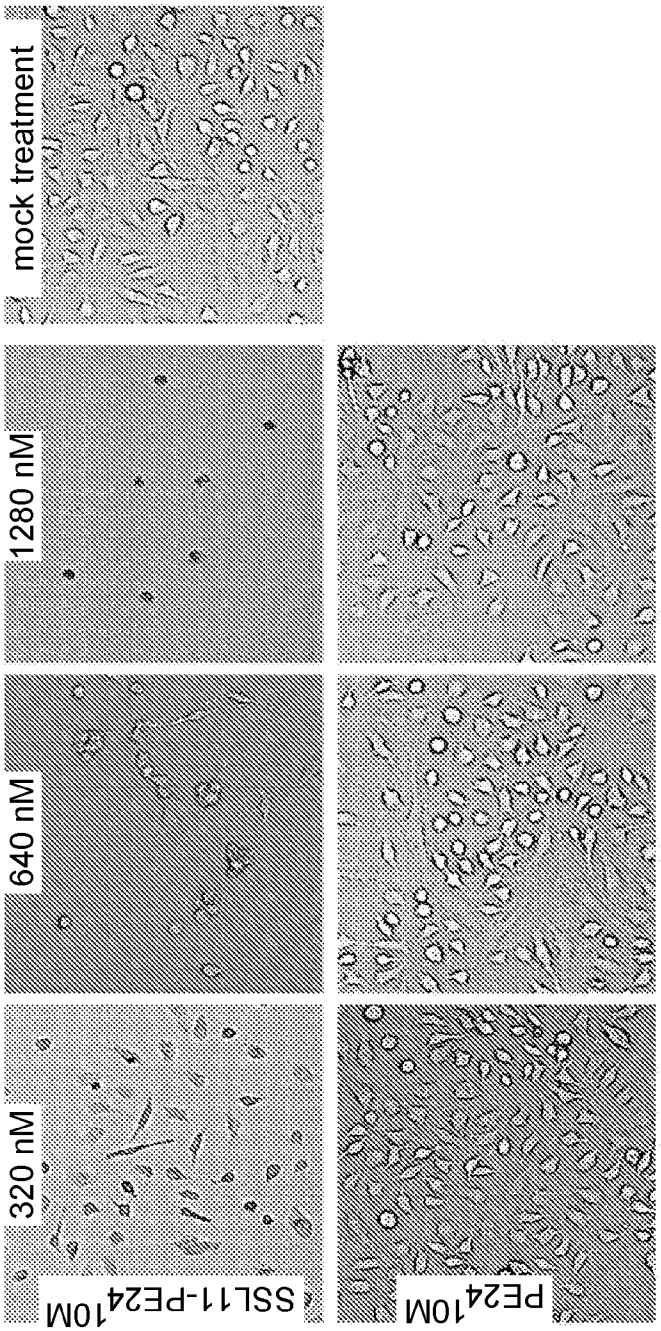

FIG. 3A

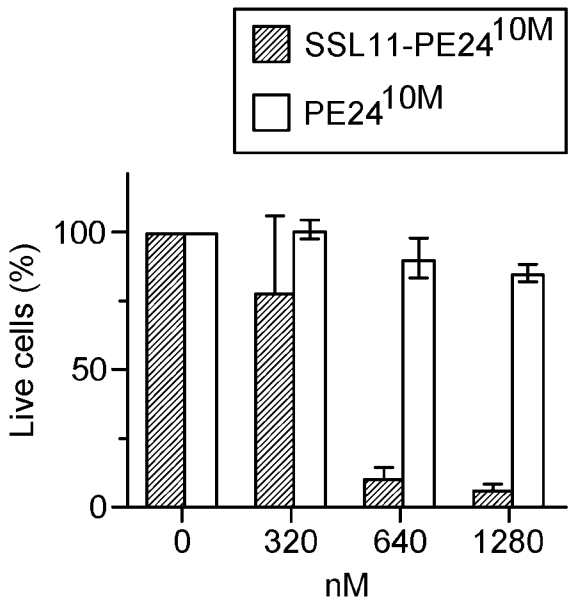

FIG. 3B

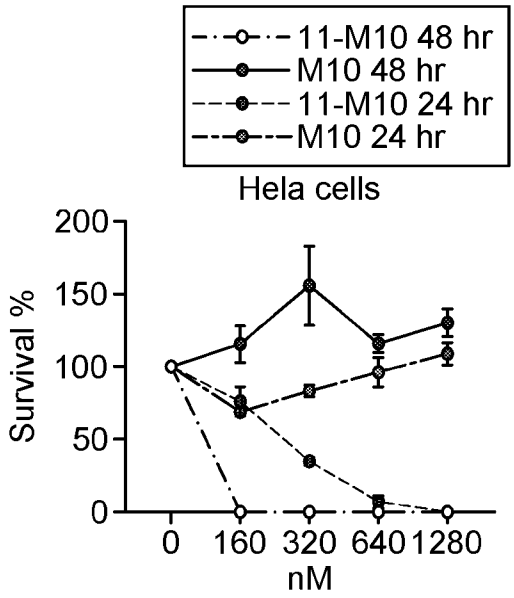
11-M10 48 hr
M10 48 hr
11-M10 24 hr
M10 24 hr
Hela cells
Survival %
200
150
100
50
0
0
160
320
640
1280
nM

FIG. 4A

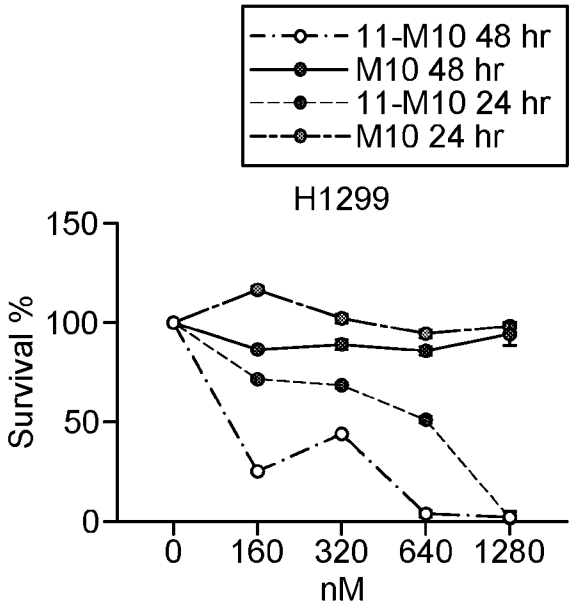
11-M10 48 hr
M10 48 hr
11-M10 24 hr
M10 24 hr
H1299
Survival %
150
100
50
0
0
160
320
640
1280
nM

FIG. 4B

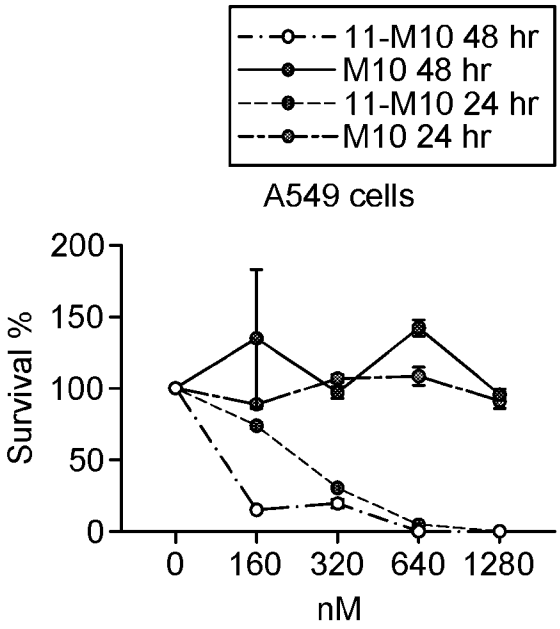
11-M10 48 hr
M10 48 hr
11-M10 24 hr
M10 24 hr
A549 cells
Survival %
200
150
100
50
0
0
160
320
640
1280
nM

FIG. 4C

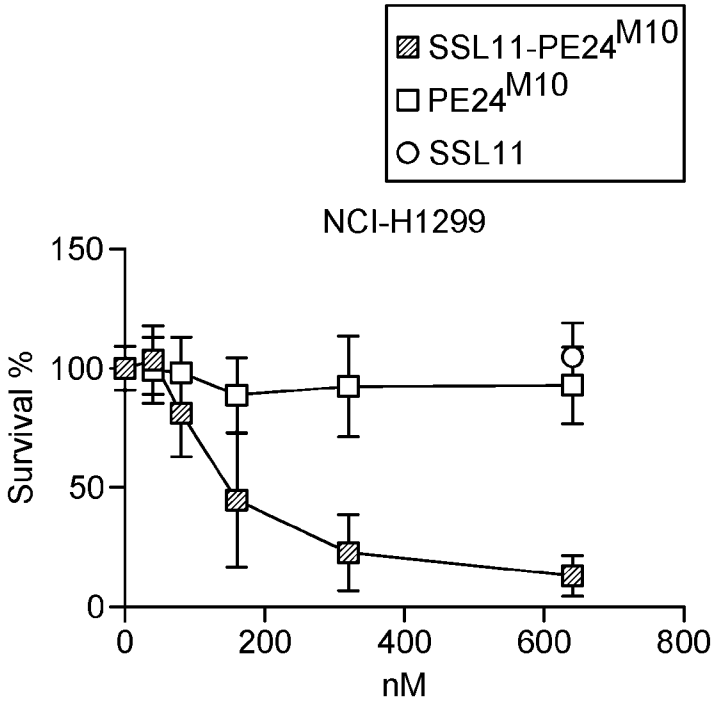
SSL11-PE24M10
PE24M10
SSL11
NCI-H1299
Survival %
150
100
50
0
0
200
400
600
800
nM

FIG. 4D

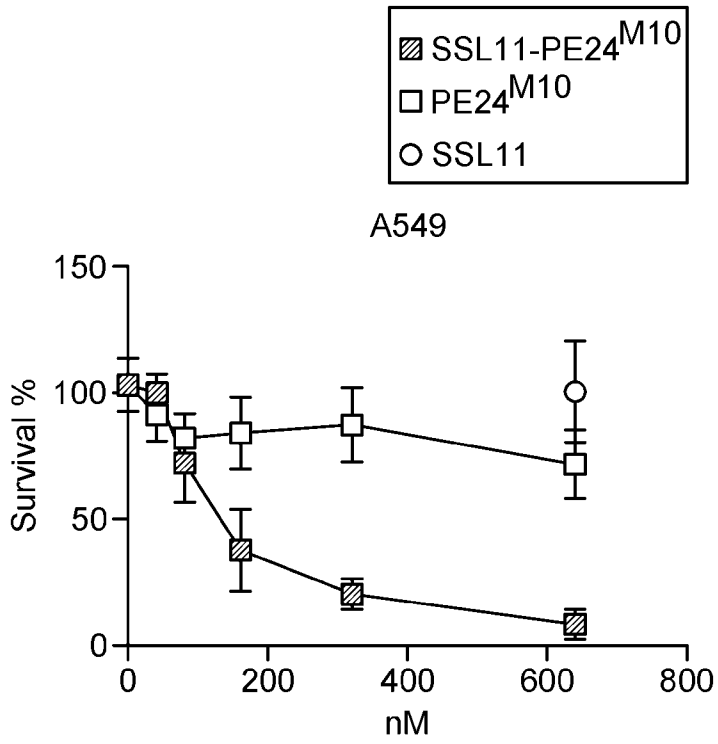
SSL11-PE24M10
PE24M10
SSL11
A549
Survival %
150
100
50
0
0
200
400
600
800
nM

FIG. 4E

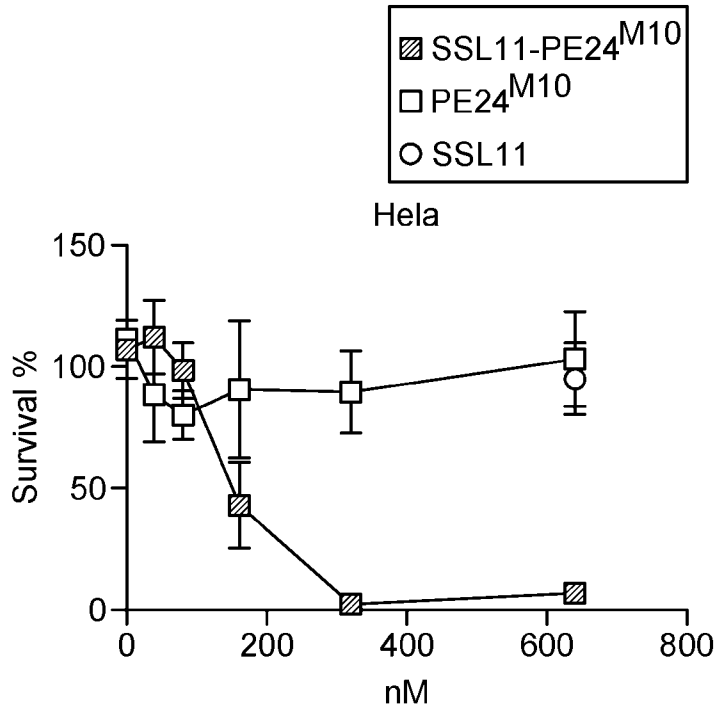
SSL11-PE24M10
PE24M10
SSL11
Hela
Survival %
150
100
50
0
0
200
400
600
800
nM

FIG. 4F

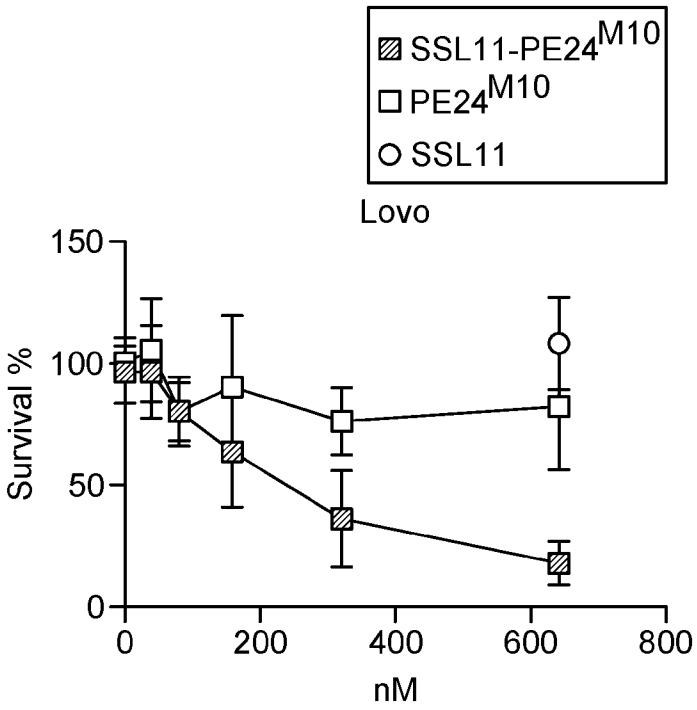
SSL11-PE24M10
PE24M10
SSL11
Lovo
150
100
50
0
Survival %
0
200
400
600
800
nM

FIG. 4G

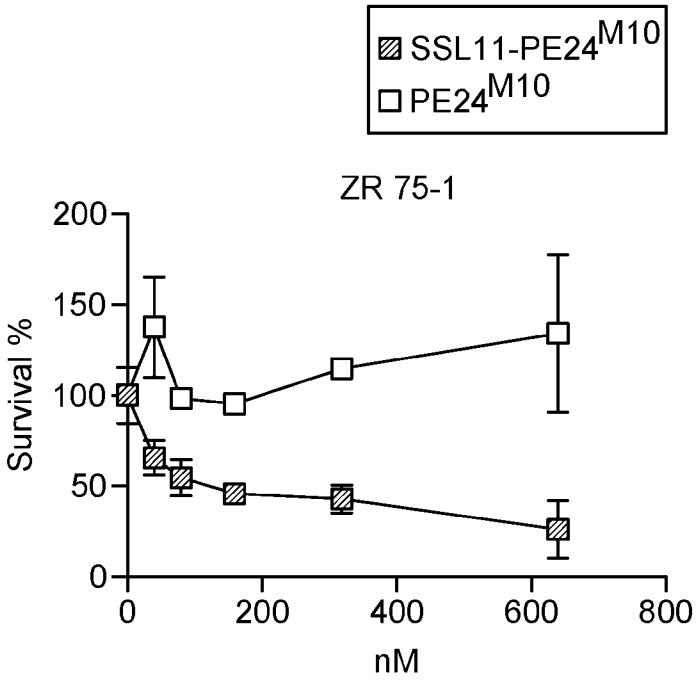
SSL11-PE24M10
PE24M10
ZR 75-1
200
150
100
50
0
Survival %
0
200
400
600
800
nM

FIG. 4H

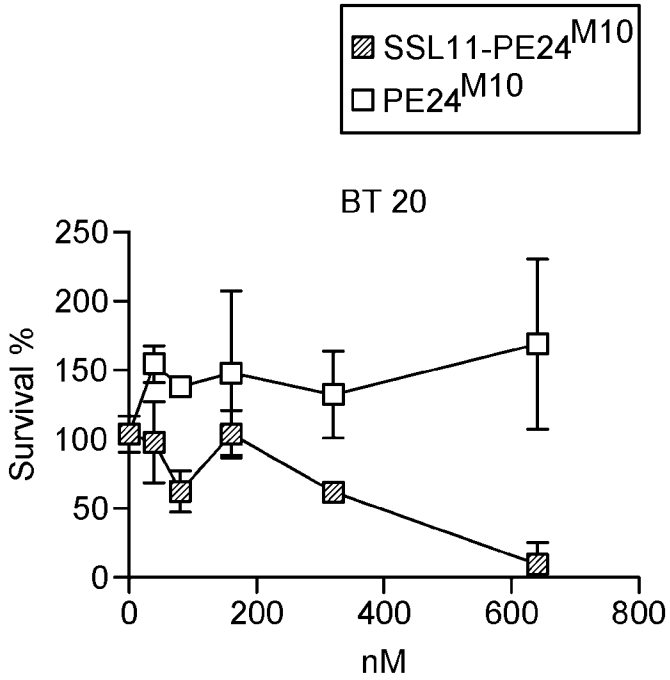
SSL11-PE24M10
PE24M10
BT 20
Survival %
250
200
150
100
50
0
0
200
400
600
800
nM

FIG. 4I

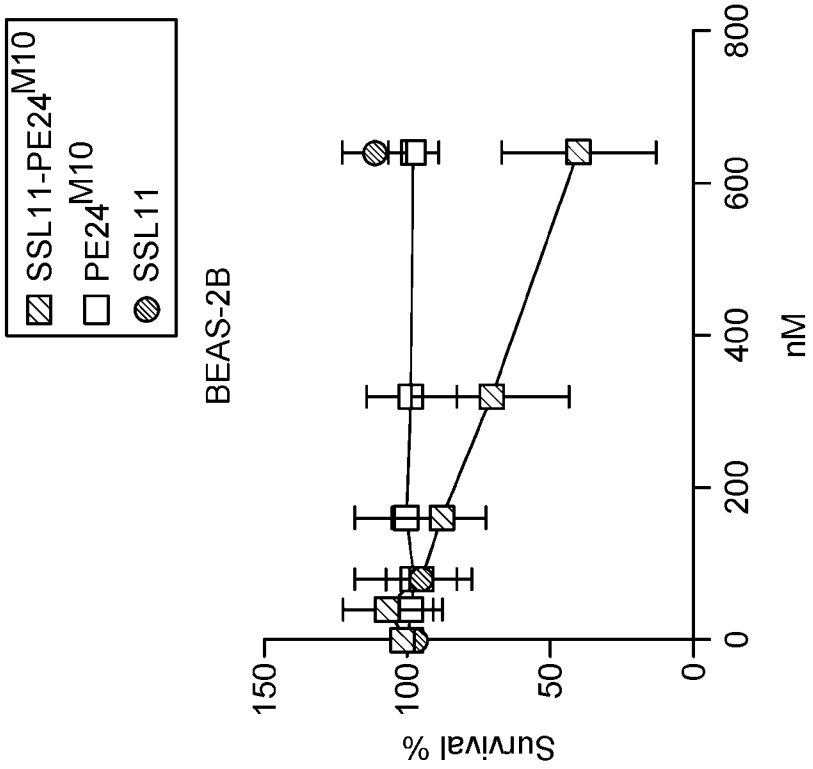
SSL11-PE24M10
PE24M10
SSL11
BEAS-2B
Survival %
150
100
50
0
0
200
400
600
800
nM

FIG. 5A

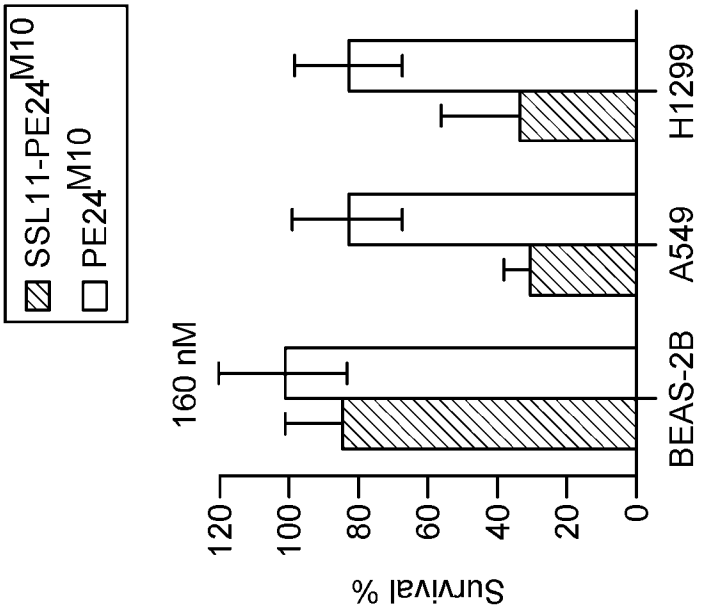
SSL11-PE24M10
PE24M10
160 nM
Survival %
120
100
80
60
40
20
0
BEAS-2B
A549
H1299

FIG. 5B

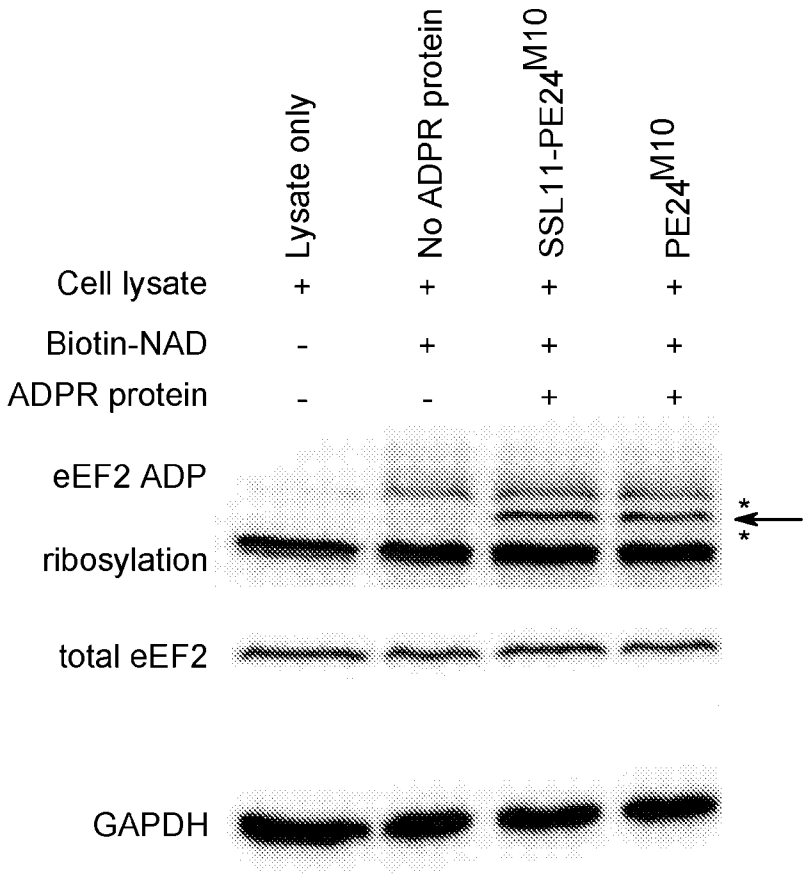
Lysate only
No ADPR protein
SSL11-PE24M10
PE24M10
Cell lysate + + + +
Biotin-NAD - + + +
ADPR protein - - + +
eEF2 ADP ribosylation
total eEF2
GAPDH

FIG. 6

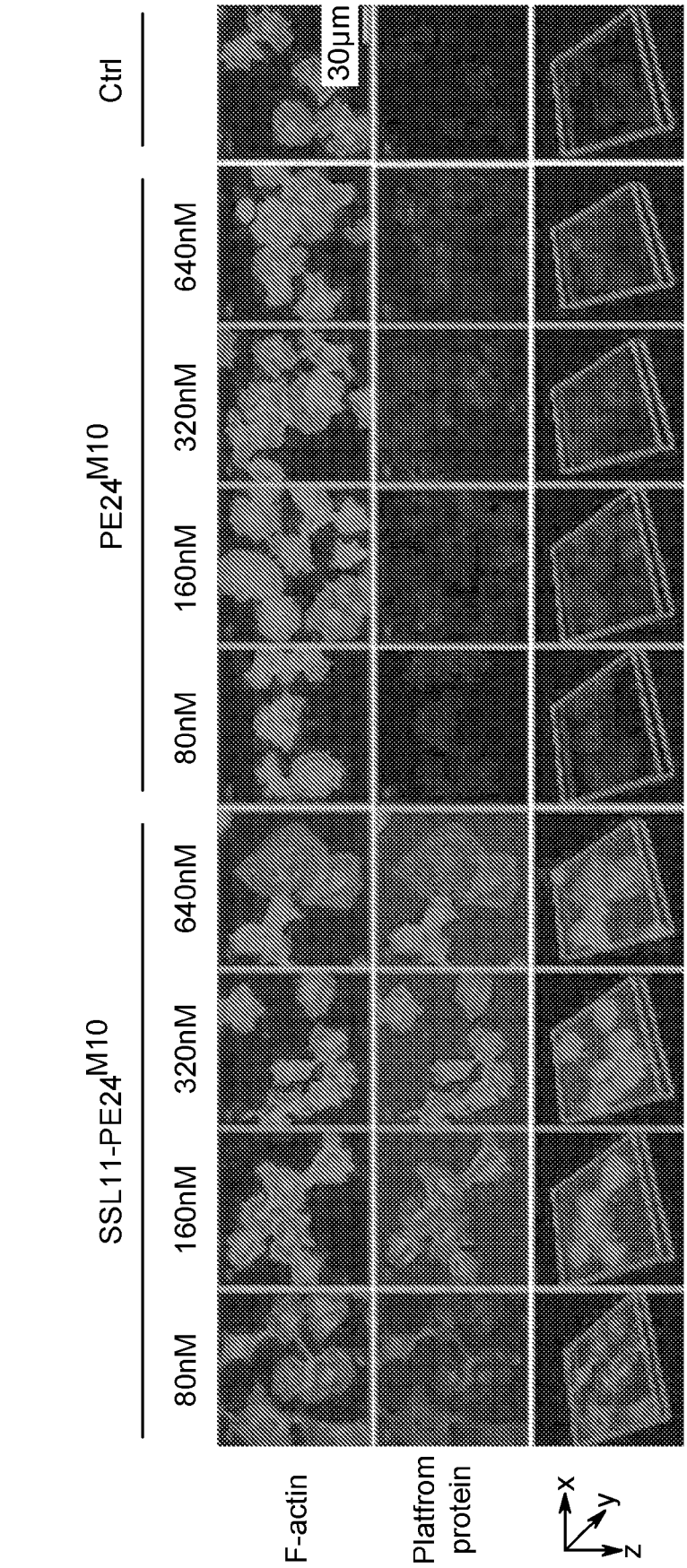
SSL11-PE24M10
80nM
160nM
320nM
640nM
PE24M10
80nM
160nM
320nM
640nM
Ctrl
30μm
F-actin
Platfrom protein
x
y
z

FIG. 7A

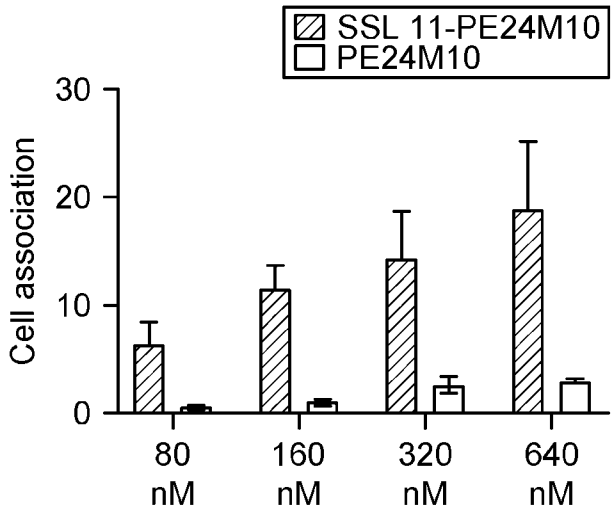
SSL 11-PE24M10
PE24M10
Cell association
30
20
10
0
80 nM
160 nM
320 nM
640 nM

FIG. 7B

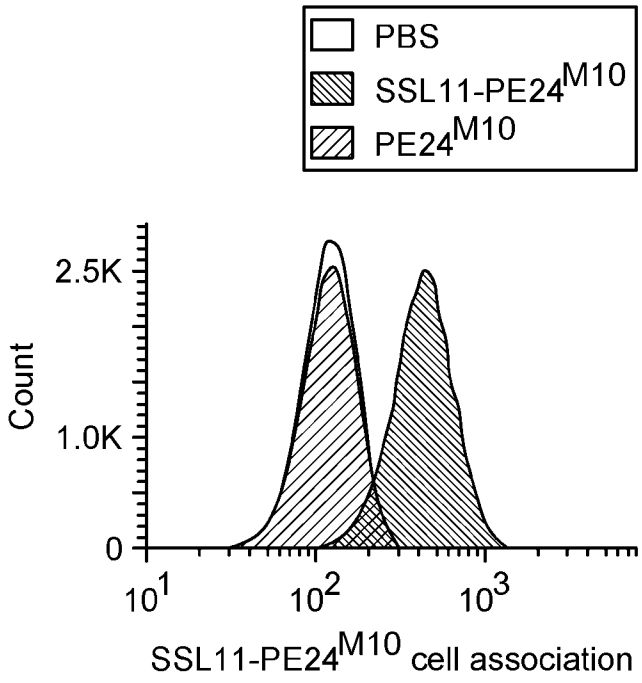
PBS
SSL11-PE24M10
PE24M10
Count
2.5K
1.0K
0
10^1
10^2
10^3
SSL11-PE24M10 cell association

FIG. 7C

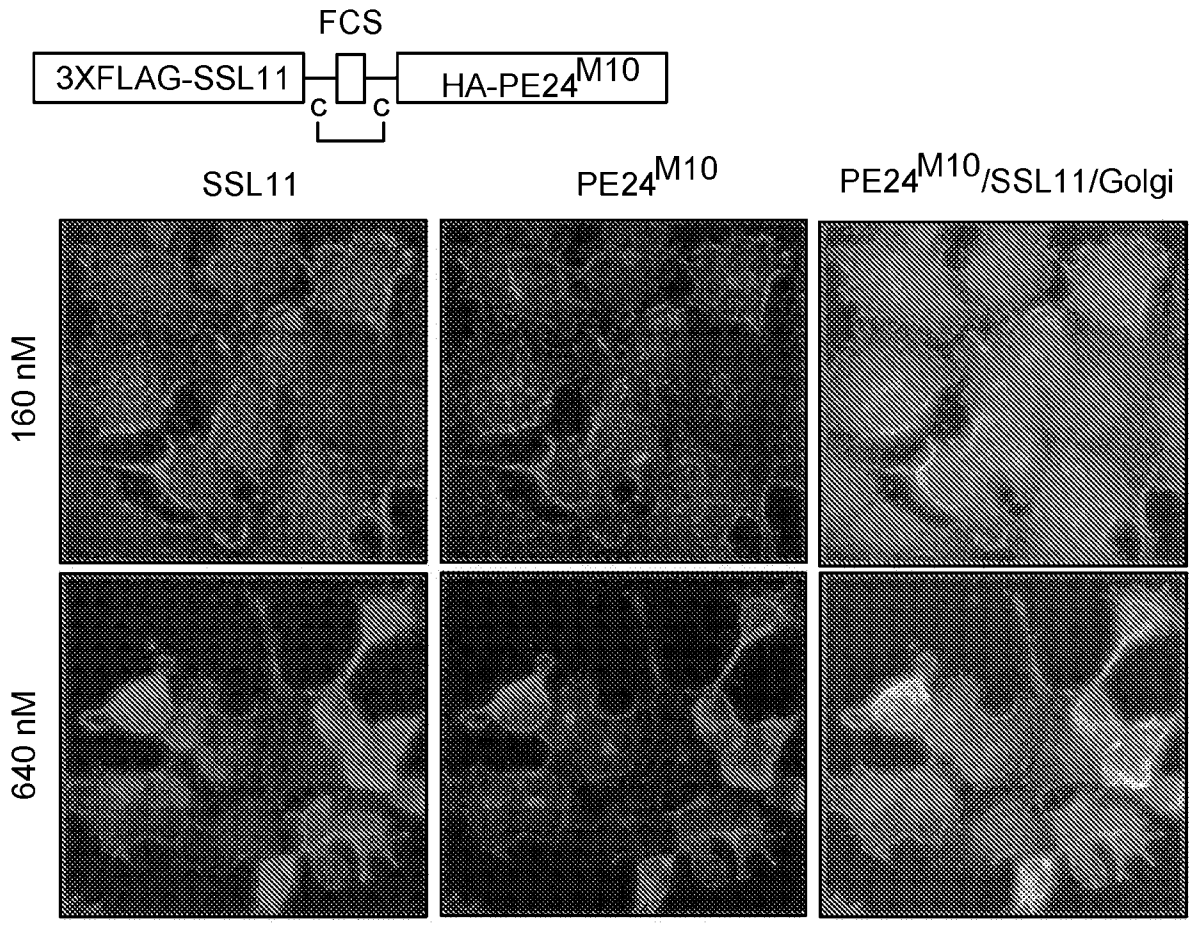
FCS
3XFLAG-SSL11
HA-PE24M10
C
C
SSL11
PE24M10
PE24M10/SSL11/Golgi
160 nM
640 nM

FIG. 8

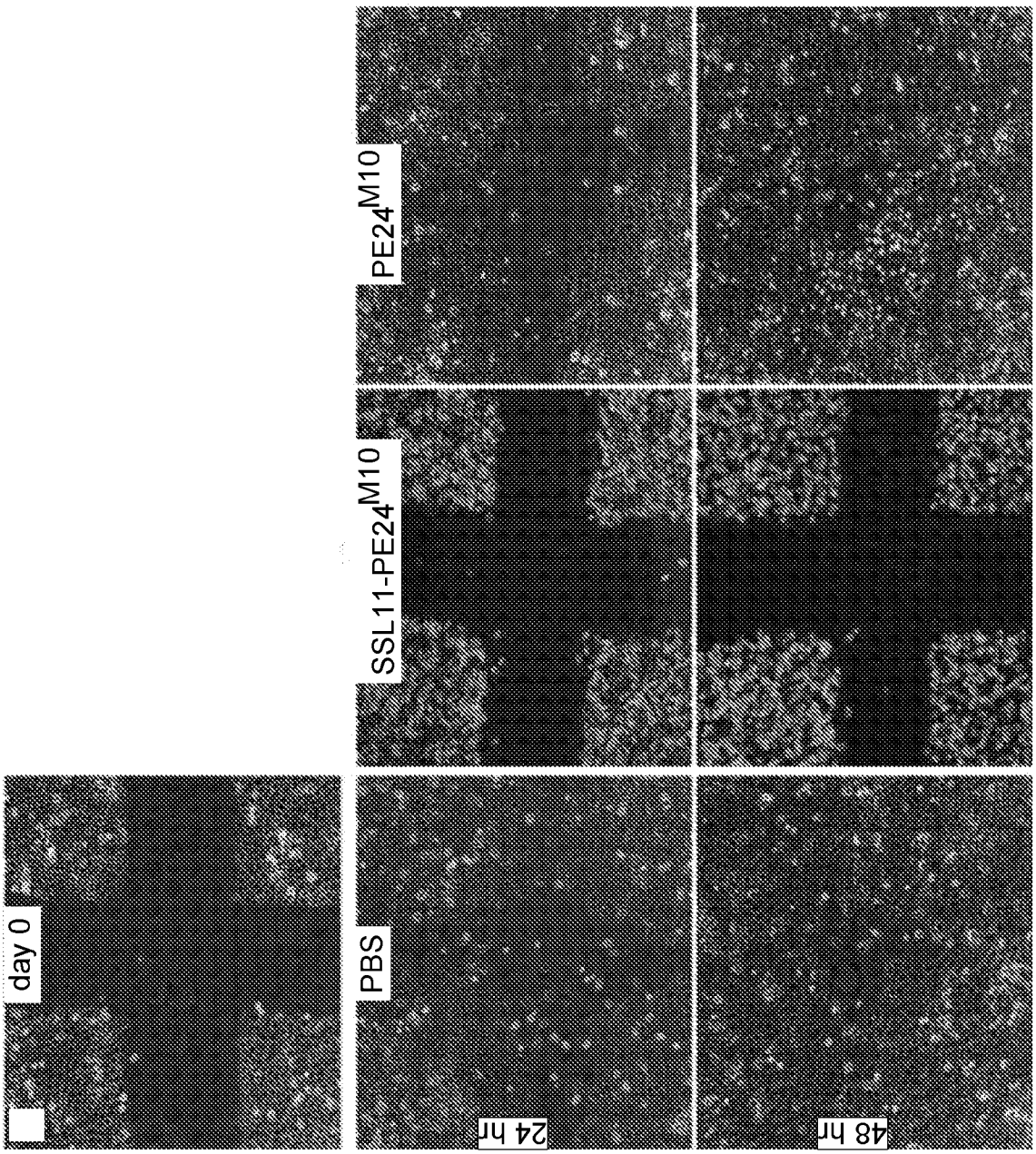

FIG. 9A

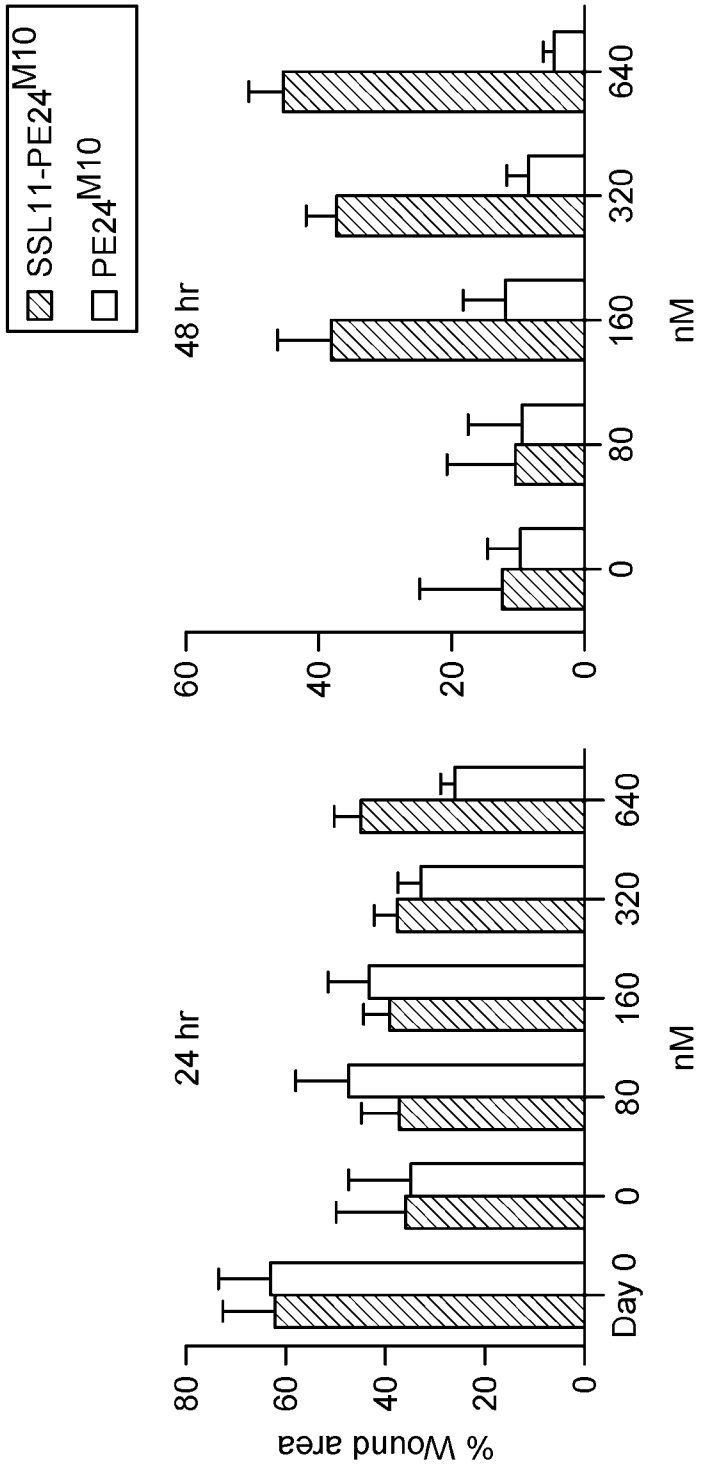
SSL11-PE24M10
PE24M10
% Wound area
0
20
40
60
80
Day 0
0
80
160
320
640
nM
24 hr
0
20
40
60
0
80
160
320
640
nM
48 hr

FIG. 9B

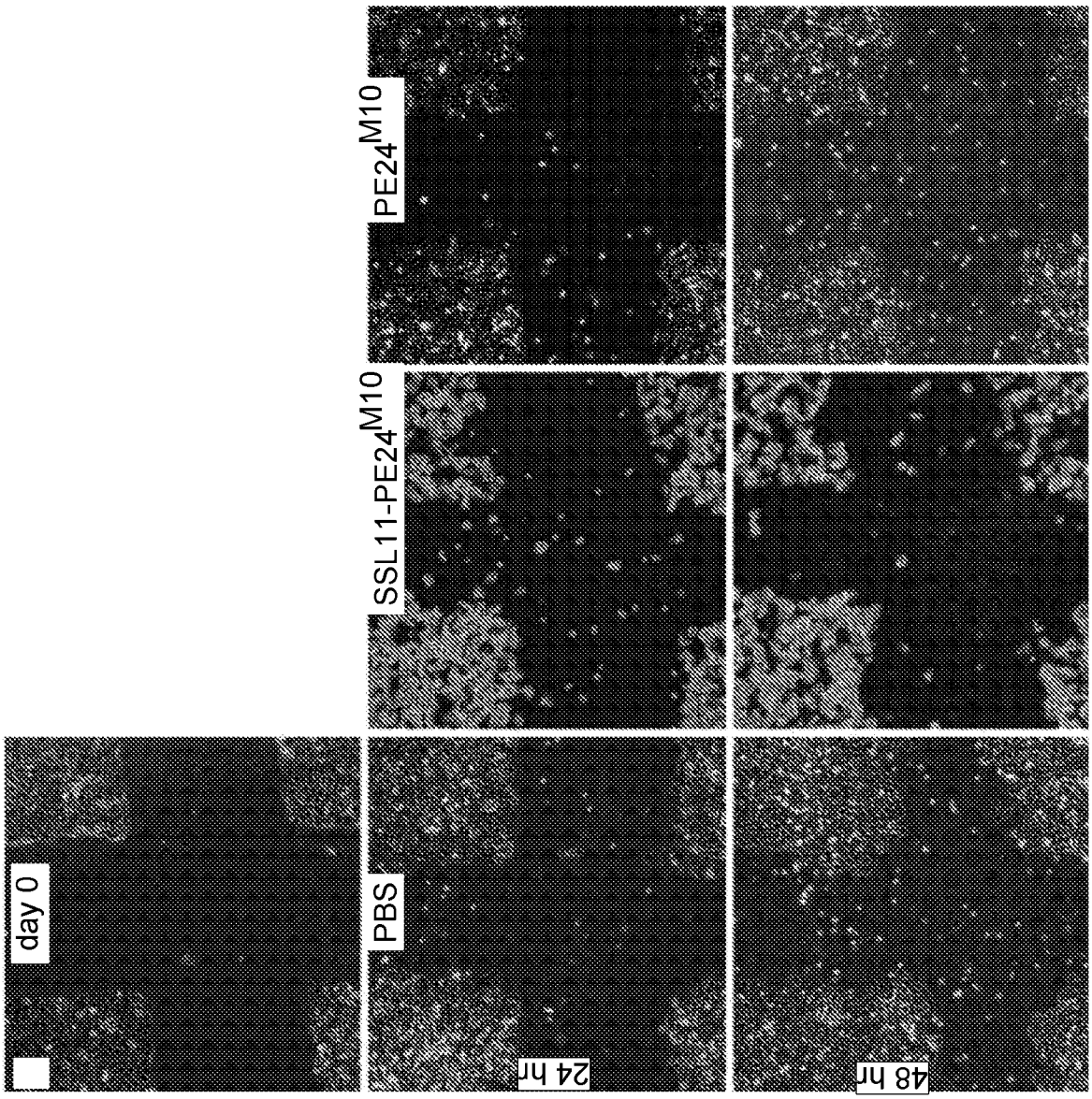

FIG. 9C

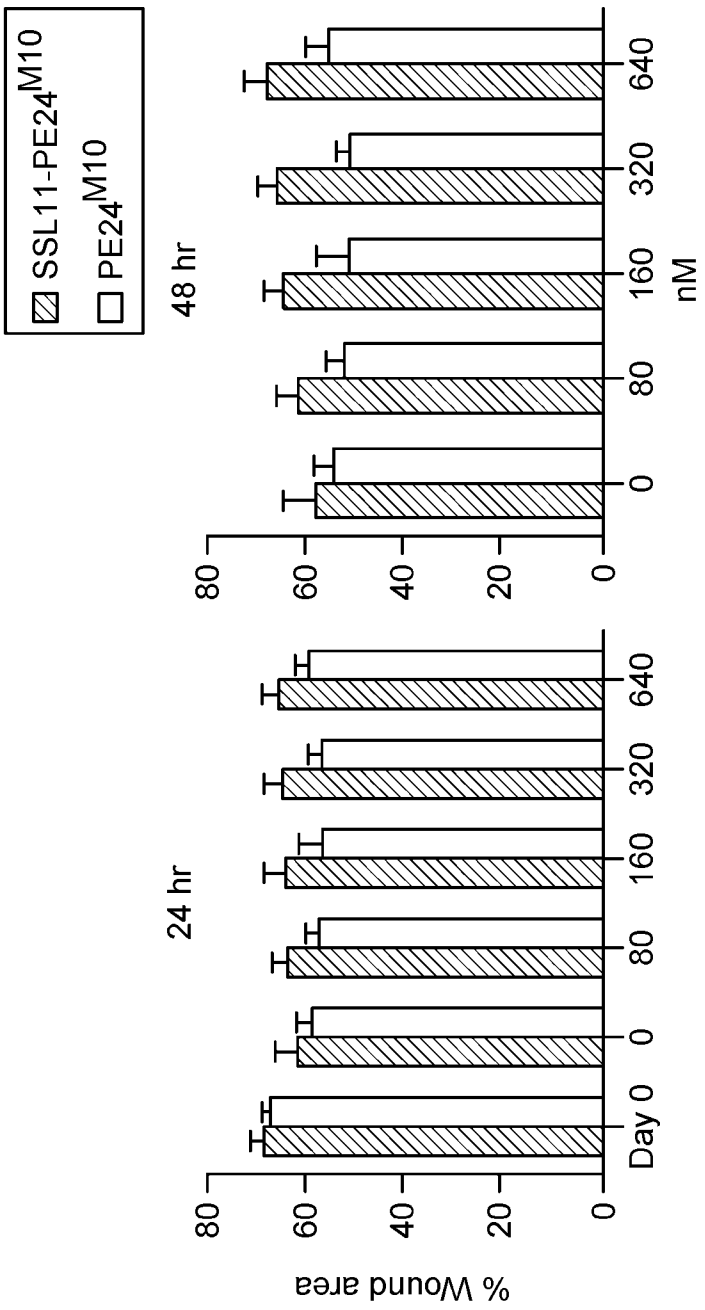
SSL11-PE24M10
PE24M10
% Wound area
24 hr
48 hr
Day 0
0
80
160
320
640
nM
0
20
40
60
80

FIG. 9D

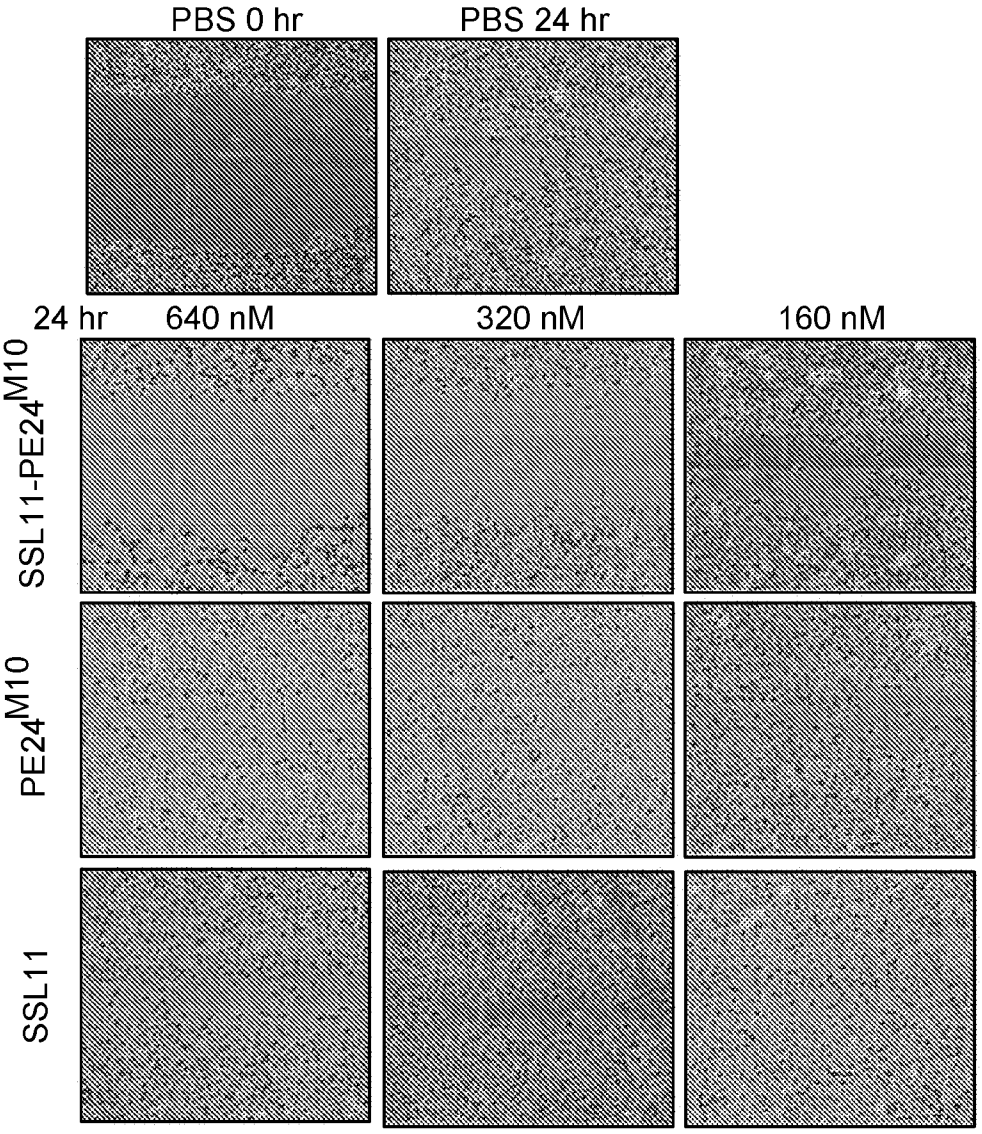

FIG. 10A

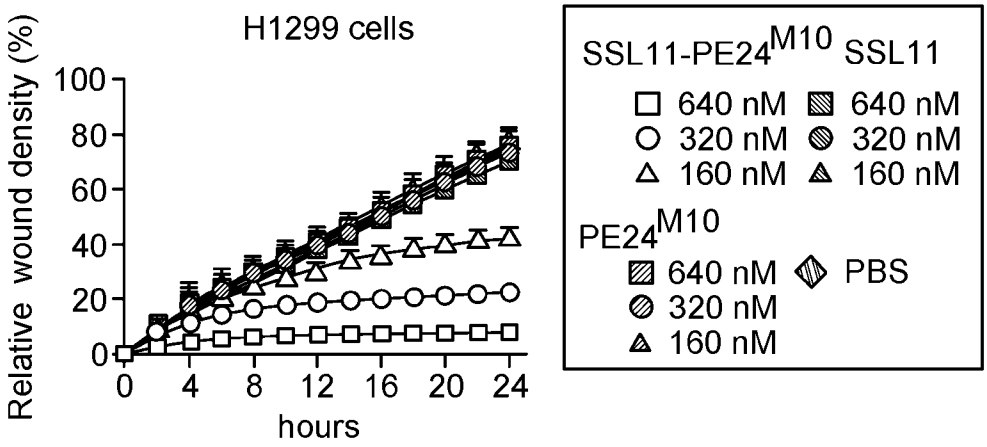

FIG. 10B

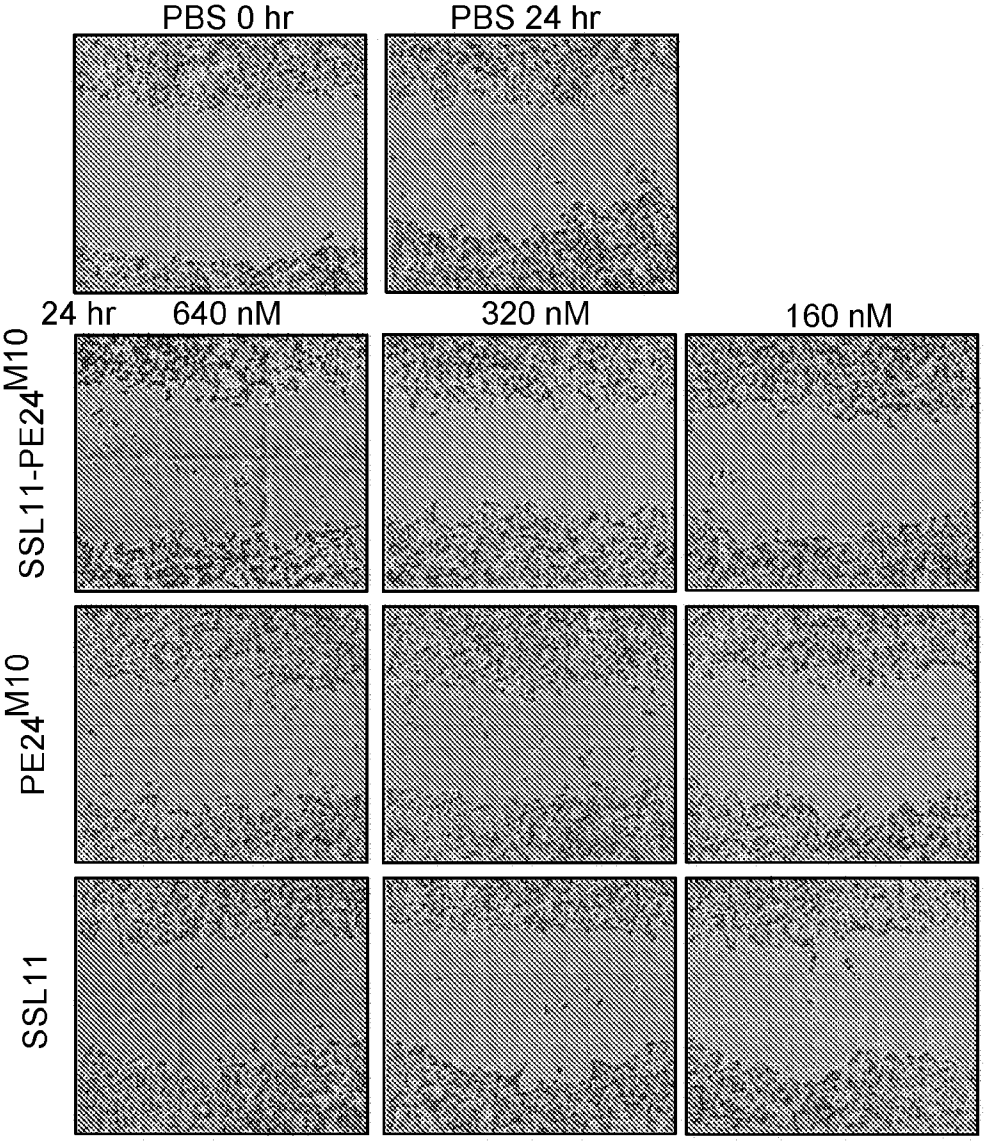

FIG. 11A

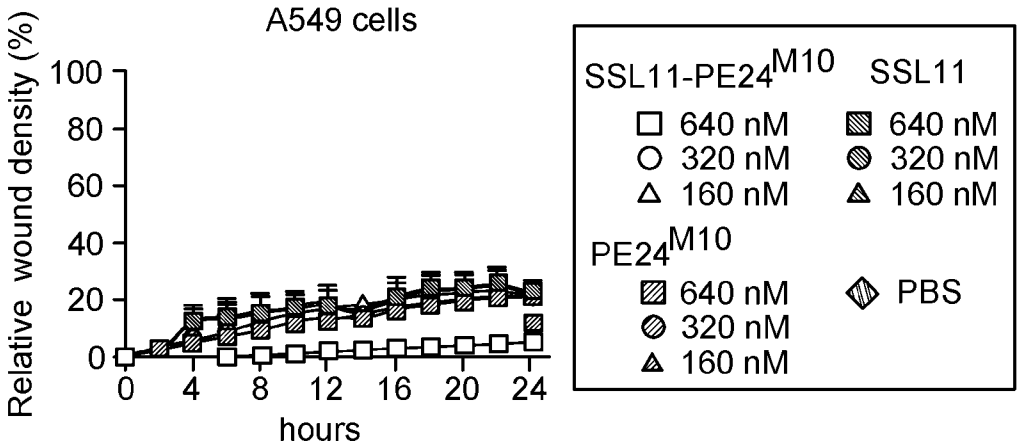

FIG. 11B

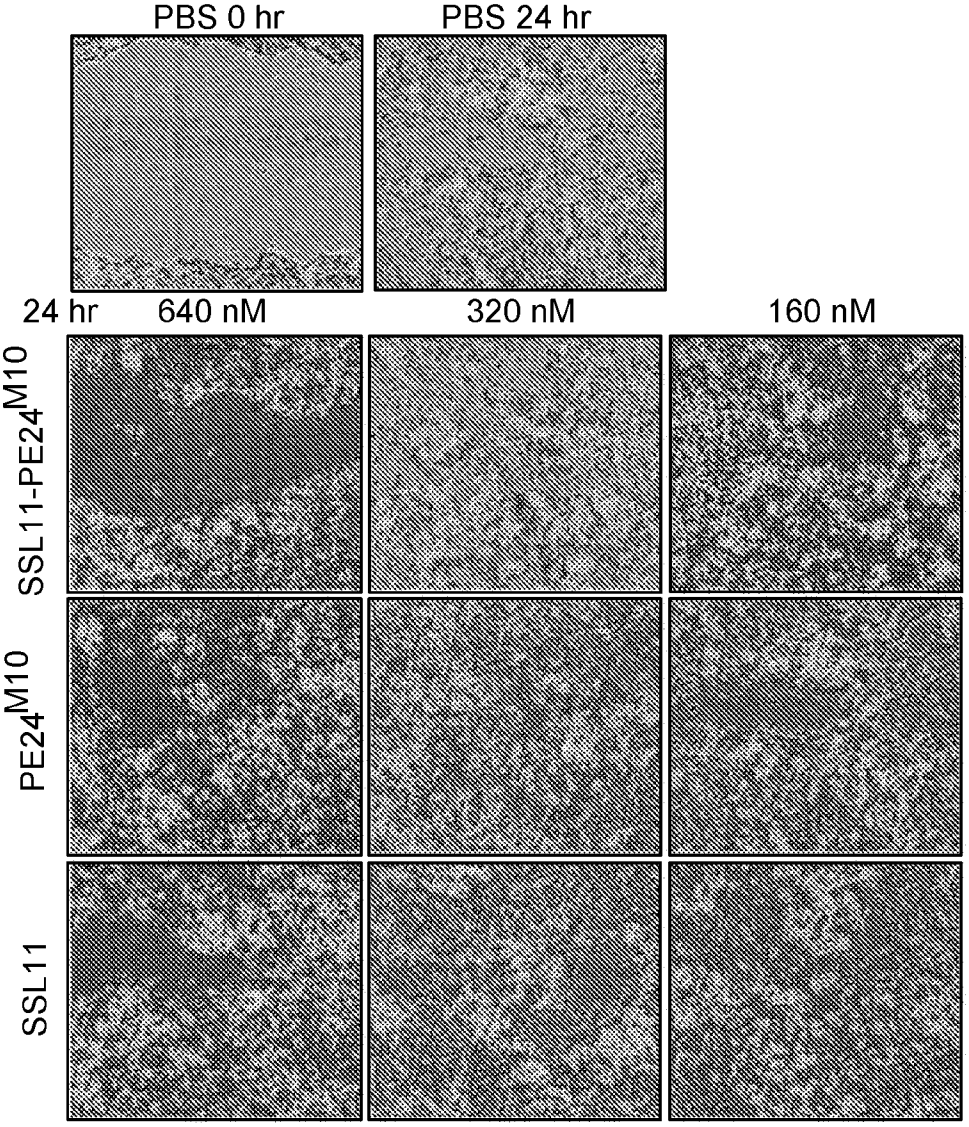

FIG. 12A

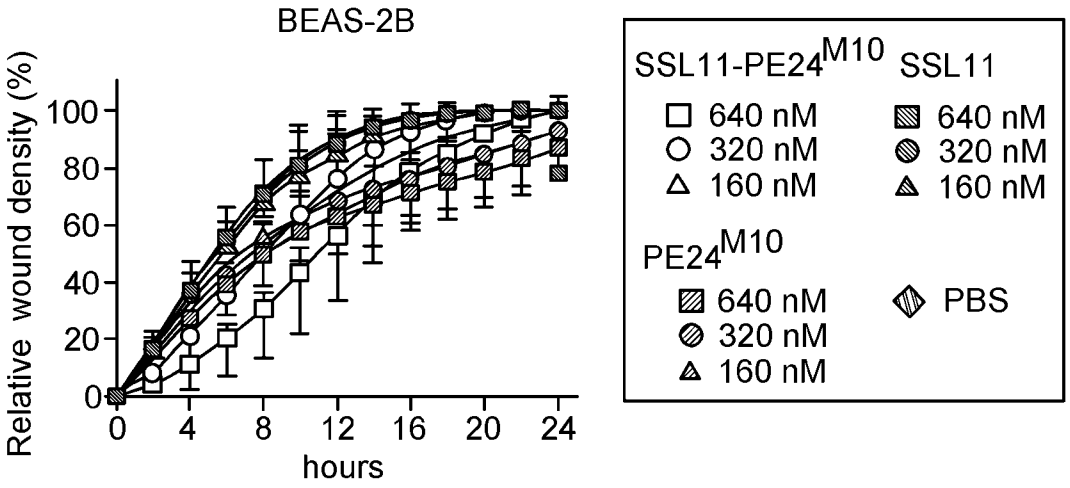

FIG. 12B


153
193
SSL 8 KGRIVINMKDENKYEIDLSDKLDFERMADVINSEQIKNIEV
SSL 9 KGRIVINMKDEKKHEIDLSEKLSFERMFDVMDSKQIKNIEV
SSL 7 YGKITINLKDGEKQEIDLGDKLQFERMGDVLNSKDINKIEV
SSL 10 DGRVKISLKDGSFYNLDLRSKLKFKYMGEVIESKQIKDIEV
SSL 3 - GTIVIKMKNGGKYTFELHKKLQEHRMA---GTNIDNIEV
SSL 4 -GKIVIKMKNGGKYTFELHKKLQENRMADVIDGTNIDNIEV
SSL 2 - GKITVKKKYYGKYTFELDKKLQEDRMSDVINVTDIDRIEI
SSL 6 KGKIVVKMEDDKFYTFELTKKLQPHRMGDTIDGTKIKEINV
SSL 5 DSKIKVIMKDGGYYTFELNKKLQTNRMSDVIDGRNIEKIEA
SSL 11 DSKIRITMKDGGFYTFELNKKLQTHRMGDVIDGRNIEKIEV
SSL 1 QGQITITMNDGTTHTIDLSQKLEKERMGESIDGTKINKILV
SSL 13 KGQIKITG-ADNNYTIDLSKRLPSTDANRYKKPQNAKIEV
SSL 14 KGQIKITA-DGNNYTIDLSKKLKLTDTNRYVKNPRNAEIEV
SSL 12 KGKLKITG-GGNNYTIDLSKRLHSDLANVYVKNPNKITVDV
Sialyl Lewis X (sLeX) binding pocket

FIG. 13

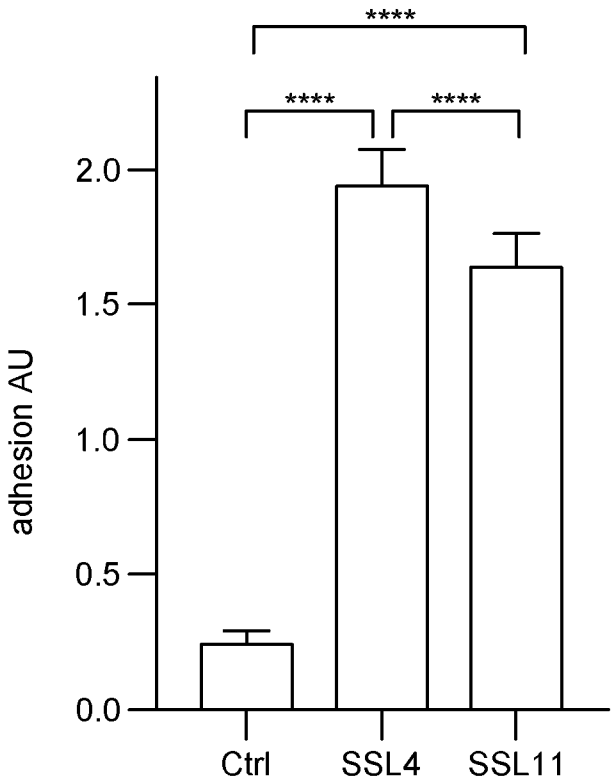

FIG. 14