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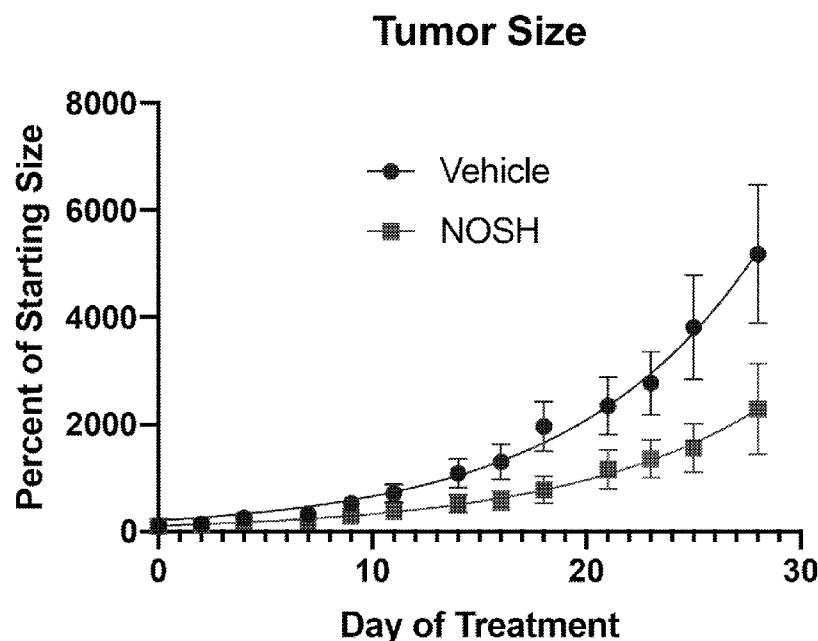


FIG. 10

(57) Abstract: Described herein is a new mitochondrial peptide. This small peptide is capable of modulating cancer, through a variety of mechanisms including autophagy/apoptosis, reduction of tumor cell viability, inducing inflammatory response in senescent cells and conversion of macrophage cell type. Administration of the peptide, its analogs and derivatives thereof, are likely to be effective treatments for cancer therapy, including generation of synthetic analogs that further enhance or abrogate activity relative to the peptide.



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MITOCHONDRIAL-DERIVED PEPTIDES AND ANALOGS THEREOF FOR USE AS A THERAPY FOR AGE-RELATED DISEASES INCLUDING CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application includes a claim of priority under 35 U.S.C. §119(e) to U.S. provisional patent application no. 63/025,495, filed May 15, 2020, the entirety of which is hereby incorporated by reference.

STATEMENT REGARDING FEDERALLY-SPONSORED RESEARCH OR DEVELOPMENT

10 This invention was made with government support under Grant Nos. AG034430 and GM090311 awarded by National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO SEQUENCE LISTING

15 The Sequence Listing submitted May 14, 2021 as a text file named "SequenceListing-065715-000103WO00_ST25" created on May 14, 2021 and having a size of 82,684 bytes, is hereby incorporated by reference.

FIELD OF THE INVENTION

Described herein are methods and compositions related to mitochondrial peptides, analogs and derivatives thereof for use as a therapy for age-related diseases, including cancer.

BACKGROUND

20 Mitochondrial peptides represent a new class of molecules for treatment of human diseases. It is now well-established that mitochondria are key actors in generating energy and regulating cell death. Mitochondria communicate back to the cell via retrograde signals that are encoded in the nuclear genome, or are secondary products of mitochondrial metabolism. 25 More recently, mitochondrial-derived peptides that are encoded by the mitochondrial genome have been identified as important actors in these regulatory processes. It is widely believed that mitochondrial-derived retrograde signal peptides will aid in the identification of genes and peptides with therapeutic and diagnostic to treat human diseases.

Therefore, it is an objective of the present invention to provide compositions based on 30 mitochondrial-derived peptides for treatment of age-related diseases, including cancer.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. NOSH (ND-One short open reading frames (sORF) in *Humans*) is safe in mice. NOSH has a sequence of MRLFGLLAVRRSGRSLMLTLIRGLSKRLG (SEQ ID NO:215).

FIG. 2. NOSH increases autophagy. The level of LC3 is increased in PC3 cells treated with 5 μ M NOSH for 6 hours.

FIG. 3. NOSH alters mitochondrial function. Membrane potential was measured by JC-1 staining.

5 FIG. 4. NOSH reduces viability of tumor cells. Viability was measured by LDH release into the media.

FIG. 5A-5E depict that NOSH acts as a senolytic peptide, preferentially reducing viability of senescent cells. FIG 5A and 5B are based on MTT assay. FIG. 5C is 10 μ M NOSH treatment for 24hr in non-senescent (NS) and senescent cells. Alternative
10 representations of FIG. 5A and 5B are shown in FIG. 5D and 5E, respectively.

FIG. 6. NOSH induces an inflammatory response in senescent cells (primary dermal fibroblast (HDFa)). Doxorubicin induced the senescent cells. The non-senescent cells and senescent cells were treated with 10 μ M NOSH for 24hr. After 24hr, conditioned media were collected and measured for the cytokine levels by meso scale discovery (MSD) assay.

15 FIG 7. NOSH analogues with increased efficacy.

FIG. 8A and 8B depict that NOSH is effective in decreasing viability of a number of different cancer cell lines. FIG. 8A depicts viability of J82 cells, OVCAR3 cells, SHSY5Y cells, HepG2 cell. FIG. 8B depicts viability of HCT116 cells and MCF7 cells (upper row: 10 μ M for 24 hours; lower row: 100 μ M).

20 FIG. 9 depicts gene expression in M2 macrophages, demonstrating that NOSH converts M2 Macrophages to M1 Macrophages. THP-1 cells were treated in PMA (200ng/ml) for 24 hours. Subsequently, medium was changed and PMA was removed. Cells were washed with PBS for three times. Cells were then treated with LPS (100ng/ml) or IL-4 (20ng/ml), with different doses of NOSH for 24 hours.

25 FIG. 10. NOSH reduces tumor size in mice.

FIG. 11A is a graph depicting efficacy of 214 NOSH analogues in DU145 cells compared to NOSH.

FIG. 11B depicts the efficacy of NOSH analogs (from NOSH-1 to NOSH-78) in DU145 cells compared to NOSH, an enlarged version of relevant data in FIG. 11A.

30 FIG. 11C depicts the efficacy of NOSH analogs (from NOSH-78 to NOSH-153) in DU145 cells compared to NOSH, an enlarged version of relevant data in FIG. 11A.

FIG. 11D depicts the efficacy of NOSH analogs (from NOSH-153 to NOSH-214) in DU145 cells compared to NOSH, an enlarged version of relevant data in FIG. 11A.

FIG. 12. NOSH analogues efficacy in A549 Cells

FIG. 13. NOSH structure. NOSH has a predicted double alpha-helix structure where the alpha-helices are predicted to be in amino acids 3-11 and amino acids 18-30. The hinge region between the helices seems to be of importance as mutations in this region significantly alter function.

FIG. 14 depicts that NOSH potently inhibits induced inflammatory markers, showing mouse plasma levels of IFN γ , TNF- α , IL-12, IL-10, IL-1 β , IL-2, IL-4 and IL-6. 10-week old, male, C57Bl6/J mice were treated with water or NOSH (10 mg/kg), with or without LPS (10 mg/kg) treatment (n=8/group), and plasma was collected 2 hour later.

DETAILED DESCRIPTION OF THE INVENTION

All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology 3rd ed.*, Revised, J. Wiley & Sons (New York, NY 2006); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual 4th ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2012), provide one skilled in the art with a general guide to many of the terms used in the present application.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

“Administering” and/or “administer” as used herein refer to any route for delivering a pharmaceutical composition to a patient. Routes of delivery may include non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes, as well as parenteral routes, and other methods known in the art.

Parenteral refers to a route of delivery that is generally associated with injection, including intraorbital, infusion, intraarterial, intracarotid, intracapsular, intracardiac, intradermal, intramuscular, intraperitoneal, intrapulmonary, intraspinal, intrasternal, intrathecal, intrauterine, intravenous, subarachnoid, subcapsular, subcutaneous, transmucosal, or transtracheal. Via the parenteral route, the compositions may be in the form of solutions or suspensions for infusion or for injection, or as lyophilized powders.

“Modulation” or “modulates” or “modulating” as used herein refers to upregulation (i.e., activation or stimulation), down regulation (i.e., inhibition or suppression) of a response or the two in combination or apart.

“Pharmaceutically acceptable excipients” refer to an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous. Examples of excipients include but are not limited to starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, wetting agents, emulsifiers, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservatives, antioxidants, plasticizers, gelling agents, thickeners, hardeners, setting agents, suspending agents, surfactants, humectants, carriers, stabilizers, pH buffering agents and combinations thereof.

“Promote” and/or “promoting” as used herein refer to an augmentation in a particular behavior of a cell or organism.

“Subject”, “individual”, or “patient”, as used herein includes all animals, including mammals and other animals, including, but not limited to, companion animals, farm animals and zoo animals. The term “animal” can include any living multi-cellular vertebrate organisms, a category that includes, for example, a mammal, a bird, a simian, a dog, a cat, a horse, a cow, a rodent, and the like. Likewise, the term “mammal” includes both human and non-human mammals. In various embodiments, a subject, individual or patient refers to a human being.

A “patient in need of” or “subject in need” of treatment for a particular disease, disorder, or condition may be a subject suspected of having that disease, disorder, or condition, diagnosed as having that disease, disorder, or condition, already treated or being treated for that disease, disorder, or condition, not treated for that disease, disorder, or condition, or at risk of developing that disease, disorder, or condition.

“Therapeutically effective amount” as used herein refers to the quantity of a specified composition, or active agent in the composition, sufficient to achieve a desired effect in a subject being treated. A therapeutically effective amount may vary depending upon a variety of factors, including but not limited to the physiological condition of the subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, desired clinical effect) and the route of administration. One skilled in the clinical and pharmacological arts will be able to determine a therapeutically effective amount through routine experimentation.

“Treat,” “treating” and “treatment” as used herein refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted condition, disease or disorder (collectively “ailment”) even if the treatment

is ultimately unsuccessful. Those in need of treatment may include those already with the ailment as well as those prone to have the ailment or those in whom the ailment is to be prevented.

“Senescence” occurs when a cell is too damaged to continue dividing and therefore its growth is curtailed. Senescent cells may begin leaching out harmful proteins and other compounds that can damage cells around them, leading to inflammation and eventually cell death. Senolytics are a class of drugs which selectively induce death of senescent cells. In some embodiments, senolytics reverse damages done by senescent cells.

“Retrograde signaling” refers to a process where a signal travels backwards from a target source to its original source. For example, mitochondrial retrograde signaling is a pathway of communication from mitochondria to the nucleus.

Mitochondria are thought to have transferred their genome to the host nucleus, leaving chromosomal “doppelgangers”, through the process of Nuclear Mitochondrial DNA-Transfer or nuclear insertions of mitochondrial origin (NUMTs). NUMTs come in various sizes from all parts of the mitochondrial DNA (mtDNA) with various degrees of homology with the original sequences. Entire mtDNA can be found in the nuclear genome, although in most cases with substantial sequence degeneration. Most NUMTs are small insertions of <500 bp and only 12.85% are >1500 bp. The percentage identity is inversely correlated with size and the mean percentage between NUMTs and mtDNA is 79.2% with a range of 63.5% to 100% identity.

Mitochondrial DNA (mtDNA) replication and transcription starts are regulated by nuclear-encoded proteins and is thought to be transcribed as a single polycistronic precursor that is processed into individual genes by excising the strategically positioned 22 tRNAs (tRNA punctuation model), giving rise to two rRNAs and 13 mRNA.

The human mitochondrion has two promoters in the heavy strand (major and minor) in close proximity, and one in the light strand, thereby giving rise to three different single polycistronic transcripts. The heavy major promoter is responsible for 80% of all mitochondrial RNA (mtRNA) transcripts. Although the entire gene is thought to be transcribed as a single transcript, the abundance of individual rRNA, tRNA, and mRNA transcripts varies greatly, and the rRNAs are the most abundant. This processing structure indicates an unexplored class of posttranscriptional processing in the mitochondria.

Importantly, many of the mRNA species identified from the mitochondria are discrete smaller length ones that do not map to the traditional mitochondrial protein encoding genes. For example, multiple such mRNAs are observed from the 16S rRNA. Parallel analysis of RNA ends (PARE) reveals a plethora of cleavage sites for the mitochondria. The majority of

tRNAs and mRNA have distinct dominant cleavage sites at the 5' termini, but intragenic cleavage sites are especially abundant in rRNAs. Notably, there is compelling evidence from the emerging field of small peptides showing biologically active peptides of 11–32 amino acids in length which are encoded by small open reading frames (sORFs) from a polycistronic mRNA.

Described herein are methods and compositions of new mitochondrial-derived peptides and synthetic analogs thereof and methods of using them in cancerous cells, inflammatory cells, or subjects with a cancer and/or an inflammatory disease. While looking for biologically active small, mitochondrially derived peptides (also referred to mitochondrial-derived peptides or mitochondrial peptides), the Inventors identified NOSH (ND-One sORF in Humans) with a potent biological activity against cancer cells while being safe for healthy cells. NOSH (ND-One sORF in Humans) is a mitochondrially encoded open reading frame that leads to the production of a new polypeptide that we call NOSH, which has biological effects affecting mitochondria, cell growth, and cell death. This peptide is implicated in cancer, senescence, and aging. As such, NOSH as well as synthetic NOSH analogues could be used in slower the progression of cancer, reducing tumor sizes, and treating other age-related diseases such as inflammatory diseases, obesity, and metabolic disorders. These peptides are key factors in retrograde mitochondrial signaling as well as mitochondrial gene expression. Compared to the human nuclear genome, mitochondria have a modest sized circular genome of ~16,570 bp, which ostensibly includes only 13 protein coding genes, which are all structural components of the electron transport chain system.

This invention includes the composition of matter of a family of peptide analogues of NOSH. This invention includes methods of use for these peptides in these indications. The invention also includes antibodies and assays for the detection of the levels of the NOSH peptide in the circulation and tissues of humans.

Various embodiments provide a composition comprising a mitochondrial-derived peptide or a synthetic or recombinant analog thereof. In some embodiments, a composition comprises a mitochondrial-derived peptide that comprises, or consists of, a peptide with an amino acid sequence of SEQ ID NO:215, or set forth in SEQ ID NO:215. In some embodiments, a composition comprises two or more mitochondrial-derived peptides selected from SEQ ID NOs: 1-215. In some embodiments, a composition includes a peptide analog of SEQ ID NO:215, a peptide derivative of SEQ ID NO:215, or a combination thereof.

In one embodiment, the mitochondrial peptide is about 12-65, 14-40, 16-35, 18-32, 20-32, or 25-32 amino acids in length. In a particular embodiment, the mitochondrial peptide is

about 32 amino acids in length. In one embodiment, the mitochondrial peptide includes a synthetic amino acid. In one embodiment, the mitochondrial peptide possesses at least 25%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more percentage identity to MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215).

5 In some embodiments, the mitochondrial peptide possesses at least 87.5% identity to SEQ ID NO:215; or, the mitochondrial peptide is 28-36 amino acids in length or contains no more than four amino acids difference (including substitution, deletion, and/or insertion) compared to SEQ ID NO: 215. In some embodiments, the mitochondrial peptide possesses at least 93% identity to SEQ ID NO:215; or, the mitochondrial peptide is 30-34 amino acids in length or
10 contains no more than two amino acids difference (including substitution, deletion, and/or insertion) compared to SEQ ID NO: 215. In some embodiments, the mitochondrial peptide possesses at least 80% identity to SEQ ID NO:215; or, the mitochondrial peptide is 26-38 amino acids in length or contains no more than six amino acids difference (including substitution, deletion, and/or insertion) compared to SEQ ID NO: 215. In some embodiments,
15 amino acids 3-11 and amino acids 18-30 are not replaced or deleted in SEQ ID NO:215. In various embodiments, substitution of amino acids that disrupt the alpha-helices (glycine, proline) are bad for activity; and therefore, in some aspects, the amino acid residues of SEQ ID NO:215 are not replaced with glycine, not replaced with proline, or not replaced with either glycine or proline; and in further aspects, the amino acid residues 3 through 11 and residues 18
20 through 30 of SEQ ID NO:215 are not replaced with glycine, not replaced with proline, or not replaced with either glycine or proline. In various embodiments, replacement with alpha-helix enhancers (alanine, leucine) improve activity of NOSH (SEQ ID NO:215); and therefore in some aspects, one or more residues of SEQ ID NO:215 are independently replaced with alanine, leucine, or both; and in further aspects, one or more residues 3 through 11 and residues
25 18 through 30 of SEQ ID NO:215 are independently replaced with alanine, leucine, or both.

Mitochondrial-derived peptide of SEQ ID NO: 215, in some embodiments, can be considered as an analog of one or more peptides of SEQ ID NOs: 1-214. In some embodiments, a composition comprising a peptide of any of SEQ ID NOs: 1-214 is provided, as well as its analogs or derivatives. In one embodiment, a composition includes a peptide of SEQ ID NO:1,
30 and/or a peptide that possesses at least 25%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more percentage identity to (SEQ ID NO:1). In one embodiment, a composition includes a peptide of SEQ ID NO:2, and/or a peptide that possesses at least 25%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more percentage identity to (SEQ ID NO:2).

One of ordinary skill in the art can establish percentage identity according to methods known in the art, including establishing a comparison window between a reference amino acid sequence and a second amino sequence, to establish the degree of percentage identity.

In some aspects, the mitochondrial-derived peptide and/or its analogs possesses a post-translational modification or other type of modification such as an artificial modification. In various embodiments, this includes for example, pegylation, fatty-acid conjugation lipidation, repeat polypeptide extension, the fragment crystallizable region of immunoglobulin G (IgG-Fc), camptothecin (CPT), human serum albumin (HAS), elastin-like polypeptide (ELP), transferrin, or albumin modification, among others.

In various embodiments described herein is a peptide, and the peptide is 12-65 amino acids in length. In various embodiments, the peptide at position 1 (i.e., first N-terminal amino acid) is X1, position 2 is (X2) and so on (X3, X4, X5, X6, etc.), wherein X1, X2, X3, X4, X5, X6, etc. is independently selected from a group consisting of a natural or synthetic amino acid. In some embodiments, the mitochondrial peptide (e.g., SEQ ID NO:215) possesses a post-translational modification or other type of modification such as an artificial modification. For example, modifications could include formylation, phosphorylation, acetylation at corresponding X1, X2, X3, X4, X5, and/or X6, etc. positions in analogs thereof. In various embodiments, the peptide possesses at least 25%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more percentage identity to MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215). In various embodiments, the peptide is 75%, 80%, 85%, 90%, 95% or more percentage identity to a portion of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), including for example, three or more, five or more, ten or more, fifteen or more, twenty or more, twenty five or more amino acids of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), wherein the portion begins at X1, X2, X3, X4, etc. This includes, any of the sequences in Table 1. In various embodiments, the peptide includes one or more of the aforementioned portions of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), further including about 6-9 amino acids of X11 to X18 of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215).

Various embodiments provide methods of treating a disease and/or condition using a mitochondrial-derived peptide and/or analogs, derivatives thereof including administering a quantity of the mitochondrial-derived peptide and/or analogs, derivative thereof, e.g., in a pharmaceutical composition with one or more pharmaceutically acceptable excipient, to a subject in need of treatment. In one embodiment, the mitochondrial peptide is a

MRLFGLLLA VRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), an analog or derivative thereof. In one embodiment, the peptide is about 12-65 amino acids in length. In one embodiment, the mitochondrial peptide is about 32 amino acids in length. In other embodiments, the peptide administered possesses at least 80%, 87.5%, 90%, 93% sequence identity to SEQ ID NO:215. In one embodiment, the peptide administered is selected from Table 1.

In one embodiment, the quantity of the mitochondrial peptide administered is a therapeutically effective amount of the mitochondrial peptide. In one embodiment, the subject is a mammal. In one embodiment, the subject is a human.

In various embodiments, administration of NOSH or its analogs decreases mitochondrial function.

In various embodiments, administration of NOSH and/or its analogues decreases cancer cell viability.

In various embodiments, administration of NOSH or its analogs to healthy mice has no grossly toxic effects.

In various embodiments, administration of NOSH or its analogs induces autophagy.

In various embodiments, one or more NOSH and its analogs are administered as a senolytic drug, which selectively kills cells that are senescent.

In various embodiments, one or more of NOSH and its analogs are administered as an immunomodulator, increasing the immune response.

In various embodiments, the disease and/or condition suitable for treatment with the mitochondrial peptide or analogue composition described includes cancer. In various embodiments, cancers are lung cancer, prostate cancer, colorectal cancer, stomach cancer, breast cancer, colorectal cancer, cervical cancer, melanoma, skin cancer, among others.

In various embodiments, disease and/or condition suitable for treatment with the mitochondrial peptide or analogue composition described includes cancer. In various embodiments, cancers are lung cancer, prostate cancer, colorectal cancer, stomach cancer, breast cancer, colorectal cancer, cervical cancer, melanoma, skin cancer, among others. In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue composition described includes prostate cancer. In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue composition described includes ovarian cancer. In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue composition described includes glioblastoma; or a malignant glioblastoma (e.g., grade IV). In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue

composition described includes neuroblastoma. In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue composition described includes bladder cancer. In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue composition described includes liver cancer. In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue composition described includes colon cancer. In some embodiments, the cancer for treatment with the mitochondrial peptide or analogue composition described includes breast cancer.

In some embodiments, a subject suitable for treatment with the mitochondrial peptide or analogue composition described has, suffers from, or has been diagnosed with one or more of ovarian cancer, glioblastoma, neuroblastoma, bladder cancer, liver cancer, colon cancer, and breast cancer.

In other embodiments, a subject suitable for treatment with the mitochondrial peptide or analogue composition described has, suffers from, or has been diagnosed with one or more of prostate cancer, glioblastoma, neuroblastoma, bladder cancer, liver cancer, and colon cancer.

In further embodiments, a subject suitable for treatment with the mitochondrial peptide or analogue composition described, or a subject in need of a treatment, a diagnosis, and/or an assay described, is a human at an age above 40 years old, above 50 years old, above 55 years old, above 60 years old, above 65 years old, above 70 years old, above 75 years old, above 80 years old, or above 85 years old.

In further embodiments, the disease and/or condition suitable for treatment with the mitochondrial peptide or analogue composition described includes an inflammatory disease. In some embodiments, the inflammatory disease is inflammatory bowel disease, pelvic inflammatory disease, Crohn's disease, costochondritis, conjunctivitis, bursitis, contact dermatitis, sarcoidosis, bronchiolitis, seroma, or chronic sinusitis. In other embodiments, the inflammatory disease is in the nervous system, cardiovascular system, respiratory system, digestion system, accessory digestive organs, integumentary system, musculoskeletal system, urine system, reproductive system, endocrine system, or lymphatic system.

In additional embodiments, disease and/or condition suitable for treatment with the mitochondrial peptide or analogue composition described includes age-related disease or condition. In various embodiments, age-related diseases or conditions are neurodegenerative disorders. In some embodiments, age-related diseases are atherosclerosis and cardiovascular disease, arthritis, cataracts, osteoporosis, type 2 diabetes, hypertension, or Alzheimer's disease.

In various embodiments, the subject does not express the peptide MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215). In various

embodiments, the subject expresses low amounts of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215) relative to a healthy normal subject. In other embodiments, the subject possesses a metabolic signature of low NOSH activity. In other embodiments, the subject possesses a metabolic signature of high or aberrant NOSH activity. In various embodiments, the subject is administered a dominant negative analog and/or derivative of NOSH. A dominant negative analog or derivative generally refers to an analog or derivative with a mutation/substitution/modification resulting in an adverse effect on the normal, wild-type molecule within the same cell. This usually occurs if the product can still interact with the same elements as the wild-type product, but block some aspect of its function.

Described herein is a method of diagnosing an individual for a disease and/or condition. In various embodiments, the method includes selecting a subject, detecting the presence, absence, or expression level of one or more biomarkers, and diagnosing the subject for a disease and/or condition, based on the presence, absence, or expression level of the one or more biomarkers. In various embodiments, the biomarker includes a mitochondrial peptide. In various embodiments, the biomarker includes MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), or any of those peptides in SEQ ID NOs: 1-214. For example, the subject may be diagnosed if expressing a low, high, or aberrant amount of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215) relative to a healthy normal subject. In various embodiments, detection of the presence, absence, or expression level of the biomarker includes antibody detection of the one or more biomarkers, including the use of, for example, a monoclonal antibody, polyclonal antibody, antisera, other immunogenic detection, and mass spectrometry detection methods.

In another embodiment, the biomarker includes a single nucleotide polymorphism (SNP). One of ordinary skill in the art is apprised of the methods capable of SNP detection.

The present invention further provides a method of enhancing efficacy of a treatment disease and/or condition using a mitochondrial peptide, including the steps of selecting a subject in need of treatment, and administering a quantity of the mitochondrial peptide to a subject receiving treatment a disease and/or condition, wherein the mitochondrial peptide enhancing the efficacy of the disease and/or condition, thereby enhancing efficacy of the treatment. In one embodiment, the mitochondrial peptide is administered simultaneously with a composition capable of treating a cancer. In one embodiment, the mitochondrial peptide is administered sequentially, before or after administration, of a composition capable of treating a disease and/or condition. In one embodiment, the subject is a human. For example, the

mitochondrial peptides and analog compositions of the invention can be co-administered with other therapeutic agents for the treatment of cancer, including for example cancers such as lung cancer, prostate cancer, colorectal cancer, stomach cancer, breast cancer, colorectal cancer, cervical cancer, melanoma, skin cancer, among others. Co-administration can be simultaneous, e.g., in a single pharmaceutical composition or separate compositions. The compositions of the invention can also be administered separately from the other therapeutic agent(s), e.g., on an independent dosing schedule.

In various embodiments, the present invention further provides a pharmaceutical composition. In one embodiment, the pharmaceutical composition includes a mitochondrial peptide and a pharmaceutically acceptable carrier. In one embodiment, the mitochondrial peptide is MRLFGLLLA VRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215). In various embodiments, the peptide is 75%, 80%, 85% or more percentage identity to a portion of MRLFGLLLA VRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), including for example, three or more, five or more, ten or more, fifteen or more, twenty or more, twenty-five or more amino acids of MRLFGLLLA VRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), wherein the portion begins at X1, X2, X3, X4, etc. This includes, any of the sequences in Table 1. In various embodiments, the peptide includes one or more of the aforementioned portions of MRLFGLLLA VRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), further including about 6-9 amino acids of X11 to X18 of MRLFGLLLA VRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215).

In some embodiments, the bioactive mitochondrial peptide is as small as about 6-9 amino-acids, about 9-15 amino acids, about 15-20 amino acid, about 20-25 amino acids, about 25-35 amino acids, about 30-40 amino acids, as well as some that are about 12-65 amino acids in length. In one embodiment, the mitochondrial peptide is about 32 amino acids in length. In one embodiment, the mitochondrial peptide in the pharmaceutical composition includes a therapeutically effective amount of the mitochondrial peptide. In one embodiment, pharmaceutical composition includes one or more mitochondrial peptides and a pharmaceutically acceptable carrier. In some embodiments, an amount of the mitochondrial-derived peptide of 0.1-20 μ M is administered or added to a sample containing tumor cells. In some embodiments, an amount of 0.1-0.5, 0.5-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-12, 12-14, 14-16, 16-18, or 18-20 μ M, or any range comprising an upper end and a lower end selected from these numbers, is administered or added to a sample. In some embodiments, a pharmaceutical composition includes a mitochondrial-derived peptide at 1-10 mg, 10-50 mg, 50-100 mg, 100-300 mg, 300-500 mg, 500 mg-1 g, 1 g-5 g, or 5 g-10 g. In some embodiments,

the amount of a mitochondrial-derived peptide is administered at a dose 0.1-1 mg/kg, 1-10 mg/kg, 10-50 mg/kg, 50-100 mg/kg, 100-200 mg/kg, 200-300 mg/kg, 300-400 mg/kg, 400-500 mg/kg, 500-600 mg/kg, 600-700 mg/kg, 700-800 mg/kg, 800-900 mg/kg, 0.9-1 g/kg, 1-5 g/kg, 5-10 g/kg to a subject.

5 In various embodiments, the present invention further provides a method of manufacturing a mitochondrial peptide. In one embodiment, the method of manufacturing includes the steps of providing one or more polynucleotides encoding a mitochondrial peptide, expressing the one or more polynucleotides in a host cell, and extracting the mitochondrial peptide from the host cell. In one embodiment, the method of manufacturing includes the steps
10 of expressing the one or more polynucleotides in a host cell, and extracting the mitochondrial peptide from the host cell. In one embodiment, the one or more polynucleotides are a sequence encoding MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), or a mitochondrial peptide possessing at least 25%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more percentage identity to
15 MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215). In various embodiments, the peptide is 75%, 80%, 85%, 90%, 95% or more percentage identity to a portion of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215), including for example, three or more, five or more, ten or more, fifteen or more, twenty or more, twenty five or more amino acids of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID
20 NO:215), wherein the portion begins at X1, X2, X3, X4, etc.

In another embodiment, the method of manufacturing includes the steps of peptide synthesis using liquid-phase synthesis or solid-phase synthesis. In one embodiment, the solid-phase synthesis is Fmoc or BOC synthesis.

EXAMPLES

25 Described herein are non-limiting examples of the claimed invention.

Example 1

A family of new peptide analogues were prepared for use as a therapy for age-related diseases, including cancer. While looking for biologically active small, mitochondrially derived peptides, the Inventors found NOSH (ND-One sORF in Humans) that had potent
30 biological activity.

Example 2.

Preliminary results

AS shown in Figure 1. NOSH was safe in mice. Further, NOSH increased autophagy. The level of autophagy-related protein microtubule-associated protein 1 light chain 3 (LC3) was increased in PC3 cells treated with 5 μ M NOSH for 6h. Figure 2.

Example 3

5

Mitochondrial function

Without being bound by any theory, preliminary results showed that Nosh altered mitochondrial function. This was further confirmed by measurements of membrane potential by JC-1 staining. Figure 3.

Moreover, as measured by lactate dehydrogenase (LDH) release into the media, NOSH reduced Cell Viability in Tumor Cells. Figure 4. PC-3 is a human prostate cancer cell line. OV90 is an ovarian cancer cells. U251 cell line was derived from a malignant glioblastoma tumor.

Example 4

Functional Properties

15 NOSH acts as a senolytic peptide, preferentially reducing viability of senescent cells. Figure 5A and Figure 5B. Treatment with 10 μ M NDDP4 for 24hr in non-senescent (NS) and senescent cells showed a higher caspase-3/7 activation in senescent cells than in non-senescent cells. Activation of caspase-3 is an essential event during apoptosis. Figure 5C.

20 NOSH induced an inflammatory response in senescent cells, for example in doxorubin induced senescent primary dermal fibroblast (HDFa). 10 μ M NOSH were incubated for 24hr in both non senescent cells and senescent cells. After 24hr, conditioned media collected and measured the cytokine levels by meso scale discovery (MSD) assay. Figure 6.

Example 5

25

Analogs

Modifications allowed for generation of NOSH Analogues with increased efficacy. Figure 7. A table of analogs, along with sequences are shown in Table 1. These analogs were applied in the platforms described herein.

Example 6

30

Role in Cancer Biology

As described, NOSH was effective in decreasing viability in a number of different cancer cell lines. Figures 8A and 8B. J82 cells are human bladder cancer cells; OVCAR3 is a cell line modeling ovarian carcinoma; SHSY5Y is a thrice-subcloned cell line derived from the SK-N-SH neuroblastoma cell line; HepG2 is a human liver cancer cell line; HCT116 is

HCT116 is a human colon cancer cell line; and MCF7 is a breast cancer cell line isolated from a 69-year-old woman. Moreover, NOSH converted M2 Macrophages to M1 Macrophages, measured by gene expression in M2 macrophages. Monocytic cells derived from a acute monocytic leukemia patient, i.e., THP-1 cells, were treated with phorbol 12-myristate 13-acetate (PMA) (200 ng/ml) for 24 hours. PMA at this concentration induces differentiation of THP-1 monocytes into macrophages. Medium was changed and PMA removed. Cells were washed with PBS for three times. Cells were then treated with lipopolysaccharide (LPS) (100ng/ml) or IL-4 (20ng/ml), with different doses of NOSH for 24 hours. Figure 10.

These results were affirmed by observations that NOSH reduces tumor size in mice. Figure 11A. When applied across cell lines, one could observe the effects of NOSH analogs in difference cell lines, including NOSH Analogues in DU145 Cells and NOSH Analogues in A549 Cells. Figure 11A and 12, respectively.

Example 7

NOSH Biochemical Structure

Figure 13 depicts NOSH structure. NOSH has a predicted double alpha-helix structure where the alpha-helices are predicted to be in AA 3-11 and AA 18-30. The hinge region between the helices seem to be of importance as mutations in this region significantly alter function.

Example 8

NOSH inhibits inflammatory markers

Figure 14 depicts the plasma levels of inflammatory markers induced in mice following LPS (10 mg/kg) administration with or without NOSH (10 mg/kg). Compared to LPS-treated mice, mice administered with NOSH following/concurrently with LPS had a statistically significantly lowered level of inflammatory markers.

Table 1 – NOSH Analogs

NOSH	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:215
NOSH-1	MRLFGLLLAVRRDGRSLSLMLTLIRGLSKRLG	SEQ ID NO:1
NOSH-2	MRLFGLLLAVRRSGRSLSLMLTLIRGLDKRLG	SEQ ID NO:2
NOSH-3	MRLFGLLLAVRRDGRSLSLMLDLIRGLDKRLG	SEQ ID NO:3
NOSH-4	GLLAVRRSGRSLDLMLTLI	SEQ ID NO:4
NOSH-5	MRLFGLLLAVRRSGRS	SEQ ID NO:5
NOSH-6	LSLMLTLIRGLSKRLG	SEQ ID NO:6
NOSH-7	MRLFGLLLAVGGSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:7

NOSH-8	MRLFGLLLAVGGSGGSLSLMLTLIRGLSKRLG	SEQ ID NO:8
NOSH-9	formylated-MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:9
NOSH-10	MRLFGLLLAVRRDGRDLDLMLDLIRGLDKRLG	SEQ ID NO:10
NOSH-11	MRLFGLLLAVRRDGRDLDLMLDLIRGLSKRLG	SEQ ID NO:11
NOSH-12	MRLFGLLLAVRRSGRDLDLMLDLIRGLDKRLG	SEQ ID NO:12
NOSH-13	MRLFGLLLAVRRDGRSLDLMLDLIRGLDKRLG	SEQ ID NO:13
NOSH-14	MRLFGLLLAVRRDGRDLSLMLDLIRGLDKRLG	SEQ ID NO:14
NOSH-15	MRLFGLLLAVRRDGRDLDLMLTLIRGLDKRLG	SEQ ID NO:15
NOSH-16	MRLFGLLLAVRRDGRDLDLMLTLIRGLSKRLG	SEQ ID NO:16
NOSH-17	MRLFGLLLAVRRDGRDLSLMLDLIRGLSKRLG	SEQ ID NO:17
NOSH-18	MRLFGLLLAVRRDGRDLSLMLTLIRGLDKRLG	SEQ ID NO:18
NOSH-19	MRLFGLLLAVRRDGRSLDLMLDLIRGLSKRLG	SEQ ID NO:19
NOSH-20	MRLFGLLLAVRRDGRSLDLMLTLIRGLDKRLG	SEQ ID NO:20
NOSH-21	MRLFGLLLAVRRSGRDLDLMLDLIRGLSKRLG	SEQ ID NO:21
NOSH-22	MRLFGLLLAVRRSGRDLDLMLTLIRGLDKRLG	SEQ ID NO:22
NOSH-23	MRLFGLLLAVRRSGRDLSLMLDLIRGLDKRLG	SEQ ID NO:23
NOSH-24	MRLFGLLLAVRRSGRSLDLMLDLIRGLDKRLG	SEQ ID NO:24
NOSH-25	MRLFGLLLAVRRDGRDLSLMLTLIRGLSKRLG	SEQ ID NO:25
NOSH-26	MRLFGLLLAVRRDGRSLDLMLTLIRGLSKRLG	SEQ ID NO:26
NOSH-27	MRLFGLLLAVRRDGRSLSLMLDLIRGLSKRLG	SEQ ID NO:27
NOSH-28	MRLFGLLLAVRRDGRSLSLMLTLIRGLDKRLG	SEQ ID NO:28
NOSH-29	MRLFGLLLAVRRSGRDLDLMLTLIRGLSKRLG	SEQ ID NO:29
NOSH-30	MRLFGLLLAVRRSGRDLSLMLDLIRGLSKRLG	SEQ ID NO:30
NOSH-31	MRLFGLLLAVRRSGRDLSLMLTLIRGLDKRLG	SEQ ID NO:31
NOSH-32	MRLFGLLLAVRRSGRSLDLMLDLIRGLSKRLG	SEQ ID NO:32
NOSH-33	MRLFGLLLAVRRSGRSLDLMLTLIRGLDKRLG	SEQ ID NO:33
NOSH-34	MRLFGLLLAVRRSGRSLSLMLDLIRGLDKRLG	SEQ ID NO:34
NOSH-35	ARLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:35
NOSH-36	MALFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:36
NOSH-37	MRAFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:37
NOSH-38	MRLAGLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:38
NOSH-39	MRLFALLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:39
NOSH-40	MRLFALLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:40
NOSH-41	MRLFGLALAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:41

NOSH-42	MRLFGLLAAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:42
NOSH-43	MRLFGLLLAARRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:43
NOSH-44	MRLFGLLLAVARRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:44
NOSH-45	MRLFGLLLAVRASGRSLSLMLTLIRGLSKRLG	SEQ ID NO:45
NOSH-46	MRLFGLLLAVRRAGRSLSLMLTLIRGLSKRLG	SEQ ID NO:46
NOSH-47	MRLFGLLLAVRRSARSLSLMLTLIRGLSKRLG	SEQ ID NO:47
NOSH-48	MRLFGLLLAVRRSGASLSLMLTLIRGLSKRLG	SEQ ID NO:48
NOSH-49	MRLFGLLLAVRRSGRALSLMLTLIRGLSKRLG	SEQ ID NO:49
NOSH-50	MRLFGLLLAVRRSGRSASLMLTLIRGLSKRLG	SEQ ID NO:50
NOSH-51	MRLFGLLLAVRRSGRSLALMLTLIRGLSKRLG	SEQ ID NO:51
NOSH-52	MRLFGLLLAVRRSGRSLSAMTLIRGLSKRLG	SEQ ID NO:52
NOSH-53	MRLFGLLLAVRRSGRSLSLATLIRGLSKRLG	SEQ ID NO:53
NOSH-54	MRLFGLLLAVRRSGRSLSLMATLIRGLSKRLG	SEQ ID NO:54
NOSH-55	MRLFGLLLAVRRSGRSLSLMLALIRGLSKRLG	SEQ ID NO:55
NOSH-56	MRLFGLLLAVRRSGRSLSLMLTAIRGLSKRLG	SEQ ID NO:56
NOSH-57	MRLFGLLLAVRRSGRSLSLMLTLARGLSKRLG	SEQ ID NO:57
NOSH-58	MRLFGLLLAVRRSGRSLSLMLTLIAGLSKRLG	SEQ ID NO:58
NOSH-59	MRLFGLLLAVRRSGRSLSLMLTLIRALSKRLG	SEQ ID NO:59
NOSH-60	MRLFGLLLAVRRSGRSLSLMLTLIRGASKRLG	SEQ ID NO:60
NOSH-61	MRLFGLLLAVRRSGRSLSLMLTLIRGLAKRLG	SEQ ID NO:61
NOSH-62	MRLFGLLLAVRRSGRSLSLMLTLIRGLSARLG	SEQ ID NO:62
NOSH-63	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKALG	SEQ ID NO:63
NOSH-64	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRAG	SEQ ID NO:64
NOSH-65	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLA	SEQ ID NO:65
NOSH-66	RLFGLLLAVGGSGGSLSLMLTLIRGLSK	SEQ ID NO:66
NOSH-67	RLFGLLLAVGGLSLMLTLIRGLSK	SEQ ID NO:67
NOSH-68	RLFGLLLAVGGSGGSGGLSLMLTLIRGLSK	SEQ ID NO:68
NOSH-69	MRLFGLLLAVGGSGGS	SEQ ID NO:69
NOSH-70	GGSGGSLSLMLTLIRGLSKRLG	SEQ ID NO:70
NOSH-71	LLAVGGSGGSLSLMLTL	SEQ ID NO:71
NOSH-72	LLAVGGSGGSLSLMLTLIRGLSK	SEQ ID NO:72
NOSH-73	MRLFGLLLAVPGSGGSLSLMLTLIRGLSKRLG	SEQ ID NO:73
NOSH-74	MRLFGLLLAVPGSGPSLSLMLTLIRGLSKRLG	SEQ ID NO:74
NOSH-75	MRLFGLLLAVRRSGGSLSLMLTLIRGLSKRLG	SEQ ID NO:75

NOSH-76	LLAVGGSGDLDLMLTLIRGLDK	SEQ ID NO:76
NOSH-77	MPLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:77
NOSH-78	MRPFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:78
NOSH-79	MRLPGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:79
NOSH-80	MRLFPLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:80
NOSH-81	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:81
NOSH-82	MRLFGLPLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:82
NOSH-83	MRLFGLLPVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:83
NOSH-84	MRLFGLLLPVRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:84
NOSH-85	MRLFGLLLAPRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:85
NOSH-86	MRLFGLLLAVPRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:86
NOSH-87	MRLFGLLLAVRPSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:87
NOSH-88	MRLFGLLLAVRRPGRSLSLMLTLIRGLSKRLG	SEQ ID NO:88
NOSH-89	MRLFGLLLAVRRSPRSLSLMLTLIRGLSKRLG	SEQ ID NO:89
NOSH-90	MRLFGLLLAVRRSGPSLSLMLTLIRGLSKRLG	SEQ ID NO:90
NOSH-91	MRLFGLLLAVRRSGRPLSLMLTLIRGLSKRLG	SEQ ID NO:91
NOSH-92	MRLFGLLLAVRRSGRSPSLMLTLIRGLSKRLG	SEQ ID NO:92
NOSH-93	MRLFGLLLAVRRSGRSLPLMLTLIRGLSKRLG	SEQ ID NO:93
NOSH-94	MRLFGLLLAVRRSGRSLSPMLTLIRGLSKRLG	SEQ ID NO:94
NOSH-95	MRLFGLLLAVRRSGRSLSLPLTLIRGLSKRLG	SEQ ID NO:95
NOSH-96	MRLFGLLLAVRRSGRSLSLMPTLIRGLSKRLG	SEQ ID NO:96
NOSH-97	MRLFGLLLAVRRSGRSLSLMLPLIRGLSKRLG	SEQ ID NO:97
NOSH-98	MRLFGLLLAVRRSGRSLSLMLTPIRGLSKRLG	SEQ ID NO:98
NOSH-99	MRLFGLLLAVRRSGRSLSLMLTLPRGLSKRLG	SEQ ID NO:99
NOSH-100	MRLFGLLLAVRRSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:100
NOSH-101	MRLFGLLLAVRRSGRSLSLMLTLIRPLSKRLG	SEQ ID NO:101
NOSH-102	MRLFGLLLAVRRSGRSLSLMLTLIRGPSKRLG	SEQ ID NO:102
NOSH-103	MRLFGLLLAVRRSGRSLSLMLTLIRGLPKRLG	SEQ ID NO:103
NOSH-104	MRLFGLLLAVRRSGRSLSLMLTLIRGLSPRLG	SEQ ID NO:104
NOSH-105	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKPLG	SEQ ID NO:105
NOSH-106	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRPG	SEQ ID NO:106
NOSH-107	MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLP	SEQ ID NO:107
NOSH-108	MRLAALLAARRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:108
NOSH-109	MRLFALLAARRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:109

NOSH-110	MRLAGLLLAARRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:110
NOSH-111	MRLAALLLAVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:111
NOSH-112	MRLFGLLLAVRRSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:112
NOSH-113	MRLFGLLLAVRRSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:113
NOSH-114	MRLFGLLLAVRRSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:114
NOSH-115	MRLFGLLLAVRRSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:115
NOSH-116	MRLFGLLLAVRRSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:116
NOSH-117	MRLFGLLLAVRRSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:117
NOSH-118	MRLFGLLLAVRRSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:118
NOSH-119	MRLFGLLLAVRRSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:119
NOSH-120	MRLAALLLAARRSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:120
NOSH-121	MRLAALLLAARRSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:121
NOSH-122	MRLAALLLAARRSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:122
NOSH-123	MRLAALLLAARRSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:123
NOSH-124	MRLAALLLAARRSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:124
NOSH-125	MRLAALLLAARRSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:125
NOSH-126	MRLAALLLAARRSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:126
NOSH-127	MRLAALLLAARRSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:127
NOSH-128	MRLFALLLAARRSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:128
NOSH-129	MRLFALLLAARRSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:129
NOSH-130	MRLFALLLAARRSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:130
NOSH-131	MRLFALLLAARRSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:131
NOSH-132	MRLFALLLAARRSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:132
NOSH-133	MRLFALLLAARRSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:133
NOSH-134	MRLFALLLAARRSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:134
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NOSH-136	MRLAGLLLAARRSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:136
NOSH-137	MRLAGLLLAARRSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:137
NOSH-138	MRLAGLLLAARRSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:138
NOSH-139	MRLAGLLLAARRSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:139
NOSH-140	MRLAGLLLAARRSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:140
NOSH-141	MRLAGLLLAARRSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:141
NOSH-142	MRLAGLLLAARRSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:142
NOSH-143	MRLAGLLLAARRSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:143

NOSH-144	MRLAALLLAVRRSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:144
NOSH-145	MRLAALLLAVRRSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:145
NOSH-146	MRLAALLLAVRRSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:146
NOSH-147	MRLAALLLAVRRSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:147
NOSH-148	MRLAALLLAVRRSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:148
NOSH-149	MRLAALLLAVRRSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:149
NOSH-150	MRLAALLLAVRRSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:150
NOSH-151	MRLAALLLAVRRSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:151
NOSH-152	MPLFGLLLPVPPSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:152
NOSH-153	MPLFGLLLPVPRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:153
NOSH-154	MPLFGLLLPVRRSGRSLSLMLTLIRGLSKRLG	SEQ ID NO:154
NOSH-155	MRLFGLLLAVRRSGRSLSLMLALIRALSKRLA	SEQ ID NO:155
NOSH-156	MRLFGLLLAVRRSGRSLSLMLTLIRALSKRLA	SEQ ID NO:156
NOSH-157	MRLFGLLLAVRRSGRSLSLMLALIRALSKRLG	SEQ ID NO:157
NOSH-158	MRLFGLLLAVRRSGRSLSLMLALIRGLSKRLA	SEQ ID NO:158
NOSH-159	MPLFGLLLPVPPSGRSLSLMLALIRALSKRLA	SEQ ID NO:159
NOSH-160	MPLFGLLLPVPPSGRSLSLMLTLIRALSKRLA	SEQ ID NO:160
NOSH-161	MPLFGLLLPVPPSGRSLSLMLALIRALSKRLG	SEQ ID NO:161
NOSH-162	MPLFGLLLPVPPSGRSLSLMLALIRGLSKRLA	SEQ ID NO:162
NOSH-163	MPLFGLLLPVPRSGRSLSLMLALIRALSKRLA	SEQ ID NO:163
NOSH-164	MPLFGLLLPVPRSGRSLSLMLTLIRALSKRLA	SEQ ID NO:164
NOSH-165	MPLFGLLLPVPRSGRSLSLMLALIRALSKRLG	SEQ ID NO:165
NOSH-166	MPLFGLLLPVPRSGRSLSLMLALIRGLSKRLA	SEQ ID NO:166
NOSH-167	MPLFGLLLPVRRSGRSLSLMLALIRALSKRLA	SEQ ID NO:167
NOSH-168	MPLFGLLLPVRRSGRSLSLMLTLIRALSKRLA	SEQ ID NO:168
NOSH-169	MPLFGLLLPVRRSGRSLSLMLALIRALSKRLG	SEQ ID NO:169
NOSH-170	MPLFGLLLPVRRSGRSLSLMLALIRGLSKRLA	SEQ ID NO:170
NOSH-171	MPLFGLLLPVPPSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:171
NOSH-172	MPLFGLLLPVPPSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:172
NOSH-173	MPLFGLLLPVPPSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:173
NOSH-174	MPLFGLLLPVPPSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:174
NOSH-175	MPLFGLLLPVPPSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:175
NOSH-176	MPLFGLLLPVPPSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:176
NOSH-177	MPLFGLLLPVPPSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:177

NOSH-178	MPLFGLLLPVPPSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:178
NOSH-179	MPLFGLLLPVPRSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:179
NOSH-180	MPLFGLLLPVPRSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:180
NOSH-181	MPLFGLLLPVPRSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:181
NOSH-182	MPLFGLLLPVPRSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:182
NOSH-183	MPLFGLLLPVPRSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:183
NOSH-184	MPLFGLLLPVPRSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:184
NOSH-185	MPLFGLLLPVPRSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:185
NOSH-186	MPLFGLLLPVPRSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:186
NOSH-187	MPLFGLLLPVRRSGRSPSPMPTPIPGPSKPPG	SEQ ID NO:187
NOSH-188	MPLFGLLLPVRRSGRSPSLMPTPIPGPSKPPG	SEQ ID NO:188
NOSH-189	MPLFGLLLPVRRSGRSPSLMPTLIPGPSKPPG	SEQ ID NO:189
NOSH-190	MPLFGLLLPVRRSGRSPSLMPTLIPGLSKPPG	SEQ ID NO:190
NOSH-191	MPLFGLLLPVRRSGRSPSLMPTLIPGLSKPLG	SEQ ID NO:191
NOSH-192	MPLFGLLLPVRRSGRSLSLMPTLIPGLSKPLG	SEQ ID NO:192
NOSH-193	MPLFGLLLPVRRSGRSLSLMPTLIPGLSKRLG	SEQ ID NO:193
NOSH-194	MPLFGLLLPVRRSGRSLSLMLTLIPGLSKRLG	SEQ ID NO:194
NOSH-195	MRLAALLLAARRSGRSLSLMLALIRALSKRLA	SEQ ID NO:195
NOSH-196	MRLAALLLAARRSGRSLSLMLTLIRALSKRLA	SEQ ID NO:196
NOSH-197	MRLAALLLAARRSGRSLSLMLALIRALSKRLG	SEQ ID NO:197
NOSH-198	MRLAALLLAARRSGRSLSLMLALIRGLSKRLA	SEQ ID NO:198
NOSH-199	MRLFALLLAARRSGRSLSLMLALIRALSKRLA	SEQ ID NO:199
NOSH-200	MRLFALLLAARRSGRSLSLMLTLIRALSKRLA	SEQ ID NO:200
NOSH-201	MRLFALLLAARRSGRSLSLMLALIRALSKRLG	SEQ ID NO:201
NOSH-202	MRLFALLLAARRSGRSLSLMLALIRGLSKRLA	SEQ ID NO:202
NOSH-203	MRLAGLLLAARRSGRSLSLMLALIRALSKRLA	SEQ ID NO:203
NOSH-204	MRLAGLLLAARRSGRSLSLMLTLIRALSKRLA	SEQ ID NO:204
NOSH-205	MRLAGLLLAARRSGRSLSLMLALIRALSKRLG	SEQ ID NO:205
NOSH-206	MRLAGLLLAARRSGRSLSLMLALIRGLSKRLA	SEQ ID NO:206
NOSH-207	MRLAALLLAVRRSGRSLSLMLALIRALSKRLA	SEQ ID NO:207
NOSH-208	MRLAALLLAVRRSGRSLSLMLTLIRALSKRLA	SEQ ID NO:208
NOSH-209	MRLAALLLAVRRSGRSLSLMLALIRALSKRLG	SEQ ID NO:209
NOSH-210	MRLAALLLAVRRSGRSLSLMLALIRGLSKRLA	SEQ ID NO:210
NOSH-211	MRLFGLLLAVRRSGRSLSLMLDLIRGLSKRLG	SEQ ID NO:211

NOSH-212	MRLFGLLLAVRRSGRSLSLMLRLIRGLSKRLG	SEQ ID NO:212
NOSH-213	MRLFGLLLAVRRSGRSLSLMLWLIRGLSKRLG	SEQ ID NO:213
NOSH-214	MRLFGLLLAVRRSGRSLSLMLKLIRGLSKRLG	SEQ ID NO:214

The various methods and techniques described above provide a number of ways to carry out the invention. Of course, it is to be understood that not necessarily all objectives or advantages described may be achieved in accordance with any particular embodiment described herein. Thus, for example, those skilled in the art will recognize that the methods
5 can be performed in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objectives or advantages as may be taught or suggested herein. A variety of advantageous and disadvantageous alternatives are mentioned herein. It is to be understood that some preferred embodiments specifically include one, another, or several advantageous features, while others specifically exclude one, another,
10 or several disadvantageous features, while still others specifically mitigate a present disadvantageous feature by inclusion of one, another, or several advantageous features.

Furthermore, the skilled artisan will recognize the applicability of various features from different embodiments. Similarly, the various elements, features and steps discussed above, as well as other known equivalents for each such element, feature or step, can be mixed and
15 matched by one of ordinary skill in this art to perform methods in accordance with principles described herein. Among the various elements, features, and steps some will be specifically included and others specifically excluded in diverse embodiments.

Although the invention has been disclosed in the context of certain embodiments and examples, it will be understood by those skilled in the art that the embodiments of the invention
20 extend beyond the specifically disclosed embodiments to other alternative embodiments and/or uses and modifications and equivalents thereof.

Many variations and alternative elements have been disclosed in embodiments of the present invention. Still further variations and alternate elements will be apparent to one of skill in the art. Among these variations, without limitation, are the compositions and methods
25 related to peptides, mitochondrial peptides, their analogs and derivatives thereof s, methods and compositions related to use of the aforementioned compositions, techniques and composition and use of solutions used therein, and the particular use of the products created through the teachings of the invention. Various embodiments of the invention can specifically include or exclude any of these variations or elements.

30 In some embodiments, the numbers expressing quantities of ingredients, properties such as concentration, reaction conditions, and so forth, used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term “about.” Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the

desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

In some embodiments, the terms “a” and “an” and “the” and similar references used in the context of describing a particular embodiment of the invention (especially in the context of certain of the following claims) can be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. “such as”) provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

Preferred embodiments of this invention are described herein, including the best mode known to the inventor for carrying out the invention. Variations on those preferred embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. It is contemplated that skilled artisans can employ such variations as appropriate, and the invention can be practiced otherwise than specifically described herein. Accordingly, many embodiments of this invention include all modifications and equivalents of

the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

5 Furthermore, numerous references have been made to patents and printed publications throughout this specification. Each of the above cited references and printed publications are herein individually incorporated by reference in their entirety.

10 In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that can be employed can be within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention can be utilized in accordance with the teachings herein. Accordingly, embodiments of the present invention are not limited to that precisely as shown and described.

THE CLAIMS

1. A composition comprising:

a mitochondrial-derived peptide, which comprises an amino acid sequence of any of SEQ ID NO:215 and SEQ ID NOs:1-214, or a derivative or analog thereof.

2. The composition of claim 1, wherein the mitochondrial-derived peptide comprises an amino acid sequence of MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215).

3. The composition of claim 1, wherein the mitochondrial-derived peptide comprises an amino acid sequence with about 75%, 80%, 85%, 90%, 95% or more percentage identity to MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215).

4. The composition of claim 1 or 3, wherein the mitochondrial-derived peptide is about 12-65 amino acids in length.

5. The composition of any of claims 1-4, wherein the mitochondrial-derived peptide possesses a post-translational or artificial modification, wherein the artificial modification comprises pegylation, fatty-acid conjugation, polypeptide extension, IgG-Fc, camptothecin (CPT), human serum albumin (HSA), elastin-like polypeptide (ELP), transferrin, or albumin modification.

6. A pharmaceutical composition, comprising a mitochondrial-derived peptide of any of claims 1-5 and a pharmaceutically acceptable excipient.

7. A method of treating a disease and/or condition comprising:

selecting a subject in need of treatment; and

administering a quantity of a mitochondrial-derived peptide to a subject in need of treatment, wherein the mitochondrial-derived peptide is capable of treating the disease and/or condition, and the mitochondrial-derived peptide comprises an amino acid sequence of any of SEQ ID NO:215 and SEQ ID NOs:1-214, or a derivative or analog thereof.

8. The method of claim 7, wherein the mitochondrial peptide comprises an amino acid sequence MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215).

9. The method of claim 7, wherein the mitochondrial-derived peptide comprises an amino acid sequence with about 75%, 80%, 85%, 90%, 95% or more percentage identity to MRLFGLLLAVRRSGRSLSLMLTLIRGLSKRLG (SEQ ID NO:215).

10. The method of claim 7, wherein the mitochondrial-derived peptide is 12-65 amino acids in length.
11. The method of any of claims 7-10, wherein the disease and/or condition comprises cancer, or an inflammatory disease.
12. The method of claim 11, wherein the cancer is lung cancer, prostate cancer, colorectal cancer, stomach cancer, breast cancer, colorectal cancer, cervical cancer, melanoma, or skin cancer.
13. The method of any of claims 7-12, wherein the mitochondrial-derived peptide is administered in a quantity effective for reducing tumor cell viability.
14. The method of any of claims 7-12, wherein the mitochondrial-derived peptide is administered in a quantity effective for increasing apoptosis of at least 50% senescent cells while maintaining at least 80% viability of non-senescent cells.
15. The method of any of claims 7-12, wherein the mitochondrial-derived peptide is administered in a quantity effective for inducing an inflammatory response in senescent cells.
16. The method of any of claims 7-12, wherein the mitochondrial-derived peptide is capable of converting M2 macrophages to M1 macrophage.
17. The method of any of claims 7 and 9-16, wherein the mitochondrial-derived peptide is selected from the group consisting of SEQ ID NOs: 1-214.
18. A method of detecting a mitochondrial-derived peptide in an individual in need thereof, comprising:
 - detecting the presence, absence, or expression level of one or more mitochondrial-derived peptides selected from SEQ NO:215 and SEQ ID NOs:1-214 in a biological sample obtained from the individual.
19. The method of claim 18, wherein the individual has a cancer and/or an inflammation.
20. The method of claim 18, wherein detecting the presence, absence, or expression level comprises an immunoassay, RNA sequencing, northern blot, in situ hybridization, hybridization array, serial analysis of gene expression (SAGE), reverse transcription polymerase chain reaction (PCR), real-time PCR, real time reverse transcription PCR, quantitative PCR, microarray, mass spectrometry, and combinations thereof.

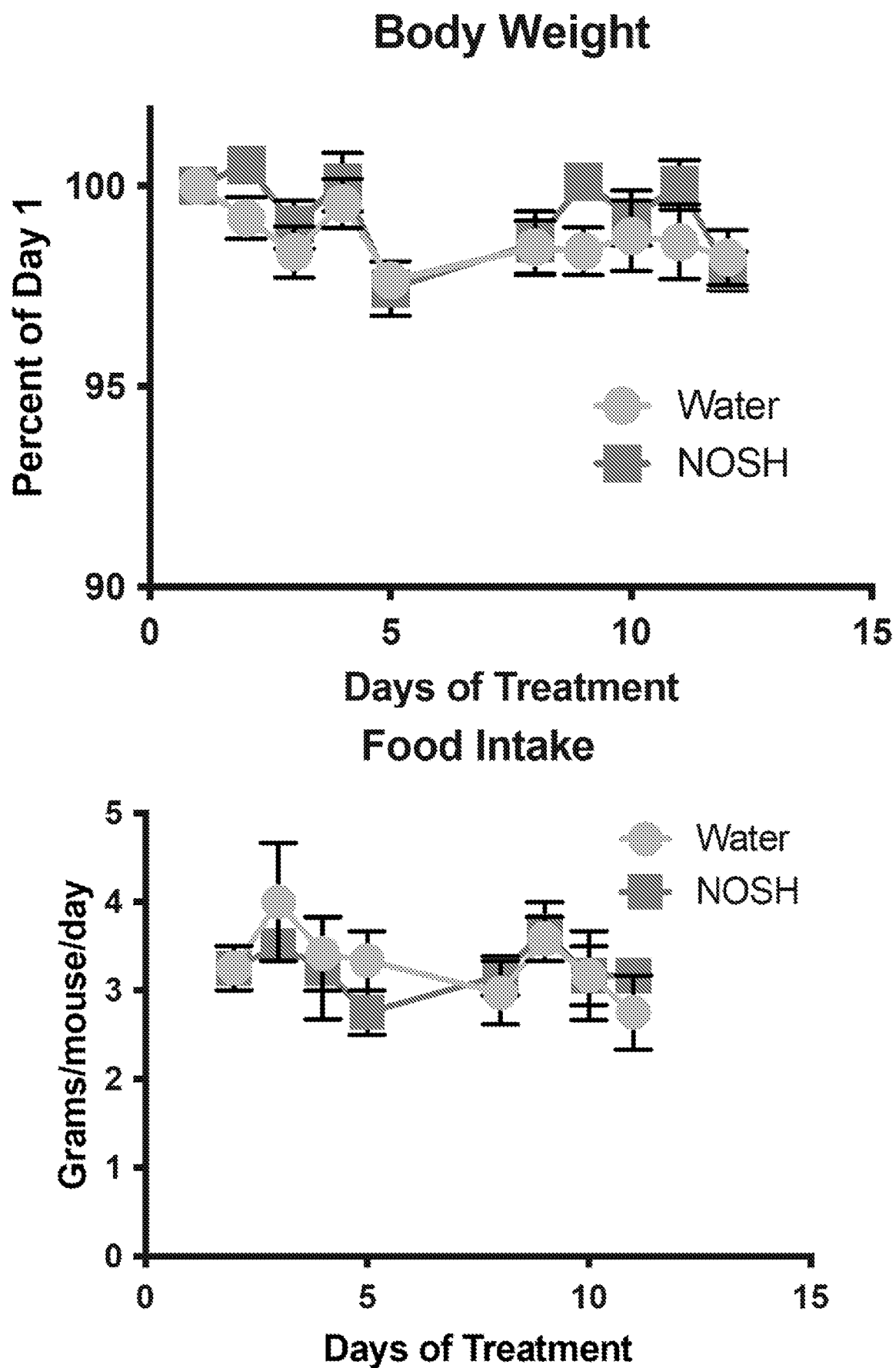


FIG. 1

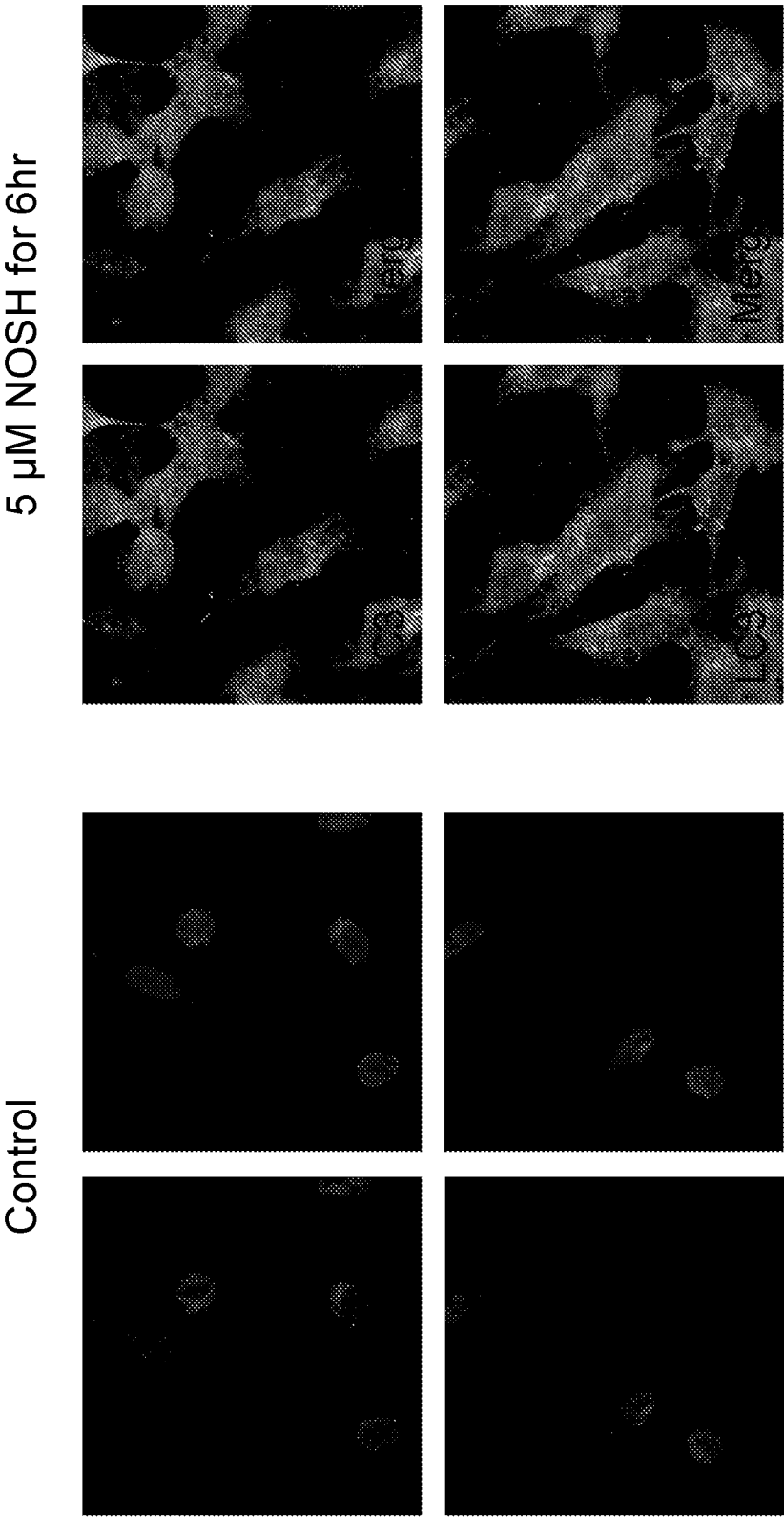


FIG. 2

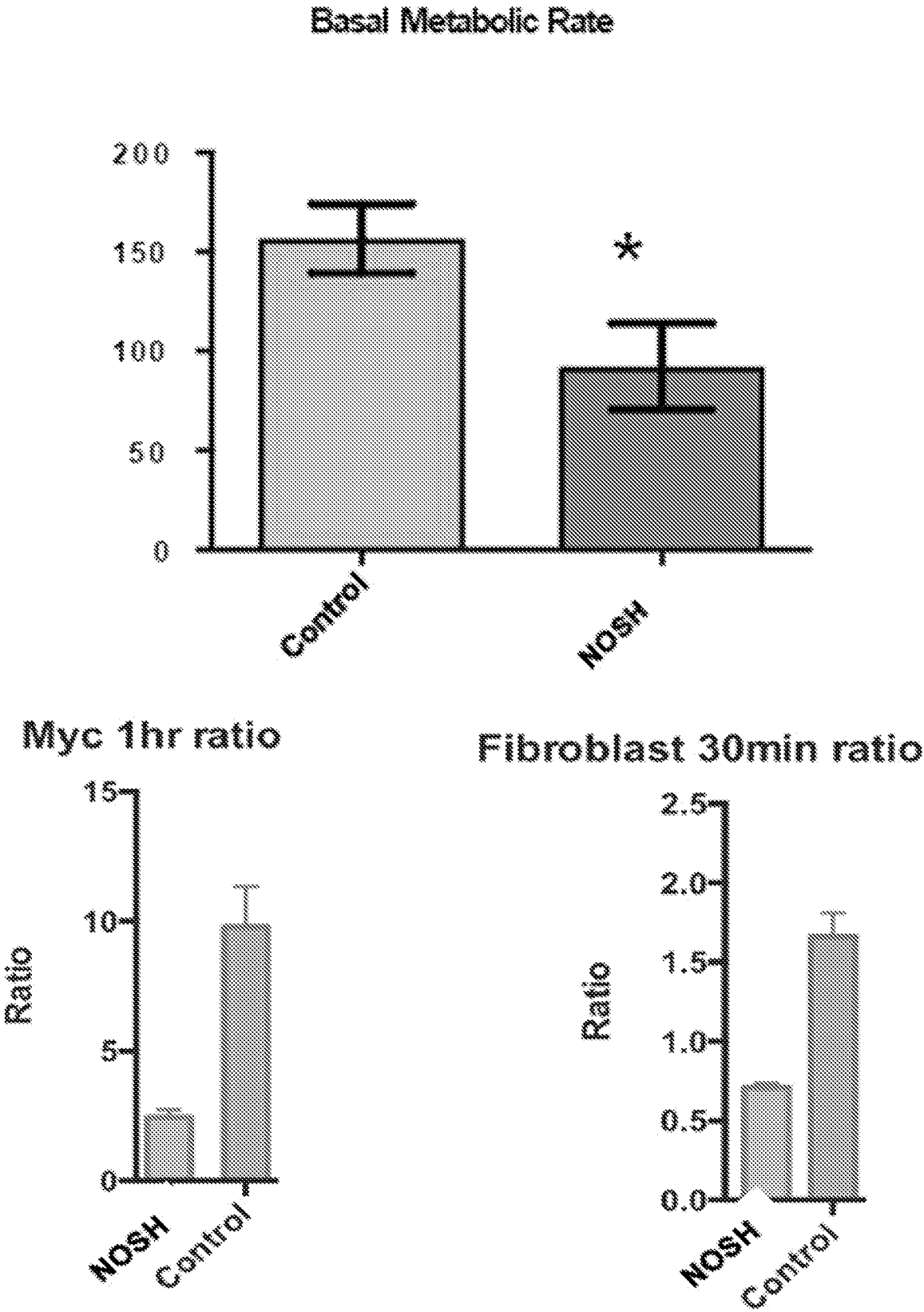


FIG. 3

Viability of Cancer Cell Lines

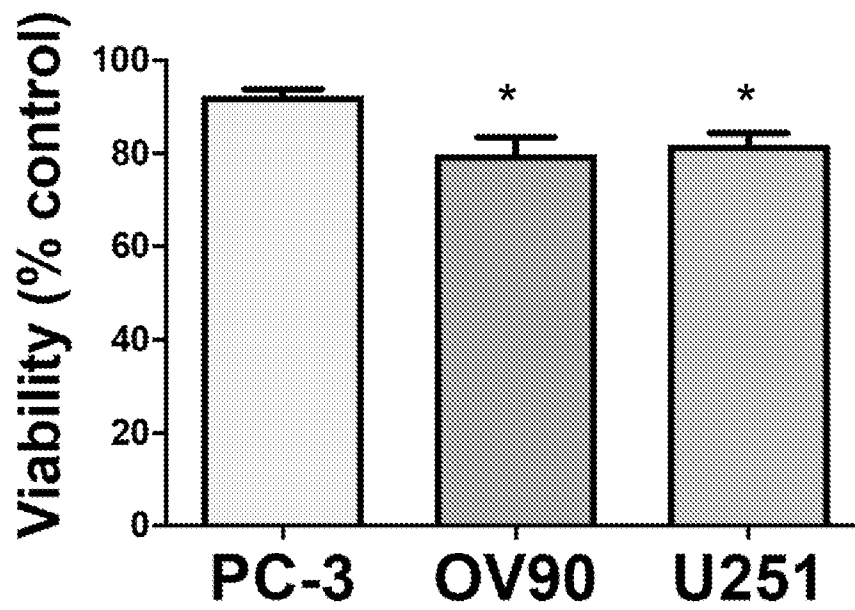


FIG. 4

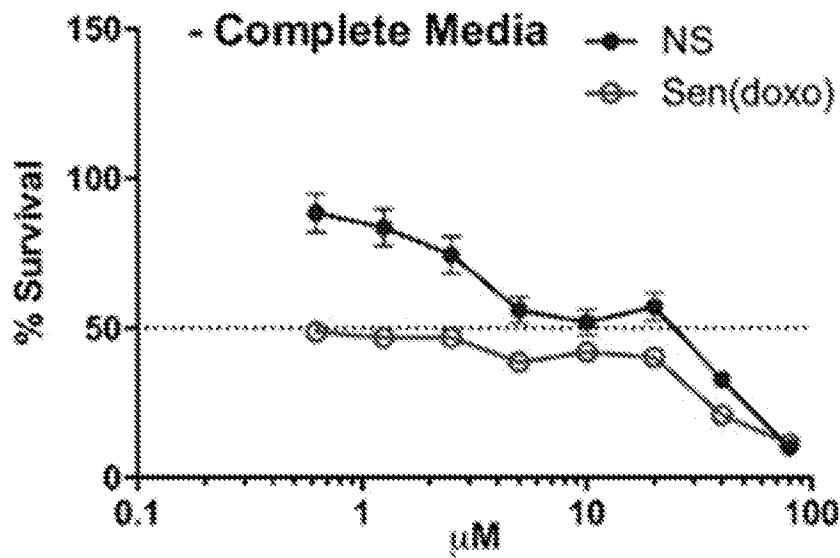


FIG. 5A

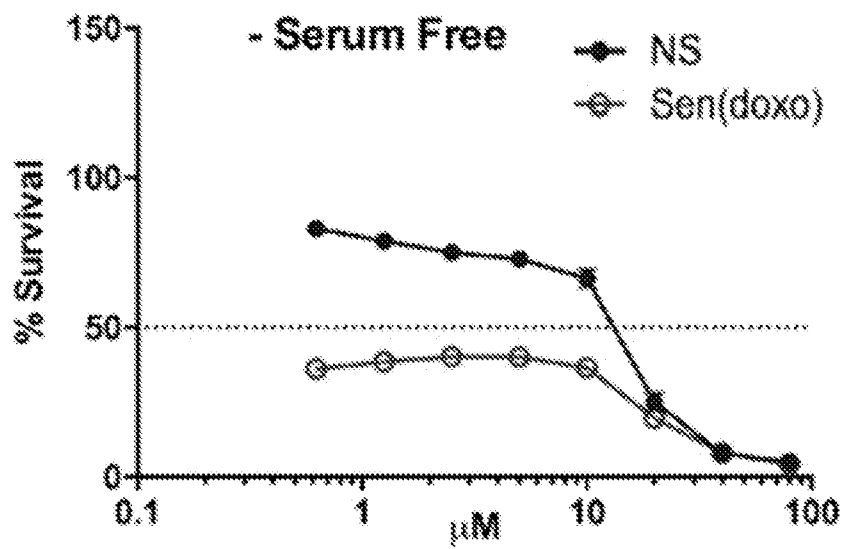


FIG. 5B

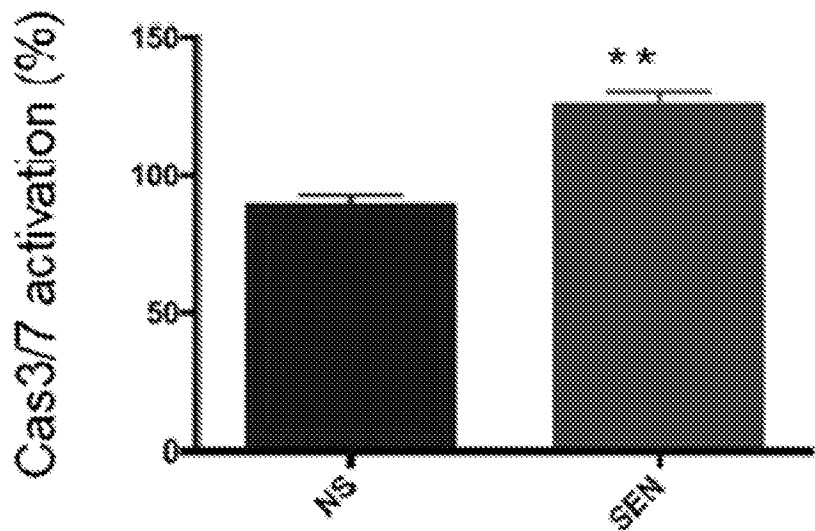


FIG. 5C

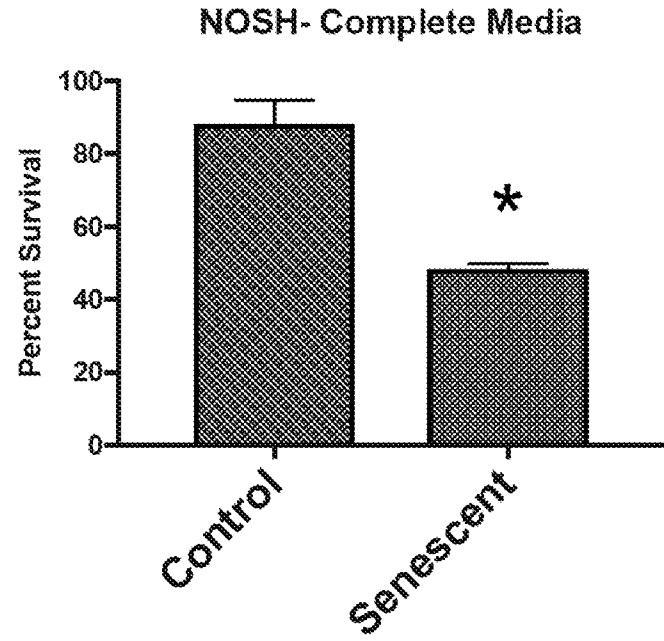


FIG. 5D

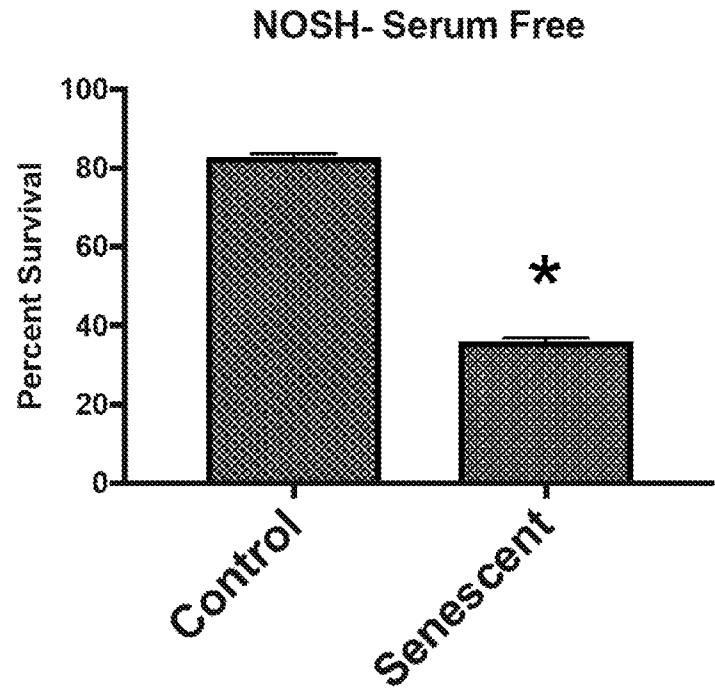


FIG. 5E

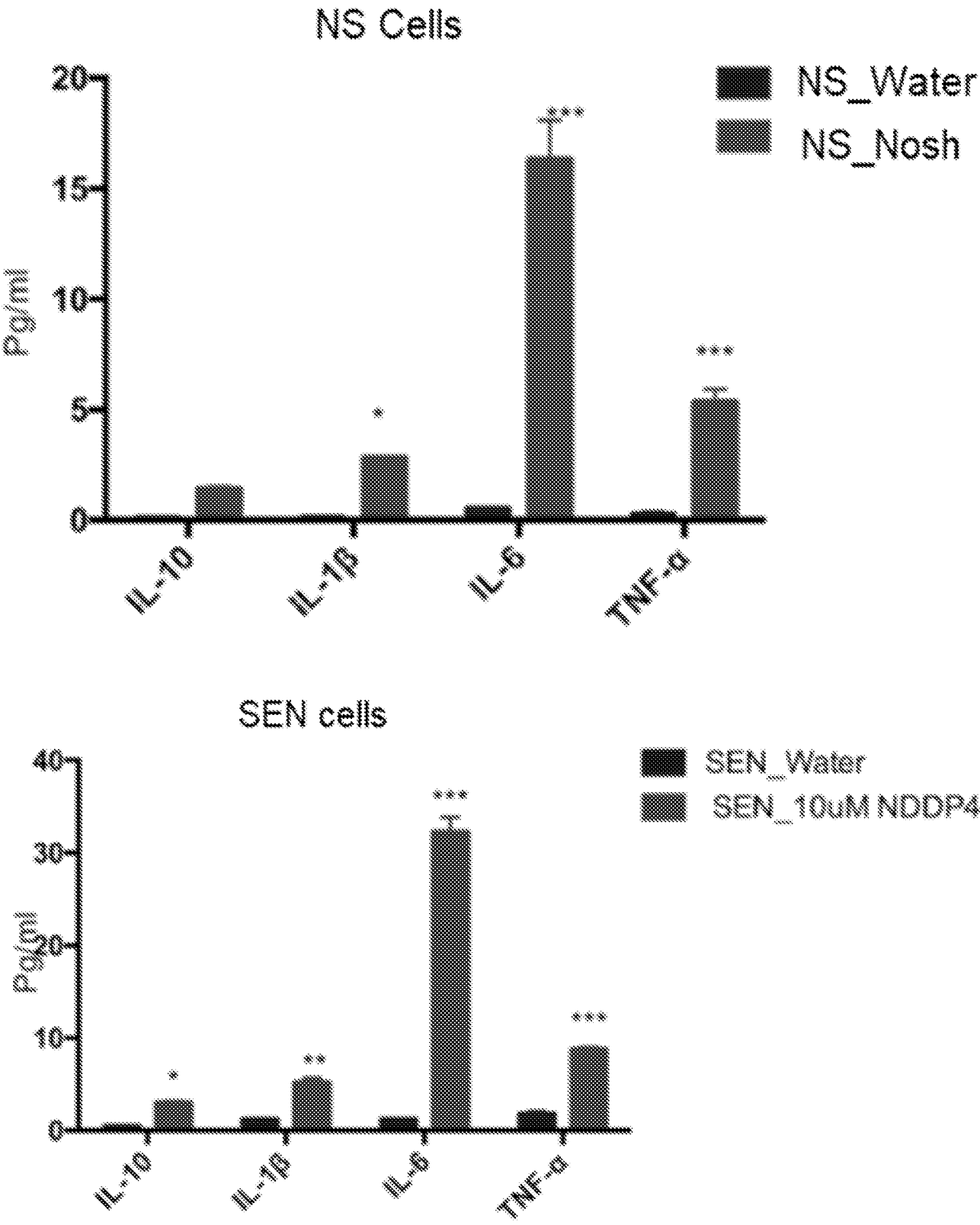


FIG. 6

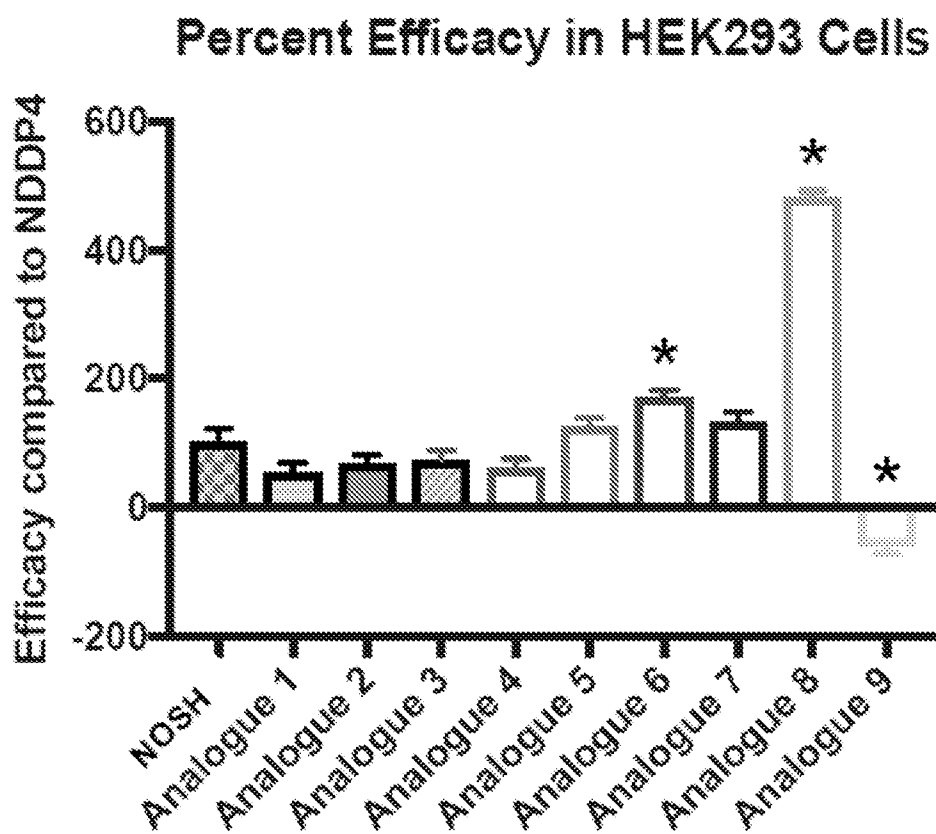
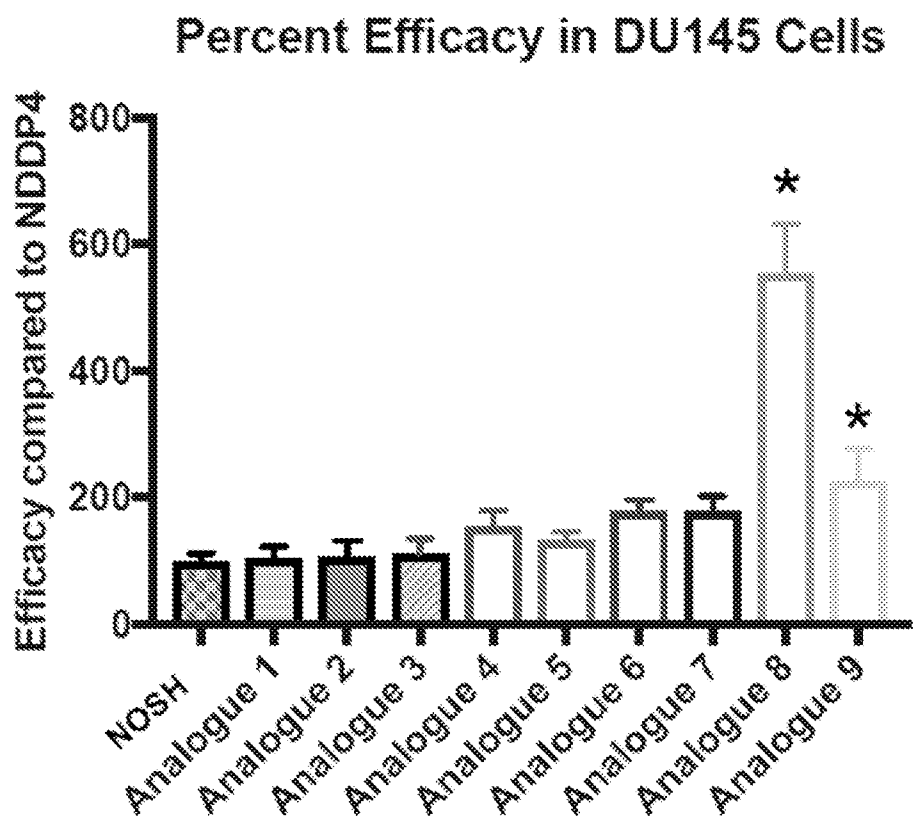


FIG. 7

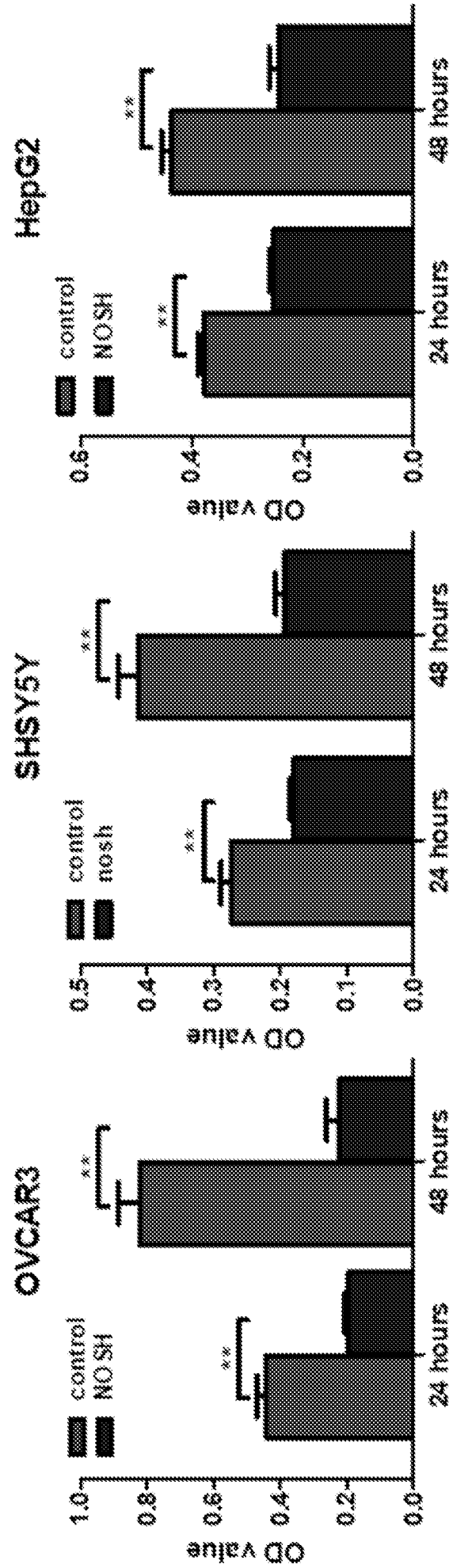
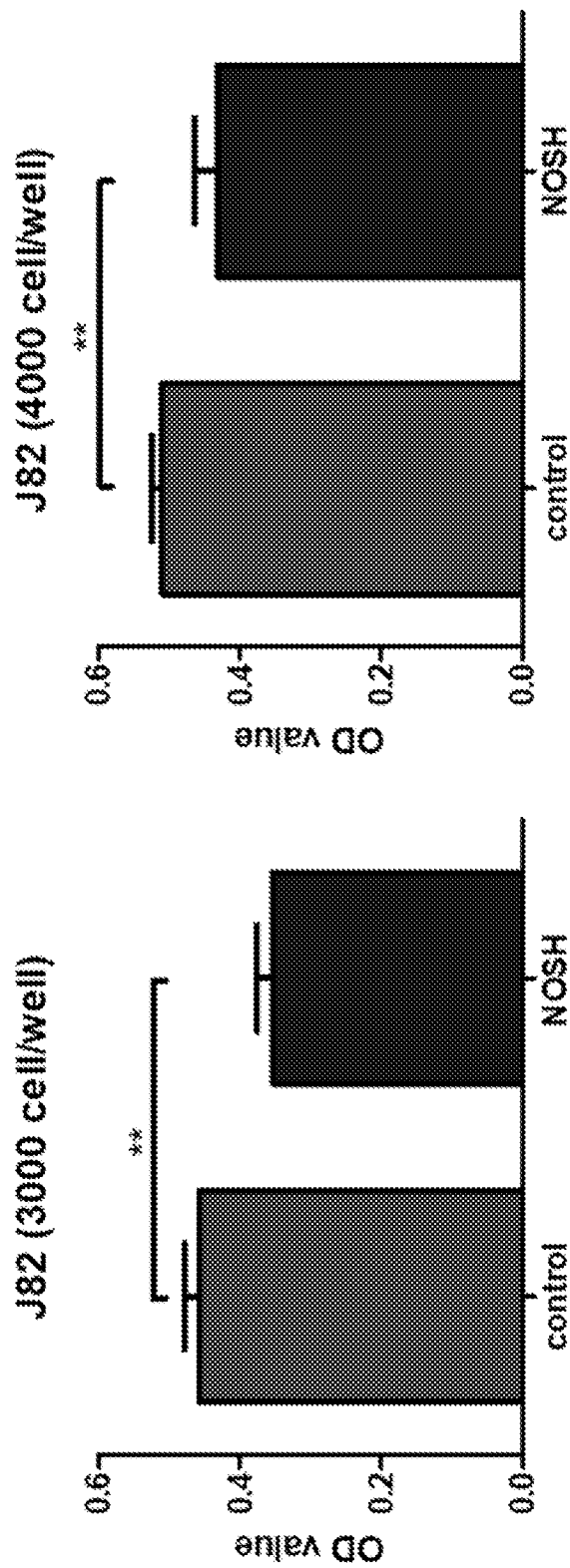


FIG. 8A

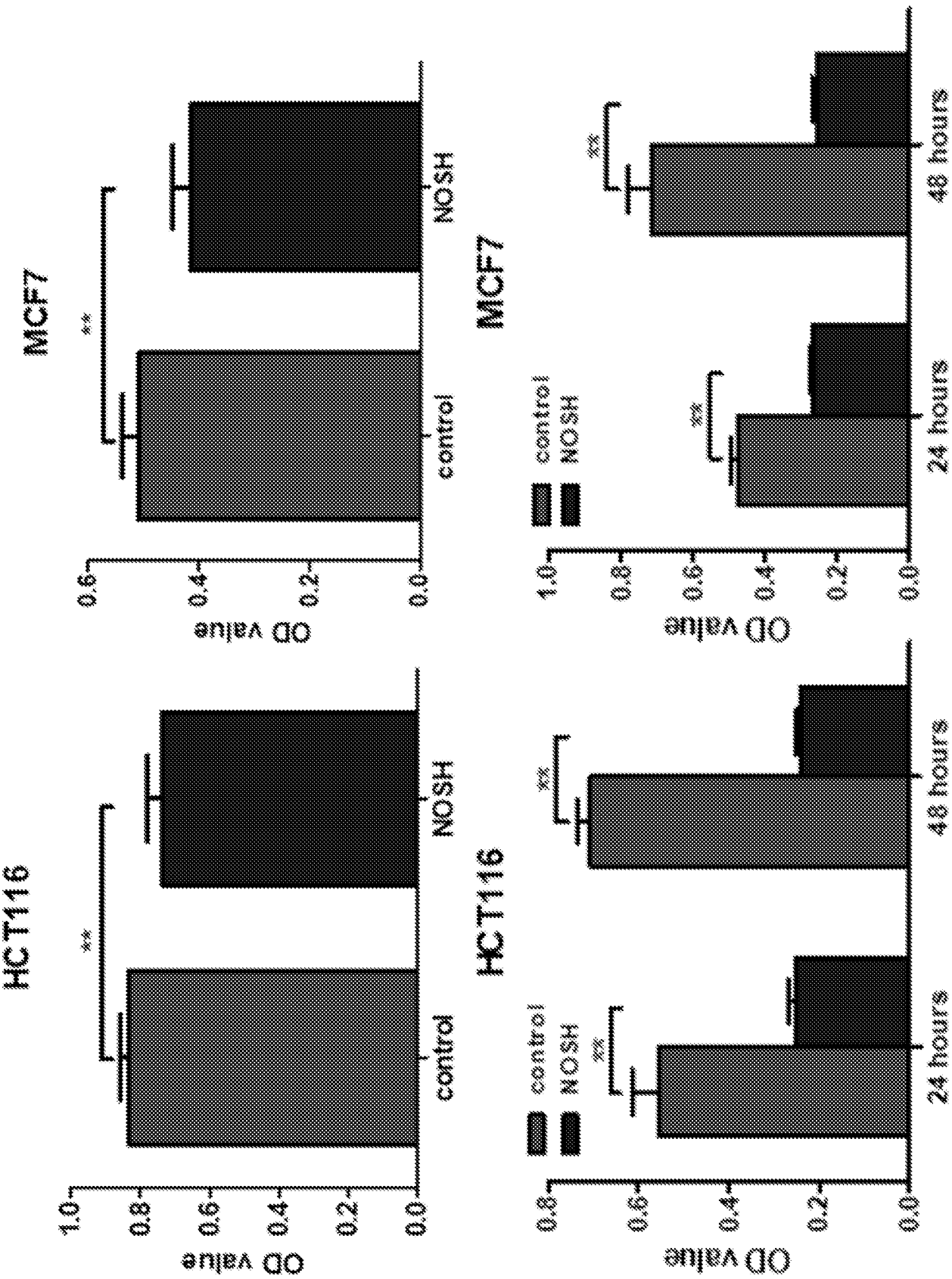


FIG. 8B

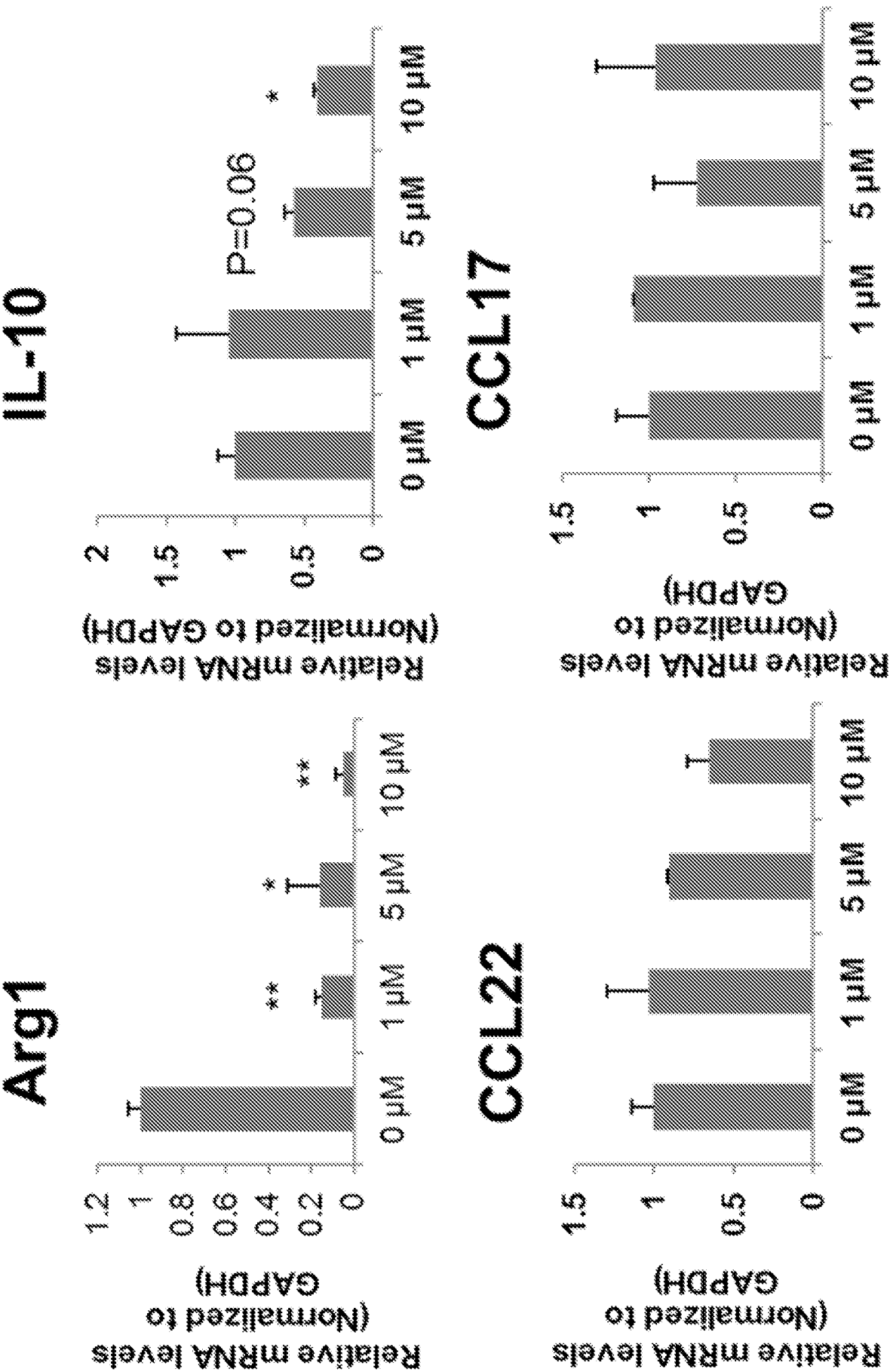


FIG. 9

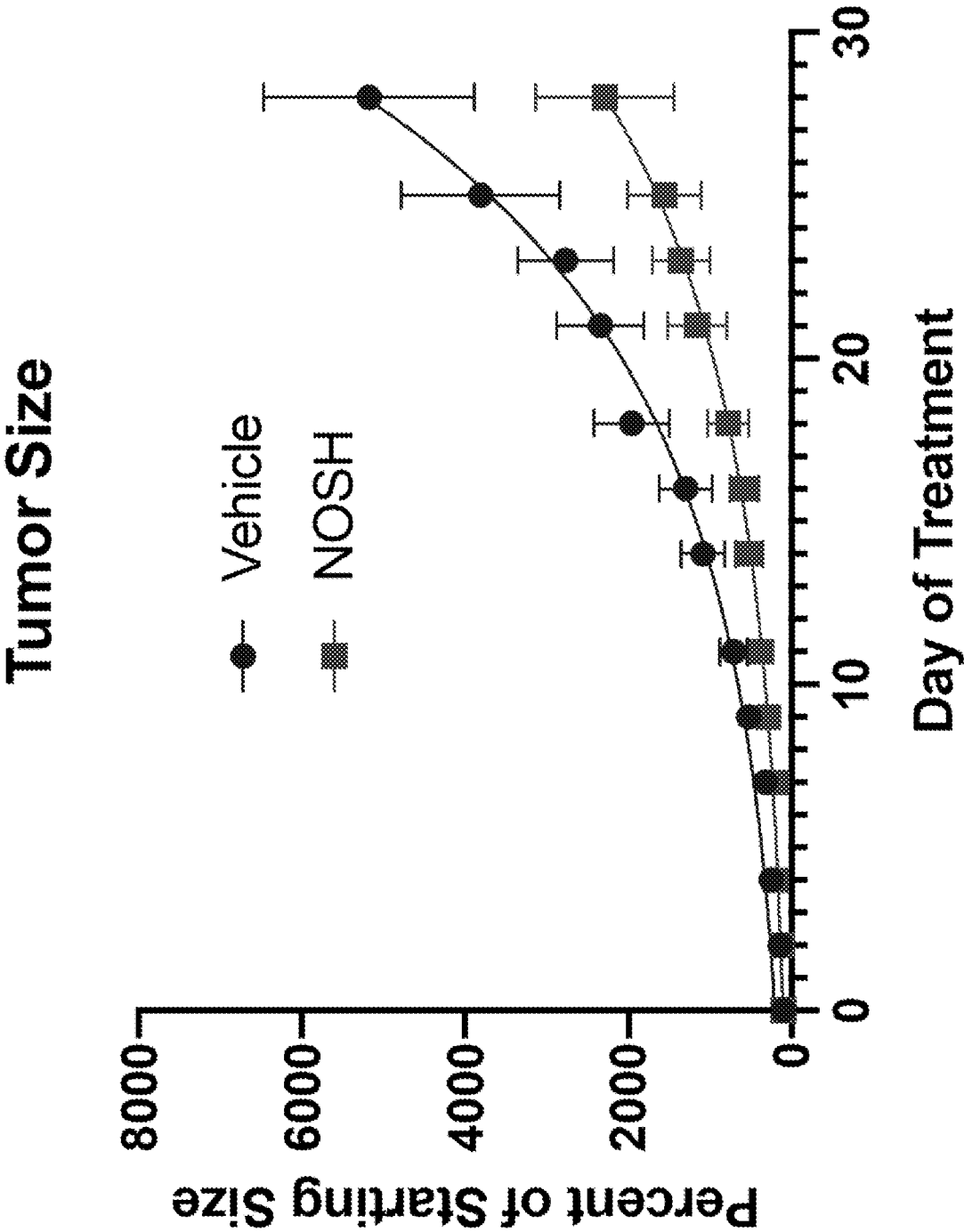


FIG. 10

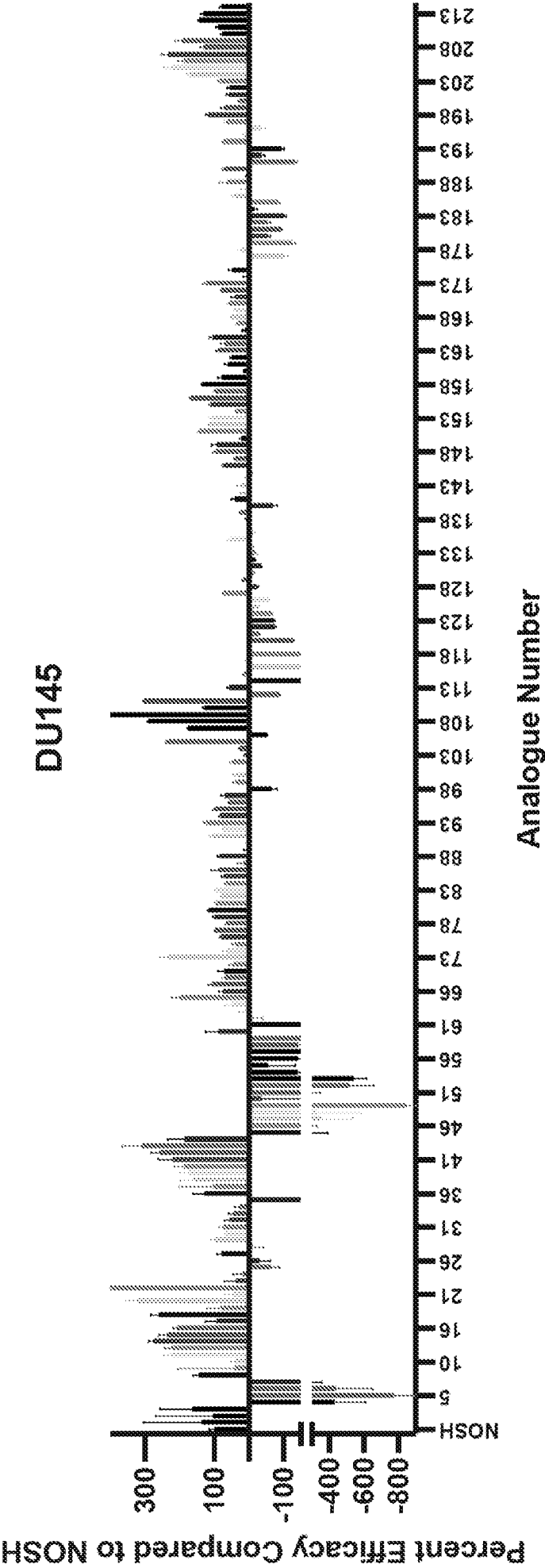


FIG. 11A

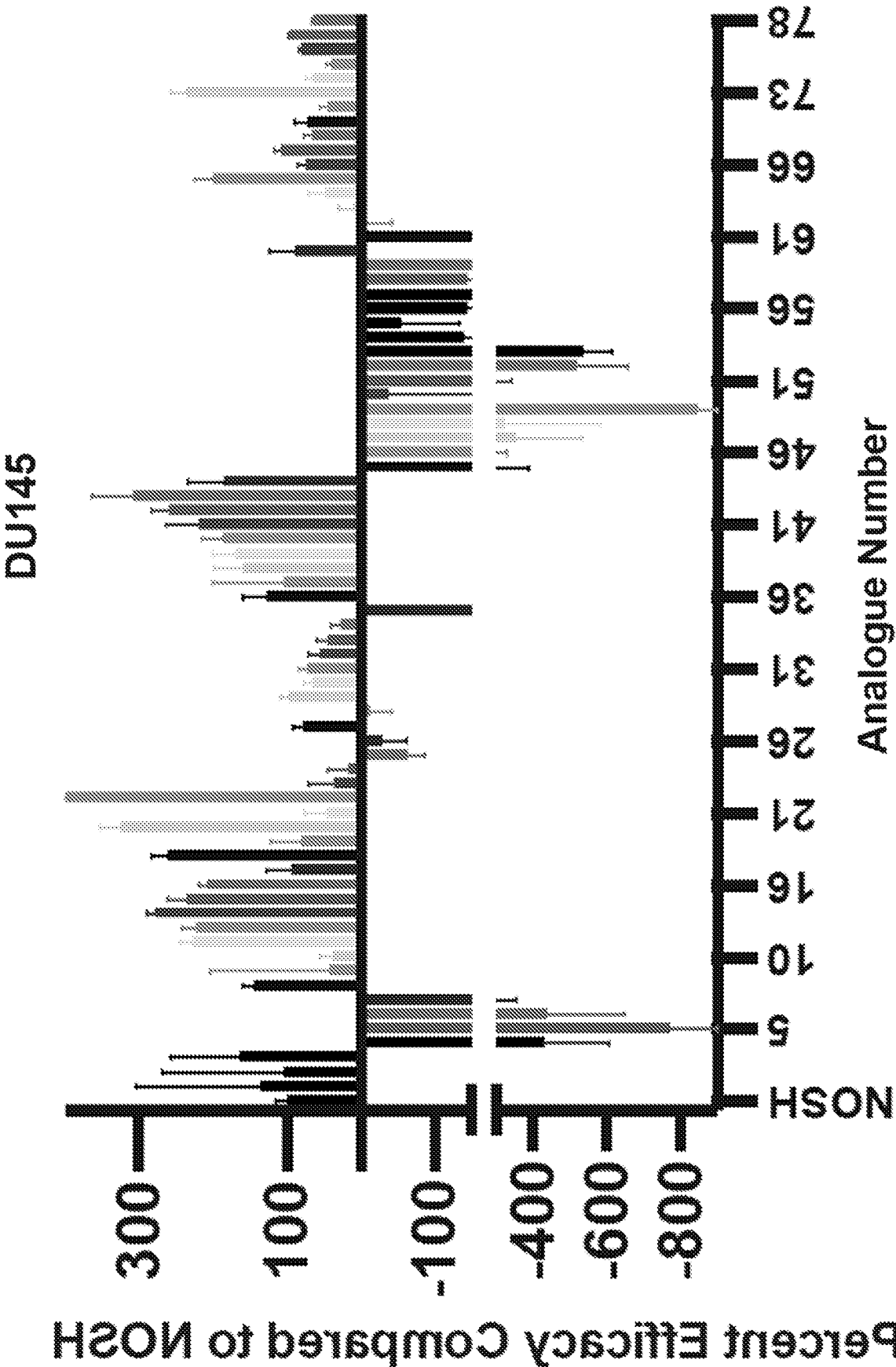


FIG. 11B

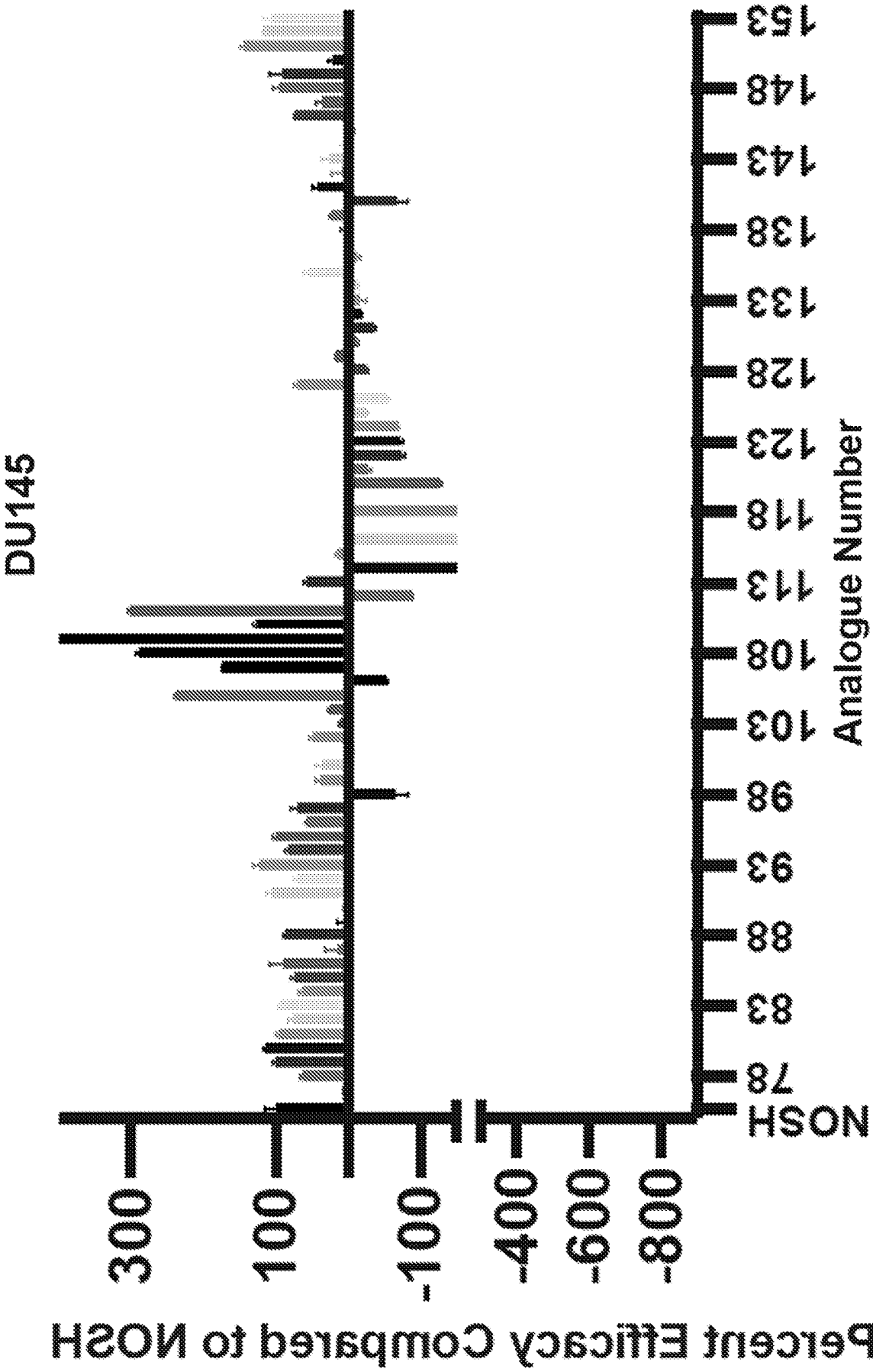


FIG. 11C

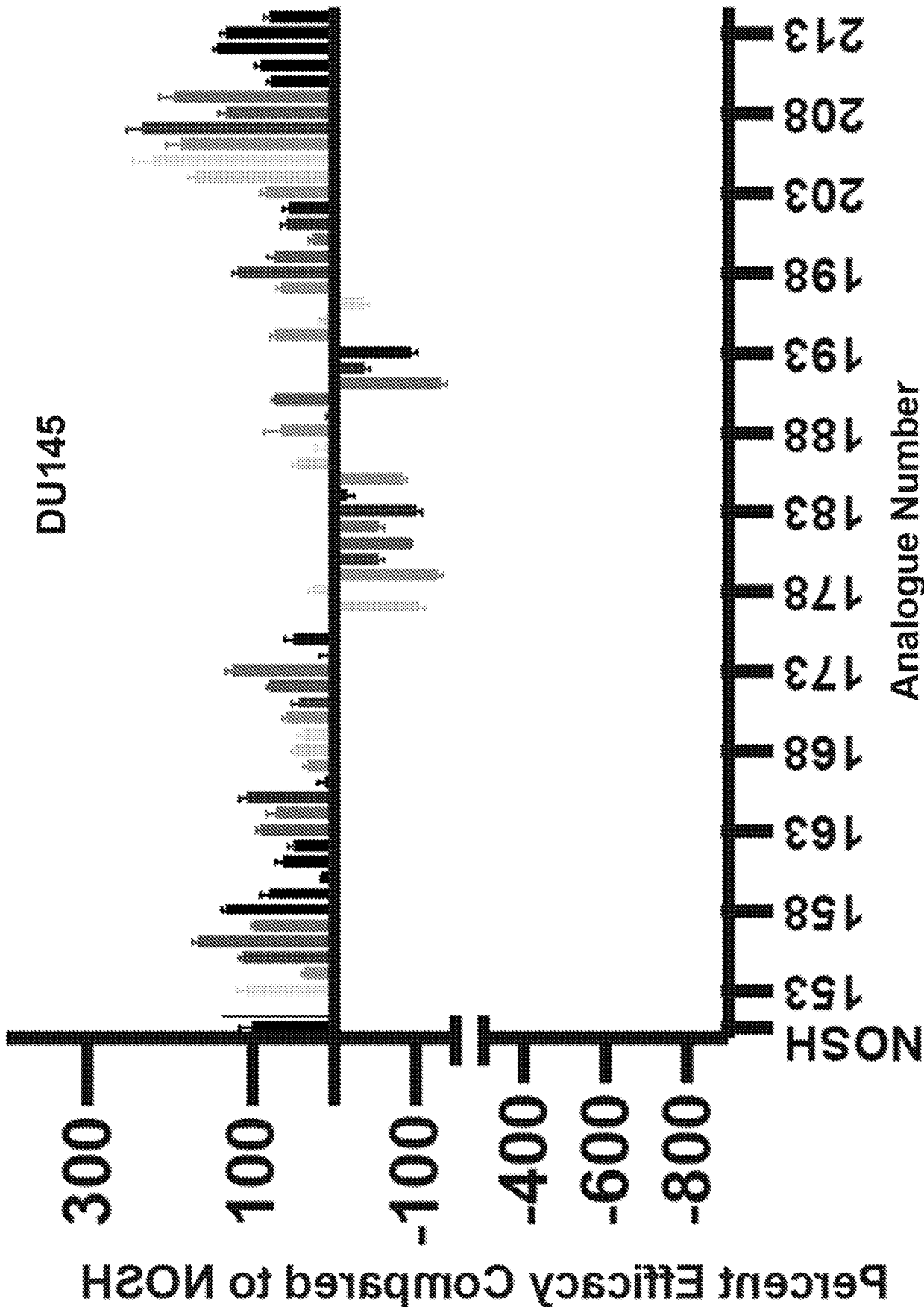


FIG. 11D

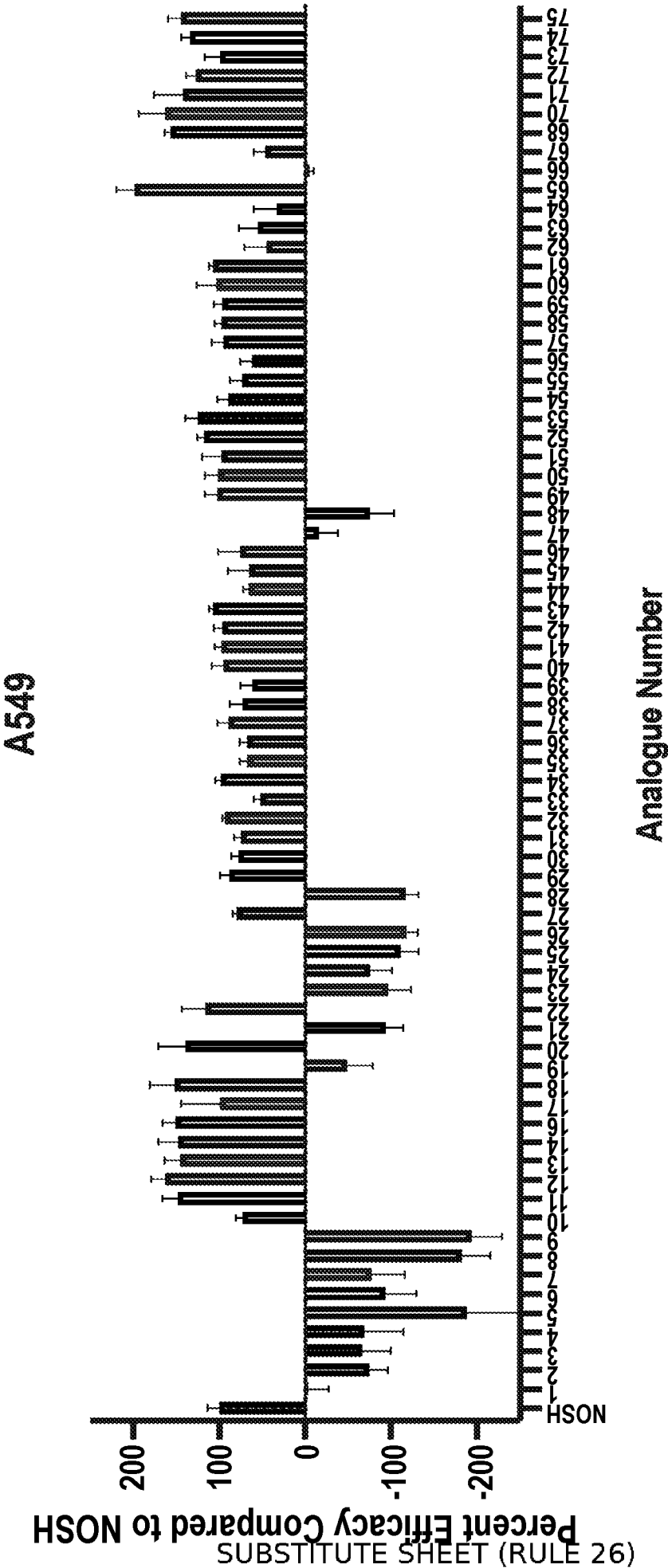


FIG. 12

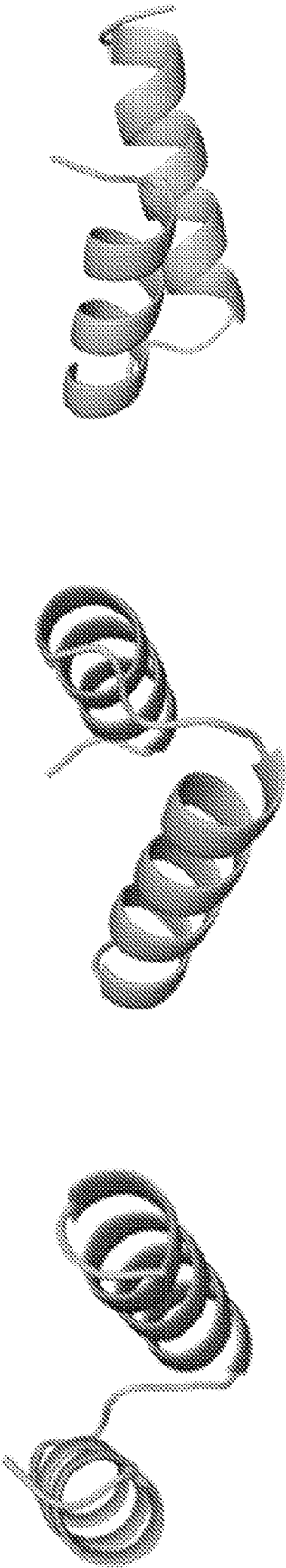


FIG. 13

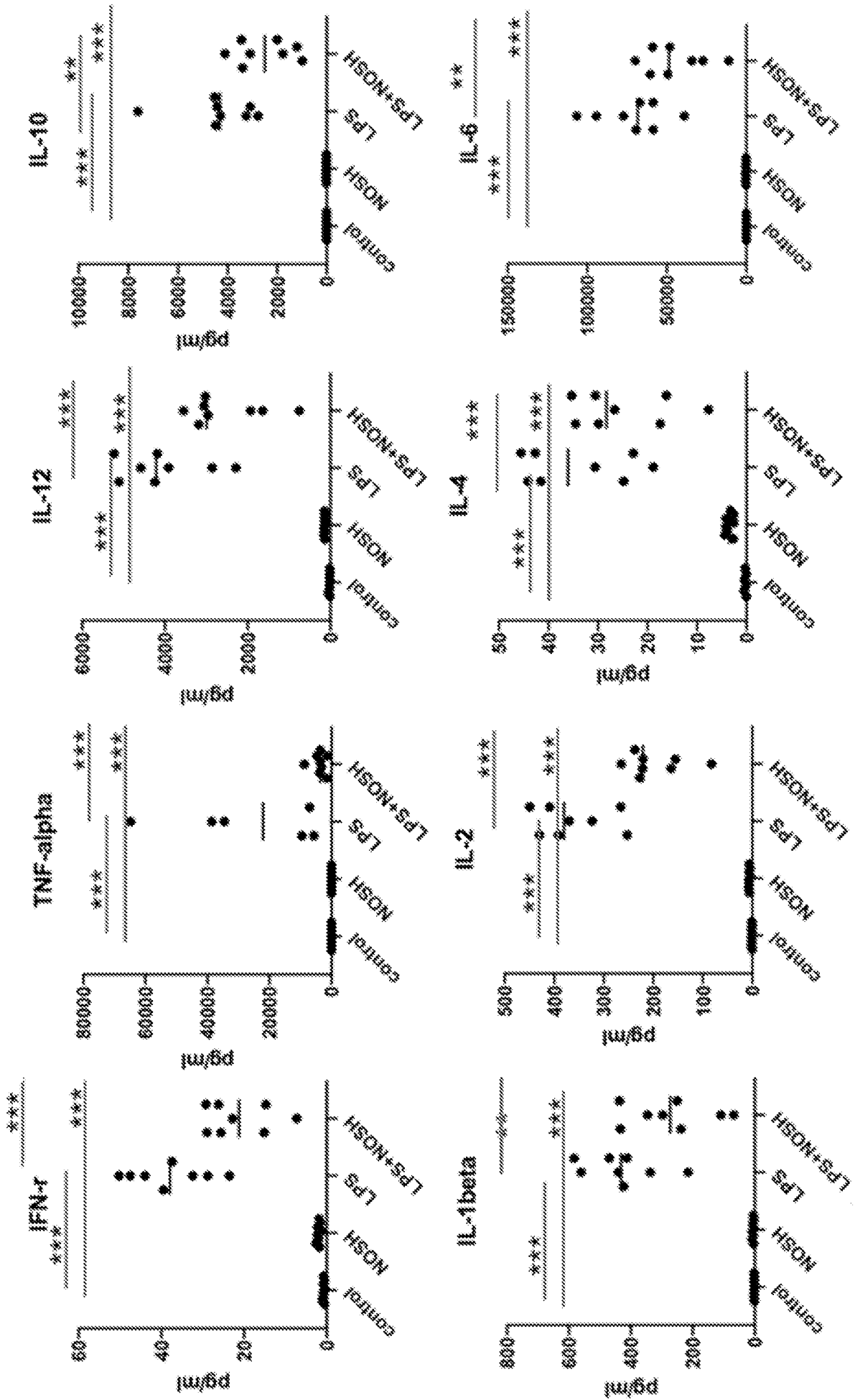


FIG. 14