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(54) Title: PEPTIDES FOR CANCER TREATMENT

(57) Abstract: The invention pertains to cancer treatment or prevention field. Particularly, the invention provides peptide fragments from SLIT2 protein and their applications in inhibition of cancer growth, invasion and metastasis.

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PEPTIDES FOR CANCER TREATMENT

Field of the Invention

[0001] The invention pertains to cancer treatment or prevention. Particularly, the invention provides peptide fragments from SLIT2 protein and their applications in inhibition of cancer growth, invasion and metastasis.

Background of the Invention

[0002] Anti-cancer peptides have gained increased attention in recent years. However, most conventional anti-cancer drugs have low bioactivity and high toxicity. Therefore, there is a need to develop an anti-cancer bioactive peptide having high bioactivity and low toxicity.

Summary of the Invention

[0003] The present disclosure provides a peptide comprising an amino acid sequence as described herein. Certain embodiments include the peptide comprising an amino acid sequence of FHIVELLA or FHAVELLA. Other certain embodiments include cyclic peptide, pegylated peptide and fluorenylmethyloxycarbonyl-peptide as described herein.

[0004] The present disclosure provides a pharmaceutical composition, comprising a peptide of as described herein.

[0005] The present disclosure provides a method for inhibiting cancer growth, invasion and/or metastasis, comprising administering an effective amount of a peptide as described herein.

[0006] The present disclosure provides a method for preventing, treating, or ameliorating a cancer, comprising administering an effective amount of a peptide as described herein.

Brief Description of the Drawings

[0007] Figure 1 shows the effect of alanine substitution of Fmoc-LT1-3 (LT1-3) on cell proliferation.

[0008] Figure 2 shows the effect of base substitutions of Fmoc-LT-A3 (LT-A3) on cell proliferation,

[0009] Figure 3 shows dose effect of Fmoc- LT-peptides (LT-peptides) in growth inhibition in CL1-5 cells.

[0010] Figure 4 shows growth inhibition activity of Fmoc-LT1-3 and Fmoc-LT-A3 in various lung cancer lines.

[0011] Figures 5 (A) to (C) show dose effect of Fmoc-LT1-3 (A), Fmoc-LT-A3 (B) and Fmoc-LT-A3-PEG3 (C) on invasion inhibition.

[0012] Figure 6 shows early tumor formation rate in mice treated with different amount of LT1-3 peptide at day 18.

[0013] Figure 7 shows the effect of Fmoc-LT1-3 on growth of tumor in xenograft model.

[0014] Figure 8 shows size of tumor treated with different levels of LT1-3 peptide.

[0015] Figure 9 shows relative organ weight of animal treated with different levels of Fmoc-LT1-3.

[0016] Figure 10 shows the effect of Fmoc-LT1-3 on lung metastasis of CL1-5 cells injected through tail vein.

[0017] Figure 11 shows survival proportions of control, CDDP, LT1-3, CDDP+LT1-3 for maintenance therapy and CDDP+LT1-3 for combined therapy.

[0018] Figure 12 shows the effect of LT-1, -3, -4, -5 on invasion inhibition of CL1-5 cells.

[0019] Figure 13 shows the effect of LT1-3 peptide on cell invasion inhibition in CL1-5 cells.

[0020] Figure 14 shows the effect of LT-A3 peptide on cell invasion inhibition in CL1-5 cells.

[0021] Figure 15 shows the effect of N- or C-terminal modification of C-peptides on cell invasion in CL1-5 cells.

[0022] Figure 16 shows the effect of LT-A3-PEG peptide on cell invasion in CL1-5 cells.

[0023] Figure 17 shows the effect of LT1-3-PEG on cell invasion in CL1-5 cells.

[0024] Figure 18 shows the effect of LT1-3-PEG on cell invasion in A549 cells.

[0025] Figure 19 shows the effect of LT1-3-PEG on cell invasion in H1299 cells.

[0026] Figures 20 (A) and (B) show the effect of N- or C-terminal modification of peptide on cell proliferation in CL1-5 cells; (A), PEG-LT1-3, PEG-LT-A3 and LT-A3-PEG; (B), LT1-3 and LT1-3-PEG.

[0027] Figure 21 shows the effect of LT1-3-PEG on proliferation of various lung cancer cell lines.

[0028] Figure 22 shows LT1-3 does not inhibit growth of Beas2B, HUVECs and MRC5 normal cell lines.

[0029] Figure 23 shows the effect of LT1-3-PEG on proliferation of MCF-7, T-47D , MDA-MB-453 and MDA231 breast cancer cells.

[0030] Figure 24 shows that PKA inhibitor (H89) enhances growth inhibition activity of LT1-3 peptides on CL1-5 cells.

[0031] Figure 25 shows that JNK inhibitor (Sp600125) enhances growth inhibition activity of LT1-3 peptides on CL1-5 cells.

[0032] Figure 26 shows that knockdown of the expression of PKACA enhances LT1-3-PEG-mediated growth inhibition activity on CL1-5 cells.

[0033] Figure 27 shows that knockdown of the expression of JNK1 enhances LT1-3-PEG-mediated growth inhibition activity on CL1-5 cells.

Detailed Description of the Invention

[0034] It will be understood that the various aspects and embodiments described herein are merely intended to provide illustration and do not serve to limit the scope of the claims.

[0035] Articles "a" and "an" are used herein to refer to one or to more than one (i.e. at least one) of the grammatical object of the article.

[0036] Abbreviations for amino acids are used throughout this disclosure and follow the standard nomenclature known in the art. For example, as would be understood by those of ordinary skill in the art, alanine is Ala or A; arginine is Arg or R; asparagine is Asn or N; aspartic acid is Asp or D; cysteine is Cys or C; glutamic acid is Glu or E; glutamine is Gln or Q; glycine is Gly or G; histidine is His or H; isoleucine is Ile or I; leucine is Leu or L; lysine is Lys or K; methionine is Met or M; phenylalanine is Phe or F; proline is Pro or P; serine is Ser or S; theonine is Thr or T; tryptophan is Trp or W; tyrosine is Tyr or Y; and valine is Val or V.

[0037] An amino acid having a hydrophobic side chain includes the non-limiting examples of alanine (A), isoleucine (I), leucine (L), methionine (M), phenylalanine (F), tryptophan (W), tyrosine (Y), and valine (V). An amino acid having a hydrophilic side chain includes the non-limiting examples of glycine (G), asparagine (N), glutamine (Q), serine (S), threonine (T), and cysteine (C). An amino acid having a basic side chain includes the non-limiting examples of histidine (H), lysine (K) and arginine (R). An amino acid having an acidic side chain includes the non-limiting examples of aspartic acid (D), and glutamic acid (E). An amino acid having a positively charged side chain, under typical physiological conditions, includes the non-limiting examples of arginine (R), histidine (H), and lysine (K). An amino acid having a negatively charged side chain, under typical physiological conditions, includes the non-limiting examples of aspartic acid (D) and glutamic acid (E). An amino acid having a polar uncharged side chain

includes the non-limiting examples of serine (S), threonine (T), asparagine (N), and glutamine (Q).

[0038] Unnatural amino acids include, but are not limited to, azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, naphthylalanine ("naph"), aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisbutyric acid, 2-aminopimelic acid, tertiary-butylglycine ("tBuG"), 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethylglycine, N-ethylasparagine, homoproline ("hPro" or "homoP"), hydroxylysine, allo-hydroxylysine, 3-hydroxyproline ("3Hyp"), 4-hydroxyproline ("4Hyp"), isodesmosine, allo-isoleucine, N-methylalanine ("MeAla" or "Nime"), N-alkylglycine ("NAG") including N-methylglycine, N-methylisoleucine, N-alkylpentylglycine ("NAPG") including N-methylpentylglycine. N-methylvaline, naphthylalanine, norvaline ("Norval"), norleucine ("Norleu"), octylglycine ("OctG"), ornithine ("Orn"), pentylglycine ("pG" or "PGly"), pipecolic acid, thioproline ("ThioP" or "tPro"), homoLysine ("hLys"), and homoArginine ("hArg").

[0039] As used herein, "wild-type" (wt) is a term referring to the natural form, including sequence, of a polynucleotide, polypeptide or protein in a species. A wild-type form is distinguished from a mutant form of a polynucleotide, polypeptide or protein arising from genetic mutation(s).

[0040] As used herein, the term "amino acid" refers to natural amino acids, unnatural amino acids, and amino acid analogs, all in their D and L stereoisomers, unless otherwise indicated, if their structures allow such stereoisomeric forms.

[0041] As used herein, the term "peptide" or "oligopeptide" refers to organic compounds composed of amino acids, which may be arranged in either a linear chain (joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues) prior to cyclization, or in a cyclic form or in a constrained (e.g., "macrocycle") form.

[0042] As used herein, the term "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use, including but not limited to metal salts (e.g., sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

[0043] As interchangeably used herein, the terms "individual," "subject," "host," and "patient," refer to a mammal, including, but not limited to, murines (rats, mice), non-human

primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc.

[0044] As used herein, the term "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease, for the purpose of decreasing the risk of developing pathology. The compounds or compositions of the disclosure may be given as a prophylactic treatment to reduce the likelihood of developing pathology or to minimize the severity of the pathology, if developed.

[0045] As used herein, the terms "treatment," "treating," and the like, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0046] As used herein, the term "therapeutically effective amount" or "efficacious amount" refers to the amount of a subject peptide that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment of the disease.

[0047] The present invention surprisingly found that fragments of SLIT2 protein have advantageous effects in inhibiting tumor growth and invasion. Particularly, the present invention found a fragment consisting of 91 amino acids from SLIT2 (ASAIYSVETINDGNFHIVELLALDQSLSLSVDGGNPKIITNLSKQSTLNFDSPLYVGGMP GKSNVASLRQAPGQNGTSFHGCIRNLYINSE) (SEQ ID NO:1), which has the effect in inhibiting both tumor growth and invasion. The present invention unexpected found that the following five overlapping peptide sequences in the SEQ ID NO:1 are effective in tumor growth and/or invasion.

LT-1: ASAIYSVETINDGNFHIVELLA (SEQ ID NO:2)

LT-2: FHIVELLALDQSLSLSVDGGNPKIIT (SEQ ID NO:3)

LT-3: KIITNLSKQSTLNFDSPLYVGG (SEQ ID NO:4)

LT-4: GGMPGKSNVASLRQAPGQNGTSF (SEQ ID NO:5)

LT-5: LRQAPGQNGTSFHGCIRNLYINSE (SEQ ID NO:6)

[0048] LT-1 possesses an inhibitory effect on growth and invasion activity while LT-3, LT-4 and LT-5 have the inhibitory effect on cell invasion only. The N-terminal sequence of LT-2

contains the sequence of LT1-3 (SEQ ID NO:4), and thus it is highly possible that LT-2 has the inhibitory effect on growth and invasion activity.

[0049] Accordingly, the present disclosure provides a peptide comprising an amino acid sequence as shown in SEQ ID NO: 1, or a fragment thereof. In some embodiments, the fragment of the peptide comprises an amino acid sequence as shown in SEQ ID NO:2, 3, 4, 5 or 6.

[0050] The present invention also unexpectedly found that three fragments, a 10-amino acid fragment LT1-1 (ASAIYSVETI (SEQ ID NO:7), a 10-amino acid fragment LT1-2 (ETINDGNFHI (SEQ ID NO:8)) and an 8-amino acid fragment LT1-3 (FHIVELLA (SEQ ID NO:9), can be identified within the 22-amino acid fragment of LT-1 and that LT1-3 has a superior effect in inhibiting both tumor growth and invasion.

[0051] In one aspect, the invention provides a peptide comprising the following Formula I,

$$X_1\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}X_6\text{-}X_7\text{-}X_8 \qquad (I)$$

wherein X_1 is selected from an amino acid having a hydrophobic side chain; X_2 is selected from an amino acid having a basic side chain, an amino acid having an acidic side chain or an amino acid having a hydrophobic side chain; X_3 is selected from an amino acid having a hydrophobic side chain; X_4 is selected from an amino acid having a hydrophobic side chain; X_5 is selected from an amino acid having a basic side chain, an amino acid having an acidic side chain or an amino acid having a hydrophobic side chain; X_6 is selected from an amino acid having a hydrophobic side chain; X_7 is selected from an amino acid having a hydrophobic side chain or an amino acid having a hydrophilic side chain; and X_8 is selected from an amino acid having a hydrophobic side chain or an amino acid having a hydrophilic side chain;
or a modified peptide or a salt thereof.

[0052] In some embodiments, X_1 is selected from F, A, Y and W; X_2 is selected from H, K, R, A and E; X_3 is selected from I or A; X_4 is selected from V and A; X_5 is selected from E, D, H, Orn, A and H; X_6 is selected from L and A; X_7 is selected from L and S and X_8 is selected from A and S; or a modified peptide or a salt thereof.

[0053] In some embodiments, the peptide comprises FHIVELLA (LT1-3) (SEQ ID NO:9), FHAVELLA (LT-A3) (SEQ ID NO:10), FKAVELLA (LT-K2) (SEQ ID NO:11), FRAVELLA (LT-R2) (SEQ ID NO:12), FHAVDLLA (LT-D5) (SEQ ID NO:13), FHAVHLLA (LT-H5) (SEQ ID NO:14), FHAVOLLA (LT-Orn5) (SEQ ID NO:15), AHAVELLA (LT-A13) (SEQ ID NO:16), FHAAELLA (LT-A34) (SEQ ID NO:17), FHAVEALA (LT-A36) (SEQ ID NO:18), FHAAEALA

(LT-A346) (SEQ ID NO:19), FAIVALLA (LT-A25) (LT-A2) (SEQ ID NO:20), FEAVHLLA (LT-E2H5) (SEQ ID NO:21), YHAVELLA (LT-Y1) (SEQ ID NO:22), WHAVELLA (LT-W1) (SEQ ID NO:23), FHAVELSA (LT-S7) (SEQ ID NO:24), FHAVELLS (LT-S8) (SEQ ID NO:25), **DGN**FHIVELLA (LT-11AA) (SEQ ID NO:26), **A**HIVELLA (LT-A1) (SEQ ID NO:27), FAIVELLA (LT-A2) (SEQ ID NO:28), FHI**A**ELLA (LT-A4) (SEQ ID NO:29), FHIV**A**LLA (LT-A5) (SEQ ID NO:30), FHIVE**A**LA (LT-A6) (SEQ ID NO:31), FHIVEL**A**A (LT-A7) (SEQ ID NO:32), or a peptide comprising an amino acid sequence having one or several amino acid substitutions, deletions and/or additions in the amino acid sequence set forth in any of SEQ ID NOs: 9 to32, and having an activity equal to or higher than that/those of the amino acid sequence set forth in any of SEQ ID NOs: 9-32; or a modified peptide or a salt thereof.

[0054] In further embodiments, the peptide of the present disclosures is FHAVELLA (SEQ ID NO:10); or a modified peptide or a salt thereof.

[0055] The peptide according to the present disclosure encompasses a peptide comprising an amino acid sequence having one or several amino acid substitutions, deletions and/or additions in the amino acid sequence set forth in Formula (I) (hereinafter also referred to as a "modified peptide"). It is a widely known fact for one of ordinary skill in the art that a peptide consisting of an amino acid sequence having one or several amino acid substitutions, deletions and/or additions in a certain amino acid sequence can maintain the biological activity of the original peptide.

[0056] In this context, in a case where one or several amino acids are substituted with other amino acids, the properties of amino acid side chains are preferably conserved before and after substitution. The properties of amino acid side chains include hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), amino acids having an aliphatic side chain (G, A, V, L, I, P), amino acids having a hydroxy group-containing side chain (S, T, Y), amino acids having a sulfur atom-containing side chain (C, M), amino acids with a side chain having carboxylic acid or amide (D, N, E, Q), amino acids having a base-containing side chain (R, K, H), amino acids having an aromatic-containing side chain (H, F, Y, W) (each of the letters in parenthesis represents the one-letter notation for amino acids.

[0057] Further, the homology between a modified peptide and the original peptide is preferably 60% or more, more preferably 75% or more, even more preferably 85% or more, in particular preferably 95% or more, and most preferably 98% or more.

[0058] The peptides of the present disclosure can be modified to improve solubility or stability. In some embodiments, the modified peptide is a cyclic peptide, pegylated peptide or fluorenylmethyloxycarbonyl-peptide. For example, it is possible to arbitrarily add a set of polar residues or PEG (polyethylene glycol) to the peptide of the present disclosure to improve its solubility. The PEG can be added to the C terminal or N terminal of the peptide of the present disclosure. In one embodiment, the PEG is added to the N- or C- terminal of the peptide of the present disclosure. In some embodiments, the modified peptide is a PEG-peptide, wherein PEG binds to N terminal or C terminal of the peptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos:9 to 32. In an embodiment, the PEG-peptide is PEG-LT1-3 or PEG-LT-A3 (i.e. PEG binds to C terminal or N terminal of the peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:9 or 10).

[0059] In one embodiment, a fluorenylmethyloxycarbonyl (Fmoc) is added to the peptide of the present disclosure to improve the stability. In some embodiments, the modified peptide is a Fmoc-peptide, wherein Fmoc binds to N terminal or C terminal of the peptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos:9 to 32. In an embodiment, the Fmoc-peptide is Fmoc-LT1-3 or Fmoc-LT-A3 (i.e. Fmoc binds to N terminal of the peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:9 or 10).

[0060] The peptides can be produced using any means for making polypeptides known in the art, including, e.g., synthetic and recombinant methods. Because of their relatively small size, the peptides of the present disclosures may be directly synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. For example, in some embodiments the peptides can be synthesized using synthetic chemistry techniques such as solid-phase synthesis, Merrifield-type solid-phase synthesis, t-Boc solid-phase synthesis, Fmoc solid-phase synthesis, BOP solid-phase synthesis, and solution-phase synthesis. In other embodiments, the peptides can be produced, for example, by expressing the peptide from a nucleic acid encoding the peptide in a cell or in a cell-free system according to recombinant techniques familiar to those of skill in the art. The peptides can incorporate any of the various modifications and protective groups known to those of skill in the art.

[0061] In one embodiment, the modified peptide of the present disclosure is in a cyclic form. In one embodiment, the modified peptide of the present disclosure is a cyclic that is formed by adding one or more hydrophilic amino acid, optionally one or more hydrophobic amino acid, at the N terminal of the peptide. In one embodiment, the cyclic peptide comprises two hydrophilic amino acids and one hydrophobic amino acid bonding to the N terminal of the peptide of the present disclosure.

[0062] In one embodiment, the peptide of the present disclosure is a cyclic form of the peptide of Formula I. In a further embodiment, the present disclosure provides a cyclic peptide having the following Formula II,

$$X_{a1}\text{-}X_{a2}\text{-}X_{a3}\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}X_6\text{-}X_7\text{-}X_8 \quad (II)$$

wherein

X_{a1} bonds to X_8 as head to tail cyclization, and X_{a1} is D; X_{a2} is G; X_{a3} is N; and X_1, X_2, X_3, X_4, X_5, X_6, X_7 and X_8 are each an amino acid as defined herein

[0063] The peptide compound having a cyclic portion according to the present disclosure refers to a compound formed by the amide bonds or ester bonds of amino acids and/or amino acid analogs and has a cyclic portion resulting from covalent bond-mediated cyclization such as amide bond or carbon-carbon bond formation reaction.

[0064] The modified peptide compound having a cyclic portion according to the present disclosure is not particularly limited as long as the peptide is cyclized at the cyclic site. The posttranslational cyclization site is required to be a cyclization unit that forms functional groups providing for the compatibility of membrane permeability and metabolic stability (druglikeness). Any such cyclization method can be used without particular limitations. Examples of such methods include amide bond formation from carboxylic acid and amine and carbon-carbon bond formation using a transition metal as a catalyst, such as Suzuki reaction, Heck reaction and Sonogashira reaction. Thus, the peptide compound of the present disclosure contains at least one set of functional groups capable of such bond formation reaction.

[0065] The cyclic portion in the peptide compound of the present disclosure is preferably, for example, a cyclic portion formed by cyclization by chemical reaction after translational synthesis. Also, the cyclic portion is preferably a cyclic portion that can be formed even under reaction conditions not influencing nucleic acids such as RNA or DNA after translation.

[0066] In a further embodiment, the cyclic peptide of the present disclosure comprises an amino acid sequence of **DGN**FHIVELLA (SEQ ID NO:26), wherein D bonds to A as head to tail cyclization (Cyclic-LT-11AA).

[0067] The peptides of the present disclosure exhibit inhibition of both of tumor growth and invasion and thus can be used to treat a cancer.

[0068] In another aspect, the present disclosure provide a pharmaceutical composition, comprising a peptide described herein. In one embodiment, the composition comprises a second anti-cancer agent. For example, the second anti-cancer agent includes, but is not limited to, erlotinib, afatinib, gefitinib, bevacizumab, ramucirumab, gefitinib, lapatinib, erlotinib, cisplatin, paclitaxel, methotrexate, cyclophosphamide, ifosfamide, chlorambucil, carmustine, carboplatin, vincristine, vinblastine, thiotepa, lomustine, semustine, 5-fluorouracil, corticosteroids, calcineurin inhibitors, NSAIDs, inhibitors of 5-lipoxygenase, and cytarabine.

[0069] Some embodiments relating to pharmaceutical compositions for therapeutic or prophylactic treatment provide for formulations specific for any of mucosal (oral, nasal, inhalation, rectal, vaginal, tracheal, etc.), parenteral, topical, or local administration. In some embodiments, the pharmaceutical compositions are suitably administered parenterally, e.g., intravenously, subcutaneously, intradermally, or intramuscularly. The pharmaceutical compositions of the present disclosures can be formulated according to conventional methods (for example, Remington's Pharmaceutical Science, latest edition, Mark Publishing Company, Easton, U.S.A.), and may also contain pharmaceutically acceptable carriers and additives. Examples include, but are not limited to, surfactants, excipients, coloring agents, flavoring agents, preservatives, stabilizers, buffers, suspension agents, isotonic agents, binders, disintegrants, lubricants, fluidity promoting agents, and corrigents, and other commonly used carriers can be suitably used. Specific examples of the carriers include light anhydrous silicic acid, lactose, crystalline cellulose, mannitol, starch, carmellose calcium, carmellose sodium, hydroxypropyl cellulose, hydroxypropyl methylcellulose, polyvinylacetal diethylaminoacetate, polyvinylpyrrolidone, gelatin, medium-chain triglyceride, polyoxyethylene hardened castor oil 60, saccharose, carboxymethyl cellulose, corn starch, inorganic salt, and such.

[0070] In another aspect, the present disclosure provides a method for preventing, treating, or ameliorating a cancer, comprising administering an effective amount of a peptide described herein to a subject.

[0071] In another aspect, the present disclosure provides a method for inhibiting cancer growth, invasion and/or metastasis, comprising administering an effective amount of a peptide described herein to a subject.

[0072] In one embodiment, the method of the present disclosure further comprises a step of administering a second anti-cancer agent as described herein. In another embodiment, the peptide of the present disclosures and the second anti-cancer agent are administered concurrently or separately.

[0073] For example, the cancers treatable by the peptides, compositions and methods of the present disclosure include but are not limited to the group consisting of: neuroblastoma; bile duct cancer; lung cancer; non-small cell lung carcinoma; hepatocellular carcinoma; head and neck squamous cell carcinoma; squamous cell cervical carcinoma; lymphoma; nasopharyngeal carcinoma; gastric cancer; colon cancer; uterine cervical carcinoma; gall bladder cancer; prostate cancer; breast cancer; testicular germ cell tumors; colorectal cancer; glioma; thyroid cancer; basal cell carcinoma; gastrointestinal stromal cancer; hepatoblastoma; endometrial cancer; ovarian cancer; pancreatic cancer; renal cell cancer, Kaposi's sarcoma, chronic leukemia, sarcoma, rectal cancer, throat cancer, melanoma, colon cancer, bladder cancer, mastocytoma, mammary carcinoma, mammary adenocarcinoma, pharyngeal squamous cell carcinoma, testicular cancer, gastrointestinal cancer, or stomach cancer and urothelial cancer.

[0074] Subjects to be treated by the methods described herein encompass mammalian subjects, including both human subjects and non-human (animal) subjects such as dogs, cats, rabbits, goats, horses, pigs, cattle, etc. The term "concurrently administered" as used herein means that two compounds are administered sufficiently close in time to achieve a combined (immunological) effect. Concurrent administration may thus be carried out by sequential administration or simultaneous administration (e.g., simultaneous administration in a common, or the same, carrier).

[0075] In some embodiments, the disclosed peptides and compositions may be administered by any suitable route of administration, including, but not limited to, injection (subcutaneous, intraperitoneal, intravenous, intrathecal, intramuscular, intracerebroventricular, and spinal injection), intranasal, oral, transdermal, parenteral, inhalation, nasopharyngeal or transmucosal absorption. Administration encompasses providing at least one peptide as described herein formulated as a pharmaceutical composition. Pharmaceutically acceptable carriers are well

known to those of skill in the art. Determination of particular pharmaceutical formulations and therapeutically effective amounts and dosing regimen for a given treatment is within the ability of one of skill in the art taking into consideration, for example, patient age, weight, sex, ethnicity, organ (e.g., liver and kidney) function, the extent of desired treatment, the stage and severity of the disease and associated symptoms, and the tolerance of the patient for the treatment.

[0076] In embodiments relating to therapeutic applications, the administration can be performed on a subject already suffering from the disorder of interest. Those in the incubation phase or the acute phase of the disease can be treated by the methods described herein, either alone or in conjunction with other treatments, as suitably based on the particular disease/condition, patient, and combination. One of skill in the art will be able to determine when a combination treatment is or is not suitable. In therapeutic methods and uses, the peptides and composition described herein can be administered to a subject in an amount sufficient to treat, or at least partially arrest, symptoms and/or complications. An amount adequate to accomplish this is often referred to as "therapeutically effective dose." Amounts effective for this use will depend in part on the peptide, composition, the manner of administration, the stage and severity of the condition being treated, the age, weight, and general health of the patient, and the judgment of the prescribing physician.

[0077] Without further elaboration, it is believed that one skilled in the art can utilize the present disclosures to its fullest extent on the basis of the preceding description. The following examples are, therefore, to be construed as merely illustrative and not a limitation of the scope of the present disclosures in any way.

Examples

Example 1 Preparation of LT-peptides.

[0078] Dimethyl sulfoxide (DMSO; >99.9%) and γ-cyclodextrin (γ-CD; 2.5 mM in RPMI-1640 (serum free)) were used as solvents to dissolved LT-peptides. LT peptides were dissolved in DMSO or γ-CD to a final 2.5 mM concentration and vortexed for 30 min. The peptide solutions were further diluted to 1 mM, 0.5 mM, and 50 μM using RPMI medium. The LT1-3-PEG peptide can be dissolved in RPMI-1640 directly. The peptide solutions were observed under microscope to examine their solubility.

Example 2 Cell proliferation assay

[0079] The peptides described below were Fmoc modified and dissolved in DMSO and γ-CD and then used in cell proliferation assay. CL1-5 cells were cultured to 80% confluence in RPMI medium containing 10% FBS. After detachment, 3 x 10^4 cells were seeded onto 12-well plates. After 16 h of incubation, cells were either harvested before treatment (designated as 0 h), or treated with LT peptides for 24 h (designated 24 h), and viable cells were counted. Relative fold-change in cell number or percent increase in cell growth (%) was calculated by following formula: (Cell number $_{24h}$ – Cell number $_{0h}$) / (Cell number of control $_{24h}$ – Cell number of control $_{0h}$).

[0080] The results showed that Fmoc-LT-A3 had the best growth inhibition capability. F-moc-LT-A1, LT-A2, LT-A4, LT-A6, and LT-A7 also had better inhibition activity than F-moc-LT1-3 peptide (Figure 1). Further modifications on LT-A3 peptide revealed less growth inhibition activities than Fmoc-LT-A3 peptide (Figure 2). Dose effect experiment also demonstrated that Fmoc-LT-A3 has the best growth inhibition capability (Figure 3). We also determined the effect of Fmoc-LT1-3 and Fmoc-LT-A3 on growth inhibition in different lung cancer cell lines. The results showed that Fmoc-LT1-3 and Fmoc-LT-A3 greatly inhibited CL1-5, H1355 and H460 lung cancer cell lines, partially inhibited H1299 cell line, and had no effect on A549 cell line (Figure 4). The trends of growth inhibition are similar between Fmoc-LT1-3 and Fmoc-A3, suggesting that these two peptides function specifically in growth inhibition.

Example 3 Cell invasion assay.

[0081] Fmoc-LT1-3 peptides were used in cell invasion assay. A 24-well modified Boyden chamber with a polycarbonate membrane (8-μm pore size) was used for in vitro invasion assays. The membrane was coated with 18 μg Matrigel (Becton Dickinson, diluted in RPMI-1640). CL1-5 cells (3×10^4 cells/well) in RPMI medium with various concentrations of peptide were seeded onto the upper chamber. RPMI-1640 medium alone was used in the lower chamber. After 24 h of incubation, the cells were fixed with ice-cold 100% methanol and stained with 10% Giemsa. Following staining, cells on the upper side of the membrane were removed with a cotton swab. The number of invaded cells attached to the lower surface of the polycarbonate membranes was counted under a light microscope. All experiments were performed in triplicate. As shown in Figure 5A and 5B, both Fmoc-LT1-3 and Fmoc-LT-A3 inhibited cell invasion, however Fmoc-LT1-3 had better invasion inhibition capability than Fmoc-LT-A3. Interestingly, C-terminal PEGylation on Fmoc-LT-A3 greatly increased invasive inhibition activity (Figure 5C).

Example 4 The effect of Fmoc-LT1-3 on tumor growth in Xenograft animal model

[0082] Six-week old immune deficient BALB/CAnN.Cg-Foxn1^{nu}/CrlBltw mice were housed in individual ventilated chambers (IVC). A total of 2x 10^6 CL1-5 lung cancer cells in 100 μL PBS were mixed with 100 μL of 10x Matrigel. The lung cancer cells/Matrigel mix was injected subcutaneously to the lower back of the mice. Three days later, the mice were treated with 0, 10, 20, or 30 nmol of Fmoc-LT-1-3 subcutaneously everyday. The Fmoc-LT-1-3 peptide was dissolved in 100% DMSO and diluted to the desired concentration in PBS from day 3 to day 17. On the 18th day, we observed that 80% of control group animal, 40% of 10 nmol group animal, 20% of 20 nmol group animal, and 40% of 30 nmol group animal developed tumor. Percent of tumor free animal increased with increasing level of Fmoc-LT1-3 between 10 nmol and 20 nmol. However, 30 nmol group did not exhibit further increased percent of tumor free animal (Figure 6). This phenomenon may be due to lower solubility of Fmoc-LT1-3 at higher concentration. We thus decided to replace the solvent from DMSO with γ-CD, and diluted the peptide to the desired concentration for injection on the 18th day and thereafter. The volume of tumor was measured periodically. The results showed that 20 nmol of Fomc-LT1-3 has greatest effect on inhibiting tumor growth (Figure 7). Although 10 nmol and 30 nmol of Fmoc-LT1-3/DMSO had the same initial tumor developing rate by day 18 as shown in Figure 6, 30 nmol of Fmoc-LT1-3/γ-CD treatment group significant suppressed tumor growth after replacement the solvent with γ-CD (Figure 7). On the 39th day, animals were sacrificed. The tumor and organs were weighted, fixed and embedded in paraffin. Embedded tissue were sectioned and stained with hematoxylin-eosin for histologic analysis. As shown in Figure 8, 20 nmol and 30 nmol of Fmoc-LT-1-3 significantly inhibited tumor weight when compared to the vesicle control group. Figure 9 shows no significant differences of the relative weight of organs between treatment groups and the control group. The histologic analysis did not reveal obvious toxicity in animals when treated with Fmoc-LT1-3.

Example 5 The effect of Fmoc-LT1-3 peptide on metastasis of lung cancer

[0083] Five-weeks old immune deficient BALB/CAnN.Cg-Foxn1^{nu}/CrlBltw mice were housed in IVC. CL1-5 cells were pretreated with 200 μM Fmoc-LT1-3 for 24 h and then cultured in fresh medium containing Fmoc-LT1-3 peptide 6 h before cell harvest. CL1-5 cells were detached, centrifuged, and resuspended in PBS containing 200 μM Fmoc-LT1-3 peptide for injection. Thirty minutes before tail vein injection, the mice were also treated with 20 nmol

Fmoc-LT1-3 subcutaneously. A total of $1x10^6$ CL1-5 cells were intravenously injected into the mice. The second day after tail vein injection, 20 nmol of Fmoc-LT1-3/0.5% DMSO or Fmoc-LT1-3/γ-CD was subcutaneously injected into mice daily. By day 56, animals were sacrificed, and all organs were examined for metastasis formation. The metastatic nodules on the surface of lungs were counted under dissecting microscope. Organs were fixed and embedded in paraffin. Embedded tissue was sectioned and stained with hematoxylin-eosin for histologic analysis. As shown in Figure 10, Fmoc-LT1-3 significantly inhibited metastasis of CL1-5 cells via tail vein injection in both solvents.

Example 6 Therapy of Fmoc-LT1-3 peptide and cisplatin

[0084] CL1-5 cells were washed, detached, and resuspended in PBS. Subsequently, a single-cell suspension containing $2x10^6$ cell in 80 μL PBS was mixed with 20 μL 10x Matrigel (Corning, 5117012). The cell suspension was subcutaneously injected into the upper back of the immune deficient BALB/CAnN.Cg-Foxn1nu/CrlBltw mice. The tumors were left to grow for 10 days, during which 90% of mice generated tumors. The tumor-bearing mice were divided into five groups (n=8/group). The average tumor volume was 120-140 mm^3 among five groups: Control, Fmoc-LT1-3, cisplatin, maintenance treatment and combined treatment. On day 13, the mice in the Fmoc-LT1-3 group were subcutaneously injected with 20 nmol Fmoc-LT1-3/γ-CD in the lower back daily. The mice in the cisplatin group were administered an intraperitoneal injection (IP) of 4 mg/kg cisplatin every week, 4 times in total. The mice in maintenance treatment group received IP of cisplatin 4 times and 20 nmol of Fmoc-LT1-3/γ-CD daily at the third IP injection of cisplatin. The mice in the combined treatment group were administered IP of cisplatin weekly 4 times and 20 nmol Fmoc-LT1-3/γ-CD daily. During the course of each treatment, the tumor volume was measured every 3 days. The mortality rates of mice were recorded, which included mice with tumor volume over 1500 mm^3. The percent of survival rate is recorded in Figure 11 and the median survival of each group is calculated in Table 1. The results showed that the median survival of vesicle control group was 27.5 days, cisplatin only and Fmoc-LT1-3 only groups extended median survival to 37.5 days, the combination treatment with cisplatin and Fmoc-LT1-3 further extended median survival to 47.5 days, and the maintenance treatment group also extended median survival to 44 days. Interestingly, Fmoc-LT1-3 seemed to alleviate cisplatin-induced toxicity as we observed that mice died after cisplatin injection in cisplatin only group and the first two cycles of maintenance groups but not in the

combination treatment group (Figure 11). In addition, the mice in combination treatment were more active and healthier than the cisplatin only group. Thus, Fmoc-LT1-3 not only inhibits tumor growth and metastasis but also reduces toxicity of cisplatin and significantly enhances animal survival in combination treatment with cisplatin.

Table 1. Fmoc-LT1-3 peptide elongates animal survival in combination with cisplatin treatment and in maintenance therapy.

	Con	CDDP	LT	Combination treatment	Maintenance treatment
Median survival (day)	27.5	37.5	37.5	47.5	44.0

Example 7 Cell proliferation and invasion assays of the fragments within LT (SEQ ID NO:1)

[0085] The cell proliferation assay and invasion assay of the peptide fragments LT-1, LT-2, LT-3, LT-4 and LT-5 at the concentrations of 0 μM, 12.5 μM, 25 μM and 50 μM were assayed according to the method described in Examples 2 and 3. LT-1 possesses an inhibitory effect on growth and invasion activity while LT-3, LT-4 and LT-5 have the inhibitory effect on cell invasion only. Due to cyclization effect during peptide synthesis, no full-length LT-2 peptide was obtained for final analysis. However, the N-terminal sequence of LT-2 contains the sequence of LT1-3, and thus it is highly possible that LT-2 has the inhibitory effect on growth and invasion activity (see Figure 12 and the table below).

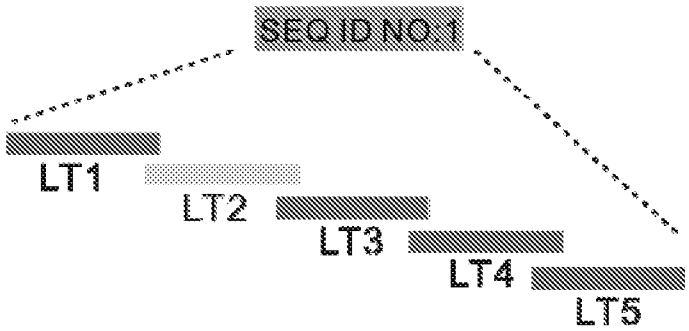


Table 2. The role of LT-1, -3, -4, -5 in growth and invasion inhibition of CL1-5 cells.

	Growth inhibition	Invasion inhibition
LT-1	Yes	Yes
LT-3	No	Yes
LT-4	No	Yes
LT-5	No	Yes

Example 8 The effect of LT-peptides on cell invasion in CL1-5 lung cancer cells

[0086] LT1-3 and LT-A3 are hydrophobic. LT-A3 has better growth inhibition activity than LT1-3 (Figure 1), while LT1-3 has better cell invasion inhibition than LT-A3 (Figure 13 and Figure 14). To increase peptide solubility PEG modification was applied on either N- or C-terminal of LT1-3 and LT-A3. The results showed that PEG3 modification on N-terminal of LT-1-3 and LT-A3 peptides (PEG-1-3 & PEG-A3) abolished invasion inhibition activity on CL1-5 cells while C-terminal PEG3 modification of LT-A3 (A3-PEG) had good invasion inhibition activity (Figure 15). Dose effect of LT-A3-PEG on invasion inhibition of lung cancer cells revealed that LT-A3-PEG greatly enhanced invasion inhibition activity of the peptide (Figure 16) when compared with LT-A3 (Figure 14). C-terminal PEG3 modified LT-1-3 (LT1-3-PEG) also enhanced invasion inhibition activity (Figure 17) when compared with LT1-3 (Figure 13).

Example 9 The effect of LT-1-3-PEG on cell invasion in A549 and H1299 lung cancer cells

[0087] It is important to examine whether LT-peptides inhibit development of lung cancer cells other than CL1-5. The results showed that LT1-3-PEG also efficiently inhibited invasion of A549 and H1299 cells (Figure 18 and 19). LT1-3-PEG also inhibited invasion of H460 and H1355 lung cancer cells and the dose effect of LT1-3-PEG on these two cell lines are currently under investigation.

Example 10 The effect of PEG-modified LT-peptides on cell proliferation in CL1-5 cells

[0088] Although LT-A3 has great growth inhibition activity in CL1-5 cells, N-terminal or C-terminal PEG3 modification on LT-A3 (PEG-A3 & A3-PEG) and N-terminal PEG3 modification on LT1-3 (PEG-1-3) abolished growth inhibition activity (Figure 20A). Fortunately, C-terminal PEG3 modified LT1-3 (LT1-3-PEG) retained growth inhibition activity (Figure 20B).

Example 11 The effect of LT1-3-PEG on cell proliferation in various lung cancer cell lines

[0089] The dose effect of LT1-3-PEG on cell proliferation in various cell lines was examined. The results showed that LT1-3-PEG effectively inhibited CL1-5, A549, H460 and H1355 proliferation but not that in H1299 (Figure 21).

Example 12 The effect of LT-peptides on normal cell types

[0090] It is important to investigate how LT-peptides affect normal cell lines. We treated SV40 transformed human bronchial epithelial cells Beas2B, human embryonic lung fibroblast MRC5, and HUVECs with LT-peptides and perform cell proliferation assay. Our preliminary results showed that LT-peptides did not inhibit growth of these normal cells (Figure 22).

Example 13 The effect of LT1-3-PEG on proliferation of breast cancer cells

LT1-3-PEG also inhibited proliferation of MCF-7, T-47D, MDA-MB-231 and MDA-MB-453 breast cancer cell lines (Figure 23).

Example 14 Blocking of PKA and JNK activity enhanced LT-peptides-mediated growth inhibition

[0091] PKA inhibitor (H89) and JNK inhibitor (SP600125) enhanced LT1-3 and LT1-3-PEG-mediated growth inhibition in CL1-5 lung cancer cells (Figures 24 and 25). To confirm these observations, the catalytic subunit of PKA and JNK1 were interfered with siRNA in CL1-5 cells and the cells were followed by LT-peptides treatment. The results proved that decrease in the activity of PKA and JNK enhances an inhibitory effect on LT-1-3-peptides-mediated growth (Figures 26 and 27).

Claims

What is claimed is:

1. A peptide comprising an amino acid sequence as shown in the following Formula I,

$$X_1\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}X_6\text{-}X_7\text{-}X_8 \qquad (I)$$

wherein X_1 is selected from an amino acid having a hydrophobic side chain; X_2 is selected from an amino acid having a basic side chain, an amino acid having an acidic side chain or an amino acid having a hydrophobic side chain; X_3 is selected from an amino acid having a hydrophobic side chain; X_4 is selected from an amino acid having a hydrophobic side chain; X_5 is selected from an amino acid having a basic side chain, an amino acid having an acidic side chain or an amino acid having a hydrophobic side chain; X_6 is selected from an amino acid having a hydrophobic side chain; X_7 is selected from an amino acid having a hydrophobic side chain or an amino acid having a hydrophilic side chain; and X_8 is selected from an amino acid having a hydrophobic side chain or an amino acid having a hydrophilic side chain;

or a modified peptide or a salt thereof.

2. The peptide of Claim 1, wherein X_1 is selected from F, A, Y and W; X_2 is selected from H, K, R, A and E; X_3 is selected from I or A; X_4 is selected from V and A; X_5 is selected from E, D, H, Orn, A and H; X_6 is selected from L and A; X_7 is selected from L and S and X_8 is selected from A and S; or a modified peptide or a salt thereof.

3. The peptide of Claim 1, which comprises FHIVELLA (LT1-3) (SEQ ID NO:9), FHAVELLA (LT-A3) (SEQ ID NO:10), FKAVELLA (LT-K2) (SEQ ID NO:11), FRAVELLA (LT-R2) (SEQ ID NO:12), FHAVDLLA (LT-D5) (SEQ ID NO:13), FHAVHLLA (LT-H5) (SEQ ID NO:14), FHAVOLLA (LT-Orn5) (SEQ ID NO:15), AHAVELLA (LT-A13) (SEQ ID NO:16), FHAAELLA (LT-A34) (SEQ ID NO:17), FHAVEALA (LT-A36) (SEQ ID NO:18), FHAAEALA (LT-A346) (SEQ ID NO:19), FAIVALLA (LT-A25) (LT-A2) (SEQ ID NO:20), FEAVHLLA (LT-E2H5) (SEQ ID NO:21), YHAVELLA (LT-Y1) (SEQ ID NO:22), WHAVELLA (LT-W1) (SEQ ID NO:23), FHAVELSA (LT-S7) (SEQ ID NO:24), FHAVELLS (LT-S8) (SEQ ID NO:25), **DGN**FHIVELLA (LT-11AA) (SEQ ID NO:26), **A**HIVELLA (LT-A1) (SEQ ID NO:27), FAIVELLA (LT-A2) (SEQ ID NO:28), FHI**A**ELLA (LT-A4) (SEQ ID NO:29), FHIV**A**LLA (LT-A5) (SEQ ID NO:30), FHIVE**A**LA (LT-A6) (SEQ ID NO:31), FHIVEL**A**A (LT-A7) (SEQ ID NO:32), or a peptide comprising an amino acid sequence having one or several amino acid substitutions, deletions and/or additions in the amino acid sequence set forth in any of SEQ ID

NOs: 9 to 32, and having an activity equal to or higher than that/those of the amino acid sequence set forth in any of SEQ ID NOs: 9-32; or a modified peptide or a salt thereof.

4. The peptide of Claim 1, which comprises an amino acid sequence of FHIVELLA (SEQ ID NO:9) or FHAVELLA (SEQ ID NO:10); or a modified peptide or a salt thereof.

5. The peptide of any of Claims 1 to 4, wherein the modified peptide has an improved stability or solubility.

6. The peptide of any of Claims 1 to 4, wherein the modified peptide is a cyclic peptide, pegylated peptide or fluorenylmethyloxycarbonyl (Fmoc)-peptide.

7. The peptide of any of Claims 1 to 4, wherein the modified peptide is a Fmoc-peptide, wherein Fmoc binds to N terminal or C terminal of the peptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 9 to 32.

8. The peptide of any of Claims 1 to 4, wherein the modified peptide is a PEG-peptide, wherein PEG binds to N terminal or C terminal of the peptide comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 9 to 32.

9. The peptide of any of Claim 6, wherein the cyclic peptide comprises one or more hydrophilic amino acid, optionally one or more hydrophobic amino acid, at N terminal of the peptide of any of Claims 1 to 4.

10. The peptide of any of Claim 6, wherein the cyclic peptide comprises two hydrophilic amino acids and one hydrophobic amino acid bonding to the N terminal of the peptide of any of Claims 1 to 4.

11. A cyclic peptide having the following Formula II,

$$X_{a1}\text{-}X_{a2}\text{-}X_{a3}\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}X_6\text{-}X_7\text{-}X_8 \quad (II)$$

wherein X_{a1} bonds to X_8 as head to tail cyclization, and X_{a1} is D; X_{a2} is G; X_{a3} is N; and X_1, X_2, X_3, X_4, X_5, X_6, X_7 and X_8 are an amino acid as defined in Claim 1.

12. The cyclic peptide of Claim 11, wherein the cyclic peptide comprises an amino acid sequence of **DGN**FHIVELLA (SEQ ID NO:26), wherein D bonds to A as head to tail cyclization.

13. A peptide comprising an amino acid sequence of ASAIYSVETINDGNFHIVELLALDQSLSLSVDGGNPKIITNLSKQSTLNFDSPLYVGGMPG KSNVASLRQAPGQNGTSFHGCIRNLYINSE) (SEQ ID NO:1) or a fragment thereof.

14. The peptide of Claim 13, wherein the fragment of the peptide comprises an amino acid sequence as shown in SEQ ID NO:2, 3, 4, 5 or 6.

15. A pharmaceutical composition, comprising a peptide of any of Claims 1 to 14 and a pharmaceutically acceptable carrier.

16. The pharmaceutical composition of Claim 15, which further comprises a second anti-cancer agent.

17. The pharmaceutical composition of Claim 16, wherein the second anti-cancer agent is erlotinib, afatinib, gefitinib, bevacizumab, ramucirumab, gefitinib, lapatinib, erlotinib, cisplatin, paclitaxel, methotrexate, cyclophosphamide, ifosfamide, chlorambucil, carmustine, carboplatin, vincristine, vinblastine, thiotepa, lomustine, semustine, 5-fluorouracil, corticosteroids, calcineurin inhibitors, NSAIDs, inhibitors of 5-lipoxygenase, or cytarabine.

18. A method for preventing, treating, and/or ameliorating a cancer or inhibiting cancer growth, invasion and/or metastasis, comprising administering an effective amount of a peptide of any of Claims 1 to 14 to a subject.

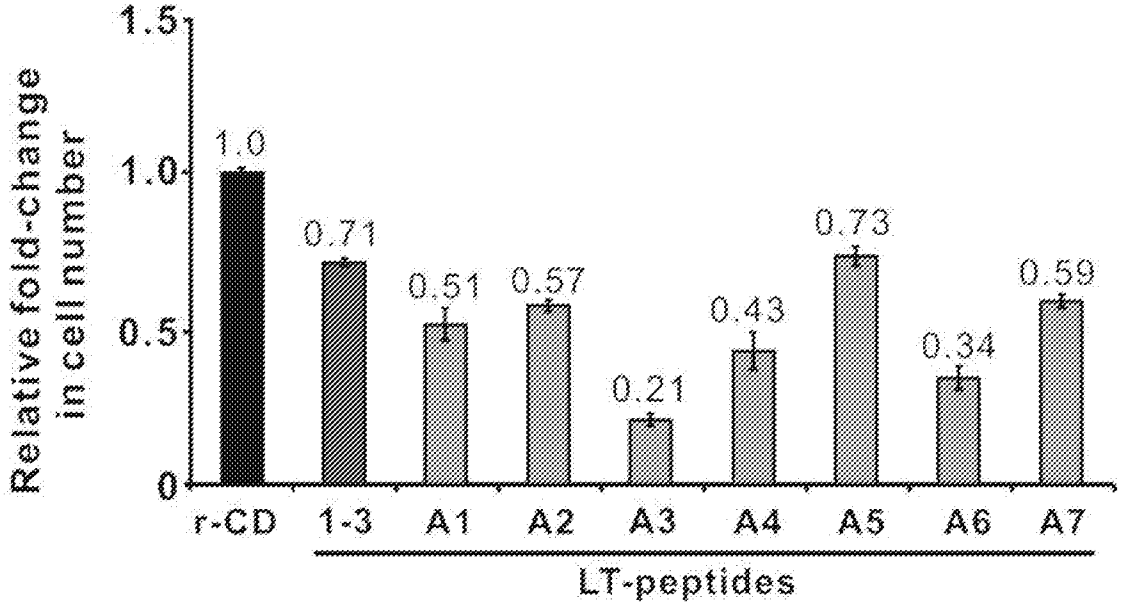
19. The method of Claim 18, which further comprises a step of administering a second anti-cancer agent.

20. The method of Claim 19, wherein the second anti-cancer agent is erlotinib, afatinib, gefitinib, bevacizumab, ramucirumab, gefitinib, lapatinib, erlotinib, cisplatin, paclitaxel, methotrexate, cyclophosphamide, ifosfamide, chlorambucil, carmustine, carboplatin, vincristine, vinblastine, thiotepa, lomustine, semustine, 5-fluorouracil, corticosteroids, calcineurin inhibitors, NSAIDs, inhibitors of 5-lipoxygenase, or cytarabine.

21. The method of Claim 19, which is a combined treatment or a maintenance treatment.

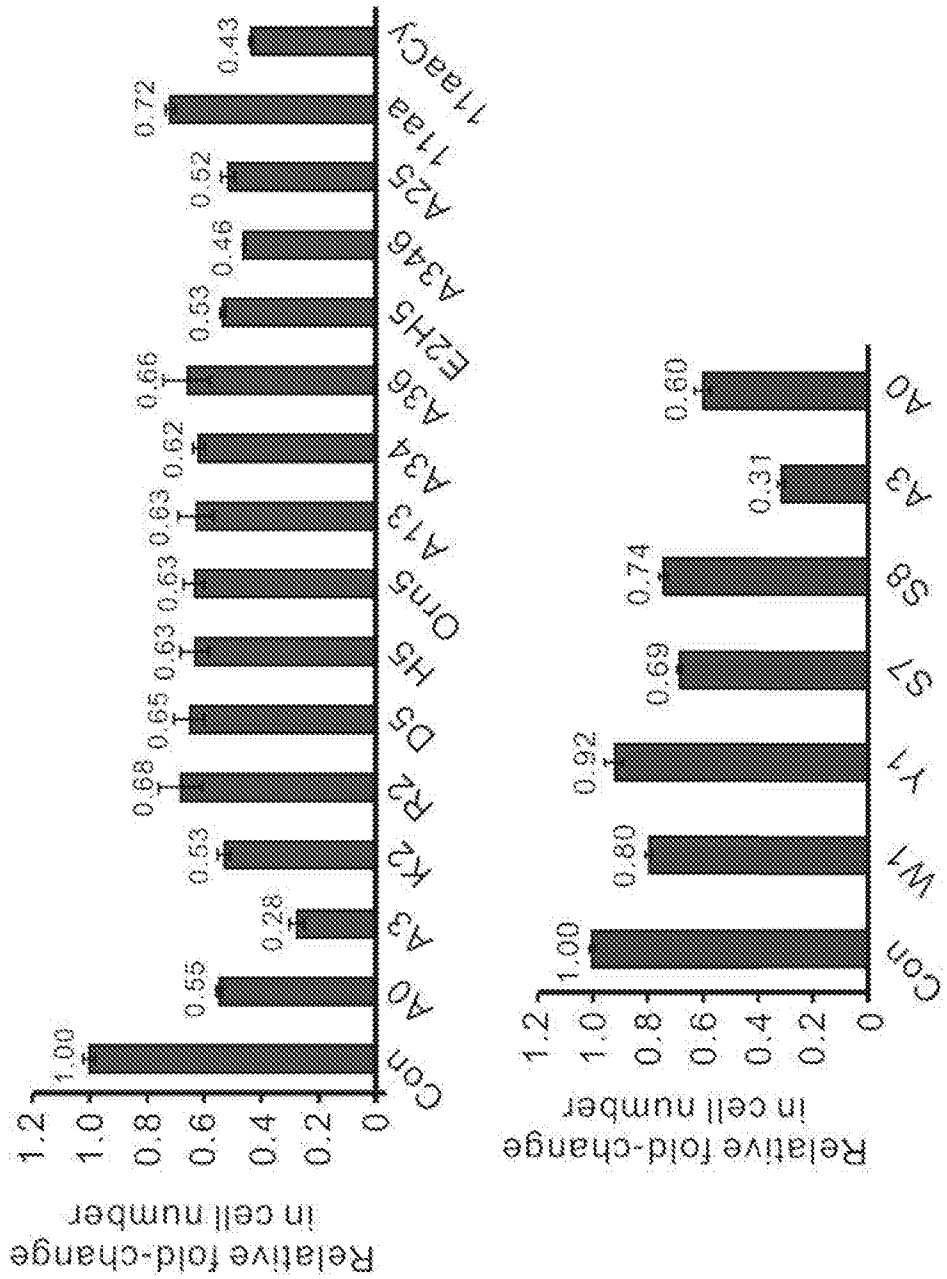
LT-1 peptide: ASAIYSVETINDGNFHIVELLA

LT1-3: FHIVELLA
LT-A1: **A**HIVELLA
LT-A2: F**A**IVELLA
LT-A3: FH**A**VELLA
LT-A4: FHI**A**ELLA
LT-A5: FHIV**A**LLA
LT-A6: FHIVE**A**LA
LT-A7: FHIVEL**A**A



All peptides were Fmoc modified at N-terminal.

Fig. 1



LT1-3(A0): FHIVELLA
A3: FHAVELLA
K2: FKAVELLA
R2: FRAVELLA
D5: FHAVDLLA
H5: FHAVHLLA
Orn5: FHAVOLLA
A13: AHAVELLA
A34: FHAAELLA
A36: FHAVEALA
A346: FHAAEALA
A25: FAIVALLA
E2H5: FEAVHLLA
Y1: YHAVELLA
W1: WHAVELLA
S7: FHAVELSA
S8: FHAVELLS
11AA: DGNFHIVELLA
Cyclic-11: DGNFHIVELLA

Fig. 2

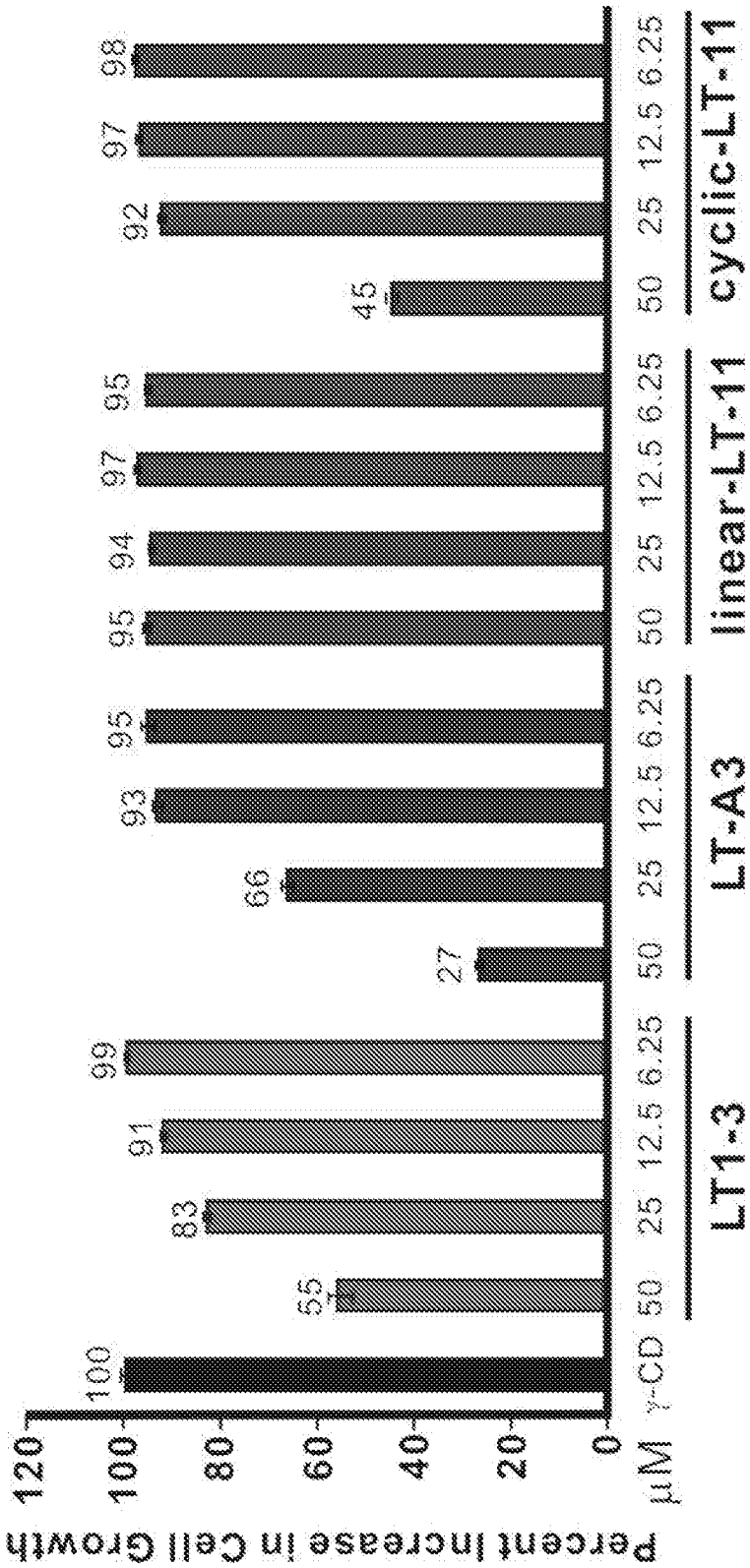

Percent Increase in Cell Growth
120
100
80
60
40
20
0
μM γ-CD 50 25 12.5 6.25 50 25 12.5 6.25 50 25 12.5 6.25 50 25 12.5 6.25 50 25 12.5 6.25
100 55 83 91 99 27 66 93 95 95 94 97 95 45 92 97 98
LT1-3
LT-A3
linear-LT-11
cyclic-LT-11

Fig. 3

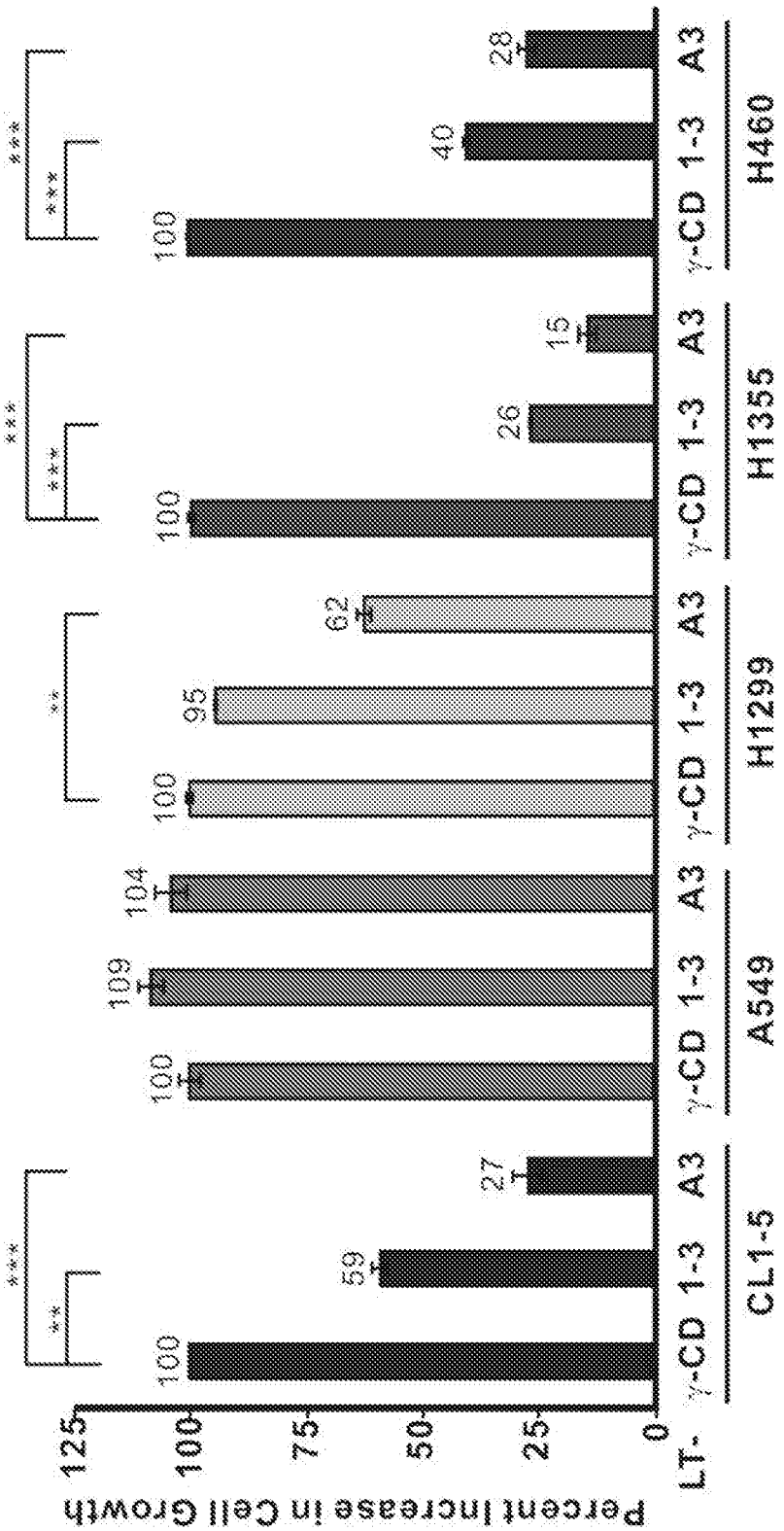
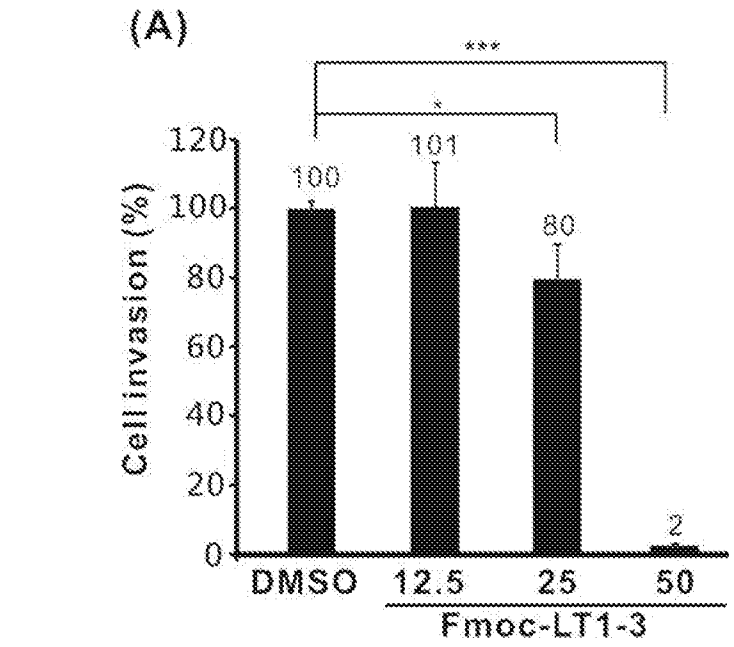

Percent Increase in Cell Growth
125
100
75
50
25
0
LT-
γ-CD 1-3
A3
CL1-5
A549
H1299
H1355
H460
100
59
27
100
109
104
100
95
62
100
26
15
100
40
28
**
***
**
***
**
***
***

Fig. 4


(A)
***
*
120
100
80
60
40
20
0
Cell invasion (%)
100
101
80
2
DMSO
12.5
25
50
Fmoc-LT1-3

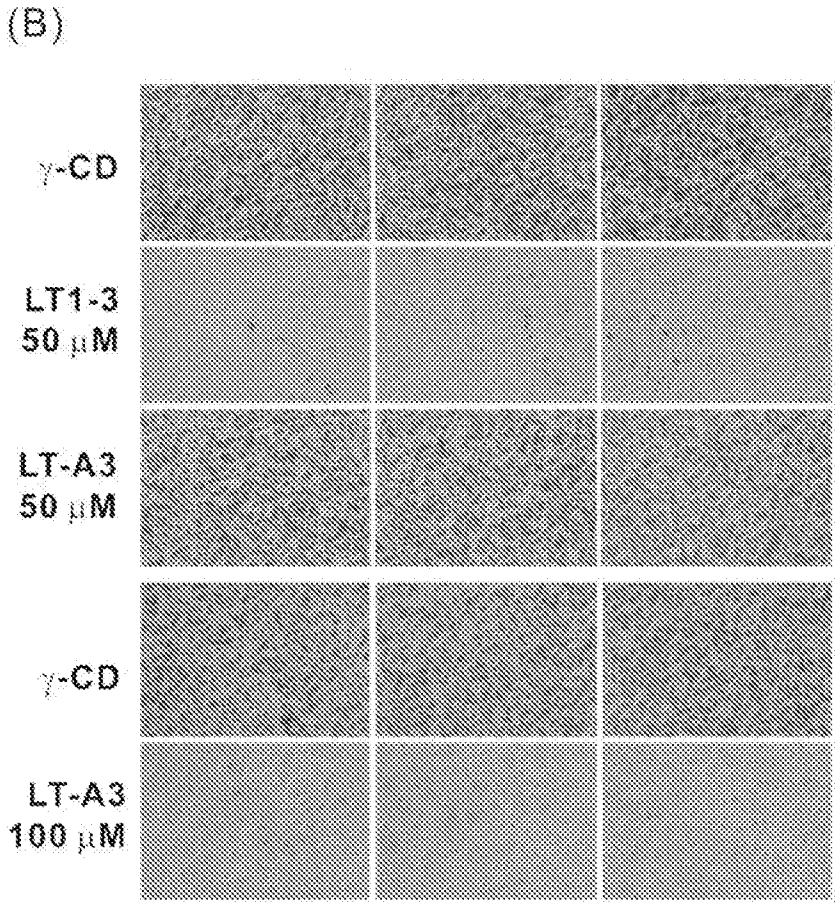

(B)
γ-CD
LT1-3
50 μM
LT-A3
50 μM
γ-CD
LT-A3
100 μM

Fig. 5

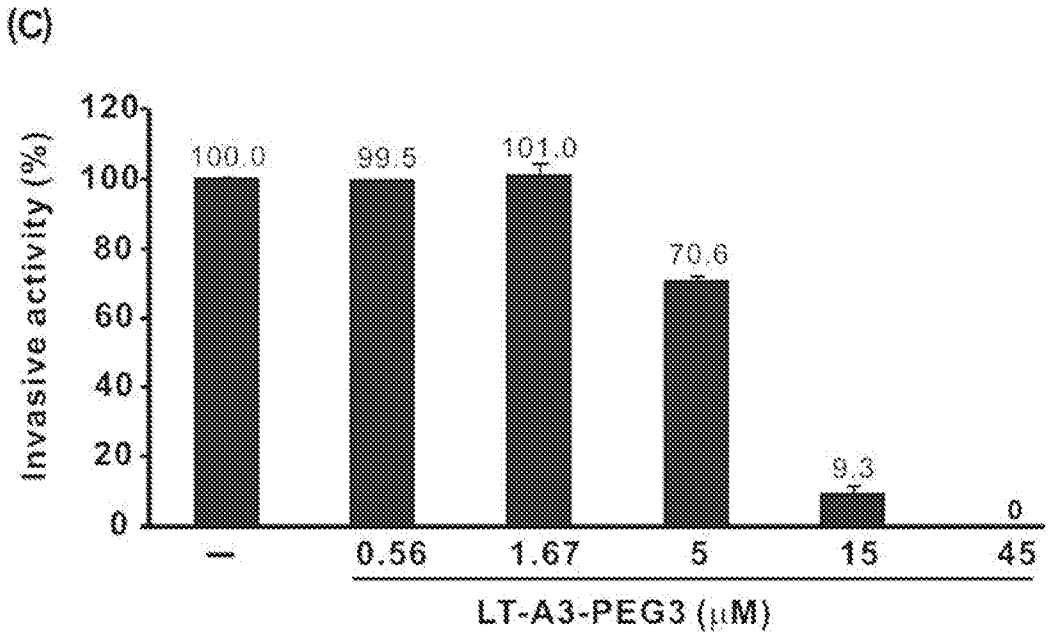

(C)
Invasive activity (%)
120
100
80
60
40
20
0
100.0
99.5
101.0
70.6
9.3
0
–
0.56
1.67
5
15
45
LT-A3-PEG3 (μM)

Fig. 5

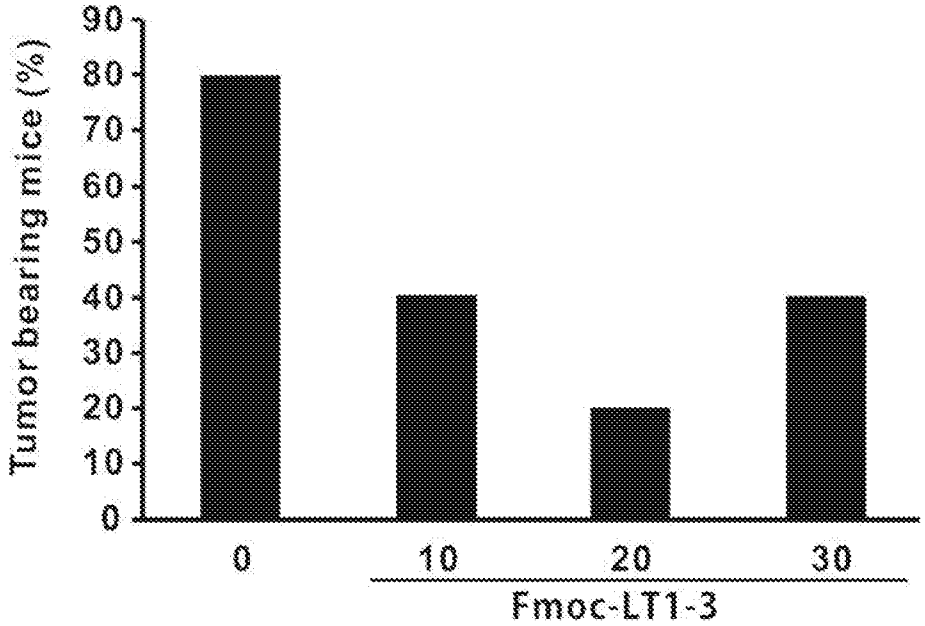

Tumor bearing mice (%)
90
80
70
60
50
40
30
20
10
0
0
10
20
30
Fmoc-LT1-3

Fig. 6

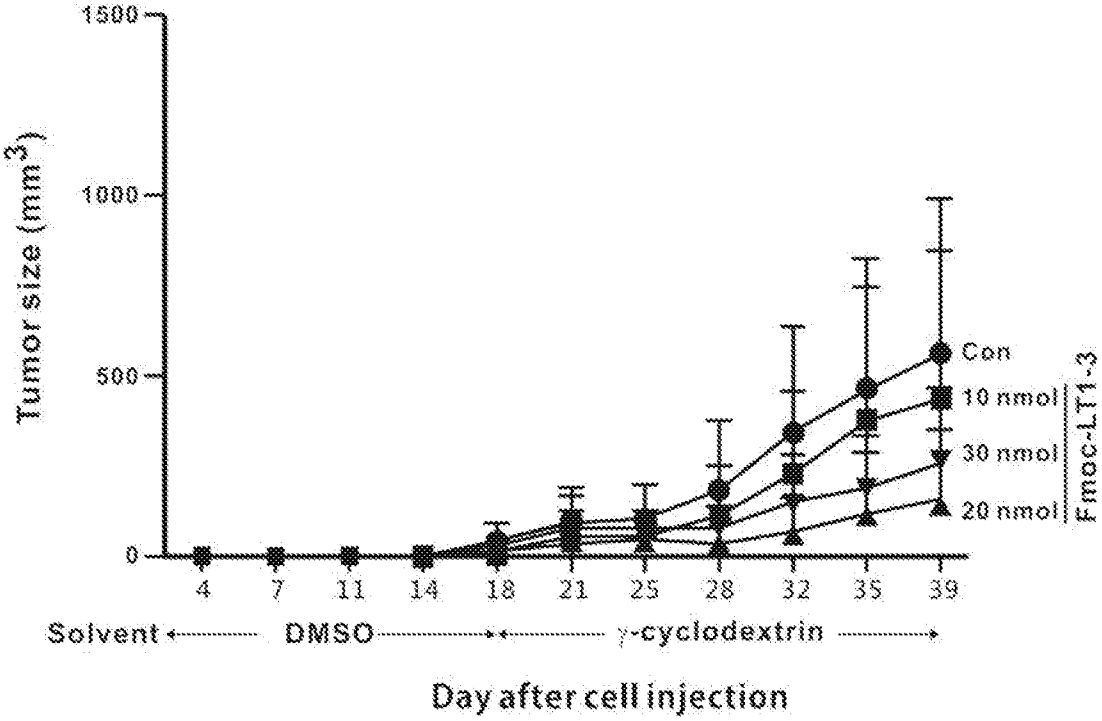
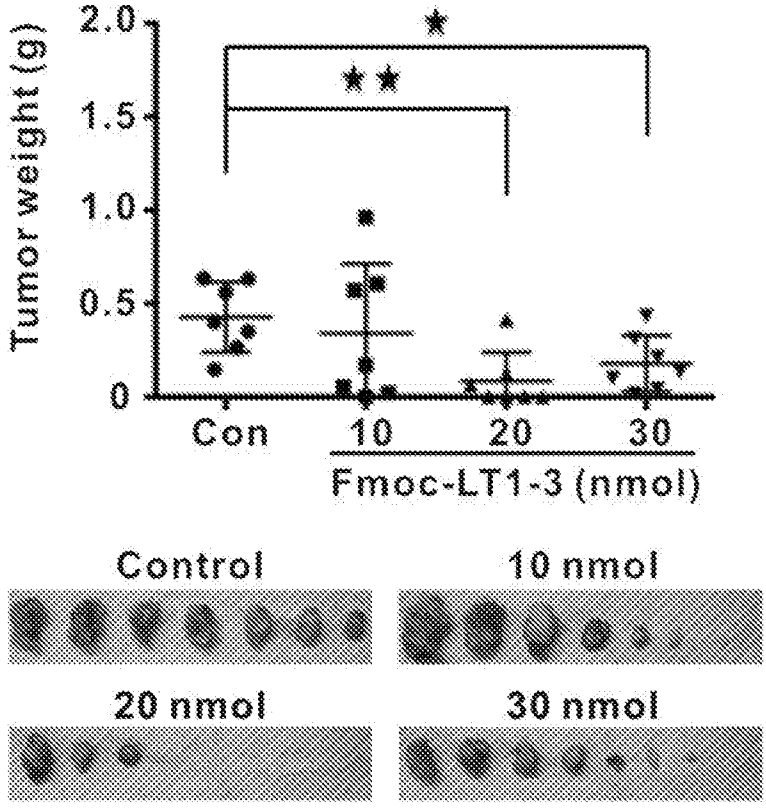

1500
1000
500
0
Tumor size (mm3)
4
7
11
14
18
21
25
28
32
35
39
Con
10 nmol
30 nmol
20 nmol
Fmoc-LT1-3
Solvent
DMSO
γ-cyclodextrin
Day after cell injection

Fig. 7


2.0
1.5
1.0
0.5
0
Tumor weight (g)
Con
10
20
30
Fmoc-LT1-3 (nmol)
Control
10 nmol
20 nmol
30 nmol

Fig, 8

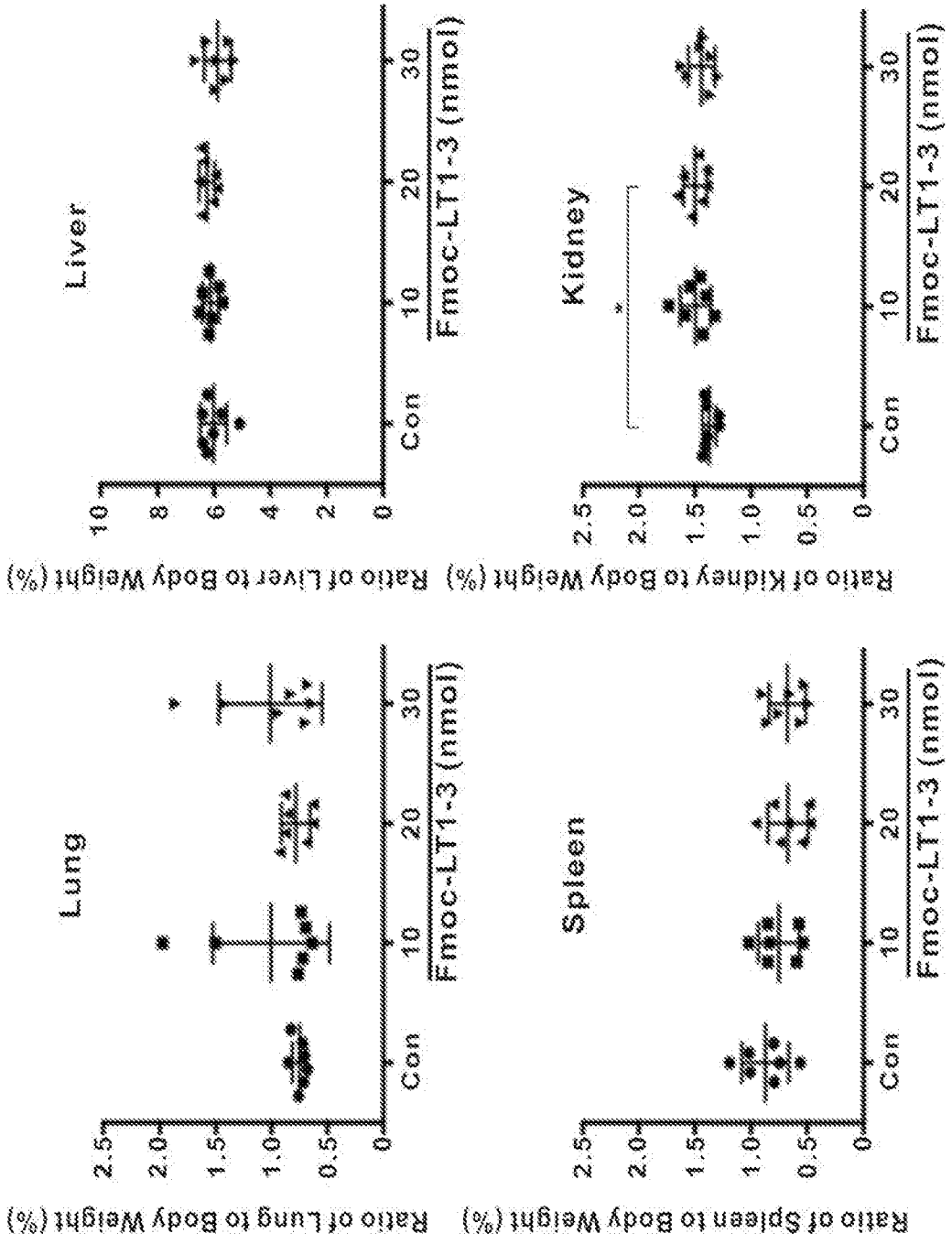

Liver
Ratio of Liver to Body Weight (%)
Kidney
Ratio of Kidney to Body Weight (%)
Lung
Ratio of Lung to Body Weight (%)
Spleen
Ratio of Spleen to Body Weight (%)
Con
10
20
30
Fmoc-LT1-3 (nmol)

Fig. 9

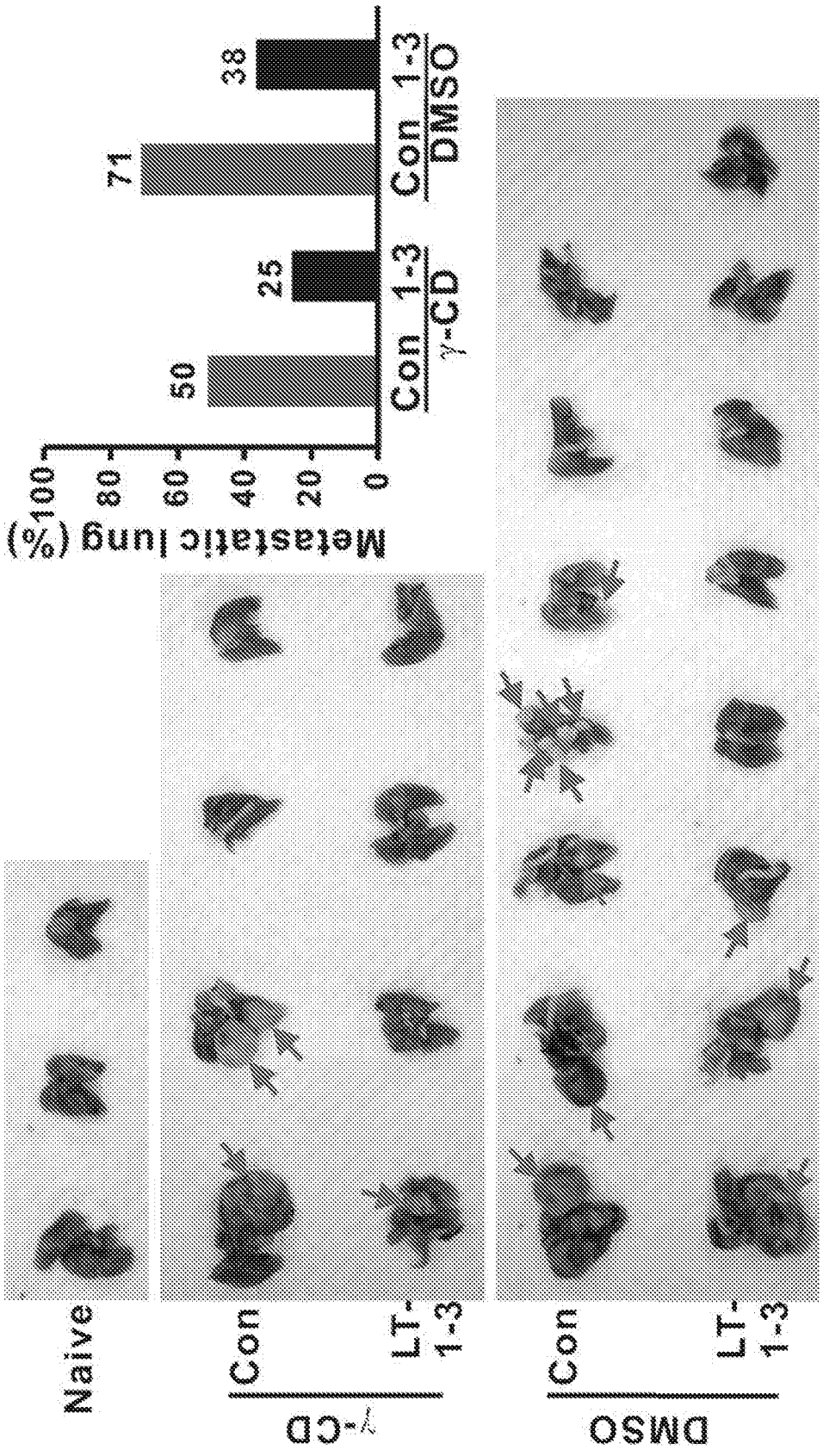

Metastatic lung (%)
100
80
60
40
20
0
50
25
71
38
Con 1-3
γ-CD
Con 1-3
DMSO
Naive
Con
LT-1-3
γ-CD
Con
LT-1-3
DMSO

Fig. 10

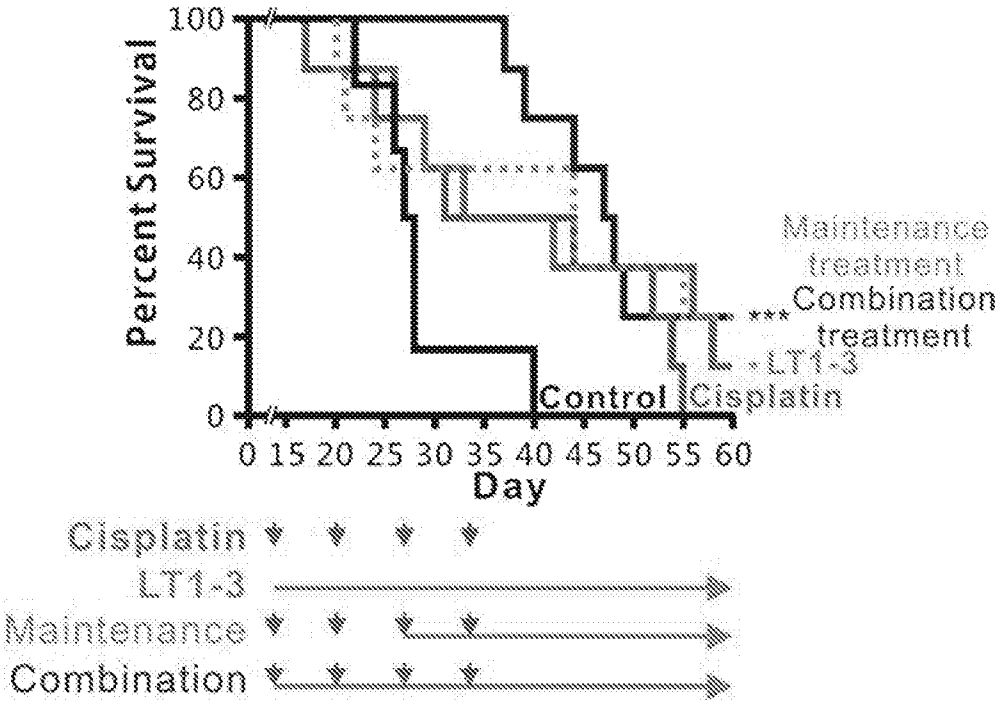

Percent Survival
100
80
60
40
20
0
0 15 20 25 30 35 40 45 50 55 60
Day
Maintenance treatment
Combination treatment
LT1-3
Control
Cisplatin
Cisplatin
LT1-3
Maintenance
Combination

Fig. 11

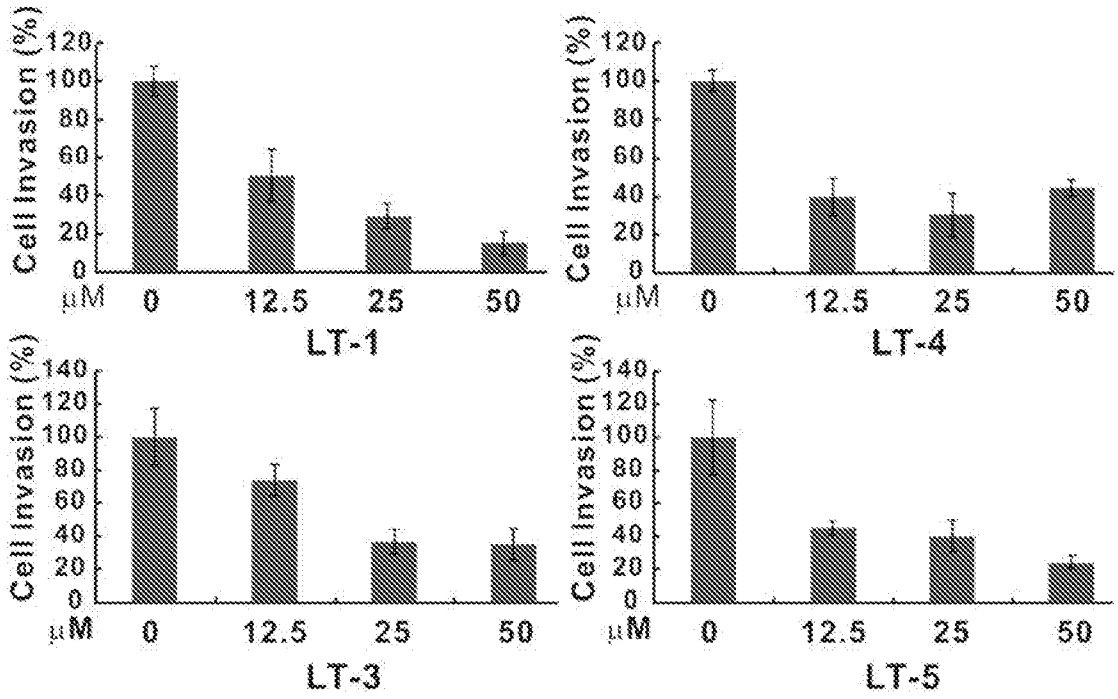

Cell Invasion (%)
120
100
80
60
40
20
0
μM
0
12.5
25
50
LT-1
LT-4
140
LT-3
LT-5

Fig. 12

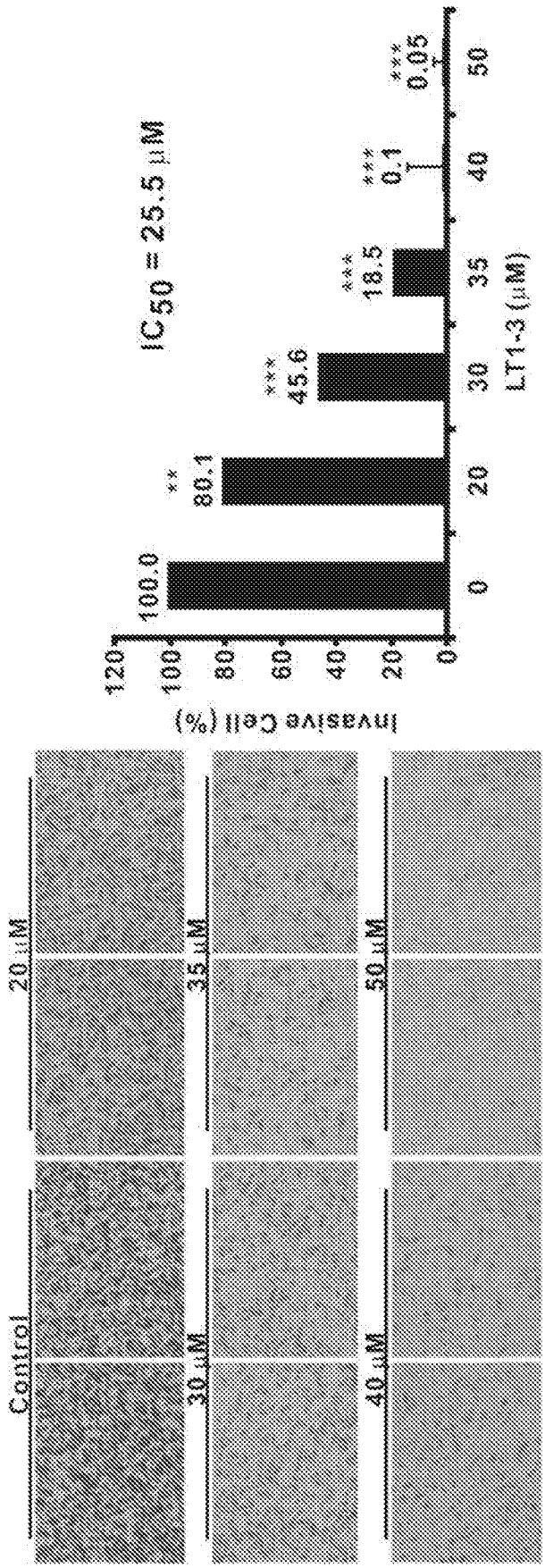

Invasive Cell (%)
120
100
80
60
40
20
0
100.0
**
80.1
***
45.6
***
18.5
***
0.1
***
0.05
0
20
30
35
40
50
LT1-3 (μM)
IC50 = 25.5 μM
Control
20 μM
35 μM
50 μM
30 μM
40 μM

Fig. 13

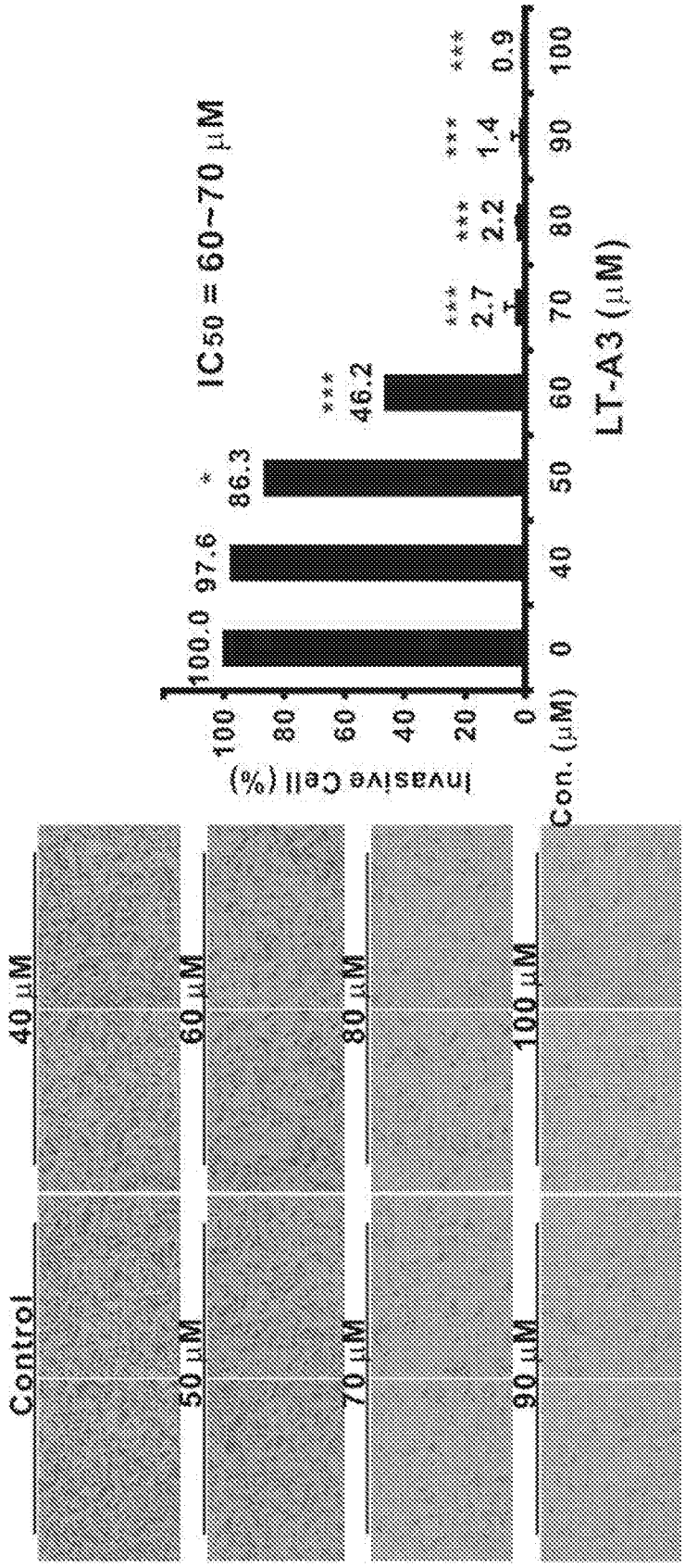

Control
40 μM
60 μM
80 μM
100 μM
50 μM
70 μM
90 μM
Invasive Cell (%)
100
80
60
40
20
0
Con. (μM)
0
40
50
60
70
80
90
100
LT-A3 (μM)
IC50 = 60~70 μM
100.0
97.6
86.3
46.2
2.7
2.2
1.4
0.9
*
***
***
***
***

Fig. 14

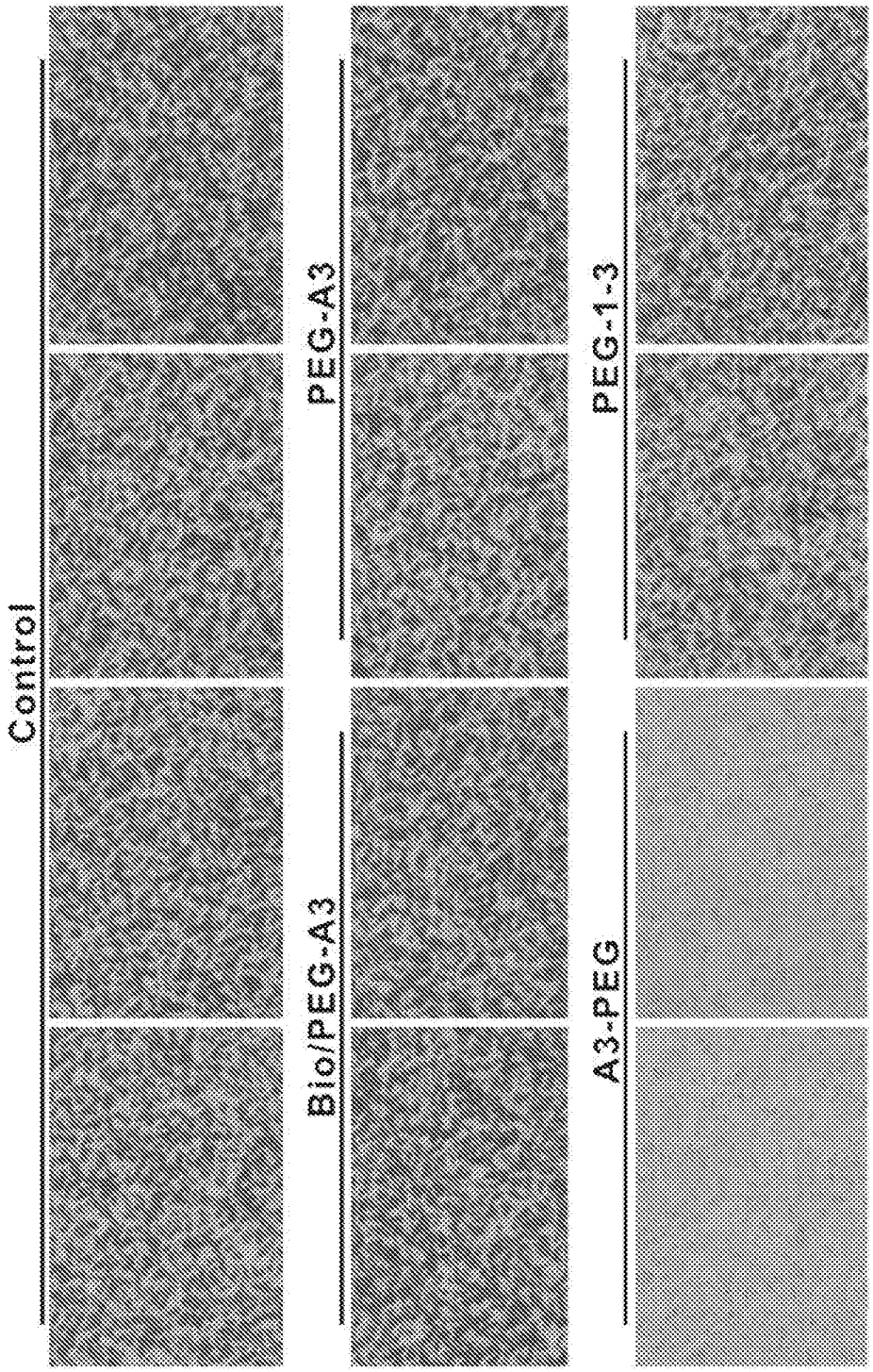


Fig. 15

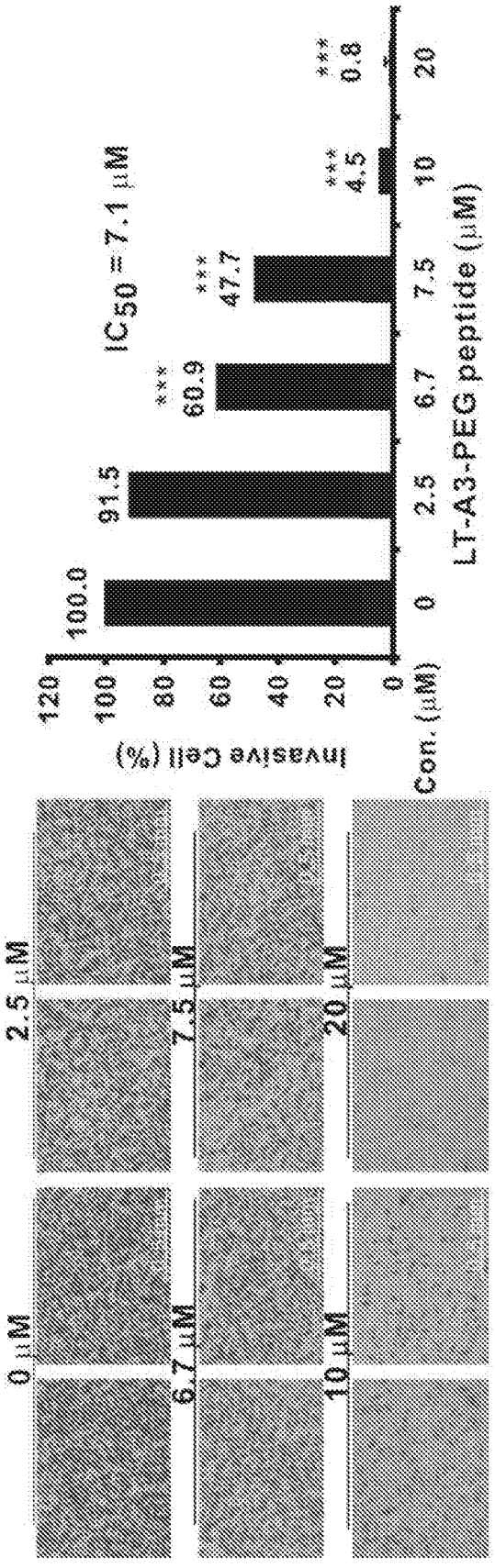

Invasive Cell (%)
120
100
80
60
40
20
0
100.0
91.5
***
60.9
***
47.7
***
4.5
***
0.8
IC50 = 7.1 μM
Con. (μM)
0
2.5
6.7
7.5
10
20
LT-A3-PEG peptide (μM)
0 μM
2.5 μM
6.7 μM
7.5 μM
10 μM
20 μM

Fig. 16

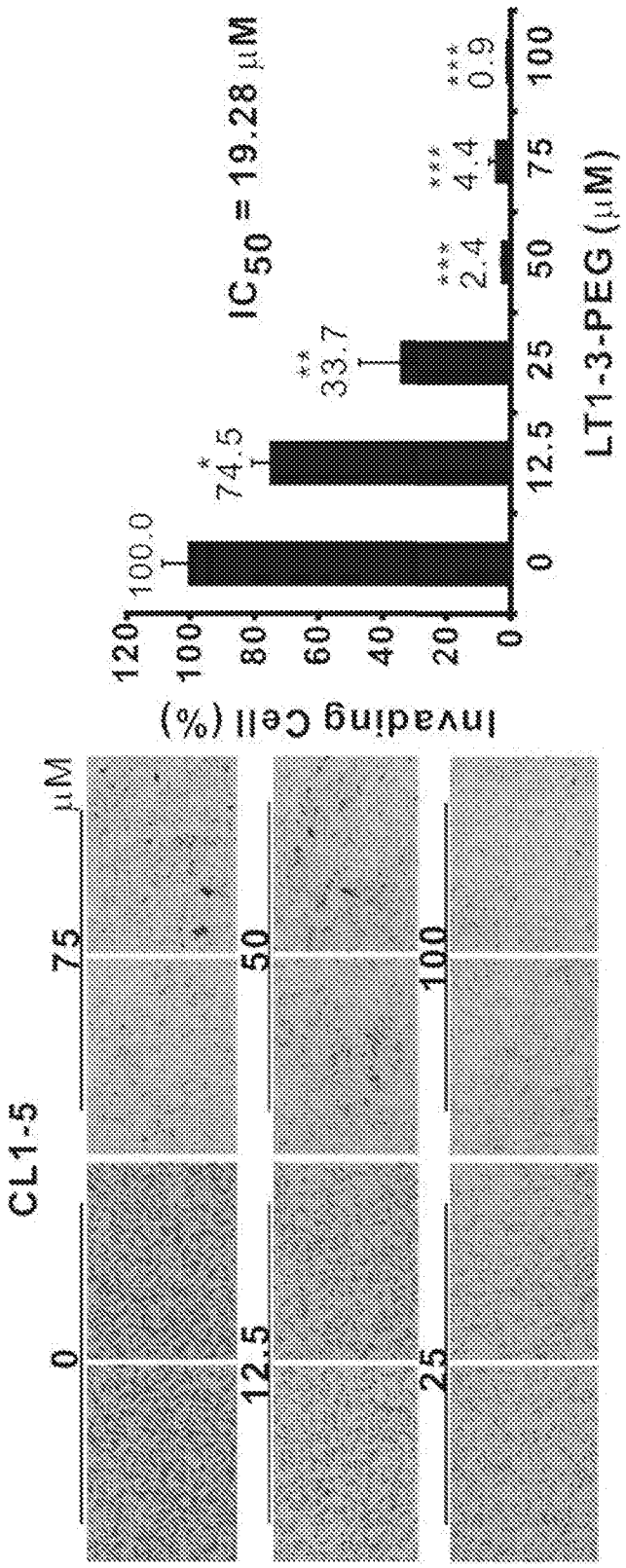

CL1-5
0
12.5
25
50
75
100
μM
Invading Cell (%)
120
100
80
60
40
20
0
100.0
74.5
33.7
2.4
4.4
0.9
*
**
***
***
***
IC50 = 19.28 μM
0
12.5
25
50
75
100
LT1-3-PEG (μM)

Fig. 17

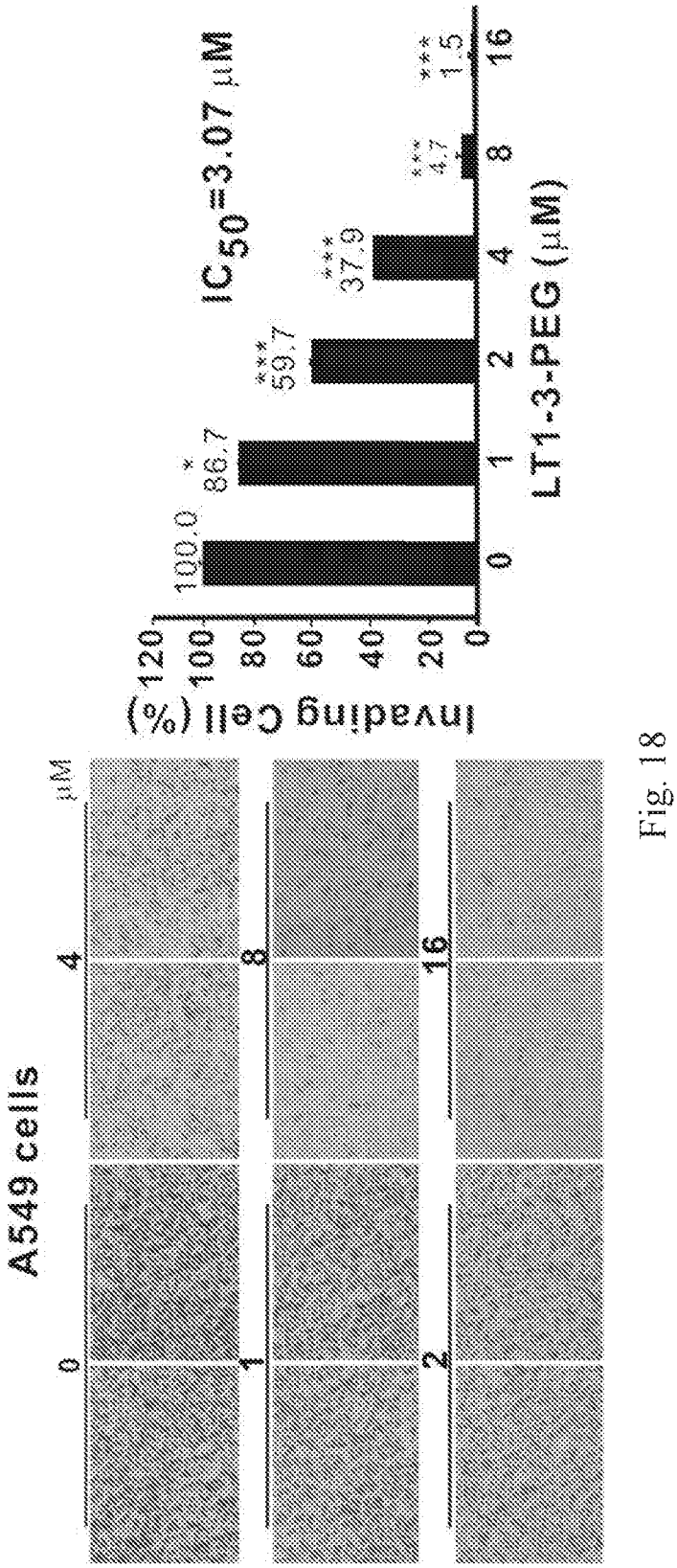

A549 cells
μM
0
1
4
2
8
16
Invading Cell (%)
120
100
80
60
40
20
0
100.0
*
86.7
***
59.7
***
37.9
***
4.7
*
***
1.5
IC50=3.07 μM
0
1
2
4
8
16
LT1-3-PEG (μM)

Fig. 18

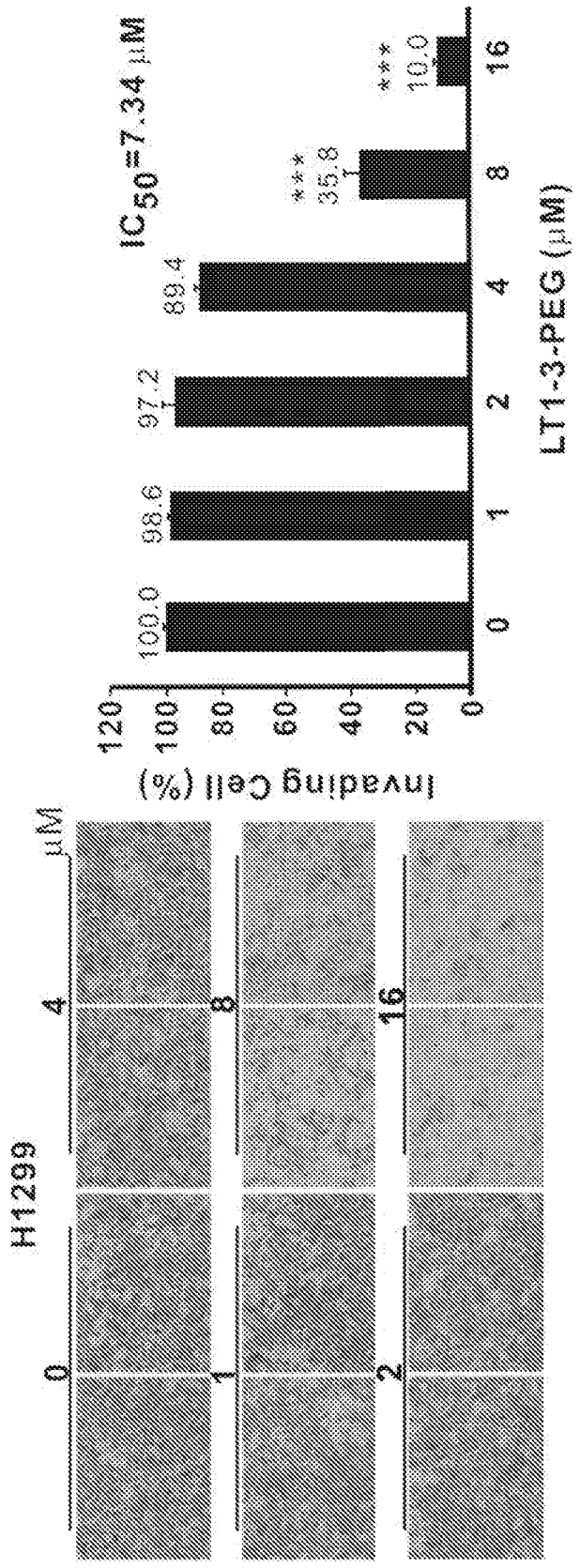

H1299
0
1
2
4
8
16
μM
Invading Cell (%)
120
100
80
60
40
20
0
100.0
98.6
97.2
89.4
35.8
10.0
***
***
IC50=7.34 μM
0
1
2
4
8
16
LT1-3-PEG (μM)

Fig. 19

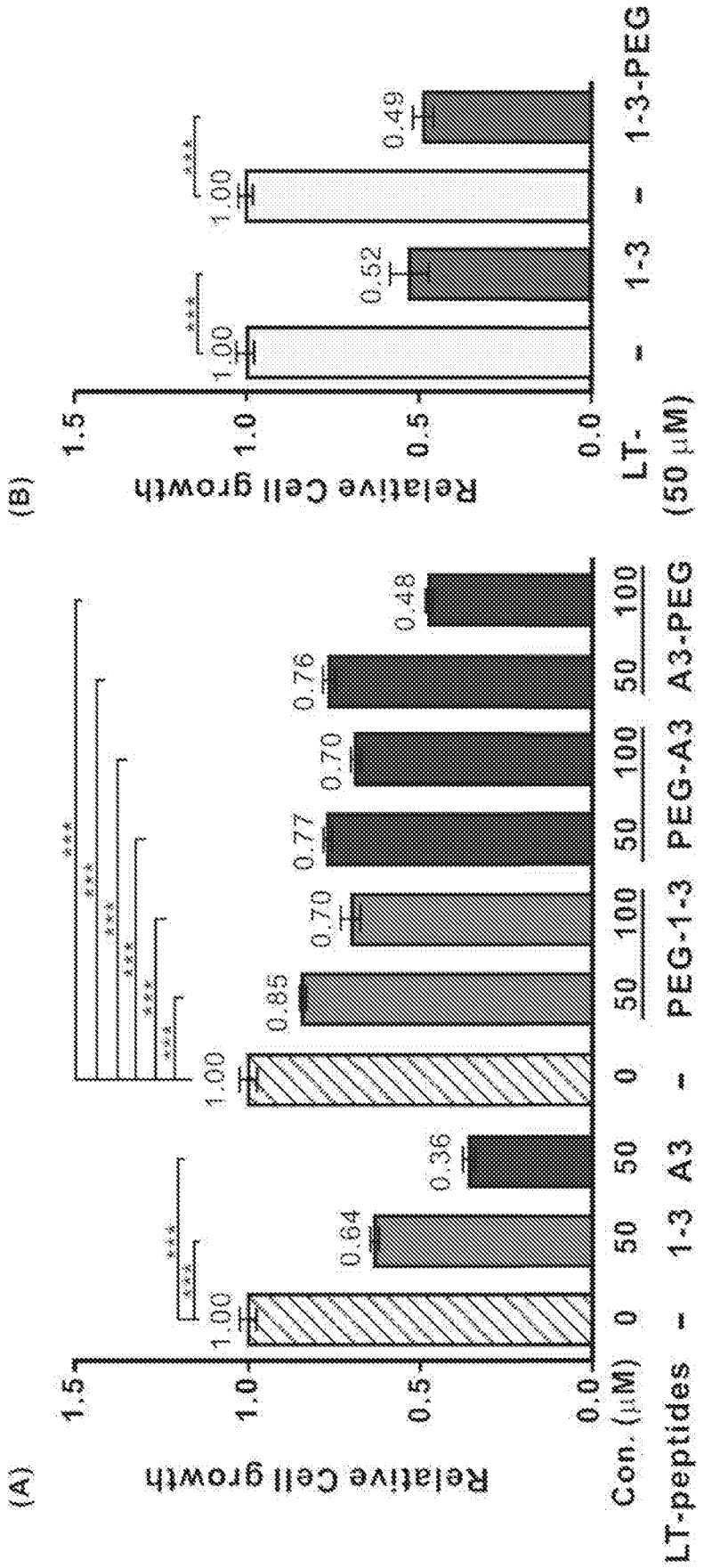

(A)
Relative Cell growth
1.5
1.0
0.5
0.0
LT-peptides - 1-3 A3 - PEG-1-3 PEG-A3 A3-PEG
Con. (μM) 0 50 50 0 50 100 50 100 50 100
1.00
0.64
0.36
0.85
0.70
0.77
0.70
0.76
0.48
***
(B)
Relative Cell growth
1.5
1.0
0.5
0.0
LT- - 1-3 - 1-3-PEG
(50 μM)
1.00
0.52
1.00
0.49
***

Fig. 20

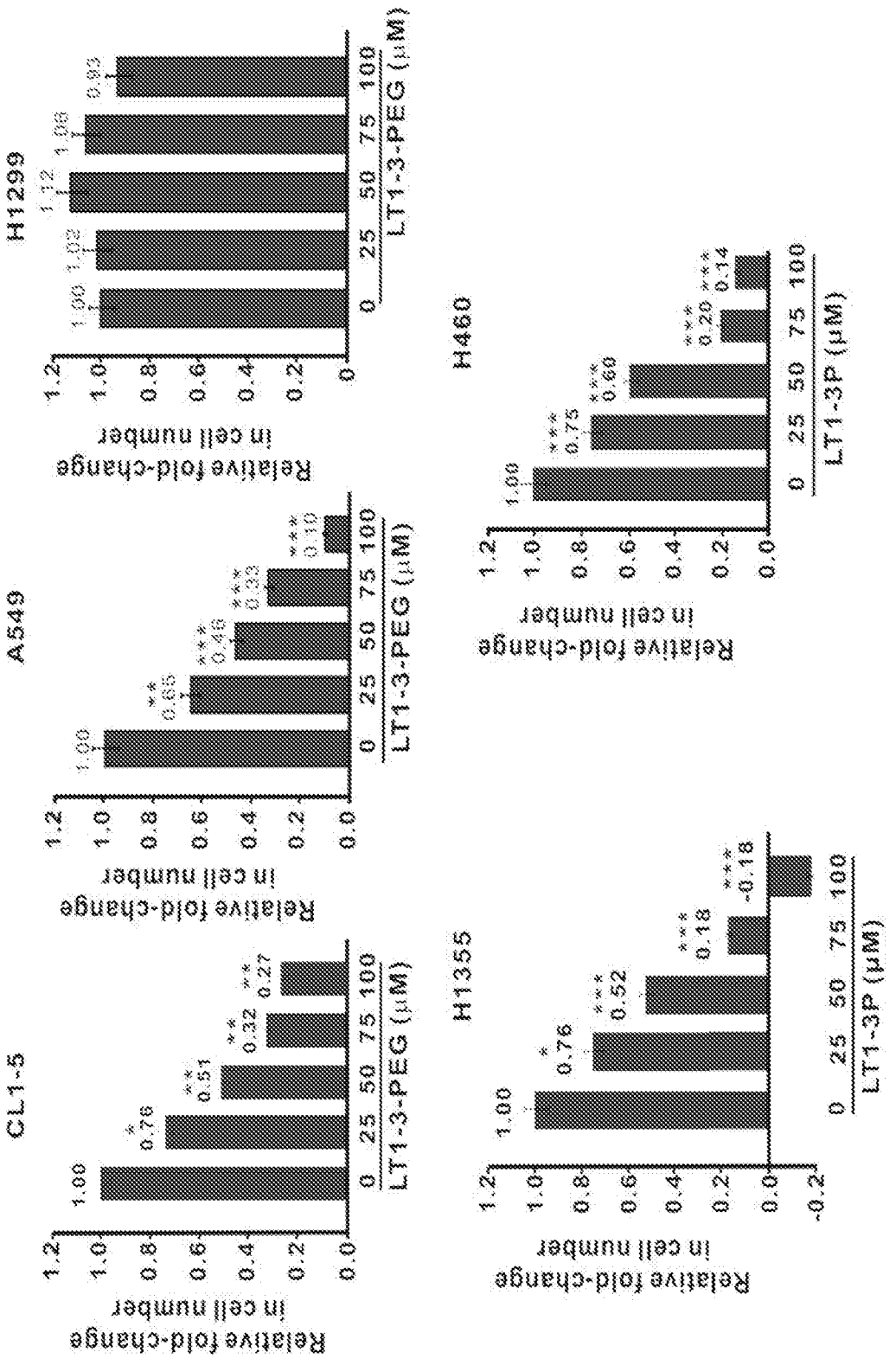

CL1-5
A549
H1299
H460
H1355
Relative fold-change in cell number
LT1-3-PEG (μM)
LT1-3P (μM)
0 25 50 75 100
1.00
0.76
0.51
0.32
0.27
0.65
0.46
0.33
0.10
1.00
1.02
1.12
1.08
0.93
0.75
0.60
0.20
0.14
0.52
0.18
-0.18

Fig. 21

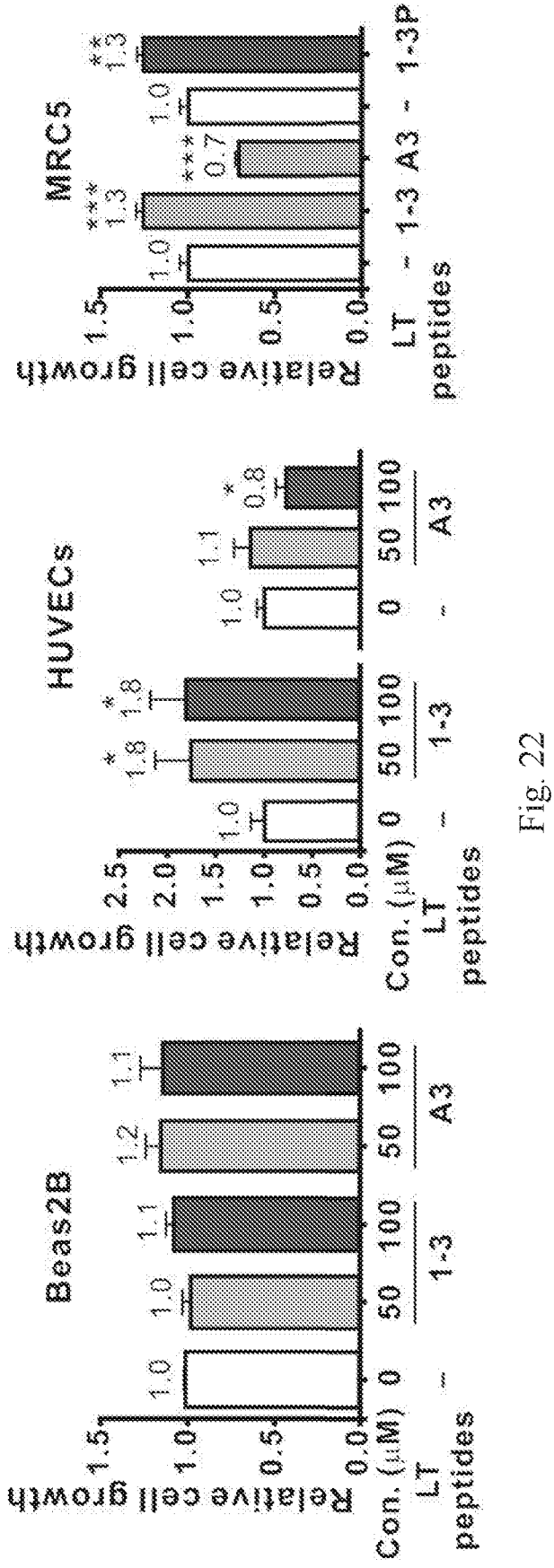

Beas2B
HUVECs
MRC5
Relative cell growth
Con. (μM) 0 50 100 50 100 0 50 100
LT peptides – 1-3 A3
LT – 1-3 A3 – 1-3P peptides
1.0
1.0
1.1
1.2
1.1
1.0
1.8
1.8
1.0
1.1
0.8
1.0
1.3
0.7
1.0
1.3
*
*
*
*
**
***
***

Fig. 22

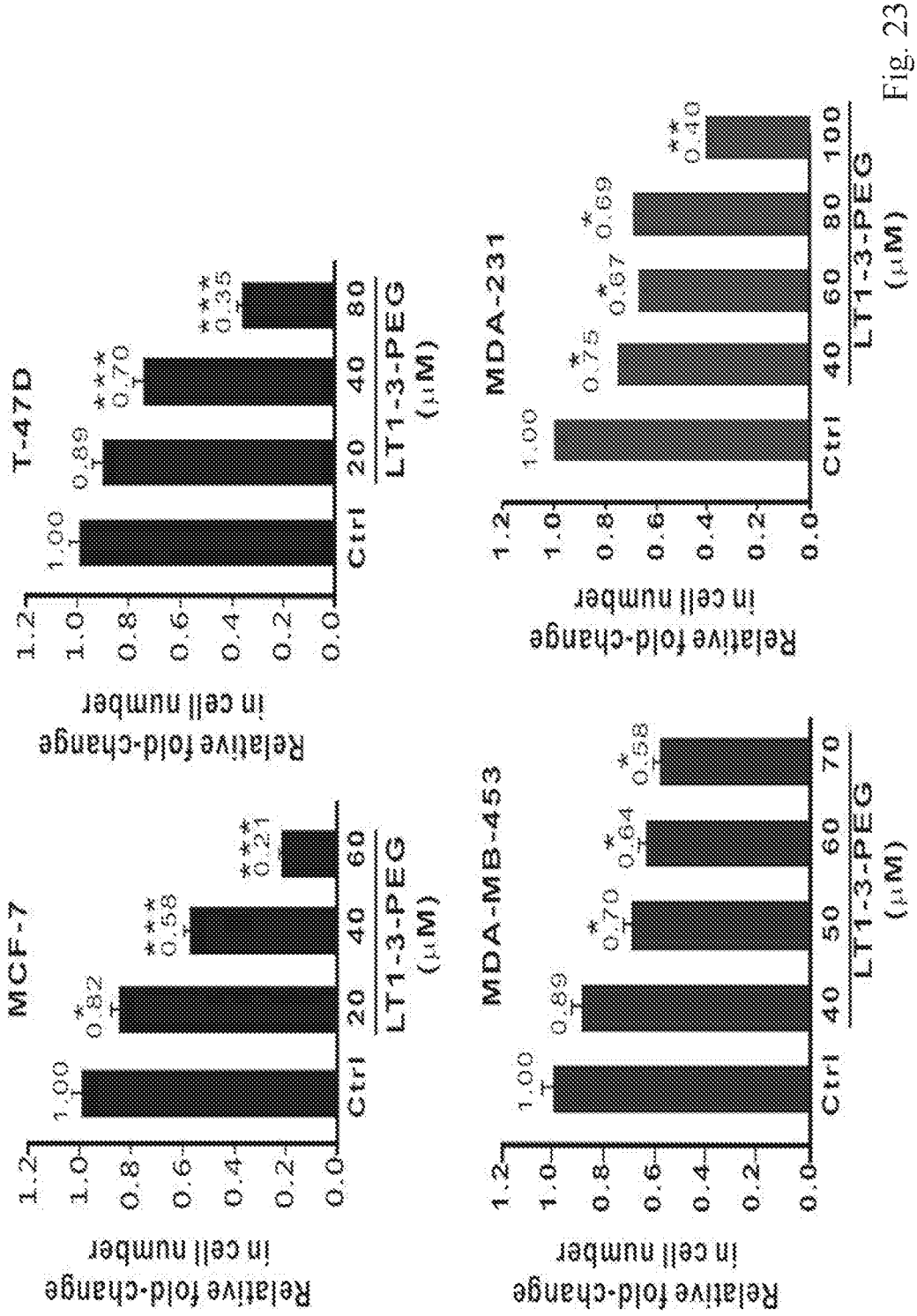

MCF-7
T-47D
MDA-MB-453
MDA-231
Relative fold-change in cell number
LT1-3-PEG (μM)
Ctrl
1.00
0.82*
0.58***
0.21***
0.89
0.70***
0.35***
0.70*
0.64*
0.58*
0.75*
0.67*
0.69*
0.40**

Fig. 23

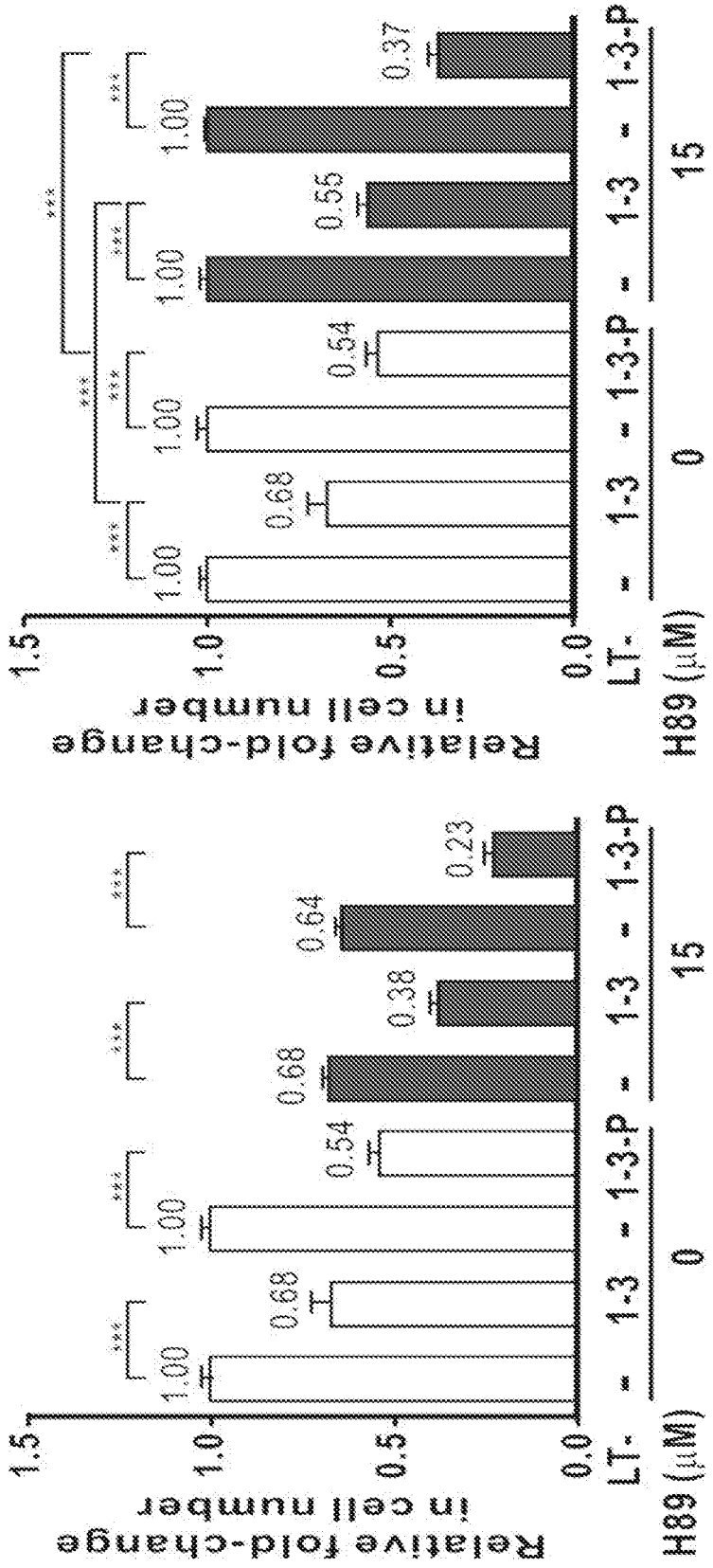

Relative fold-change in cell number
1.5
1.0
0.5
0.0
1.00
0.68
1.00
0.54
1.00
0.55
1.00
0.37
0.23
0.64
0.38
0.68
0.54
1.00
0.68
1.00
***
LT- = 1-3 = 1-3-P = 1-3 = 1-3-P
H89 (μM) 0 15

Fig. 24

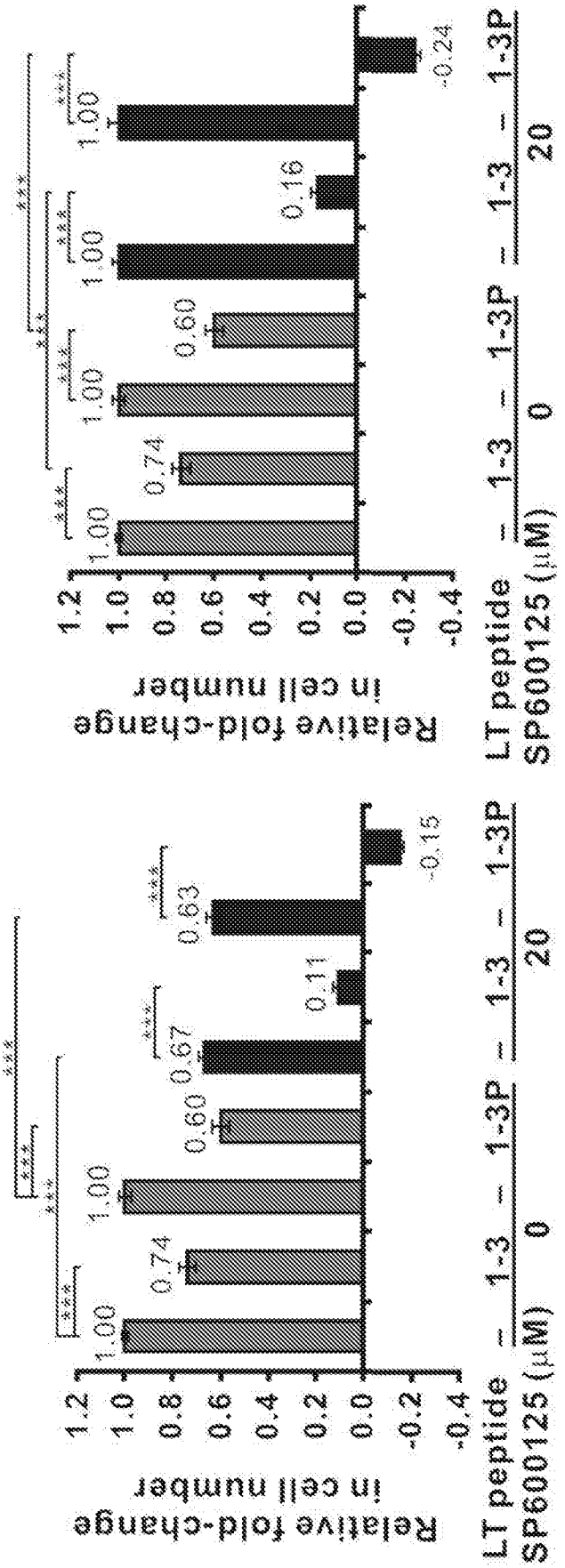

Relative fold-change in cell number
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
1.00
0.74
1.00
0.60
0.67
0.11
0.63
-0.15
1.00
0.74
1.00
0.60
0.16
1.00
-0.24
***
LT peptide
SP600125 (μM)
–
0
1-3
1-3P
20

Fig. 25

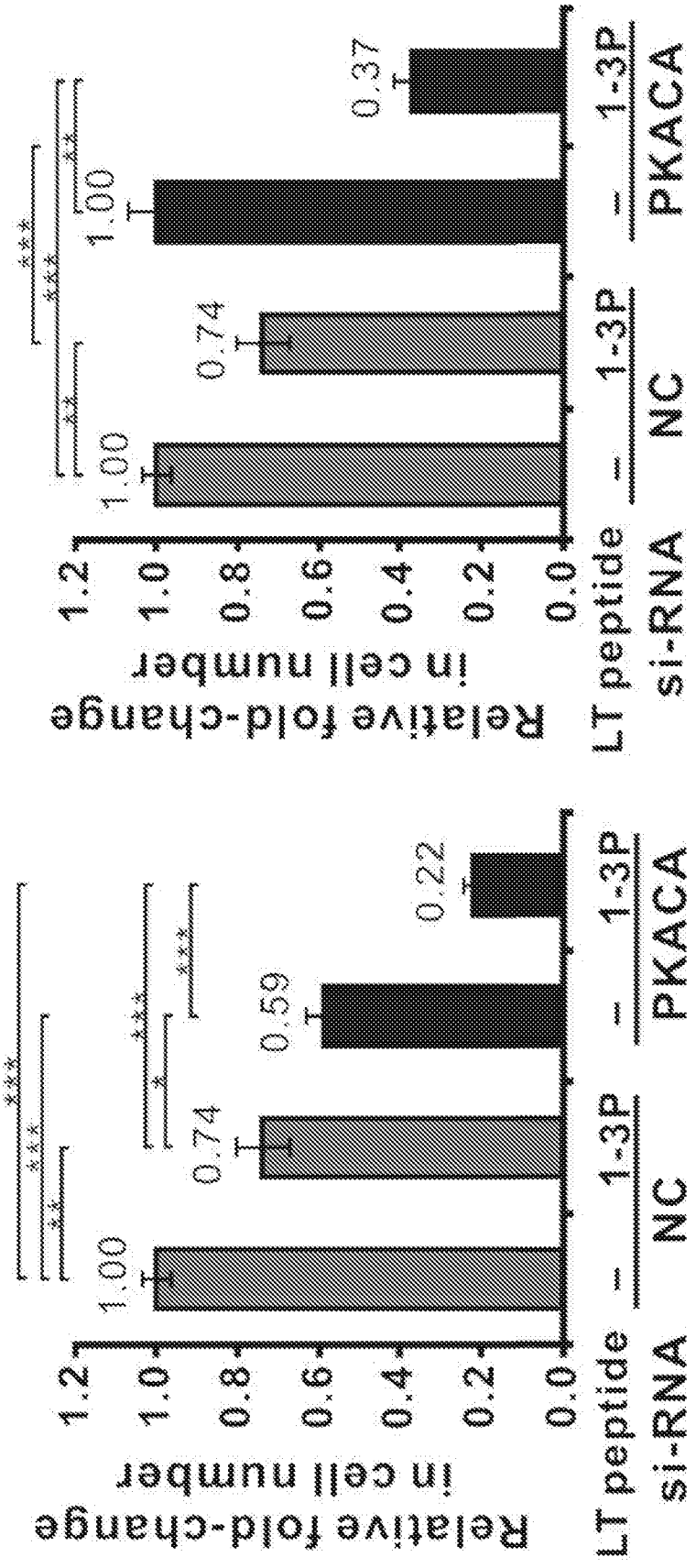

Relative fold-change in cell number
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.00
0.74
0.59
0.22
0.37
***
**
*
LT peptide
si-RNA
–
1-3P
NC
PKACA

Fig. 26

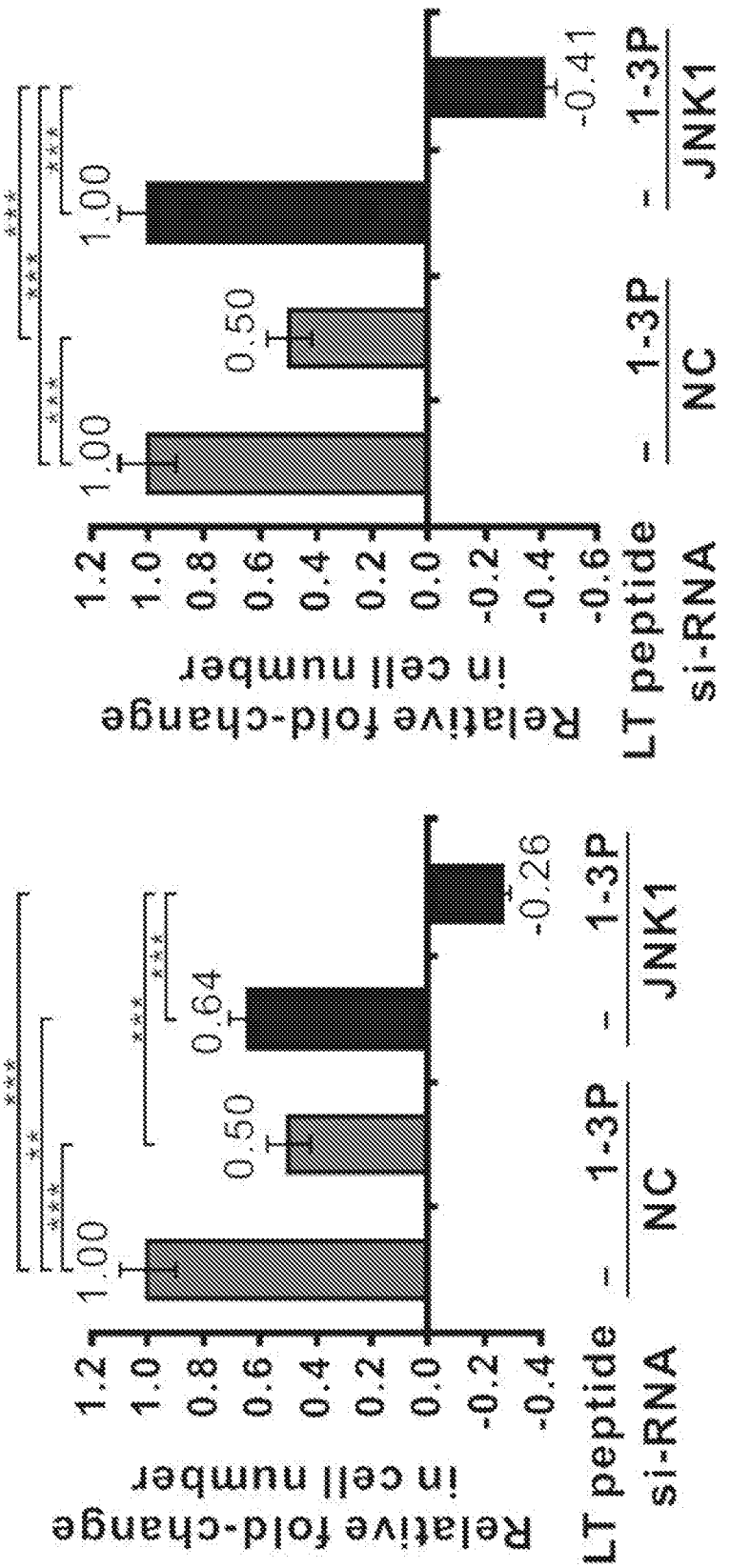

Relative fold-change in cell number
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
1.00
0.50
-0.41
***
***
***
LT peptide
si-RNA
–
NC
1-3P
–
JNK1
1-3P
Relative fold-change in cell number
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
1.00
0.50
0.64
-0.26
***
**
***
***
LT peptide
si-RNA
–
NC
1-3P
–
JNK1
1-3P

Fig. 27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/29733

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 7/64, 7/00, 14/00, 1/06 (2018.01)
CPC - C07K 7/64, 7/00, 14/00, 1/06, 1/063

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	UniProt database H0Y968, 15 February 2017 [online]. [Retrieved 2 July 2018]. Retrieved from the internet: <URL: https://www.uniprot.org/uniprot/H0Y968.txt?version=36>. Especially pg 1.	1-4, 13, 14 ---------- 5-8, 11, 12
Y	US 2016/0168200 A1 (IMMATICS BIOTECHNOLOGIES GMBH) 16 June 2016 (16.06.2016) para [0203], [0313], [0314].	5-8
Y	US 6,008,058 A (SPATOLA et al.) 28 December 1999 (28.12.1999) col 12 ln 33-41	11, 12

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 July 2018	26 JUL 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/29733

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
GenCore ver 6.4.1 SEQ ID NOs: 1-3, 9, 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/29733

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9, 10, 15-21
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.