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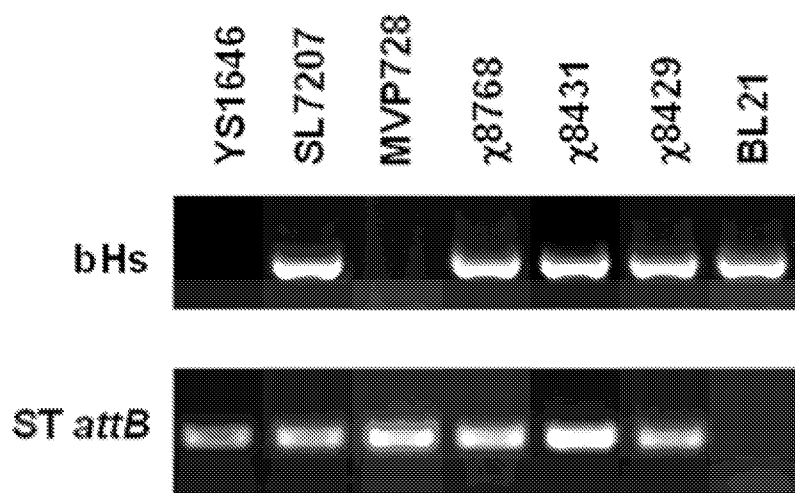
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(54) Title: COMPOSITIONS AND METHODS FOR TARGETING TUMOR-ASSOCIATED EXTRACELLULAR MATRIX COMPONENTS TO IMPROVE DRUG DELIVERY

FIG. 1A



(57) Abstract: Provided herein are compositions and methods to treat tumors that include attenuated facultative anaerobic bacterium. The bacterium includes a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.



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Compositions and Methods for Targeting Tumor-Associated Extracellular Matrix Components to Improve Drug Delivery

CROSS-REFERENCED APPLICATIONS

[0001] This application claims priority benefit to U.S. provisional 62/848,873 filed May 16, 2019, which is incorporated herein in its entirety.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with government support under grant nos. P30 CA033572 awarded by the National Cancer Institute. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 15, 2020 is named 048440-723001WO_SEQUENCE_LISTING_ST25.txt and is 2,799 bytes in size.

BACKGROUND

[0004] Hyaluronan, also known as hHA, is a component of pancreatic ductal adenocarcinoma (PDAC) stroma that is expressed at extremely high levels in the extracellular matrix (ECM), resulting in a biophysical barrier that significantly increases interstitial fluidic pressure, compresses blood vessels and hinders effective drug delivery. While PDAC tumors have the greatest incidence of HA overexpression in patients (>95%), other cancer types such as breast and prostate cancer express high levels. Thus, agents to degrade tumor-derived HA, and other overexpressed ECM components, to improve drug delivery and efficacy has been an area of extensive research. Various forms of bovine hyaluronidase and human PH20 hyaluronidase have been utilized to enhance the delivery of chemotherapy into solid tumors. However, because these enzymes are delivered systemically and their activity is not restricted to only tumor tissue, significant adverse events have been observed relating to HA depletion in joints and other organs, requiring lower doses or co-administration with additional agents to minimize these stresses. (See 4-22).

BRIEF SUMMARY

[0005] In view of the foregoing, there remains the need for new agents and treatment strategies to improve overall survival for patients with cancer. The present disclosure addresses this need, and provides additional benefits as well.

[0006] In an aspect, provided herein is an attenuated facultative anaerobic bacterium including a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0007] In an aspect, provided herein is a method of treating a tumor in a subject including administering to the subject an effective amount of an attenuated facultative anaerobic bacterium that includes a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0008] In an aspect, provided herein is a method of treating a tumor in a subject including administering to the subject an effective amount of an attenuated facultative anaerobic bacterium that includes a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] **FIGS. 1A-1C** presents data demonstrating transgene stability and expression of *Streptomyces koganeiensis* hyaluronidase (bHs) by attenuated ST strains. **FIG. 1A** presents polymerase chain reaction (PCR) experiments to detect for the bHs transgene contained within an inducible pBAD vector (pBAD-bHs) transformed into indicated ST strains. Representative colony PCRs shown from ≥ 8 colonies per transformed strain. A positive PCR control using ST-specific *attB* primers was performed for each colony. *E. coli* (BL21) transformed with pBAD-bHs is used as a positive PCR control for bHs and negative control for ST *attB*. **FIG. 1B** shows ST strains retaining the bHs transgene were cultured in Luria Broth (LB) containing 0% (uninduced) or 2% (induced) L-arabinose and cultured for 3 hours at 37°C. Lysates from $\sim 5 \times 10^7$ colony forming units (CFUs) were run on a 4-20% polyacrylamide gradient gel and subjected to coomassie blue staining (CB) and western blot analysis against an amino terminal His-tag fused to bHs (α -His). Predicted bHs size ~ 27 kDa (arrow). L = protein ladder. **FIG. 1C** shows that ST strains encoding His-tagged bHs were cultured in LB media containing 2% L-arabinose and immunostained (α -His) to determine localization of bHs. The cytosol is imaged by staining genomic DNA with DAPI. His-tagged bHs expression by SL7207 colocalizes with DAPI,

indicating that bHs is localized in the cytosol. In χ 8431, His-tagged bHs expression is punctate (arrows) and surrounding the nucleus, indicating bHs protein is contained within inclusion bodies. In χ 8429 and χ 8768, bHs is observed to be localized to the membrane (dotted line) outside of the cytoplasm. All data presented are representative of ≥ 3 experiments.

[0010] **FIGS. 2A-2E** demonstrate growth kinetics and viability of bHs-expressing ST strains. **FIGS. 2A-C** show optical density readings (OD_{600}) for uninduced (solid blue circles) and induced (open red circles) ST strains SL7207 (**FIG. 2A**), χ 8429 (**FIG. 2B**) and χ 8768 (**FIG. 2C**) transformed with the pBAD-bHs construct. Cultures were done in triplicate and error bars represent standard error of the mean. **FIG. 2D** are growth curves of induced bHs-expressing strains are compared. $*p < 0.05$ by ANOVA. **FIG. 2E** is data showing bacterial cells from uninduced (-) and induced (+2% L-arabinose) cultures of SL7207-bHs and χ 8768-bHs were stained at indicated time points (4 and 24 hours) with acridine orange to indicate live bacterium and imaged by fluorescence microscopy at 100X magnification. Under induced conditions (+) the majority of SL7207 at 4 and 24 hours post-induction are observed in aggregates and to have minimal (dim) staining (arrows), indicating dead bacterium. χ 8768-bHs stain strongly with acridine orange, suggesting high viability following induction. All data are representative of ≥ 3 experiments.

[0011] **FIGS. 3A-3D** demonstrate functional analysis of bHs-expressing ST strains. **FIG. 3A** shows bHs-expressing strains were cultured in LB broth containing indicated percentages of L-arabinose for 3 hours at 37°C. 1×10^8 CFUs were plated onto LB-hyaluronan-BSA agar plates overnight and then flushed with 2N acetic acid. Plates were imaged on a black background to visualize areas of clearing, indicating hyaluronan breakdown. **FIG. 3B** shows 1×10^8 CFUs of ST-bHs strains were added to LB containing 0% or 2% L-arabinose and 0.4 mg/mL HA. The cetyltrimethylammonium bromide turbidimetric method (CTM) was used to determine rate of HA breakdown over 24 hours (OD_{600}) for pBAD-bHs-transformed SL7207, (**FIG. 3C**) χ 8768 and (**FIG. 3D**) χ 8729. Error bars = standard error of the mean. All data are representative of ≥ 3 experiments.

[0012] **FIG. 4** demonstrates that induced χ 8768-bHs effectively depletes tumor-derived hyaluronan. χ 8768-bHs was grown for 3 hours in LB media containing 0% (uninduced) or 2% (induced) L-arabinose. 1×10^8 CFUs were then co-incubated with serial sections of PANC-1 tumor tissue overnight. HA was detected using biotinylated HA-binding protein (HABP)

followed by incubation with Vectastain streptavidin-HRP and ImmPACT DAB substrate. Serial sections incubated with PBS serve as an HA-positive control and specificity of HABP was confirmed through overnight incubation with 10 U/mL bovine hyaluronidase (Bov. Hs). Scale bar = 75 μ M. All images are representative of ≥ 3 experiments.

[0013] FIGS. 5A-5D demonstrate systemic delivery of χ 8768-bHs effectively degrades HA within orthotopic PANC-1 tumors. Uninduced χ 8768-bHs (2.5×10^6 CFU) was injected intravenously (i.v.) into NSG mice bearing orthotopic PANC-1 tumors (>250 mm³). After 48 hours, mice were then administered **(FIG. 5A)** PBS (uninduced) or **(FIG. 5B)** 250 mg L-arabinose (induced) by intraperitoneal injection. Tumors were isolated 16 hours later, sectioned and stained for ST and HA for subsequent immunofluorescence imaging at 10X and 100X magnification (with nuclear staining using DAPI present in overlays). Under uninduced conditions in FIG. 5A, areas colonized by ST also show presence of HA, indicating no depletion of HA. Under induced conditions in FIG. 5B, ST colonization is associated with absence of HA staining, suggesting HA depletion. **FIG. 5C** shows uninduced χ 8768-bHs (2.5×10^6 CFU) was injected intravenously (i.v.) into NSG mice bearing orthotopic PANC-1 tumors (>250 mm³). After 48 hours, mice were then administered PBS (uninduced) or 250 mg L-arabinose (induced) by intraperitoneal injection. Tumors were isolated 16 hours later, sectioned and stained for ST and DAPI for subsequent immunofluorescence imaging. Tile-scanning was performed on entire tumor sections at 10X magnification. Areas of ST colonization under uninduced conditions are limited to small concentrated areas (arrows), whereas under induced conditions, greater areas of ST staining are observed (dotted lines), indicating greater diffusion caused by HA depletion. Representative tumors are shown for uninduced and induced groups. **FIG. 5D** demonstrates percent area of tumor colonized by χ 8768-Hase under uninduced and induced conditions based on immunofluorescence. Percentage calculated using: (Area occupied by χ 8768-Hase (green)/Total tumor area (DAPI)) X 100%. Areas (μ m²) were determined using Image-Pro Plus (Media Cybernetics) analysis software. Error bars = standard error of the mean, * $p < 0.05$, t-test. All data are representative of ≥ 3 experiments.

[0014] FIGS. 6A-6C demonstrate that ST-Hase potentiates the anti-tumor effects of gemcitabine treatment in PANC-1 tumor xenografts. **FIG. 6A** shows that s.c. tumors and skin ($n=4$) were isolated 3, 7 and 11 days post-induction (dpi), sectioned and stained for HA (red) and ST (green) for subsequent immunofluorescence (IF) imaging at 5X magnification. Trichrome staining of serial sections for same tissue sample also shown to left of IF images. Representative

images shown. Arrows indicate area of ST/HA overlap. Scale bars = 50 μ m. **FIG. 6B** shows that after 2 dpi, groups of tumor-bearing mice ($n=6$) were administered either gemcitabine (40 mg/kg) or diluent control (0.9% saline) by i.p. route, followed by additional administrations twice per week. PBS only group did not receive pre-treatment with ST-HAse. Tumors were measured weekly using a digital caliper. $**p<0.01$, $***p<0.001$, ANOVA with Tukey's post hoc test. **FIG. 6C** shows that body weights were measured on indicated days following gemcitabine or control treatment and are presented as a percentage of initial body weight. *n.s.*, not significant

[0015] FIGS. 7A-7E characterize bHs-expressing ST strains. **FIG. 7A** shows that BHs, expressed by induced ST strains, is not secreted into the culture media. LB culture media from induced bHs-expressing ST strains were run on a 4-20% polyacrylamide gradient gel and subjected to coomassie blue staining (CB) and western blot analysis against an amino terminal His-tag (α -His). Predicted bHs size ~27 kDa (arrow). L = protein ladder. **FIG. 7B** shows the predicted bacterial subcellular localization of *Streptomyces koganeinsis* bHs using subcellular prediction software. Predicted (pred) locations - I: inside; i: inside tail; H: transmembrane helix; O: outside; o: outside-tail. **FIG. 7C** are optical density readings (OD_{600}) for uninduced (solid blue circles) and induced (open red circles) χ 8431-bHs. Cultures were done in triplicate and error bars represent standard error of the mean. **FIG. 7D** shows bacterial cells from uninduced (-) and induced (+2% L-arabinose) cultures of χ 8429-bHs stained at indicated time points (4 and 24 hours) with acridine orange to indicate live bacterium and imaged by fluorescence microscopy at 63X magnification. Under induced conditions (+) the majority of χ 8429-bHs at 4 and 24 hours post-induction are observed to have minimal (dim) staining (arrows), indicating dead bacterium. All data are representative of ≥ 3 experiments. In **FIG. 7E**, 1×10^8 CFUs of χ 8768-bHs were added to LB containing 0% or 2% L-arabinose and 0.4 mg/mL HA. The cetyltrimethylammonium bromide turbidimetric method (CTM) was used to determine rate of HA breakdown over 24 hours (OD_{600}). Error bars = standard error of the mean. All data are representative of ≥ 3 experiments.

[0016] FIG. 8 demonstrates that induced χ 8768-bHs effectively depletes PC-3-derived hyaluronan. χ 8768-bHs was grown for 3 hours in LB media containing 0% (uninduced) or 2% (induced) L-arabinose. 1×10^8 CFUs were then co-incubated with HA^{high} human prostate cancer (PC-3) cells overnight. HA was detected using biotinylated HA-binding protein (HABP) followed by incubation with Vectastain streptavidin-HRP and ImmPACT DAB substrate.

Specificity of HABP was confirmed through overnight incubation with 10 U/mL bovine hyaluronidase (Bov. Hs). Scale bar = 100 μ M. All images are representative of ≥ 3 experiments.

[0017] FIGS. 9A-9E demonstrate χ 8768-bHs tumor colonization and vasculature in PANC-1 tumors. **FIG. 9A** shows that recombinant χ 8768 transformed with a bacterial expression construct encoding the bioluminescent LUX cassette (χ 8768-LUX) was injected intravenously (2.5×10^6 colony forming units (CFU)) into NSG mice bearing orthotopic PANC-1 tumors ($>250 \text{ mm}^3$). LUX is constitutively expressed in viable bacteria. Mice were imaged on days 1, 3 and 5 by intravital bioluminescent imaging (representative mouse shown). **FIG. 9B** shows that PANC-1 tumor, spleen and liver were isolated from NSG mice 48 hours following intravenous injection of χ 8768-LUX (2.5×10^6 CFU) and imaged using a Lago X bioluminescent imager. **FIG. 9C** shows duct-like structures observed in PANC-1 sections of χ 8768-bHs-treated mice, uninduced or induced. Representative fields are shown at 20X magnification. Scale bar = 75 μ m. Arrows indicate blood vessels. **FIG. 9D** are trichrome stained sections from PANC1 tumors containing induced or uninduced χ 8768-bHs. Blood vessels are encircled by solid black lines. Sections serial to the trichrome slides were stained for ST showing representative patterns of concentration. ST staining under uninduced conditions are restricted to vessels (V), whereas ST staining under induced conditions can be observed outside of vessels (arrows), indicating enhance tumor permeability. **FIG. 9E** shows representative fields from trichrome stained slides that were counted for blood vessels and their diameters were measured in microns (μ m). Bar graph represents $n > 40$ observations per slide. Error bars = standard error of the mean.

**** $p < 0.0001$, t-test. All data are representative of ≥ 3 experiments.

[0018] FIGS. 10A-10C demonstrate that induced χ 8768-bHs causes no observable ST colonization or HA depletion in HA^{high} joints and does not decrease tumor cell density. Uninduced χ 8768-bHs (2.5×10^6 CFU) was injected i.v. into NSG mice bearing subcutaneous (s.c.) PANC-1 tumors ($>150 \text{ mm}^3$). After 48 hours, mice were then administered PBS (uninduced, U) or 240 mg L-arabinose (induced, I) by i.p. injection. In **FIG. 10A**, the joint between femur and tibia bones from hind leg ($n=4$) were isolated 3, 7 and 14 days post-i.p. injection (dpi), sectioned and stained for HA and ST for subsequent IF imaging at 5X magnification. Trichrome staining of serial sections for same joint shown to left of IF image. The overall amount of HA (arrows) is unchanged under induced conditions (compared to uninduced conditions), indicating that χ 8768-bHs does not deplete HA in joints. Representative images shown. B, bone. Scale bars = 100 μ m. **FIG. 10B** are enlarged areas of serial tumor sections

showing IF staining of HA and ST, trichrome (TC) and pan-cytokeratin (PC) in uninduced (U) and induced (I) conditions 3, 7 and 14 days post-i.p. injection (dpi). PC-staining is used to indicate areas of tumor cells. Regions enclosed by yellow rectangle represent magnified, high resolution images in FIG. 6A. Areas enclosed by dotted-lines are areas of PC-positive staining cells that are also colonized by χ 8768-bHs (using corresponding IF imaging). Co-staining of PC markers with ST colonization indicates that ST are colonizing areas containing pancreatic tumor cells. Tile-scanning performed at 5X magnification. Scale bars = 200 μ m. **FIG. 10C** are serial sections of induced tumors (3 and 7 dpi) were stained for HA and nuclei for subsequent IF imaging at 5X magnification. Areas enclosed by dotted lines indicate areas that have been colonized by χ 8768-bHs under induced conditions and have been depleted of HA as indicated by loss of HA staining (darker areas). Representative images shown. All images are representative of ≥ 2 experiments. Scale bars = 50 μ m.

[0019] FIGS. 11A-11C demonstrates expression, subcellular localization and toxicity of *Streptomyces omiyaensis* collagenase (CNase) expressed by attenuated VNP20009. **FIG. 11A** shows that attenuated VNP20009 transformed with the pBAD-CNase construct was cultured in Luria Broth (LB) containing 0% (uninduced) or 1% to 6% (induced) L-arabinose for up to 4 hours at 37°C. Bacterial cell lysates from $\sim 5 \times 10^7$ colony forming units (CFUs) or 20X concentrated corresponding culture media at each time point for each L-arabinose concentration were run on a 4-20% polyacrylamide gradient gel and subjected to western blot analysis against a His-tag fused to the amino terminus of CNase (α -His). Predicted CNase size ~ 31 kDa (arrow). In **FIG. 11B**, ST-CNase was cultured in LB media containing 1% L-arabinose for 1 hour and then immunostained (α -His) to determine subcellular localization of CNase. Expression of His-tagged CNase is only observed under induced conditions (arrows) and surrounding the cytoplasm. The cytosol (genomic DNA) is stained using DAPI. Representative images shown. Scale bar = 1.0 μ m. **FIG. 11C** is the growth curve of ST-CNase post-induction. Optical density readings (OD_{600}) for uninduced and induced (1% L-ara) ST-CNase cultures were measured over 24 hours. Uninduced and induced cultures were done in triplicate and error bars represent standard error of the mean. Growth curves of uninduced and induced are compared. For time points ≥ 2 hrs: $**p < 0.01$, t-test.

[0020] FIGS. 12A-12D demonstrate hydrolytic collagenase activity by ST-CNase towards various substrates. **FIG. 12A** shows that uninduced or induced (1% L-arabinose) ST-CNase was incubated on LB-gelatin plates overnight (16 hr) at 37°C. Hydrolysis of gelatin in LB agar media

is observed as opaque areas on LB-gelatin plates. Arrows indicate areas where uninduced or induced ST-CNase were spotted onto the plate. **FIG. 12B-12D** are hydrolysis reactions performed using uninduced or induced ST-CNase co-incubated with FITC-conjugated pig skin gelatin (**FIG. 12B**), bovine skin collagen type I (**FIG. 12C**) or human placenta collagen type IV (**FIG. 12D**) in 50 mM Tris-HCl (pH 8.0) containing 10 mM CaCl₂ at 37°C. Enzyme activity was measured by monitoring the fluorescence (FITC) (ex: 495 nm, em: 519 nm). Data are expressed as mean±SD of three independent experiments. * $p<0.05$, ** $p<0.01$, $p<0.001$, t -test.

[0021] FIGS. 13A-13C demonstrate that in vivo depletion of collagenase by ST-CNase increases ST spread and is restricted to tumor tissue. **FIG. 13A** is immunofluorescence staining (IF) of ST-CNase in representative Pan02 tumors isolated from mice treated under uninduced (U) or induced (+L-arabinose, I) conditions with ST-CNase for 48 hours. In left panels, under induced conditions, ST-CNase can be observed to occupy greater areas of tumor (enclosed by dotted lines) compared to uninduced conditions. In right panels, magnified areas occupied by ST-CNase under induced conditions (enclosed by squares in IF) are also observed to be depleted of collagen (enclosed by squares in TC) as indicated by greater white space (loss of collagen) between tumor cells compared to uninduced conditions. **FIG. 13B** is the percent area of tumor colonized by ST-CNase under uninduced and induced conditions based on immunofluorescence. Percentage calculated using: (Area occupied by ST-CNase (green)/Total tumor area (DAPI)) X 100%. Areas (μm²) were determined using Image-Pro Plus (Media Cybernetics) analysis software. Error bars = standard error of the mean, * $p<0.05$, t -test. All data are representative of ≥2 experiments. **FIG. 13C** is TC staining of representative skin in Pan02-tumor bearing mice administered ST-CNase and then left uninduced or induced. Tissues examined in uninduced and induced conditions were isolated 72 hours post-induction. Data showed no ST colonization in skin and no loss of collagen content under induced conditions (compared to uninduced conditions). These results indicate that ST-CNase does not deplete collagen in collagen-high tissues.

DETAILED DESCRIPTION

I. Definitions

[0022] Before the present invention is further described, it is to be understood that this invention is not strictly limited to particular embodiments described, as such may of course vary. It is also to be understood that the terminology used herein is for the purpose of describing

particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the claims.

[0023] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. It should further be understood that as used herein, the term “a” entity or “an” entity refers to one or more of that entity. For example, a nucleic acid molecule refers to one or more nucleic acid molecules. As such, the terms “a,” “an,” “one or more” and “at least one” can be used interchangeably. Similarly the terms “comprising,” “including” and “having” can be used interchangeably.

[0024] Unless defined otherwise, all 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. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates, which may need to be independently confirmed.

[0025] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0026] It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[0027] As used herein, the term “about” means a range of values including the specified value, which a person of ordinary skill in the art would consider reasonably similar to the specified value. In embodiments, about means within a standard deviation using measurements generally acceptable in the art. In embodiments, about means a range extending to +/- 10% of the specified value. In embodiments, about means the specified value.

[0028] As used herein, the term “facultative anaerobe” refers to is an organism that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation if oxygen is absent. Some examples of facultatively anaerobic bacteria are *Staphylococcus* spp., *Streptococcus* spp. *Escherichia coli*, *Salmonella*, *Listeria* spp. and *Shewanella oneidensis*. Certain eukaryotes are also facultative anaerobes, including fungi such as *Saccharomyces cerevisiae* and many aquatic invertebrates such as Nereid (worm) polychaetes.

[0029] As used herein, the term “virulence” refers to a pathogen's or microbe's ability to infect or damage a host. In the context of animals, virulence refers to the degree of damage caused by a microbe to its host. The pathogenicity of an organism - its ability to cause disease - is determined by its virulence factors. The most commonly used measurement of virulence is the lethal dose required to kill 50% of infected hosts, referred to as the LD₅₀. The LD₅₀ measurement has the advantage that it allows comparisons across microbes, and the use of host death provides a nonequivocal endpoint. Some have developed approaches for measuring virulence that are not dependent on mortality.

[0030] As used herein, the term “attenuated” refers to a reduced virulence and to procedures that weaken an agent of disease (a pathogen). An attenuated pathogen is weakened, less vigorous compared to one that is non-attenuated. Attenuation may be due to genetic mutations. Genetic mutations may be engineered or result from passaging of the pathogen in cell culture. An “attenuated facultative anaerobic bacterium,” as used herein, refers to a facultative anaerobic bacterium that has been altered to reduce virulence relative to the facultative anaerobic bacterium without the alteration, and is capable of replicating. In embodiments, the alteration is a genetic alteration of a gene that confers virulence to the unaltered facultative anaerobic bacterium.

[0031] As may be used herein, the terms “nucleic acid,” “nucleic acid molecule,” “nucleic acid oligomer,” “oligonucleotide,” “nucleic acid sequence,” “nucleic acid fragment” and “polynucleotide” are used interchangeably and are intended to include, but are not limited to, a

polymeric form of nucleotides covalently linked together that may have various lengths, either deoxyribonucleotides or ribonucleotides, or analogs, derivatives or modifications thereof.

Different polynucleotides may have different three-dimensional structures, and may perform various functions, known or unknown. Non-limiting examples of polynucleotides include a gene, a gene fragment, an exon, an intron, intergenic DNA (including, without limitation, heterochromatic DNA), messenger RNA (mRNA), transfer RNA, ribosomal RNA, a ribozyme, cDNA, a recombinant polynucleotide, a branched polynucleotide, a plasmid, a vector, isolated DNA of a sequence, isolated RNA of a sequence, a nucleic acid probe, and a primer.

Polynucleotides useful in the methods of the disclosure may comprise natural nucleic acid sequences and variants thereof, artificial nucleic acid sequences, or a combination of such sequences.

[0032] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the disclosure.

[0033] As used herein, the term "gene" means the segment of DNA involved in producing a protein; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). The leader, the trailer as well as the introns include regulatory elements that are necessary during the transcription and the translation of a gene. Further, a "protein gene product" is a protein expressed from a particular gene.

[0034] As used herein, the term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. In one embodiment, the term refers to a functional linkage between a nucleic acid expression control sequence (such as a promoter, and/or enhancer or other expression control sequence) and a second polynucleotide sequence, e.g., a polynucleotide-of-interest, wherein the expression control sequence directs transcription of the nucleic acid corresponding to the second sequence.

[0035] As used herein, the term “recombinant” generally refers to an organism, cell, or genetic material formed by recombination. Recombinant DNA (rDNA) molecules are DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome. Recombinant DNA is the general name for a piece of DNA that has been created by the combination of at least two strands. Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure, and differ only in the nucleotide sequence within that identical overall structure. The DNA sequences used in the construction of recombinant DNA molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA, and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms. Recombinant DNA differs from genetic recombination in that the former results from artificial methods in the test tube, while the latter is a normal biological process that results in the remixing of existing DNA sequences in essentially all organisms.

[0036] As used herein, the term “recombinant proteins” refers to proteins that can result from the expression of recombinant DNA within living cells. When recombinant DNA encoding a protein is introduced into a host organism, the recombinant protein is not necessarily produced. Expression of foreign proteins requires the use of specialized expression vectors and often necessitates significant restructuring by foreign coding sequences.

[0037] For specific proteins described herein, the named protein includes any of the protein’s naturally occurring forms, variants or homologs that maintain the protein transcription factor activity (e.g., within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to the native protein). In some embodiments, variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring form. In other embodiments, the protein is the protein as identified by its NCBI sequence reference. In other embodiments, the protein is the protein as identified by its NCBI sequence reference, homolog or functional fragment thereof.

[0038] The term “exogenous” refers to a molecule or substance (*e.g.*, a compound, nucleic acid or protein) that originates from outside a given cell or organism. For example, an "exogenous promoter" as referred to herein is a promoter that does not originate from the organism it is expressed by. Conversely, the term "endogenous" or "endogenous promoter" refers to a molecule or substance that is native to, or originates within, a given cell or organism.

[0039] The term "promoter" as used herein refers to a recognition site of a polynucleotide (DNA or RNA) to which an RNA polymerase binds. The term "enhancer" refers to a segment of DNA which contains sequences capable of providing enhanced transcription and in some instances can function independent of their orientation relative to another control sequence. An enhancer can function cooperatively or additively with promoters and/or other enhancer elements. The term "promoter/enhancer" refers to a segment of DNA, which contains sequences capable of providing both promoter, and enhancer functions.

[0040] As used herein, the term “inducible promoter” refers to promoters can be regulated (induced) in presence of certain abiotic or biotic factors which may include certain biomolecules. These promoters are used by genetic engineers for regulating the expression of genes cloned in any organism by simply introducing the inducer. The two ways the activity of a promoter can be regulated are positive and negative control. Examples of inducible promoters include the pLac promoter, pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter.

[0041] As used herein the term “hypoxia-inducible promoter” refers to promoters that are engineered to limit gene expression to hypoxic environments such as the tumor microenvironment. Examples of genes regulated by hypoxia-inducible promoters include *pflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*. Artificial promoters containing fumarate and nitrate reduction (FNR) regulator sites, such as FF+20* and hypoxia inducible promoter 1 (HIP1), are also examples of hypoxia-inducible promoters. See for example 61-64.

[0042] As used herein, the terms "polypeptide," "peptide" and "protein" are used interchangeably and refer to a polymer of amino acid residues, wherein the polymer may In embodiments be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A "fusion protein" refers to a chimeric protein encoding two or more separate protein sequences that are recombinantly expressed as a single moiety.

[0043] As used herein, the term “extracellular matrix” or “ECM” refers to a three-dimensional network of extracellular macromolecules, such as collagen, enzymes, and glycoproteins, that provide structural and biochemical support of surrounding cells. Because multicellularity evolved independently in different multicellular lineages, the composition of ECM varies between multicellular structures; however, cell adhesion, cell-to-cell communication and differentiation are common functions of the ECM. Components of the ECM are produced intracellularly by resident cells and secreted into the ECM via exocytosis. Once secreted, they then aggregate with the existing matrix. The ECM is composed of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs). The extracellular matrix regulates tissue development and homeostasis, and its dysregulation contributes to neoplastic progression. In addition, a number of tumors and cancers overexpress components of the extracellular matrix, creating a fibrous barrier that prevents access by therapeutics to the tumor cells (See, for example, Refs. 75-79).

[0044] As used herein, the term “extracellular matrix degrading enzyme” refers to enzymes that degrade components of the extracellular matrix. Examples include but are not limited to matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparatinase, cathepsin, lyase, trypsin, protease, plasmin, and urokinase.

[0045] As used herein, the term “recombinant extracellular matrix degrading enzyme” refers to an extracellular matrix degrading enzyme produced by recombinant DNA and/or protein expression systems.

[0046] As used herein, the term “matrix metalloproteinase” or “MMP” or “matrixins”, are metalloproteinases that are calcium-dependent zinc-containing endopeptidases; other family members are adamalysins, serralysins, and astacins. The MMPs belong to a larger family of proteases known as the metzincin superfamily. Collectively, these enzymes are capable of degrading all kinds of extracellular matrix proteins, but also can process a number of bioactive molecules. MMPs are also thought to play a major role in cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis, and host defense.

[0047] As used herein, the term “collagenase” refers to enzymes that break the peptide bonds in collagen.

[0048] As used herein, the term “hyaluronidase” refers to a family of enzymes that catalyze the degradation of hyaluronic acid (HA).

[0049] As used herein, the term “chondroitinase” refers to enzymes that catalyze the degradation of chondroitin.

[0050] As used herein, the term “heparatinase” refers to enzymes that catalyze the degradation of heparin.

[0051] As used herein, the term “cathepsin” refers to a class of proteases (enzymes that degrade proteins) found in all animals as well as other organisms. There are approximately a dozen members of this family, which are distinguished by their structure, catalytic mechanism, and which proteins they cleave. Cathepsins have a vital role in mammalian cellular turnover.

[0052] As used herein, the term “lyase” an enzyme that catalyzes the breaking (an "elimination" reaction) of various chemical bonds by means other than hydrolysis (a "substitution" reaction) and oxidation, often forming a new double bond or a new ring structure.

[0053] As used herein, the term “trypsin” refers to s a serine protease from the PA clan superfamily, found in the digestive system of many vertebrates, where it hydrolyzes proteins. Trypsin is formed in the small intestine when its proenzyme form (trypsinogen produced by the pancreas) is activated. Trypsin cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline.

[0054] As used herein, the term “protease” refers to an enzyme that helps proteolysis which is protein catabolism by hydrolysis of peptide bonds. Proteases have evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms. Proteases can be found in all forms of life and viruses.

[0055] As used herein, the term “plasmin” refers to a serine protease that degrades many blood plasma proteins, including fibrin clots. The degradation of fibrin is termed fibrinolysis, Apart from fibrinolysis, plasmin proteolyses proteins in various other systems: It activates collagenases, some mediators of the complement system, and weakens the wall of the Graafian follicle, leading to ovulation. It cleaves fibrin, fibronectin, thrombospondin, laminin, and von Willebrand factor.

[0056] As used herein, the term “urokinase” also known as” urokinase-type plasminogen activator” or “uPA” is a serine protease involved in degradation of the extracellular matrix and possibly tumor cell migration and proliferation.

[0057] The terms “disease” or “condition” refer to a state of being or health status of a patient or subject capable of being diagnosed and/or treated with compounds or methods provided herein. The disease may be a cancer.

[0058] As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals (e.g. humans). Examples of cancers that may be treated with a composition or method provided herein include solid tumors. In embodiments, the cancer is breast cancer, pancreatic cancer, or prostate cancer, or a subtype thereof. In embodiments, the pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC).

[0059] “Treating” or “treatment” as used herein (and as well understood in the art) includes any approach for obtaining beneficial or desired results in a subject’s condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease’s transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. “Treating” or “treatment” refers to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient’s physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters; including the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. In other words, "treatment" as used herein includes any cure, amelioration, or prevention of a disease.

[0060] "Treating" or "treatment" as used herein includes prophylactic treatment. Prophylactic treatment may inhibit the disease’s spread; relieve the disease’s symptoms, fully or partially remove the disease’s underlying cause, shorten a disease’s duration, or do a combination of these things. In embodiments, treating includes preventing. In embodiments, treating does not include preventing. Treatment methods include administering to a subject a therapeutically effective amount of an active agent. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the age of the patient, the

concentration of active agent, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by diagnostic assays (e.g., assays described herein or known in the art). In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient.

[0061] The term “prevent” refers to a decrease in the occurrence of disease symptoms in a patient. The prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment.

[0062] The term “patient” or “subject” refers to a living organism suffering from or prone to a disease or condition that can be treated by administration of a pharmaceutical composition. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a subject is human.

[0063] An “effective amount” is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (e.g. achieve the effect for which it is administered, treat a disease, reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which could also be referred to as a “therapeutically effective amount.” A “reduction” of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques.

[0064] The term “administering” as used herein refers to oral administration, administration as a suppository, topical contact, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal or subcutaneous administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations,

intravenous infusion, transdermal patches, etc. In embodiments, the administering does not include administration of any active agent other than the recited active agent. In embodiments, compositions described herein may be administered by intravenous, subcutaneous or intratumoral route.

[0065] The term "co-administer" as used herein refers to a composition described herein administered at the same time, just prior to, or just after the administration of one or more additional therapies. The compounds provided herein can be administered alone or can be coadministered to the patient. Co-administration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the present disclosure can be delivered transdermally, by a topical route, or formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0066] The term "cancer model organism" as used herein refers to an organism exhibiting a phenotype indicative of cancer, or the activity of cancer causing elements, within the organism. A wide variety of organisms may serve as cancer model organisms, and include for example, cancer cells and mammalian organisms such as rodents (e.g. mouse or rat) and primates. Cancer cell lines are widely understood by those skilled in the art as cells exhibiting phenotypes or genotypes similar to in vivo cancers. Cancer cell lines as used herein includes cell lines from animals (e.g. mice) and from humans.

[0067] The term "anticancer agent" is used in accordance with its plain ordinary meaning and refers to a composition (e.g. compound, drug, antagonist, inhibitor, modulator) having antineoplastic properties or the ability to inhibit the growth or proliferation of cells. In some embodiments, an anti-cancer agent is a chemotherapeutic. In some embodiments, an anti-cancer agent is an agent identified herein having utility in methods of treating cancer. In some embodiments, an anti-cancer agent is an agent approved by the FDA or similar regulatory agency of a country other than the USA, for treating cancer. Examples of anti-cancer agents include, but are not limited to, MEK (e.g. MEK1, MEK2, or MEK1 and MEK2) inhibitors (e.g. XL518, CI-1040, PD035901, selumetinib/ AZD6244, GSK1120212/ trametinib, GDC-0973, ARRY-162, ARRY-300, AZD8330, PD0325901, U0126, PD98059, TAK-733, PD318088, AS703026, BAY 869766), alkylating agents (e.g., cyclophosphamide, ifosfamide, chlorambucil, busulfan, melphalan, mechlorethamine, uramustine, thiotepa, nitrosoureas, nitrogen mustards (e.g.,

mechloretamine, cyclophosphamide, chlorambucil, melphalan), ethylenimine and methylmelamines (e.g., hexamethylmelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomustine, semustine, streptozocin), triazines (decarbazine)), anti-metabolites (e.g., 5-azathioprine, leucovorin, capecitabine, fludarabine, gemcitabine, pemetrexed, raltitrexed, folic acid analog (e.g., methotrexate), or pyrimidine analogs (e.g., fluorouracil, floxouridine, Cytarabine), purine analogs (e.g., mercaptopurine, thioguanine, pentostatin), *etc.*), plant alkaloids (e.g., vincristine, vinblastine, vinorelbine, vindesine, podophyllotoxin, paclitaxel, docetaxel, *etc.*), topoisomerase inhibitors (e.g., irinotecan, topotecan, amsacrine, etoposide (VP16), etoposide phosphate, teniposide, *etc.*), antitumor antibiotics (e.g., doxorubicin, adriamycin, daunorubicin, epirubicin, actinomycin, bleomycin, mitomycin, mitoxantrone, plicamycin, *etc.*), platinum-based compounds (e.g. cisplatin, oxaloplatin, carboplatin), anthracenedione (e.g., mitoxantrone), substituted urea (e.g., hydroxyurea), methyl hydrazine derivative (e.g., procarbazine), adrenocortical suppressant (e.g., mitotane, aminoglutethimide), epipodophyllotoxins (e.g., etoposide), antibiotics (e.g., daunorubicin, doxorubicin, bleomycin), enzymes (e.g., L-asparaginase), inhibitors of mitogen-activated protein kinase signaling (e.g. U0126, PD98059, PD184352, PD0325901, ARRY-142886, SB239063, SP600125, BAY 43-9006, wortmannin, or LY294002, Syk inhibitors, mTOR inhibitors, antibodies (e.g., rituxan), gossypol, genasense, polyphenol E, Chlorofusin, all trans-retinoic acid (ATRA), bryostatin, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), 5-aza-2'-deoxycytidine, all trans retinoic acid, doxorubicin, vincristine, etoposide, gemcitabine, imatinib (Gleevec.RTM.), geldanamycin, 17-N-Allylamino-17-Demethoxygeldanamycin (17-AAG), flavopiridol, LY294002, bortezomib, trastuzumab, BAY 11-7082, PKC412, PD184352, 20-epi-1, 25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A;

bizelesin; breflate; broprimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentantraquinones; cycloplatin; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; 9-dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorubicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium

cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylerie conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen-binding protein; sizofuran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrnan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; tricitribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine

kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinoxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer, Adriamycin, Dactinomycin, Bleomycin, Vinblastine, Cisplatin, acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; broprimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; fluorocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; iimofosine; interleukin II (including recombinant interleukin II, or rIL.sub.2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1; interferon alfa-n3; interferon beta-1a; interferon gamma-1b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedopa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazoie; nogalamycin; ormaplatin; oxisuran; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol; safingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spirolatin;

streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; tricyribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulazole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride, agents that arrest cells in the G2-M phases and/or modulate the formation or stability of microtubules, (e.g. Taxol.TM (i.e. paclitaxel), Taxotere.TM, compounds comprising the taxane skeleton, Erbulozole (i.e. R-55104), Dolastatin 10 (i.e. DLS-10 and NSC-376128), Mivobulin isethionate (i.e. as CI-980), Vincristine, NSC-639829, Discodermolide (i.e. as NVP-XX-A-296), ABT-751 (Abbott, i.e. E-7010), Altorhyrtins (e.g. Altorhyrtin A and Altorhyrtin C), Spongistatins (e.g. Spongistatin 1, Spongistatin 2, Spongistatin 3, Spongistatin 4, Spongistatin 5, Spongistatin 6, Spongistatin 7, Spongistatin 8, and Spongistatin 9), Cemadotin hydrochloride (i.e. LU-103793 and NSC-D-669356), Epothilones (e.g. Epothilone A, Epothilone B, Epothilone C (i.e. desoxyepothilone A or dEpoA), Epothilone D (i.e. KOS-862, dEpoB, and desoxyepothilone B), Epothilone E, Epothilone F, Epothilone B N-oxide, Epothilone A N-oxide, 16-aza-epothilone B, 21-aminoepothilone B (i.e. BMS-310705), 21-hydroxyepothilone D (i.e. Desoxyepothilone F and dEpoF), 26-fluoroepothilone, Auristatin PE (i.e. NSC-654663), Soblidotin (i.e. TZZT-1027), LS-4559-P (Pharmacia, i.e. LS-4577), LS-4578 (Pharmacia, i.e. LS-477-P), LS-4477 (Pharmacia), LS-4559 (Pharmacia), RPR-112378 (Aventis), Vincristine sulfate, DZ-3358 (Daiichi), FR-182877 (Fujisawa, i.e. WS-9885B), GS-164 (Takeda), GS-198 (Takeda), KAR-2 (Hungarian Academy of Sciences), BSF-223651 (BASF, i.e. ILX-651 and LU-223651), SAH-49960 (Lilly/Novartis), SDZ-268970 (Lilly/Novartis), AM-97 (Armad/Kyowa Hakko), AM-132 (Armad), AM-138 (Armad/Kyowa Hakko), IDN-5005 (Indena), Cryptophycin 52 (i.e. LY-355703), AC-7739 (Ajinomoto, i.e. AVE-8063A and CS-39.HCl), AC-7700 (Ajinomoto, i.e. AVE-8062, AVE-8062A, CS-39-L-Ser.HCl, and RPR-258062A), Vitilevuamide, Tubulysin A, Canadensol, Centaureidin (i.e. NSC-106969), T-138067 (Tularik, i.e. T-67, TL-138067 and TI-138067), COBRA-1 (Parker Hughes Institute, i.e. DDE-261 and WHI-261), H10 (Kansas State University), H16 (Kansas State University), Oncocidin A1 (i.e. BTO-956 and DIME), DDE-313 (Parker Hughes Institute), Fijianolide B, Laulimalide, SPA-2 (Parker Hughes Institute), SPA-1 (Parker Hughes Institute, i.e. SPIKET-P), 3-IAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-569), Narcosine (also known as NSC-5366), Nascapine, D-24851 (Asta

Medica), A-105972 (Abbott), Hemiasterlin, 3-BAABU (Cytoskeleton/Mt. Sinai School of Medicine, i.e. MF-191), TMPN (Arizona State University), Vanadocene acetylacetonate, T-138026 (Tularik), Monsatrol, Inanocine (i.e. NSC-698666), 3-IAABE (Cytoskeleton/Mt. Sinai School of Medicine), A-204197 (Abbott), T-607 (Tularik, i.e. T-900607), RPR-115781 (Aventis), Eleutherobins (such as Desmethyleleutherobin, Desacetyeleutherobin, Isoeleutherobin A, and Z-Eleutherobin), Caribaeoside, Caribaeolin, Halichondrin B, D-64131 (Asta Medica), D-68144 (Asta Medica), Diazonamide A, A-293620 (Abbott), NPI-2350 (Nereus), Taccalonolide A, TUB-245 (Aventis), A-259754 (Abbott), Diozostatin, (-)-Phenylahistin (i.e. NSCL-96F037), D-68838 (Asta Medica), D-68836 (Asta Medica), Myoseverin B, D-43411 (Zentaris, i.e. D-81862), A-289099 (Abbott), A-318315 (Abbott), HTI-286 (i.e. SPA-110, trifluoroacetate salt) (Wyeth), D-82317 (Zentaris), D-82318 (Zentaris), SC-12983 (NCI), Resverastatin phosphate sodium, BPR-OY-007 (National Health Research Institutes), and SSR-250411 (Sanofi)), steroids (e.g., dexamethasone), finasteride, aromatase inhibitors, gonadotropin-releasing hormone agonists (GnRH) such as goserelin or leuprolide, adrenocorticosteroids (e.g., prednisone), progestins (e.g., hydroxyprogesterone caproate, megestrol acetate, medroxyprogesterone acetate), estrogens (e.g., diethylstilbestrol, ethinyl estradiol), antiestrogen (e.g., tamoxifen), androgens (e.g., testosterone propionate, fluoxymesterone), antiandrogen (e.g., flutamide), immunostimulants (e.g., Bacillus Calmette-Guérin (BCG), levamisole, interleukin-2, alpha-interferon, *etc.*), monoclonal antibodies (e.g., anti-CD20, anti-HER2, anti-CD52, anti-HLA-DR, and anti-VEGF monoclonal antibodies), immunotoxins (e.g., anti-CD33 monoclonal antibody-calicheamicin conjugate, anti-CD22 monoclonal antibody-pseudomonas exotoxin conjugate, *etc.*), radioimmunotherapy (e.g., anti-CD20 monoclonal antibody conjugated to ^{111}In , ^{90}Y , or ^{131}I , *etc.*), triptolide, homoharringtonine, dactinomycin, doxorubicin, epirubicin, topotecan, itraconazole, vindesine, cerivastatin, vincristine, deoxyadenosine, sertraline, pitavastatin, irinotecan, clofazimine, 5-nonyloxytryptamine, vemurafenib, dabrafenib, erlotinib, gefitinib, EGFR inhibitors, epidermal growth factor receptor (EGFR)-targeted therapy or therapeutic (e.g. gefitinib (Iressa™), erlotinib (Tarceva™), cetuximab (Erbix™), lapatinib (Tykerb™), panitumumab (Vectibix™), vandetanib (Caprelsa™), afatinib/BIBW2992, CI-1033/canertinib, neratinib/HKI-272, CP-724714, TAK-285, AST-1306, ARRY334543, ARRY-380, AG-1478, dacomitinib/PF299804, OSI-420/desmethyl erlotinib, AZD8931, AEE788, pelitinib/EKB-569, CUDC-101, WZ8040, WZ4002, WZ3146, AG-490, XL647, PD153035, BMS-599626), sorafenib, imatinib, sunitinib, dasatinib, or the like.

[0068] As used herein, the term “pharmaceutically acceptable excipient” and “pharmaceutically acceptable carrier” refer to a substance that aids the administration of an active agent to and absorption by a subject and can be included in the compositions of the present invention without causing a significant adverse toxicological effect on the patient. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer’s, normal sucrose, normal glucose, binders, fillers, disintegrants, lubricants, coatings, sweeteners, flavors, salt solutions (such as Ringer's solution), alcohols, oils, gelatins, carbohydrates and the like. Such preparations can be sterilized and, if desired, mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds of the invention. One of skill in the art will recognize that other pharmaceutical excipients are useful in the present invention.

[0069] Pharmaceutical compositions provided by the present invention include compositions wherein the active ingredient (e.g. compounds described herein, including embodiments or examples) may be contained in a therapeutically effective amount, *i.e.*, in an amount effective to achieve its intended purpose. The actual amount effective for a particular application will depend, *inter alia*, on the condition being treated. When administered in methods to treat a disease, such compositions will contain an amount of active ingredient effective to achieve the desired result, e.g., reducing, eliminating, or slowing the progression of disease symptoms. Determination of a therapeutically effective amount of a compound of the invention is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure herein.

[0070] The dosage and frequency (single or multiple doses) administered to a mammal can vary depending upon a variety of factors, for example, whether the mammal suffers from another disease, and its route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated, kind of concurrent treatment, complications from the disease being treated or other health-related problems. Other therapeutic regimens or agents can be used in conjunction with the methods and compounds of Applicants' invention. Adjustment and manipulation of established dosages (e.g., frequency and duration) are well within the ability of those skilled in the art.

II. Compositions

[0071] In an aspect, provided herein is an attenuated facultative anaerobic bacterium including a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0072] In embodiments, the attenuated facultative anaerobic bacterium is selected from *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Vibrio cholerae*, *Vibrio fischeri*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Lactobacillus bulgaricus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus gallolyticus*, *Enterococcus faecium*, and *Streptococcus pyogenes*. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella bongori*. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella choleraesuis*. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella enterica*. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella enteritidis*. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella paratyphi*. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhi*. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium*. In embodiments, the attenuated facultative anaerobic bacterium is *Vibrio cholerae*. In embodiments, the attenuated facultative anaerobic bacterium is *Vibrio fischeri*. In embodiments, the attenuated facultative anaerobic bacterium is *Escherichia coli*. In embodiments, the attenuated facultative anaerobic bacterium is *Shigella boydii*. In embodiments, the attenuated facultative anaerobic bacterium is *Shigella dysenteriae*. In embodiments, the attenuated facultative anaerobic bacterium is *Shigella flexneri*. In embodiments, the attenuated facultative anaerobic bacterium is *Shigella sonnei*. In embodiments, the attenuated facultative anaerobic bacterium is *Lactobacillus bulgaricus*. In embodiments, the attenuated facultative anaerobic bacterium is *Listeria monocytogenes*. In embodiments, the attenuated facultative anaerobic bacterium is *Enterococcus faecalis*. In embodiments, the attenuated facultative anaerobic bacterium is *Enterococcus gallolyticus*. In embodiments, the attenuated facultative anaerobic bacterium is *Enterococcus faecium*. In embodiments, the attenuated facultative anaerobic bacterium is *Streptococcus pyogenes*.

[0073] In embodiments, the attenuated facultative anaerobic bacterium is a *Salmonella typhimurium* strain selected from MVP728 (see, for example, Ref. 65), YS1646 (VNP20009) (see, for example, Refs. 66-67), RE88 (see, for example, Ref. 68), LH430 (see, for example, Ref. 69), SL7207 (see, for example, Refs. 70-71), χ 8429, χ 8431 and χ 8768 (see, for example, Refs.

72-73). In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* MVP728. Y In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* S1646 (VNP20009). In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* RE88. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* LH430. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* S L7207. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* χ 8429. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* χ 8431. In embodiments, the attenuated facultative anaerobic bacterium is *Salmonella typhimurium* χ 8768. (See for example Bollen *et al.* 2008; Rodriguez *et al.* 2012)

[0074] In embodiments, the attenuated bacterium is attenuated by alteration or mutation of genes. Attenuation includes genetic mutations that prevent expression of virulence-associated genes. Genes include those that encode for proteins contributing to recombination (*recA*), nucleotide synthesis (*purI*), motility (*fliC*), cell wall composition (*msbB*, *asd*), transcription regulation (*phoP*, *phoQ*) and amino acid synthesis (*aroA*). In embodiments, attenuated bacterium have one or more gene mutations. In embodiments, attenuated bacterium have one or more mutations in a gene selected from *recA*, *purI*, *fliC*, *msbB*, *asd*, *phoP*, *phoQ*, and *aroA* (see, for example, Ref. 74).

[0075] In embodiments, the nucleic acid molecule is an expression vector or plasmid. In embodiments, the nucleic acid molecule includes elements for gene transcription and translation. In embodiments, the elements for gene transcription and translation include an origin of replication, a selectable marker, a gene, and a promoter. In embodiments, the gene is transcribed and translated to produce a functional protein or gene product.

[0076] In embodiments, the nucleic acid molecule includes a gene encoding a recombinant extracellular matrix degrading enzyme. In embodiments, the gene is transcribed and translated to produce a functional extracellular matrix degrading enzyme. In embodiments, the extracellular matrix degrading enzyme is a bacterial extracellular matrix degrading enzyme. In embodiments, the extracellular matrix degrading enzyme is a human extracellular matrix degrading enzyme. In embodiments, the extracellular matrix degrading enzyme is a parasitic extracellular matrix degrading enzyme.

[0077] In embodiments, the recombinant extracellular matrix degrading enzyme is selected from matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparitinase,

cathepsin, lyase, trypsin, protease, plasmin, and urokinase. In embodiments, the recombinant extracellular matrix degrading enzyme is matrix metalloproteinase. In embodiments, the recombinant extracellular matrix degrading enzyme is collagenase. In embodiments, the recombinant extracellular matrix degrading enzyme is hyaluronidase. In embodiments, the recombinant extracellular matrix degrading enzyme is chondroitinase. In embodiments, the recombinant extracellular matrix degrading enzyme is heparatinase. In embodiments, the recombinant extracellular matrix degrading enzyme is cathepsin. In embodiments, the recombinant extracellular matrix degrading enzyme is lyase. In embodiments, the recombinant extracellular matrix degrading enzyme is trypsin. In embodiments, the recombinant extracellular matrix degrading enzyme is protease. In embodiments, the recombinant extracellular matrix degrading enzyme is plasmin. In embodiments, the recombinant extracellular matrix degrading enzyme is urokinase. In embodiments, the recombinant extracellular matrix degrading enzyme is a protease encoded by a codon optimized nucleic acid comprising SEQ ID NO.: 2. In embodiments, the recombinant extracellular matrix degrading enzyme is a *Streptomyces omiyaensis* trypsin-like protease encoded by a codon optimized nucleic acid comprising SEQ ID NO.: 2.

[0078] In embodiments, the hyaluronidase is bacterial hyaluronidase. In embodiments, the bacterial hyaluronidase is selected from *Streptomyces koganeiensis*, *Streptomyces hyaluronolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens*. In embodiments, the bacterial hyaluronidase is from *Streptomyces koganeiensis*. In embodiments, the bacterial hyaluronidase is from *Streptomyces hyaluronolyticus*. In embodiments, the bacterial hyaluronidase is from *Staphylococcus aureus*. In embodiments, the bacterial hyaluronidase is from *Streptococcus pyogenes*. In embodiments, the bacterial hyaluronidase is from *Clostridium perfringens*. In embodiments, the bacterial hyaluronidase is encoded by a codon optimized nucleic acid sequence comprising SEQ ID NO.: 1. In embodiments, the bacterial hyaluronidase is a *Streptomyces koganeiensis* hyaluronidase encoded by a codon optimized nucleic acid sequence comprising SEQ ID NO.: 1.

[0079] In embodiments, the promoter is an inducible promoter. In embodiments, the inducible promoter is selected from a pLac promoter, pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter. In embodiments, the inducible promoter is a pLac promoter. In embodiments, the inducible promoter is a pTac promoter. In embodiments, the inducible promoter is a tetracycline-controlled promoter. In embodiments, the inducible promoter is a pBAD promoter.

[0080] In embodiments, the promoter is a tumor-specific promoter.

[0081] In embodiments, the promoter is a hypoxia-inducible bacterial promoter. In embodiments, the hypoxia-inducible bacterial promoter is selected from those regulating expression of *spflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*. In embodiments, the hypoxia-inducible bacterial promoter is a *spflE* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a *hcp* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a *menD* promoter. In embodiments, the hypoxia-inducible bacterial promoter is an *ansB* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a *mltD* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a *glpA* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a *glpT* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a *pepT* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a FF+20* promoter. In embodiments, the hypoxia-inducible bacterial promoter is a HIP1 promoter.

[0082] In embodiments, the attenuated facultative anaerobic bacterium expresses an extracellular matrix degrading enzyme under tumor-specific conditions such as hypoxia.

III. Methods of Use

[0083] In an aspect, provided herein is a method of treating a tumor in a subject including administering to the subject an effective amount of an attenuated facultative anaerobic bacterium that includes a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0084] In an aspect, provided herein is a method of treating a tumor in a subject including administering to the subject an effective amount of an attenuated facultative anaerobic bacterium and a chemotherapeutic agent. The attenuated facultative anaerobic bacterium includes a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0085] In embodiments, the tumor is a solid tumor. In embodiments, the tumor is selected from pancreatic, breast, prostate, skin, lung, and abdomen tumor. In embodiments, the tumor is a pancreatic tumor. In embodiments, the tumor is a breast tumor. In embodiments, the tumor is a prostate tumor. In embodiments, the tumor is a skin tumor. In embodiments, the tumor is lung tumor. In embodiments, the tumor is an abdomen tumor.

[0086] In embodiments, the pancreatic tumor is pancreatic ductal adenocarcinoma. In embodiments, the skin tumor is malignant melanoma. In embodiments, the skin tumor is desmoplastic squamous cell carcinoma. In embodiments, the lung tumor is small cell lung cancer. In embodiments, the lung tumor is non-small cell lung cancer. In embodiments, the abdomen tumor is desmoplastic small round cell tumor.

[0087] In embodiments, the methods provided herein include administering to a subject an effective amount of attenuated facultative anaerobic bacterium. In embodiments, compositions described herein may be administered by intravenous, intraperitoneal, subcutaneous or intratumoral route.

[0088] In embodiments, an effective amount of an attenuated facultative anaerobic bacterium is an amount such that expression of the extracellular matrix degrading enzyme is sufficient to degrade the extracellular matrix, degrade a component of the extracellular matrix, and/or increase penetration of an anti-tumor agent to the tumor. In embodiments, an effective amount of an attenuated facultative anaerobic bacterium is an amount such that expression of the extracellular matrix degrading enzyme is sufficient to degrade the extracellular matrix. In embodiments, an effective amount of an attenuated facultative anaerobic bacterium is an amount such that expression of the extracellular matrix degrading enzyme is sufficient to degrade a component of the extracellular matrix. In embodiments, an effective amount of an attenuated facultative anaerobic bacterium is an amount such that expression of the extracellular matrix degrading enzyme is sufficient to increase penetration of an anti-tumor agent to the tumor.

[0089] In embodiments, the attenuated facultative anaerobic bacterium includes a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter as described above.

[0090] In embodiments, administration of the attenuated facultative anaerobic bacterium that includes a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter as described leads to expression of extracellular matrix degrading enzyme capable of degrading components of the extracellular matrix. Degradation of components of the extracellular matrix leads to a weakness in the fibrous surroundings of the tumor and thus, access to the tumor by chemotherapeutic agents.

[0091] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application

and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

Example 1: Development of attenuated ST strains expressing bHs from *S. koganeiensis*

[0092] Various attenuated *Salmonella typhi* (ST) strains expressing bacterial hyaluronidase (bHs) from *S. koganeiensis* and referred to as bHs-ST have been developed and characterized. The data demonstrated that attenuated ST is capable of auto-displaying functional bHs that can effectively degrade purified and tumor-derived hyaluron (HA). The data confirmed bHs-ST, when delivered systemically, is capable of colonizing orthotopic human pancreatic adenocarcinoma (PDAC) tumors in mice and can cause remarkable degradation of tumor-derived HA resulting in enhanced diffusion of ST throughout the tumor. This was the first example of a tumor-specific, extracellular matrix (ECM)-degrading strategy that could significantly improve efficacy of therapies for PDAC and other desmoplastic tumor types.

[0093] Materials and methods

[0094] Animals and cell lines

[0095] Non SCID gamma (NSG) mice were obtained from breeding colonies housed at the City of Hope (COH) Animal Research Center and, for all studies, handled according to standard IACUC guidelines (approved IACUC protocol 17128). The PANC-1 and PC-3 cell lines were obtained from ATCC® (CRL1469™, CRL1435™). PC-3 cells were maintained in RPMI media containing 10% FBS, 2mM L-glutamine and pen/strep. PANC-1 cells, prior to orthotopic implantation (survival surgery) into NSG mice, were passaged ≤ 5 times and maintained at $\leq 80\%$ confluency in DMEM containing 10% FBS, 2mM L-glutamine and pen/strep.

[0096] ST strains and generation of bHs-ST

[0097] YS1646 was obtained from ATCC® (202165™). Other attenuated strains were kind gifts obtained from Roy Curtiss III (χ 8429, χ 8431, χ 8768), B.A.D Stocker (SL7207) and Michael Hensel (MVP728) (35-39). YS1646 was cultured in modified LB media containing MgSO₄ and CaCl₂ in place of NaCl. All other strains were cultured in Miller LB media (Fisher BioReagents). The *S. koganeiensis* bHs amino acid sequence (GenBank Accession no. KP313606) was used to synthesize a codon-optimized cDNA (Biomatik) inserted in-frame into a 6xHis-pBAD bacterial expression vector (kind gift from Michael Davidson, Addgene #54762) using XhoI/EcoRI sites. In-frame insertion of bHs into the pBAD vector adds a 6XHis tag to the N-terminus of the

protein. χ 8768- LUX was generated using the pAKlux2 plasmid (pAKlux2 was a gift from Attila Karsi, Addgene #14080). Plasmids were electroporated into ST strains using a BTX electroporator (1mm gap cuvettes, settings: 1.8kV, 186 ohms), spread onto LB plates containing 100 ug/mL ampicillin and incubated overnight at 37 °C. Glycerol stocks were generated for pBAD-bHs-positive clones identified by colony PCR and restriction digest of plasmid preparations.

[0098] Bacterial growth, viability and analysis of bHs expression

[0099] ST clones electroporated with pBAD-bHs were cultured in media with or without 2% (w/v) L-arabinose at 37°C, 225 rpm for time intervals ranging from 3hr - 24hr. Growth kinetics were monitored through absorbance readings at 600 nm (Genesys 30, Thermo Scientific) every 1-2 hrs, up to 24 hrs. 6XHis-tagged bHs expression was detected in bacterial lysates by western blot and localization of bHs was detected by immunofluorescence using a primary monoclonal mouse anti- 6XHis antibody (Proteintech). For immunofluorescence, uninduced and induced ST grown for ~3 hours were fixed with 4% paraformaldehyde at room temperature (RT) for 30 minutes, and permeabilized with 0.1% Triton-X 100/PBS pH=7.2 at RT for 30 minutes followed by lysozyme (Sigma, 100 ug/mL final concentration in 5 mM EDTA) at RT for 45 minutes. Fixed/permeabilized bacteria were incubated with primary antibody (1:100) for 30 minutes with shaking in a humidified 37 °C incubator followed by incubation with FITC-conjugated anti-mouse secondary (1:200, Abcam) and DAPI for 30 minutes with shaking in a humidified 37°C incubator.

[0100] Hyaluronan-BSA LB (HBL) plate and turbidimetric assays

[0101] HBL plates for evaluating hyaluronidase activity were generated as previously described (40). Briefly, LB agar plates containing final concentrations of 0.4 mg/mL HA (Sigma, H-1504), 1% bovine serum albumin fraction V (Sigma) and 100 ug/mL ampicillin (Sigma) were used for plating uninduced and induced ST strains (10% colony forming units (CFU)/5 uL drop) at 37°C for 16-24 hrs. Plates were then flooded with 2N glacial acetic acid. Clear zones were observed against a background of opaque precipitated BSA conjugated to the undigested HA. For turbidimetric quantification of HA degradation in culture media over time, the cetyltrimethylammonium bromide (CTAB) turbidimetric method was used (41). In brief, LB media containing 0.4 mg/mL HA and 100 ug/mL ampicillin, with or without 2% L-arabinose, was used to culture bHs-ST strains (2 mL starting volume) over 24 hrs at 37°C, 225 rpm. HA

content (absorbance) in culture media (100 uL aliquot) was measured every 2-3 hrs by addition of 2.5% CTAB reagent (25 uL, Sigma) and absorbance read at 600 nm.

[0102] Orthotopic tumor implantation

[0103] Previously published methods were used for survival surgery and orthotopic implantation of PANC-1 cells into the pancreas of NSG mice (42). Briefly, while anesthetized and using sterile techniques, a small incision was made in the skin and peritoneal lining and the pancreas externalized. Using a 27 gauge needle, approximately $1.5\text{--}2 \times 10^6$ PANC-1 cells in a volume of 50 uL Matrigel (BD Biosciences) were injected into the body of the pancreas. The pancreas was then reinserted into the peritoneal space and inner and outer incisions were closed using absorbable sutures and staples, respectively. Analgesics were administered pre- and post-surgery.

[0104] Immunohistochemistry/immunofluorescence (IHC/IFC) to detect HA and ST

[0105] Prior to incubation with bHs-ST *in vitro*, sections of PANC-1 tumors were de-paraffinized and rehydrated. Uninduced and induced $\chi 8768$ -bHs (10^8 CFU), PBS or bovine hyaluronidase (Sigma) were incubated on tissue sections overnight in a humidified 37°C incubator. Following treatment, specimens were incubated with a biotinylated HA binding protein (HABP, Sigma) at 5 ug/mL final concentration for 2 hours at 37°C. Slides were then washed, incubated with streptavidin-HRP at RT for 1 hr and visualized with a DAB kit (Vectastain). Hematoxylin was used for counterstaining.

[0106] PANC-1 tumor sections from NSG mice treated intravenously with $\chi 8768$ -bHs and then induced, or left uninduced, were de-paraffinized and rehydrated and stained overnight with 2.5 ug/mL HABP, 1:100 anti-ST antibody (Santa Cruz, sc-52223), or according to Masson's Trichrome protocol by the Pathology Research Services Core (City of Hope). Streptavidin-PE and anti-mouse-Cy5 were then used to visualize HA and ST by fluorescence microscopy (Zeiss Observer II), in addition to DAPI for visualizing nuclei. Tiling was performed at 10X, while high magnification to visualize ST was done at 100X (oil).

[0107] Administration and induction of $\chi 8768$ -bHs in PANC-1 tumor-bearing NSG mice

[0108] NSG mice with palpable PANC-1 tumors (>250 mm³) were intravenously injected with 2.5×10^6 $\chi 8768$ -LUX or $\chi 8768$ -bHs. Actively growing $\chi 8768$ -LUX is constitutively bioluminescent and was used to evaluate $\chi 8768$ colonization of PANC-1 tumors *in vivo* using intravital imaging (LagoX, Spectral Imaging). Two days after administering $\chi 8768$ -bHs, mice

were administered 240 mg L-arabinose or PBS (intraperitoneally). Mice were euthanized 16 hours after receiving L-arabinose or PBS and tumors were excised, fixed and sectioned to evaluate HA and ST.

[0109] Blood vessel dilation measurements

[0110] Ten to twelve fields at 10x for Trichrome stained slides from PANC1 tumors treated with χ 8768- bHs and induced with L-arabinose or uninduced in vivo were imaged using a Leica DMI8 Microscope. In each field, blood vessel diameters were measured using the Leica LasX software. A Mann-Whitney test was performed on values using the Prism 7.2 software from GraphPad.

[0111] Tightly-regulated expression of bHs by attenuated ST strains

[0112] In order to circumvent potential toxic effects of constitutive bHs expression on attenuated ST strains, a tightly-regulated inducible expression system was employed. Inducible expression in ST is possible through the use of a construct containing the P_{BAD} promoter of the araBAD (arabinose) operon and the gene encoding the positive and negative regulator of this promoter, araC (for example, ref. 43). An ST codon-optimized bHs sequence, based on the amino acid sequence of the well-characterized *S. koganeiensis* bHs, was synthesized and cloned into a previously described pBAD vector to generate pBAD-bHs (44). A single plasmid preparation of pBAD-bHs was used for electroporation into various attenuated strains of ST (**Table 1**).

[0113] Table 1. ST strains used in these studies.

ST Strain	Mutations	Parental (wildtype) ST strain	Reference(s)
SL7207	<i>aroA</i> ⁻	SL1344	(37,39)
MVP728	<i>purE</i> ⁻ / <i>htrA</i> ⁻	ATCC14028	(36)
YS1646	<i>msbB</i> ⁻ / <i>purE</i> ⁻	ATCC14028	(30)
χ8429	<i>ΔphoP/phoQ</i>	UK-1	(38)
χ8431	<i>ΔphoP/phoQ</i>	UK-1	(38)
χ8768	<i>ΔphoP233</i>	UK-1	(35)

[0114] Colony polymerase chain reaction (PCR) was performed for each transformed strain (≥ 8 colonies) to detect for retention of the bHs transgene (**Figure 1A**). All ampicillin-resistant colonies examined for YS1646 and MVP728 were completely negative for the bHs transgene in the absence of L-arabinose, suggesting loss of the transgene independent of induced protein expression. Of note, both YS1646 and MVP728 are derived from the same parental strain ATCC 14028. Culturing of pBAD-bHs-positive colonies in uninduced and induced (+2% L-arabinose) conditions, followed by coomassie blue (CB) staining and western blot (WB) of pellet lysates, revealed expression of His-tagged bHs at the correct molecular weight (27 kD) as well as tight regulation of protein expression (**Figure 1B**). No bHs was detected in culture media by CB or WB (**Figure 6A**), suggesting that bHs is not secreted by these ST strains following induction.

[0115] Using HHMTOP, PSORTb and CellP-Loc subcellular localization prediction tools, bHs is predicted to be anchored to the cytoplasmic membrane at its N-terminus, while the active region (residues 66-247) is localized to the outer membrane/extracellular space (**Figure 6B**) (For example, Refs. 45-47). To determine the subcellular location of bHs expressed by the various ST strains, immunofluorescence staining was performed utilizing a 6XHis-tag fused to the N-terminus of the bHs protein was (**Figure 1C**). His-tagged bHs expressed by induced χ8429-bHs and χ8768-bHs would reveal clear bHs localization outside of the bacterial cytoplasm, defined by DAPI staining of genomic DNA. In contrast, bHs expressed by SL7207-bHs, and to a smaller extent in χ8431-bHs, is localized to the cytoplasm, suggesting impaired transport and formation of inclusion bodies in these attenuated strains. No detectable bHs expression was observed in

uninduced cultures (data not shown). Altogether, these data confirm that expression of the ST codon-optimized bHs transgene is tightly regulated using an inducible pBAD system and that the expressed bHs protein can be autodisplayed on the bacterial surface.

[0116] Growth kinetics and viability of bHs-expressing ST strains

[0117] While tight regulation of transgene expression is important for minimizing toxicity during initial growth stages, sufficient growth and viability following induction will also be critical to maximizing bHs activity. Thus, growth kinetics of each of the bHs-ST-expressing strains over 24 hours in non-inducing and inducing ($\pm 2\%$ L-arabinose) conditions was determined. SL7207 alone is already known to have dramatically reduced growth kinetics compared to wildtype ST, reaching a maximum optical density (O.D.) 2-3 fold lower than other attenuated strains (**Figure 2**). Under induced conditions, the maximum O.D. for SL7207-bHs is significantly reduced to under 1 (**Figure 2A**), whereas other attenuated ST strains could reach maximum O.D.s 3-fold higher (**Figure 2B-D**). Both inherently poor growth kinetics of unmodified SL7207 as well as cytoplasmic accumulation of bHs (**Figure 1C**), could contribute to the significantly reduced growth kinetics of SL7207-bHs following induction. Interestingly, while $\chi 8431$ -bHs showed mixed localization of bHs by immunofluorescence, induced growth kinetics were indistinguishable from $\chi 8768$ -bHs and $\chi 8429$ -bHs. These data suggest that $\chi 8768$, $\chi 8431$ and $\chi 8429$ have greater viability upon bHs induction, which could potentially translate into more extensive HA degradation.

[0118] To further investigate bacterial viability after induction, live/dead staining was performed using a mixture of acridine orange (AO) and ethidium bromide (EB), respectively, during log phase (4 hr) and stationary phase (24 hr) of uninduced and induced cultures (For example 48, 49). As shown in **Figure 2E** and **Figure 6C**, the percentage of viable bacterial cells after induction of SL7207-bHs is significantly lower than $\chi 8768$ - and $\chi 8429$ -bHs, as indicated by highly EB-positive SL7207-Hase as early as 4 hr and continuing 24 hr after induction. These results further emphasize the deleterious toxic effects of bHs expression on the viability of attenuated strains such as SL7207 but also highlight ST strains capable of autodisplaying bHs and remaining viable during and long after initiation of induction.

[0119] BHs-ST strains degrade purified HA

[0120] To test the functionality of bHs expressed by the various attenuated ST strains, HA agar plate clearing and liquid culture turbidimetric assays (for example 40, 41) were employed. For plate clearing assays, HA and BSA are mixed into LB agar plates (HBL plates). Addition of 2N

acetic acid to HBL plates containing intact HA will form a white precipitate with BSA, while areas of HA degradation will remain clear. BHs-expressing strains were pre-induced for 3 hours in LB media containing 0 to 4% L-arabinose and then spotted (1×10^8 CFU/5 μ L) onto HBL plates overnight. After flooding HBL plates with 2N acetic acid, zones of clearing were observed for χ 8768-, χ 8431- and χ 8429-bHs, but not SL7207-bHs (**Figure 3A**). Interestingly, χ 8431-bHs, which had exhibited intermediate surface display of bHs (**Figure 1C**), also demonstrated intermediate HA degradation. These data suggest that ST strains that efficiently display bHs on their surface and exhibit greater viability are far more effective at degrading pure HA.

[0121] To determine the kinetics of HA degradation by the various bHs-ST strains, each was cultured under uninduced and induced conditions in LB media containing HA (0.4 mg/mL) and measured HA content over a 24 hour period using the cetyltrimethylammonium bromide (CTAB) turbidimetric method (39). At each time point, high molecular weight HA in culture media was precipitated with CTAB and optical density determined at a wavelength of 600 nm (**Figure 3B-D**). Data showed higher overall rates of HA degradation over the 24 hr period by χ 8768- and χ 8429- bHs (~ 0.15 O.D. units/hr), an intermediate rate for χ 8431-bHs (~ 0.10 O.D. units/hr) and no degradation by SL7207-bHs, which recapitulates activity observed for each strain on HBL plates. The highest rate of degradation was observed for χ 8768-bHs during the first 12 hours of induction (~ 0.3 O.D. units/hr), whereas χ 8429-bHs and χ 8431-bHs showed two times less activity (~ 0.15 O.D. units/hr). Overall, these data indicate that the χ 8768-bHs strain is most effective in degrading purified HA within hours of induction.

[0122] χ 8768-bHs effectively degrades tumor-derived HA

[0123] Based on its viability following induction and ability to efficiently degrade purified HA, χ 8768-bHs was selected to further determine if bHs-expressing ST could degrade tumor-derived HA. The human pancreatic cancer line PANC-1 was utilized, after confirming it expresses high levels of HA when grown orthotopically in NSG (immune-deficient) mice. First, *in vitro* HA degradation experiments were performed whereby PANC-1 tumor sections were incubated with pre-induced χ 8768- bHs. Overnight incubation of PANC-1 tumor sections with pre-induced χ 8768-bHs resulted in dramatic degradation of HA compared to sections incubated with PBS or uninduced χ 8768-bHs (**Figure 4**). Similar degradation experiments were performed using the PC-3 prostate cancer cell line, which secretes high levels of HA while in culture, and also observed considerable depletion of HA by induced χ 8768-bHs (**Figure 7**). These results

strongly suggest that χ 8768 expressing bHs could be used universally to degrade tumor-derived HA in various cancer types.

[0124] Systemically-delivered χ 8768-bHs degrades HA in orthotopic PDAC tumors

[0125] Next, the ability of χ 8768-bHs to colonize and deplete HA was determined in orthotopically implanted PANC-1 tumors when delivered intravenously into mice. First, verification that the χ 8768 strain was capable of colonizing PANC-1 tumors utilizing a constitutive bacterial reporter gene construct encoding the bioluminescent LUX operon (50) was undertaken. When recombinant χ 8768 encoding LUX (χ 8768-LUX) was injected intravenously into NSG mice bearing orthotopically implanted PANC-1 tumors (>250 mm³) observations showed bioluminescence localized to the area of the tumor, which was detected on day 3 and was undetectable on day 5 (**Fig. 8A**). To further verify tumor-specific colonization by χ 8768-LUX, tumor, spleen and liver were isolated following i.v. injection and measured bioluminescence for each tissue type. Indeed, χ 8768-LUX was highly concentrated in tumor tissue while completely absent in both spleen and liver (**Fig. 8B**). These results suggest that χ 8768-bHs is capable of colonizing orthotopic PANC-1 tumors after systemic administration and that tumor-specific colonization is achieved by day 2, which would represent an ideal time point for induction of bHs activity.

[0126] Thus, NSG mice with orthotopic PANC-1 tumors (>250 mm³) were intravenously administered 2.5×10^6 CFUs of χ 8768-bHs and then induced 2 days later by a single intraperitoneal injection of 240 mg of L-arabinose per mouse. PANC-1 tumors were excised 24 after induction (in the methods and materials you wrote 16 hours) and sectioned and stained for both HA and ST. As shown in **Figure 5A**, tumors from mice receiving only χ 8768-bHs (uninduced) were characterized by limited (punctate) ST colonization and high HA deposition in areas of ST colonization. In contrast, mice administered χ 8768-bHs and L-arabinose (induced) resulted in dramatic degradation of HA, particularly within areas colonized by χ 8768-bHs (**Figure 5B**). Remarkably, greater diffusion of χ 8768-bHs ($p < 0.05$, t test) was also observed under induced conditions (**Figure 5C, D**). Diffusion of χ 8768-bHs was observed from a number of duct-like structures (likely blood vessels) in tumors of induced mice, which were predominantly devoid of HA. In uninduced mice, these structures were less prevalent but when observed, ST were found within the duct but had not colonized the surrounding tissue which showed high HA staining (**Figure 8C**). Through trichrome staining, the blood vessels are more easily visualized by the presence of collagen and red blood cells, revealing a predominance of

larger, open vessels in induced mice compared to a majority of closed vessels in uninduced mice, reminiscent of the concentrated punctate staining of ST colonization in these tumors (**Figure 8D, E**). Altogether, these data strongly suggest that χ 8768-bHs is capable of effectively degrading tumor-derived HA *in vivo* to decrease interstitial pressure and facilitate delivery of agents as large as ST, in which a single bacterium can measure 5 μ m in length and reach a molecular weight in the hundreds of gigadaltons (for example 51-55).

[0127] DISCUSSION

[0128] Hyaluronidase administration has been shown to enhance the efficacy of gemcitabine and nAb-paclitaxel in PDAC tumor models and has had some clinical benefit in PDAC patients (for example 56, 57). However, the high risk of adverse effects associated with systemically delivered hyaluronidase still presents major concerns due to ECM degradation in healthy tissues. To reduce risk, lower doses of hyaluronidase must be given, which may not necessarily maximize the therapeutic efficacy of chemotherapy. BHs-ST is the first example of a systemically delivered ECM-degrading agent that can focus its enzymatic activity strictly to tumor tissue, potentially maximizing HA degradation and therapeutic drug delivery. Data herein established that bHs is anchored to the surface of ST, reducing the likelihood of systemic off-tumor effects resulting from circulating bHs. Previous studies have also shown that bHs expressed by *S. koganeiensis* has a unique specificity to HA, further reducing the risk of degrading other major ECM components in healthy tissue. Furthermore, the data showed replication of attenuated ST in tumor tissue, suggesting that initial input of bHs-ST could be amplified in tumors in a few days before induction of bHs to cause maximal HA degradation. The ability of attenuated ST to extensively colonize tumors also increases the window for therapeutic intervention and maximal delivery. The duration of HA degradation, the types and sizes of therapeutic agents being delivered and whether multiple administrations are possible will be ongoing studies to determine the overall utility of bHs-ST.

[0129] ST-based cancer therapy has been shown to regress tumors in pre-clinical models and this regression is heavily dependent on the ability of ST to colonize tumors (for example 58, 59). The first attenuated ST to enter clinical trials, VNP20009, was administered to patients with metastatic melanoma and head and neck cancer (for example 30). Virtually no tumor regression was observed in any patients receiving VNP20009 and only a small number of patients had tumors colonized by ST. The disclosure herein shows the ability to express bHs in ST could significantly increase tumor colonization and efficacy of ST-based therapies. Indeed, previous

work utilizing a PEGylated hyaluronidase (PEGPH20) significantly improved colonization and anti-tumor efficacy of our ST-based therapeutic.

[0130] The number of therapies being developed to treat cancer (nanoparticles, antibody-based therapies, chemotherapeutic combinations, viruses and bacteria) continues to rise and, therefore, strategies to improve penetration of these agents is also required. Currently, only a small number of ECM-targeting agents can claim to improve drug delivery (8, 18). By developing ECM-degrading agents that are more tumor-specific, one can increase the number of potential therapeutic combinations to maximize efficacy while minimizing toxicity. The data herein shows that bHs-ST is capable of targeting tumor-derived HA in PDAC tumors and increasing penetration of large particles. While bHs-ST may only be limited to patients with HA-high tumors, such as those treated with PEGPH20 (56), the ST platform described in this work could be used to develop other ECM-targeting strategies with more universal application and benefit in PDAC patients (for example 28). This suggests that delivery of relatively large particles such as bacteria could be enhanced through the use of ECM-degrading agents. Therefore, bHs-ST pre-treatment could easily be combined with virotherapy or antibodybased therapies to improve their delivery.

[0131] SEQUENCES

[0132] Salmonella typhimurium codon-optimized DNA sequences:

[0133] *Streptomyces koganeinsis* hyaluronidase (750 bp):

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ATGCCGGTTGCGCGTCGTCTGTTCTGCGGCTCTTTCACCGCGGGCGCGGTTACCGTTGCG
ACCGCGGGCGGCGACCGGCACCGCGTCTGCGGCGGGCGAAAACGGCGCGACCAACCT
TCGACGGCCCCGGTTGCGGCGGAACGTTTCTCTGCGGACACCACTGGAAGCGGCGTTC
CTGAAAACCACTCTGAAACCAACACGCGGCGACCATCTACCAGGCGGGCACCTCTGGC
GACGGCGCGGCGCTGAACGTTATCTCTGACAACCCGGGCACCTCTGCGATGTACCTGTCT
GGCACCGAAACCGCGCGCTGGCACCTGAAAATCACCCACCGTGGCTACGCGGACGGCTC
TGACAAAGACGCGGCGGCGCTGTCTCTGGACCTGCGTGTTGCGGGCACCGCGGCGCAGG
GCATCTACGTTACCGCGACCAACGGCCCCGACCAAGGCAACCTGATCGCGCTGCGTAACA
ACACCGGCCTGGACGACTTCGTTGTTAAAGGCACCGGCCGTATCGGCGTTGGCATCGACC
GTGCGGCGACCCCGCGTGCGCAGGTTACATCGTTACGCGTGGCGACGCGCTGGCGGC
GCTGCTGGTTGAAGGCTCTGTTCTGATCGGCAACGCGGCGACCGTTCCGACCTCTGTTGA
CTCTTCTGGCGGCGGCGCGCTGTACGCGTCTGGCGGCGCGCTGCTGTGGCGTGGCTCTA
ACGGCACCGTTACCAACATCGCGCCGGCGTAATAGTGA
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[0134] *Streptomyces omiyaensis* trypsin-like protease (783 bp):

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ATGCAGAAAAACCGTCTGGTTCGTACCCTGCAGAACTGGCGGCGGCGGGCGCG
GTTGCGCTGGCGGCGCTGTCTCTGCAGCCGGTTTCTTCTGCGACCGCGGCGCCG
AACCCGGTTGTTGGCGGCACCCGTGCGGCGCAGGGCGAATTTCCGTGGATGGTT
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CGTCTGTCTATGGGCTGCGGCGGCTCTCTGATCACCCCGCAGGTTGTTCTGACCG
 CGGCGCACTGCGTTGGCGCGACCGGCAACAACACCTCTATCACCGCGACCGCGG
 GCGTTGTTGACCTGCAGTCTTCTTCTGCGATCAAAGTTCGTTCTACCAAAATCTACC
 GTGCGCCGGGCTACAACGGCAAAGGCAAAGACTGGGCGCTGATCAAACCTGGCGT
 CTCCGATCACCTCTCTGCCGACCCTGAAACTGGCGGAAACCACCGCGTACAACCTC
 TGGCACCTTCACCGTTGCGGGCTGGGGCGCGGGCGCGTGAAGGCGGGCGGCCAGC
 AGCGTTACCTGCTGAAAGCGAACGTTCCGTTTCGTTTCTGACGCGTCTTGCCAGGC
 GTCTTACGGCTCTGACCTGGTTCCGTCTGAAGAAATCTGCGCGGGGCTACCCGCAG
 GGCGGGCGTTGACACCTGCCAGGGCGACTCTGGCGGCCCGATGTTCCGTAAAGAC
 AACGCGGGCGCGTGGGTTGAGTTGGCATCGTTTCTTGGGGCCAGGGCTGCGCG
 CGTCCGGACTACCCGGGCGTTTACACCGAAGTTTCTACCTTCGCGGCGGGCGATCA
 AATCTGCGGCGGGCGACCCTG

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78. Sequence: UniProtKB - A0A0U2E2J7 (A0A0U2E2J7_9ACTN) – Streptomyces koganeiensis Hyaluronidase

79. Sequence: UniProtKB - C9K160 (C9K160_9ACTN) – *Streptomyces omiyaensis* Trypsin-like protease

[0136] P-Embodiments

[0137] Embodiment P-1. An attenuated facultative anaerobic bacterium comprising a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0138] Embodiment P-2. The attenuated facultative anaerobic bacterium of Embodiment P-1 selected from *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Vibrio cholerae*, *Vibrio fischeri*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Lactobacillus bulgaricus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus gallolyticus*, *Enterococcus faecium*, and *Streptococcus pyogenes*.

[0139] Embodiment P-3. The attenuated facultative anaerobic bacterium of Embodiment P-2, wherein the *Salmonella typhimurium* is selected from MVP728, YS1646 (VNP20009), RE88, LH430, SL7207, χ 8429, χ 8431 and χ 8768.

[0140] Embodiment P-4. The attenuated facultative anaerobic bacterium of one of Embodiments P-1 to P-3 wherein the recombinant extracellular matrix degrading enzyme is selected from a human extracellular matrix degrading enzyme, a bacterial extracellular matrix degrading enzyme, and a parasitic extracellular matrix degrading enzyme.

[0141] Embodiment P-5. The attenuated facultative anaerobic bacterium of one of Embodiments P-1 to P-4, wherein the recombinant extracellular matrix degrading enzyme is selected from matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparitinase, cathepsin, lyase, trypsin, protease, plasmin, and urokinase.

[0142] Embodiment P-6. The attenuated facultative anaerobic bacterium of Embodiment P-4, wherein the recombinant extracellular matrix degrading enzyme is hyaluronidase.

[0143] Embodiment P-7. The attenuated facultative anaerobic bacterium of Embodiment P-6, wherein the hyaluronidase is bacterial hyaluronidase.

[0144] Embodiment P-8. The attenuated facultative anaerobic bacterium of Embodiment P-7, wherein the bacterial hyaluronidase is from *Streptomyces koganeiensis*, *Streptomyces hyaluronolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens*.

[0145] Embodiment P-9. The attenuated facultative anaerobic bacterium of one of Embodiments P-1 to P-5, wherein the recombinant extracellular matrix degrading enzyme is collagenase.

[0146] Embodiment P-10. The attenuated facultative anaerobic bacterium of one of Embodiments P-1 to P-9, wherein the promoter is an inducible promoter.

[0147] Embodiment P-11. The attenuated facultative anaerobic bacterium of Embodiment P-10, wherein the promoter is selected from a pLac promoter, pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter.

[0148] Embodiment P-12. The attenuated facultative anaerobic bacterium of any of Embodiments P-1 to P-9, wherein the promoter is a tumor-specific promoter.

[0149] Embodiment P-13. The attenuated facultative anaerobic bacterium of Embodiment P-2, wherein the promoter is a hypoxia-inducible bacterial promoter.

[0150] Embodiment P-14. The attenuated facultative anaerobic bacterium of Embodiment P-3, wherein the hypoxia-inducible bacterial promoter is FF+20*, HIP1, or selected from those regulating expression of *spflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*.

[0151] Embodiment P-15. A method of treating a tumor in a subject comprising administering to the subject an effective amount of an attenuated facultative anaerobic bacterium comprising a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0152] Embodiment P-16. The method of Embodiment P-15, wherein the tumor is a solid tumor.

[0153] Embodiment P-17. The method of any of Embodiments P-15 to P-16, wherein the tumor is selected from pancreatic, breast, prostate, skin, lung, and abdomen tumor.

[0154] Embodiment P-18. The method of any of Embodiment P-15 to P-17, wherein the tumor is a pancreatic ductal adenocarcinoma.

[0155] Embodiment P-19. The method of any of Embodiments P-15 to P-18, wherein the attenuated facultative anaerobic bacterium is selected from *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Vibrio cholerae*, *Vibrio fischeri*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Lactobacillus bulgaricus*, *Listeria*

monocytogenes, *Enterococcus faecalis*, *Enterococcus gallolyticus*, *Enterococcus faecium*, and *Streptococcus pyogenes*.

[0156] Embodiment P-20. The method of Embodiment P-19, wherein the *Salmonella typhimurium* is selected from MVP728, YS1646 (VNP20009), RE88, LH430, SL7207, χ 8429, χ 8431 and χ 8768.

[0157] Embodiment P-21. The method of any of Embodiments P-15 to P-20, wherein the recombinant extracellular matrix degrading enzyme is selected from a human extracellular matrix degrading enzyme, a bacterial extracellular matrix degrading enzyme, and a parasitic extracellular matrix degrading enzyme.

[0158] Embodiment P-22. The method of any of Embodiments P-15 to P-21, wherein the recombinant extracellular matrix degrading enzyme is selected from matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparatinase, cathepsin, lyase, trypsin, protease, plasmin, and urokinase.

[0159] Embodiment P-23. The method of Embodiment P-22, wherein the recombinant extracellular matrix degrading enzyme is hyaluronidase.

[0160] Embodiment P-24. The method of Embodiment P-23, wherein the hyaluronidase is bacterial hyaluronidase.

[0161] Embodiment P-25. The method of Embodiment P-24, wherein the bacterial hyaluronidase is from *Streptomyces koganeiensis*, *Streptomyces hyaluronolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens*.

[0162] Embodiment P-26. The method of any one of Embodiments P-15 to P-22, wherein the recombinant extracellular matrix degrading enzyme is collagenase.

[0163] Embodiment P-27. The method of any one of Embodiments P-15 to P-26, wherein the promoter is an inducible promoter.

[0164] Embodiment P-28. The method of Embodiment P-27, wherein the promoter is selected from a pLac promoter, a pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter.

[0165] Embodiment P-29. The method of any one of Embodiment P-15 to P-26, wherein the promoter is a tumor-specific promoter.

[0166] Embodiment P-30. The method of Embodiment P-29, wherein the promoter is a hypoxia-inducible bacterial promoter.

[0167] Embodiment P-31. The method of Embodiment P-30, wherein the hypoxia-inducible bacterial promoter is selected from FF+20*, HIP1, or those regulating expression of *spflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*.

[0168] Embodiment P-32. A method of treating tumor in a subject, comprising the step of administering to the subject a combined effective amount of an attenuated facultative anaerobic bacteria and a chemotherapeutic agent, wherein the bacteria comprises a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

[0169] Embodiment P-33. The method of Embodiment P-32, wherein the tumor is a solid tumor.

[0170] Embodiment P-34. The method of any of Embodiments P-32 to P-33, wherein the tumor is selected from pancreatic, breast, and prostate tumor.

[0171] Embodiment P-35. The method of any of claims 32-34, wherein the tumor is a pancreatic ductal adenocarcinoma.

[0172] Embodiment P-36. The method of any of Embodiment P-32 to P-35, wherein the bacteria is a species selected from *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Vibrio cholerae*, *Vibrio fischeri*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Lactobacillus bulgaricus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus gallolyticus*, *Enterococcus faecium*, and *Streptococcus pyogenes*.

[0173] Embodiment P-37. The method of Embodiment P-36, wherein the *Salmonella typhimurium* is a strain selected from strains MVP728, YS1646 (VNP20009), RE88, LH430, SL7207, χ 8429, χ 8431 and χ 8768.

[0174] Embodiment P-38. The method of any of Embodiments P-32 to P-37, wherein the recombinant extracellular matrix degrading enzyme is selected from a human extracellular matrix degrading enzyme, a bacterial extracellular matrix degrading enzyme, and a parasitic extracellular matrix degrading enzyme.

[0175] Embodiment P-39. The method of any of Embodiments P-32 to P-38, wherein the recombinant extracellular matrix degrading enzyme is selected from matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparatinase, cathepsin, lyase, trypsin, protease, plasmin, and urokinase.

[0176] Embodiment P-40. The method of Embodiment P-39, wherein the recombinant extracellular matrix degrading enzyme is hyaluronidase.

[0177] Embodiment P-41. The method of Embodiment P-40, wherein the hyaluronidase is bacterial hyaluronidase.

[0178] Embodiment P-42. The method of Embodiment P-41, wherein the bacterial hyaluronidase is from *Streptomyces koganeiensis*, *Streptomyces hyaluronolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens*.

[0179] Embodiment P-43. The method of any one of Embodiments P-32 to P-39, wherein the recombinant extracellular matrix degrading enzyme is collagenase.

[0180] Embodiment P-44. The method of any one of Embodiment P-32 to P-43, wherein the promoter is an inducible promoter.

[0181] Embodiment P-45. The method of Embodiment P-44, wherein the promoter selected is a pLac promoter, a pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter.

[0182] Embodiment P-46. The method of any one of Embodiments P-32 to P-43, wherein the promoter is a tumor-specific promoter.

[0183] Embodiment P-47. The method of Embodiment P-46, wherein the promoter is a hypoxia-inducible bacterial promoter.

[0184] Embodiment P-48. The method of Embodiment P-47, wherein the hypoxia-inducible bacterial promoter is selected from FF+20*, HIP1, or those regulating expression of *spflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*.

[0185] Embodiment P-49. The method of any one of Embodiment P-32 to P-48, wherein the chemotherapeutic agent is selected from Abraxane, asparaginase, bleomycin, busulfan, carmustine, chlorambucil, cladribine, CPT-11, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, dexamethasone, doxorubicin (commonly referred to as Adriamycin), etoposide, fludarabine, folfirinox, 5-fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, interferon- α (native or recombinant), levamisole, and lomustine, mechlorethamine, melphalan,

mercaptopurine, methotrexate, mitomycin, mitoxantrone, paclitaxel, pentostatin, prednisone, procarbazine, tamoxife, taxol-related compounds, 6-thiogaunine, topotecan, vinblastine, and vincristine.

WHAT IS CLAIMED IS:

1. An attenuated facultative anaerobic bacterium comprising a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.
2. The attenuated facultative anaerobic bacterium of claim 1 selected from *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Vibrio cholerae*, *Vibrio fischeri*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Lactobacillus bulgaricus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus gallolyticus*, *Enterococcus faecium*, and *Streptococcus pyogenes*.
3. The attenuated facultative anaerobic bacterium of claim 2, wherein the *Salmonella typhimurium* is selected from MVP728, YS1646 (VNP20009), RE88, LH430, SL7207, χ 8429, χ 8431 and χ 8768.
4. The attenuated facultative anaerobic bacterium of one of claims 1 to 3 wherein the recombinant extracellular matrix degrading enzyme is selected from a human extracellular matrix degrading enzyme, a bacterial extracellular matrix degrading enzyme, and a parasitic extracellular matrix degrading enzyme.
5. The attenuated facultative anaerobic bacterium of one of claims 1 to 4, wherein the recombinant extracellular matrix degrading enzyme is selected from matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparatinase, cathepsin, lyase, trypsin, protease, plasmin, and urokinase.
6. The attenuated facultative anaerobic bacterium of claim 5, wherein recombinant extracellular matrix degrading enzyme is a protease encoded by a codon optimized nucleic acid comprising SEQ ID NO.: 2.
7. The attenuated facultative anaerobic bacterium of claim 5, wherein the recombinant extracellular matrix degrading enzyme is hyaluronidase.
8. The attenuated facultative anaerobic bacterium of claim 7, wherein the hyaluronidase is bacterial hyaluronidase.
9. The attenuated facultative anaerobic bacterium of claim 8, wherein the bacterial hyaluronidase is from *Streptomyces koganeiensis*, *Streptomyces hyaluronolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens*.

10. The attenuated facultative anaerobic bacterium of claim 9, wherein the bacterial hyaluronidase is encoded by a codon optimized nucleic acid sequence comprising SEQ ID NO.: 1.
11. The attenuated facultative anaerobic bacterium of one of claims 1-5, wherein the recombinant extracellular matrix degrading enzyme is collagenase.
12. The attenuated facultative anaerobic bacterium of one of claims 1-11, wherein the promoter is an inducible promoter.
13. The attenuated facultative anaerobic bacterium of claim 12, wherein the promoter is selected from a pLac promoter, pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter.
14. The attenuated facultative anaerobic bacterium of any of claims 1-11, wherein the promoter is a tumor-specific promoter.
15. The attenuated facultative anaerobic bacterium of claim 14, wherein the promoter is a hypoxia-inducible bacterial promoter.
16. The attenuated facultative anaerobic bacterium of claim 15, wherein the hypoxia-inducible bacterial promoter is FF+20*, HIP1, or selected from those regulating expression of *spflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*.
17. A method of treating a tumor in a subject comprising administering to the subject an effective amount of an attenuated facultative anaerobic bacterium comprising a nucleic acid molecule encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.
18. The method of claim 17, wherein the tumor is a solid tumor.
19. The method of any of claims 17-18, wherein the tumor is selected from pancreatic, breast, prostate, skin, lung, and abdomen tumor.
20. The method of any of claims 17-19, wherein the tumor is a pancreatic ductal adenocarcinoma.
21. The method of any of claims 17-20, wherein the attenuated facultative anaerobic bacterium is selected from *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Vibrio cholerae*, *Vibrio fischeri*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Lactobacillus bulgaricus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus gallolyticus*, *Enterococcus faecium*, and *Streptococcus pyogenes*.

22. The method of claim 21, wherein the *Salmonella typhimurium* is selected from MVP728, YS1646 (VNP20009), RE88, LH430, SL7207, χ 8429, χ 8431 and χ 8768.
23. The method of any of claims 17-22, wherein the recombinant extracellular matrix degrading enzyme is selected from a human extracellular matrix degrading enzyme, a bacterial extracellular matrix degrading enzyme, and a parasitic extracellular matrix degrading enzyme.
24. The method of any of claims 17-23, wherein the recombinant extracellular matrix degrading enzyme is selected from matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparatinase, cathepsin, lyase, trypsin, protease, plasmin, and urokinase.
25. The method of claim 24, wherein the recombinant extracellular matrix degrading enzyme is a protease encoded by a codon optimized nucleic acid comprising SEQ ID NO.: 2.
26. The method of claim 24, wherein the recombinant extracellular matrix degrading enzyme is hyaluronidase.
27. The method of claim 26, wherein the hyaluronidase is bacterial hyaluronidase.
28. The method of claim 27, wherein the bacterial hyaluronidase is from *Streptomyces koganeiensis*, *Streptomyces hyaluronolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens*.
29. The method of claim 28, wherein the bacterial hyaluronidase is encoded by a codon optimized nucleic acid sequence comprising SEQ ID NO.: 1.
30. The method of any one of claims 17-24, wherein the recombinant extracellular matrix degrading enzyme is collagenase.
31. The method of any one of claims 17-30, wherein the promoter is an inducible promoter.
32. The method of claim 31, wherein the promoter is selected from a pLac promoter, a pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter.
33. The method of any one of claims 17-30, wherein the promoter is a tumor-specific promoter.
34. The method of claim 33, wherein the promoter is a hypoxia-inducible bacterial promoter.
35. The method of claim 34, wherein the hypoxia-inducible bacterial promoter is selected from FF+20*, HIP1, or those regulating expression of *spflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*.
36. A method of treating tumor in a subject, comprising the step of administering to the subject a combined effective amount of an attenuated facultative anaerobic bacteria and a chemotherapeutic agent, wherein the bacteria comprises a nucleic acid molecule

encoding a recombinant extracellular matrix degrading enzyme operably linked to a promoter.

37. The method of claim 36, wherein the tumor is a solid tumor.
38. The method of any of claims 36-37, wherein the tumor is selected from pancreatic, breast, and prostate tumor.
39. The method of any of claims 36-38, wherein the tumor is a pancreatic ductal adenocarcinoma.
40. The method of any of claims 36-39, wherein the bacteria is a species selected from *Salmonella bongori*, *Salmonella choleraesuis*, *Salmonella enterica*, *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Vibrio cholerae*, *Vibrio fischeri*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Lactobacillus bulgaricus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Enterococcus gallolyticus*, *Enterococcus faecium*, and *Streptococcus pyogenes*.
41. The method of claim 40, wherein the *Salmonella typhimurium* is a strain selected from strains MVP728, YS1646 (VNP20009), RE88, LH430, SL7207, χ 8429, χ 8431 and χ 8768.
42. The method of any of claims 36-41, wherein the recombinant extracellular matrix degrading enzyme is selected from a human extracellular matrix degrading enzyme, a bacterial extracellular matrix degrading enzyme, and a parasitic extracellular matrix degrading enzyme.
43. The method of any of claims 36-42, wherein the recombinant extracellular matrix degrading enzyme is selected from matrix metalloproteinase, collagenase, hyaluronidase, chondroitinase, heparatinase, cathepsin, lyase, trypsin, protease, plasmin, and urokinase.
44. The method of claim 43, wherein the recombinant extracellular matrix degrading enzyme is hyaluronidase.
45. The method of claim 44, wherein the hyaluronidase is bacterial hyaluronidase.
46. The method of claim 45, wherein the bacterial hyaluronidase is from *Streptomyces koganeiensis*, *Streptomyces hyaluronolyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Clostridium perfringens*.
47. The method of claim 46, wherein the bacterial hyaluronidase is encoded by a codon optimized nucleic acid sequence comprising SEQ ID NO.: 1.

48. The method of any one of claims 36-43, wherein the recombinant extracellular matrix degrading enzyme is collagenase.
49. The method of any one of claims 36-48, wherein the promoter is an inducible promoter.
50. The method of claim 49, wherein the promoter selected is a pLac promoter, a pTac promoter, a tetracycline-controlled promoter, and a pBAD promoter.
51. The method of any one of claims 36-47, wherein the promoter is a tumor-specific promoter.
52. The method of claim 51, wherein the promoter is a hypoxia-inducible bacterial promoter.
53. The method of claim 52, wherein the hypoxia-inducible bacterial promoter is selected from FF+20*, HIP1, or those regulating expression of *spflE*, *hcp*, *menD*, *ansB*, *mltD*, *glpA*, *glpT*, and *pepT*.
54. The method of any one of claims 36-53, wherein the chemotherapeutic agent is selected from Abraxane, asparaginase, bleomycin, busulfan carmustine, chlorambucil, cladribine, CPT-11, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, dexamethasone, doxorubicin (commonly referred to as Adriamycin), etoposide, fludarabine, folfirinox, 5-fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, interferon- α (native or recombinant), levamisole, and lomustine, mechlorethamine, melphalan, mercaptopurine, methotrexate, mitomycin, mitoxantrone, paclitaxel, pentostatin, prednisone, procarbazine, tamoxife, taxol-related compounds, 6-thioguanine, topotecan, vinblastine, and vincristine.

FIG. 1A

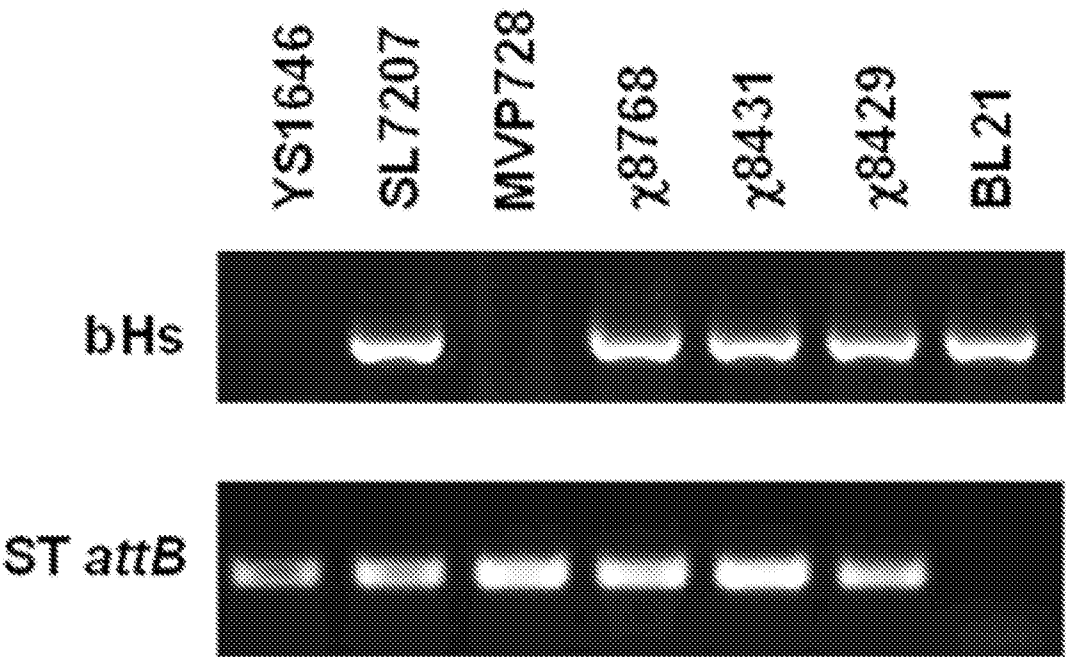


FIG. 1B

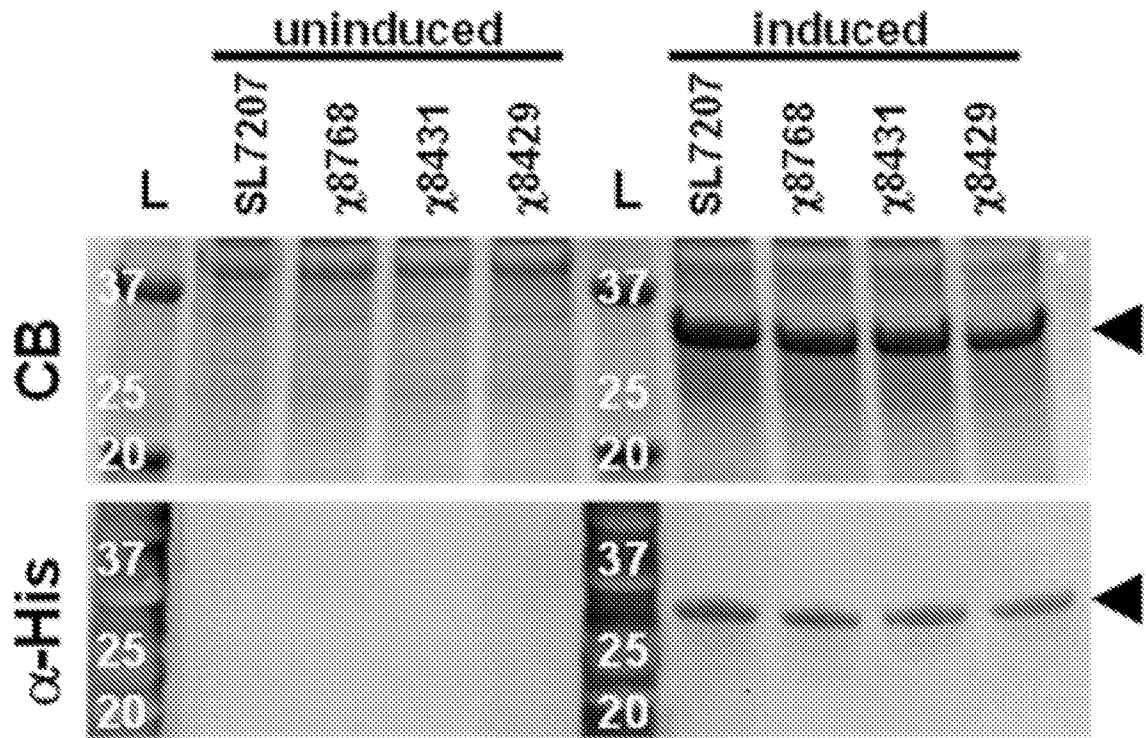


FIG. 1C

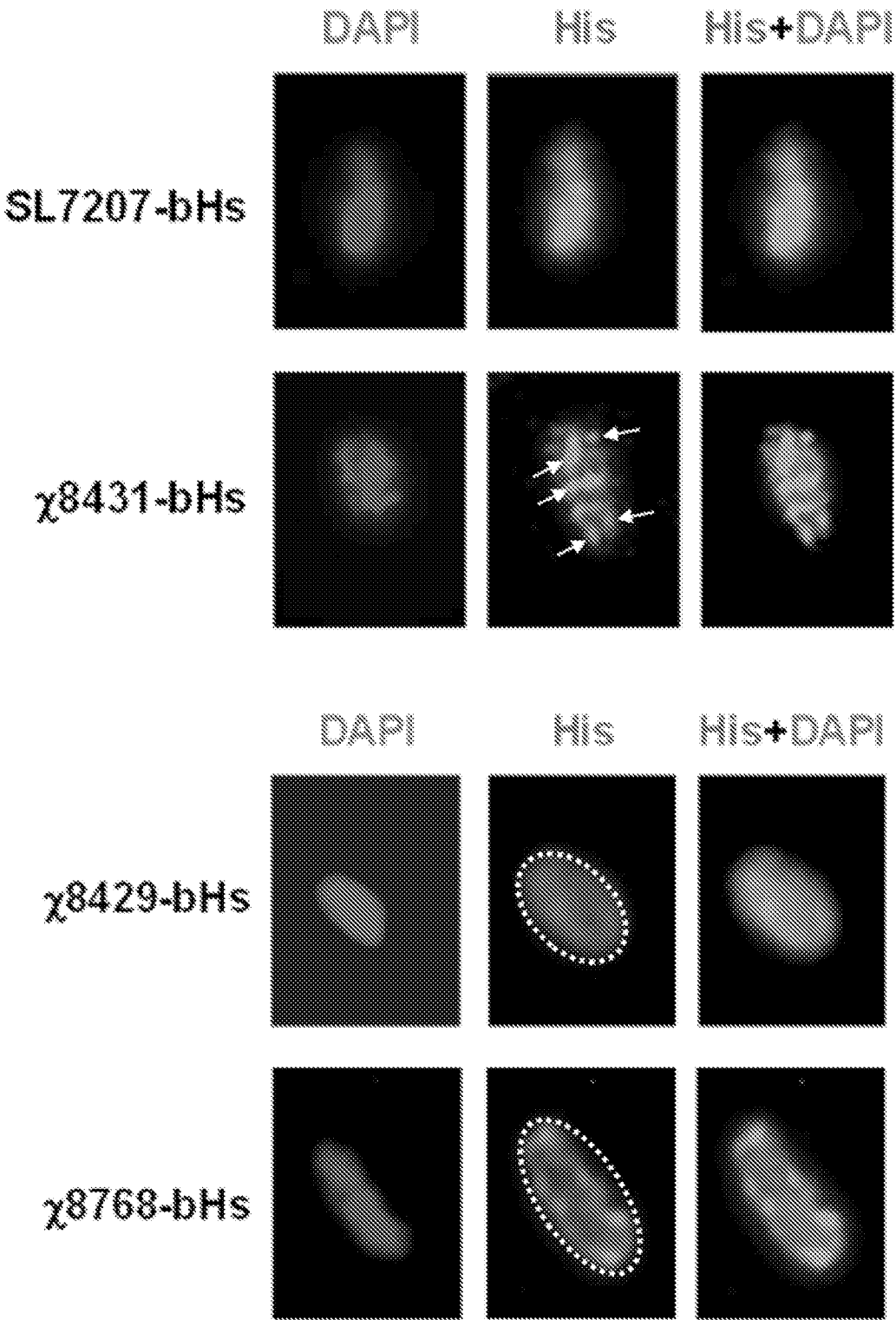


FIG. 2A

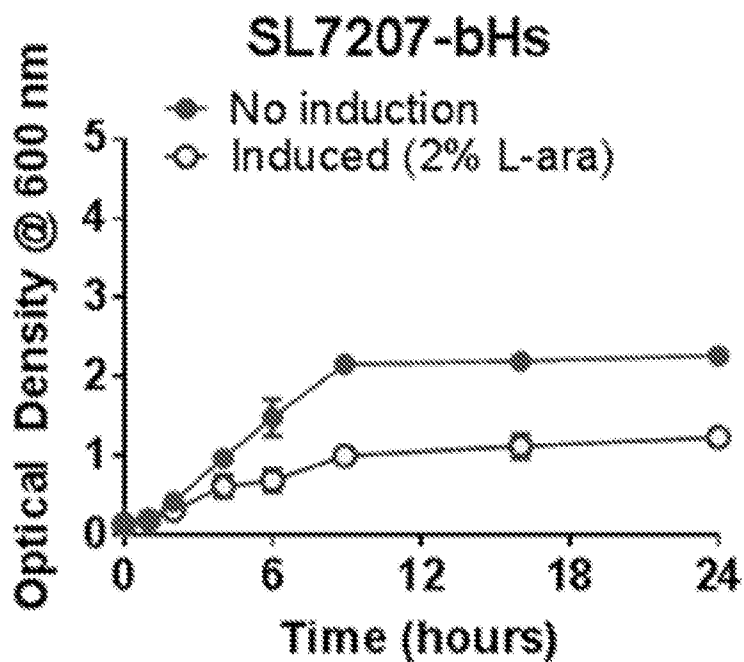


FIG. 2B

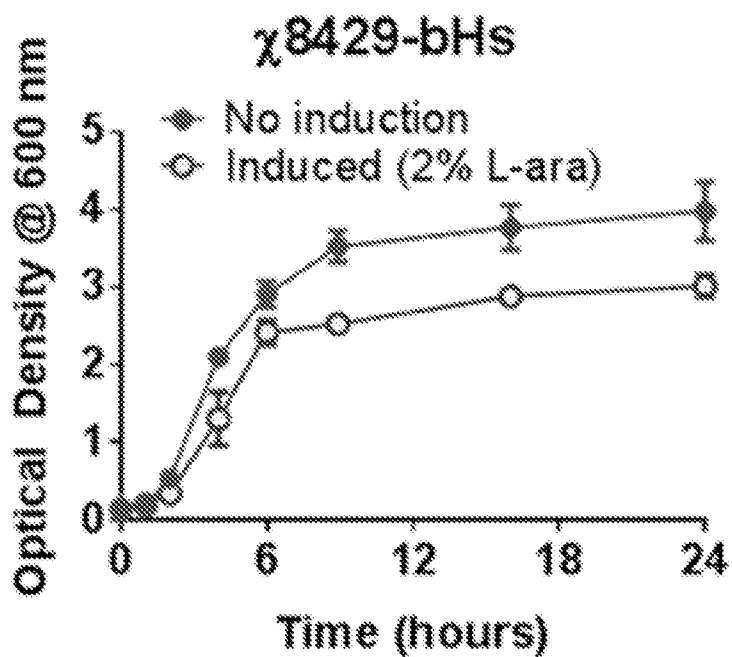


FIG. 2C

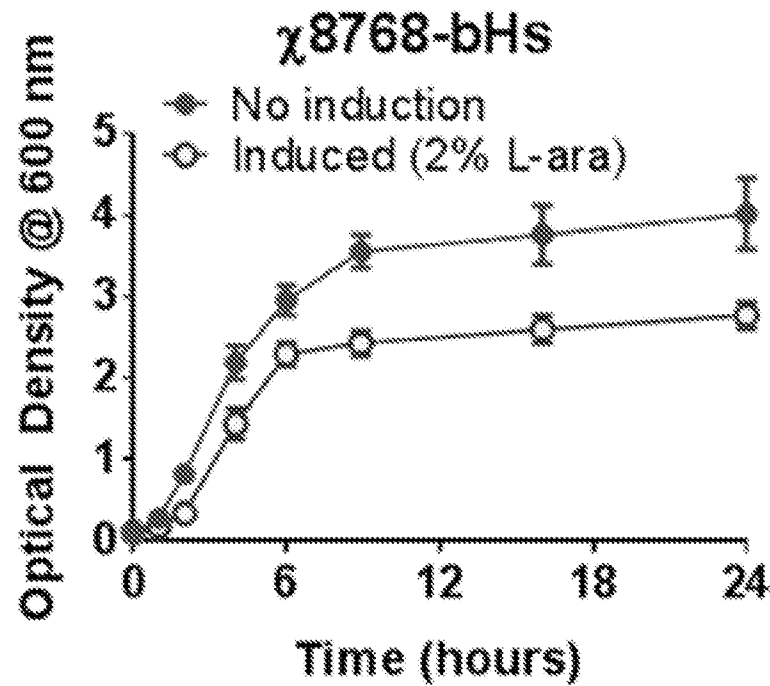
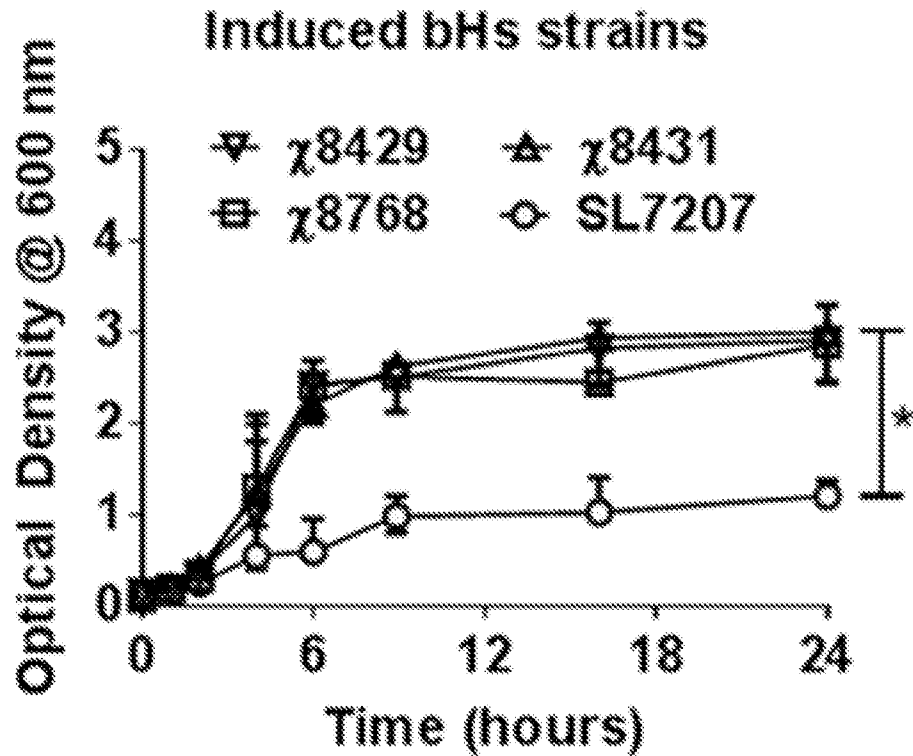


FIG. 2D



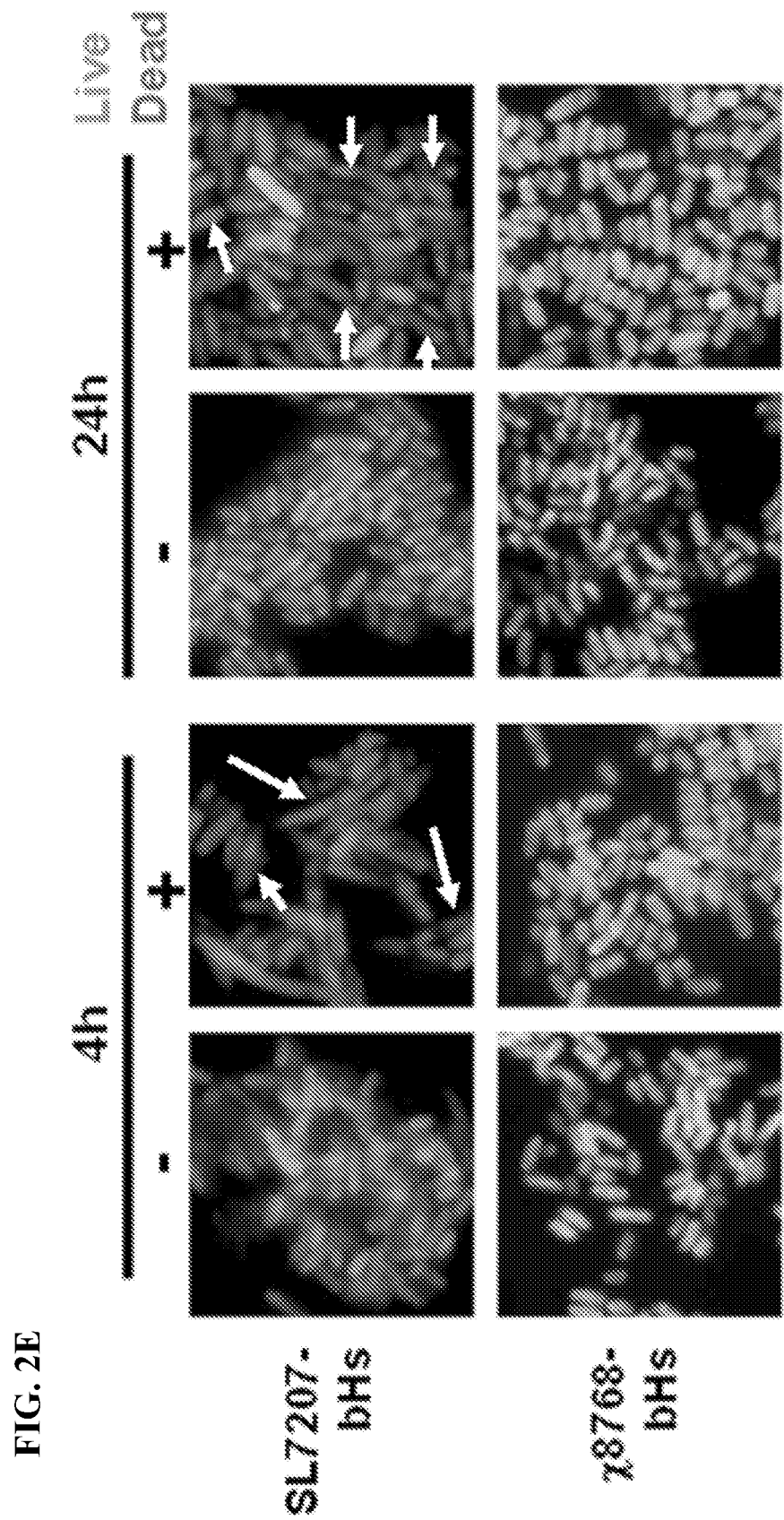


FIG. 3A

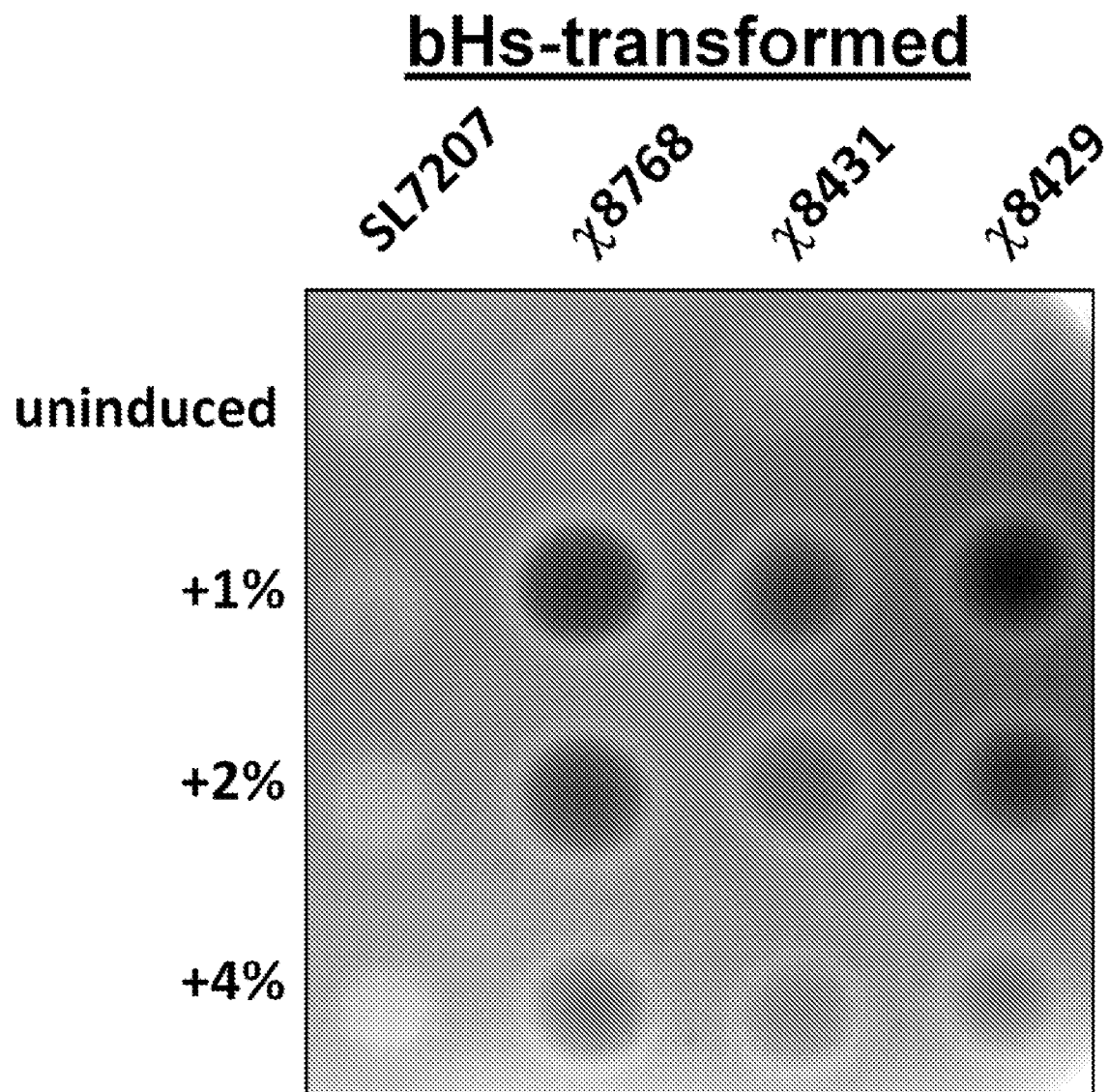


FIG. 3B

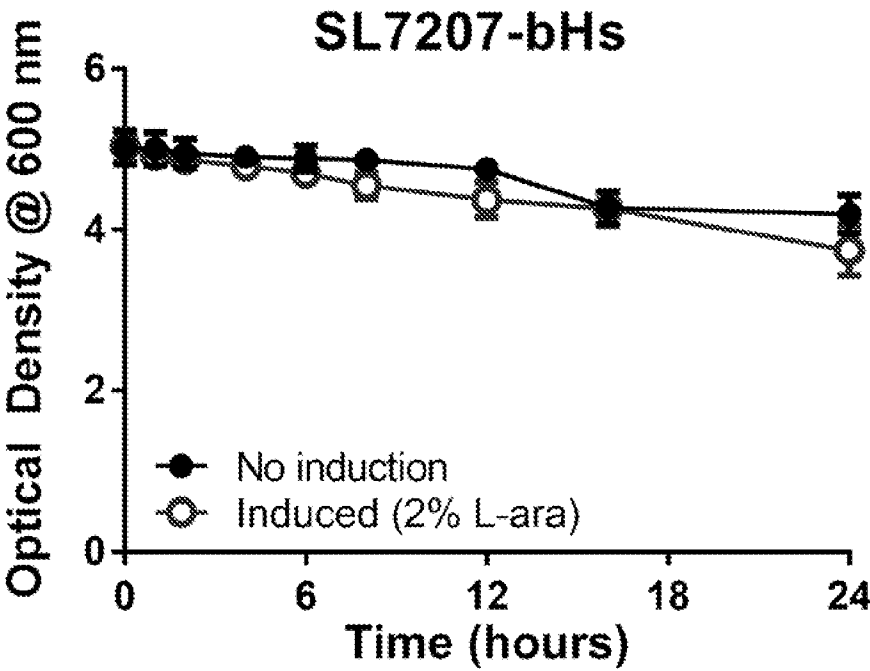


FIG. 3C

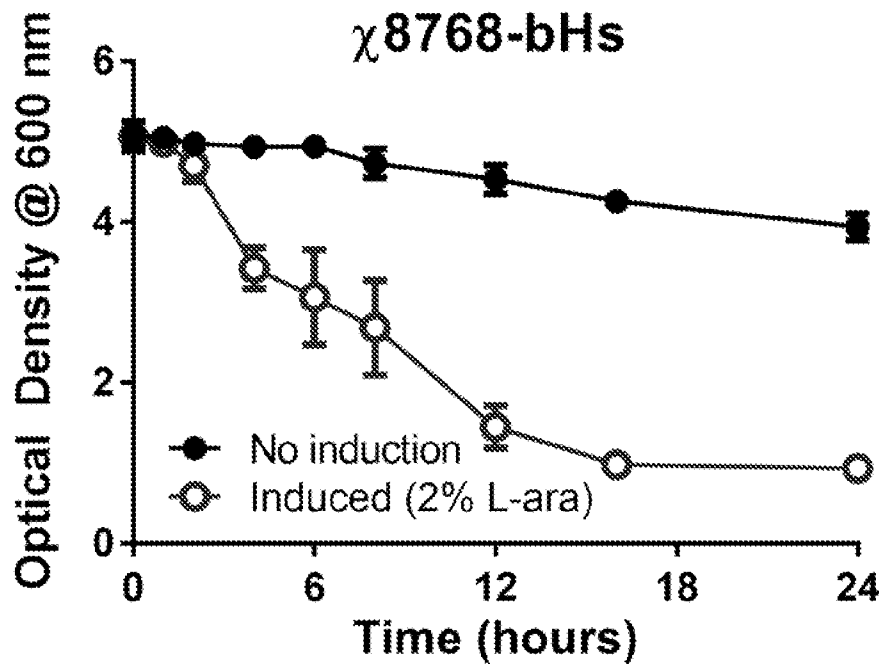


FIG. 3D

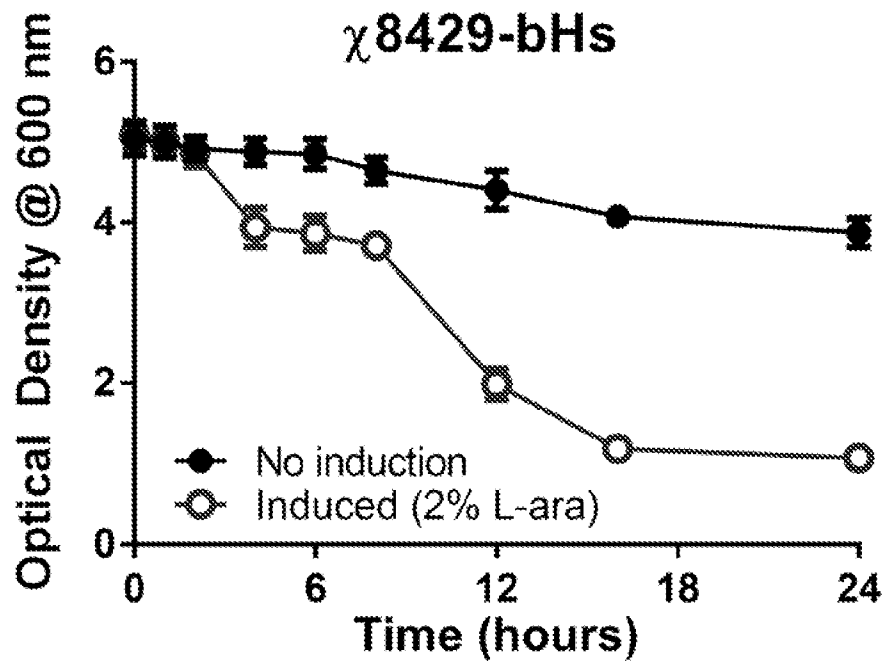


FIG. 4

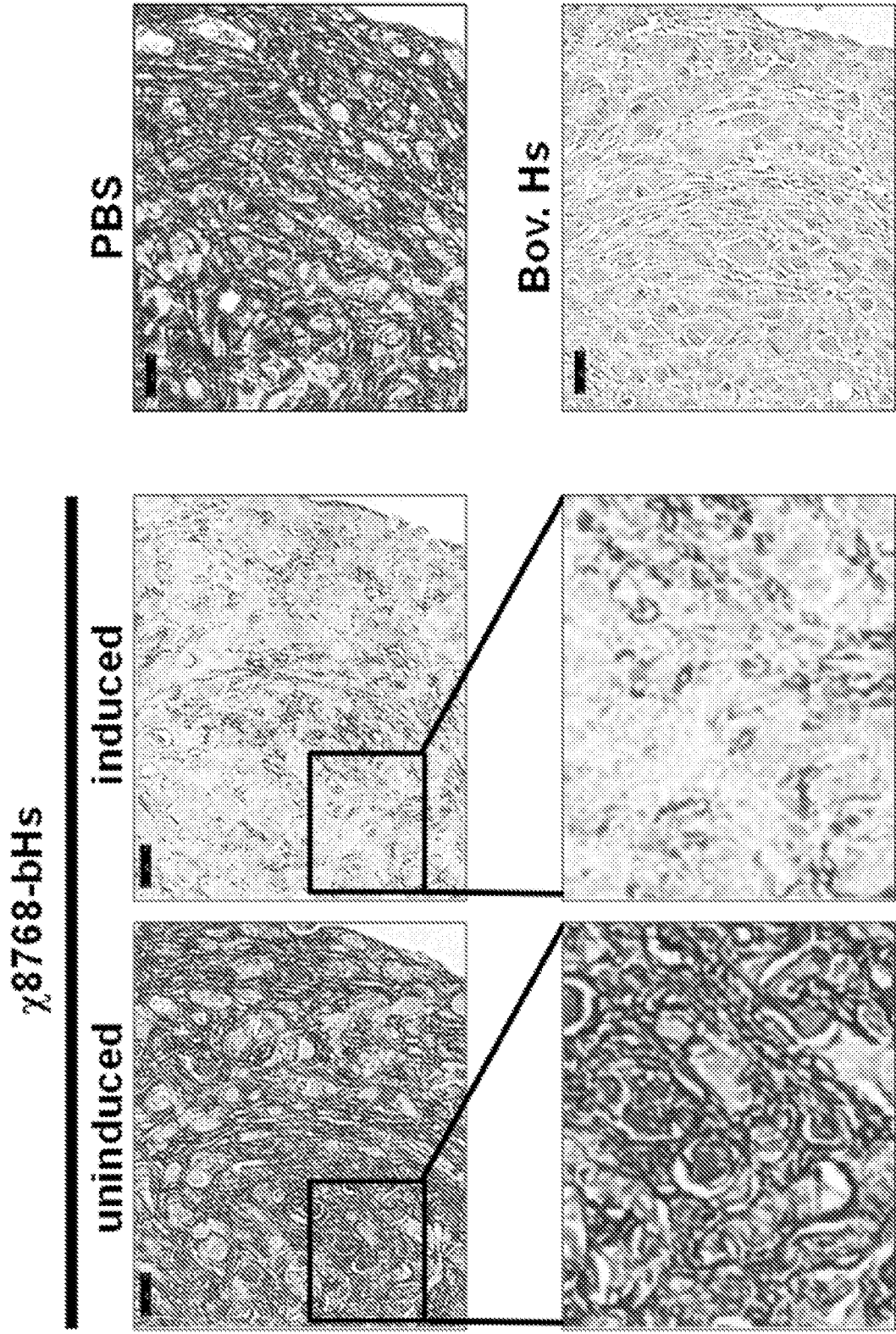


FIG. 5A

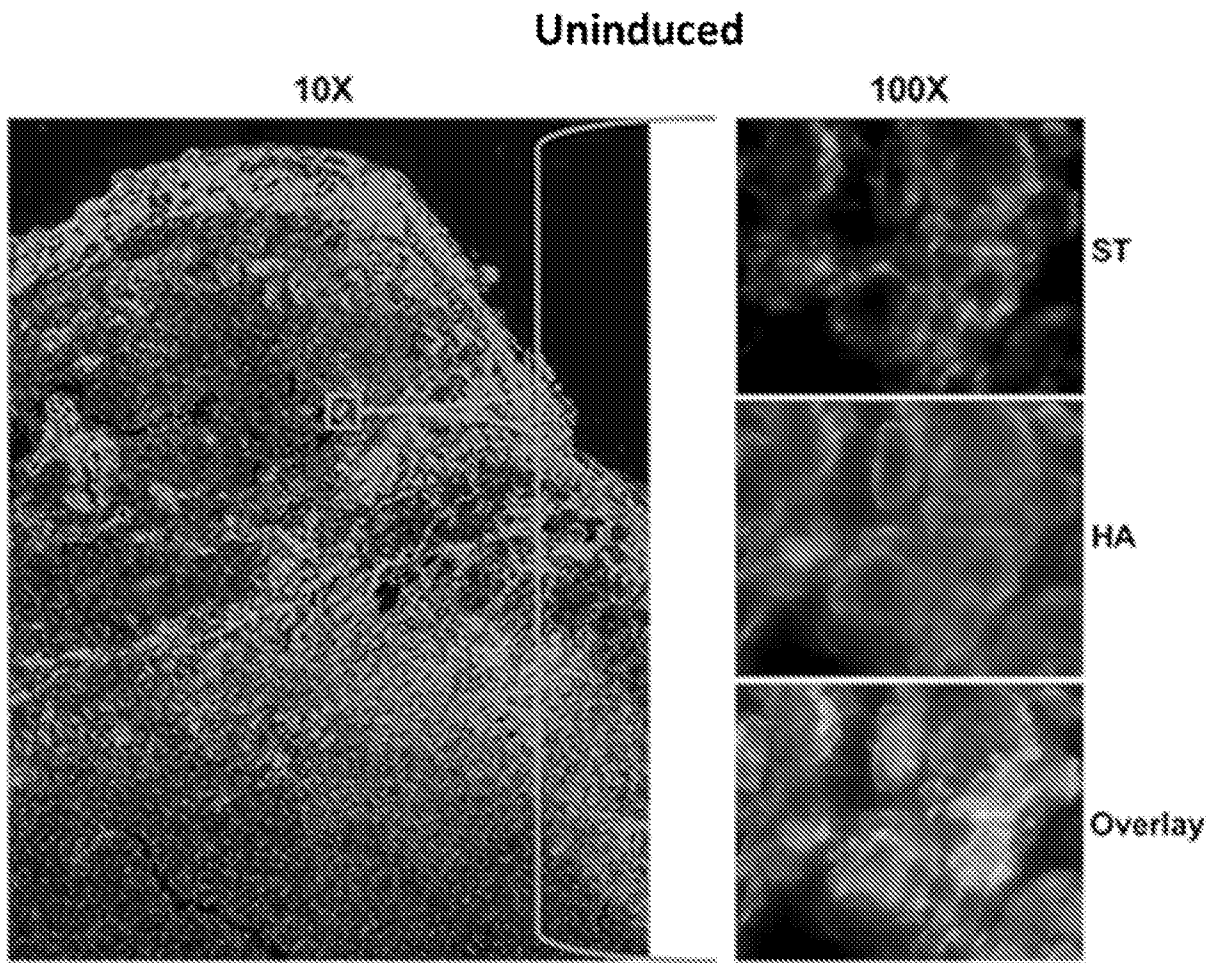


FIG. 5B

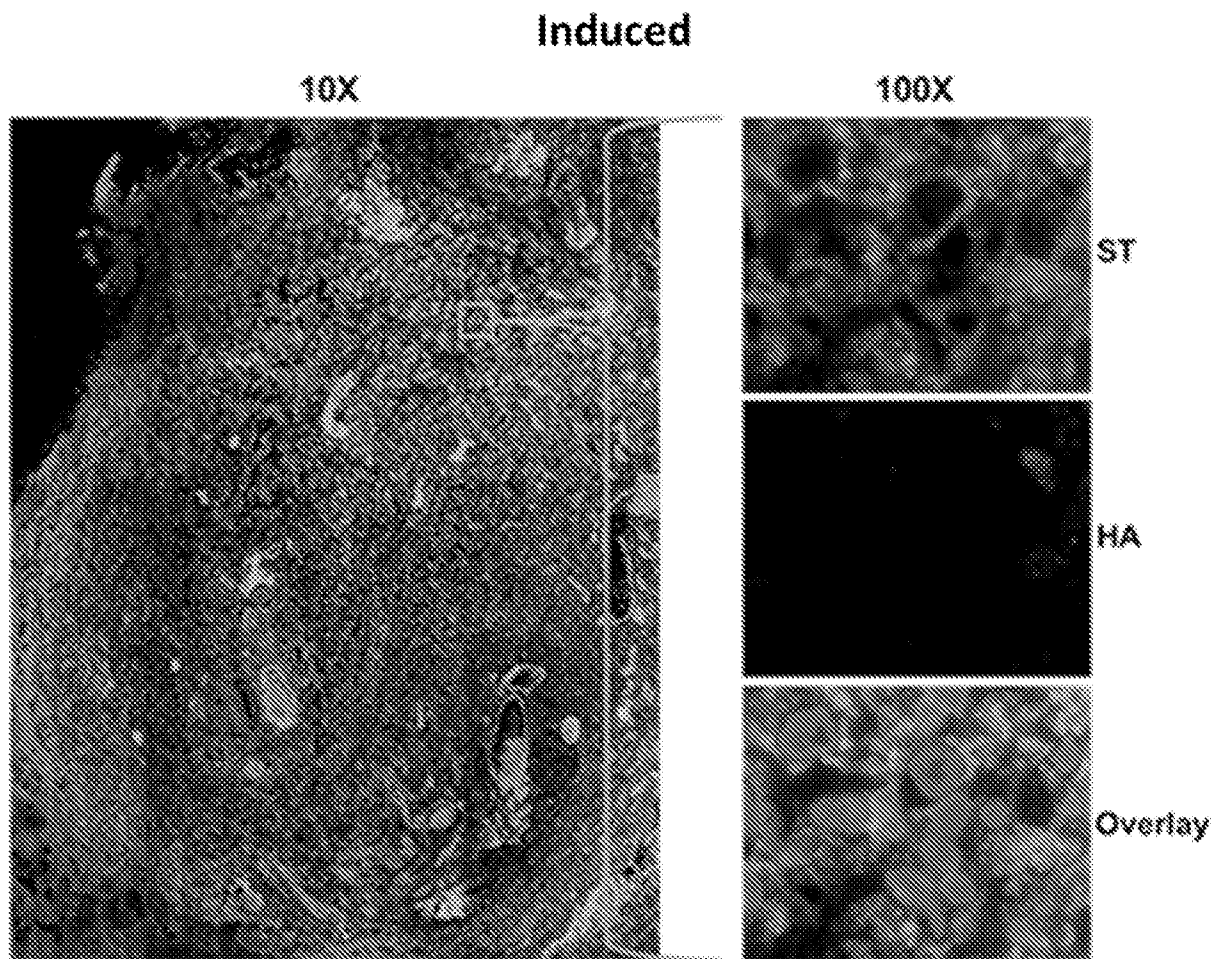


FIG. 5C

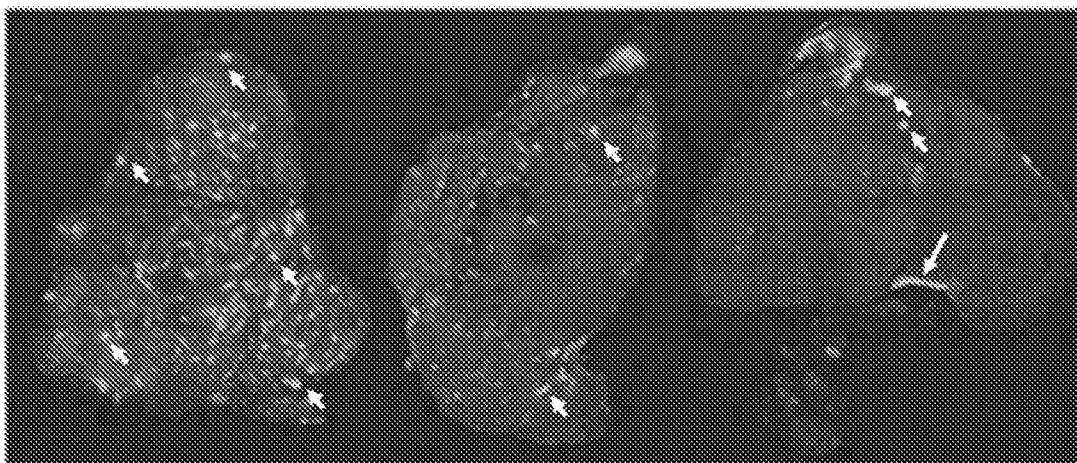
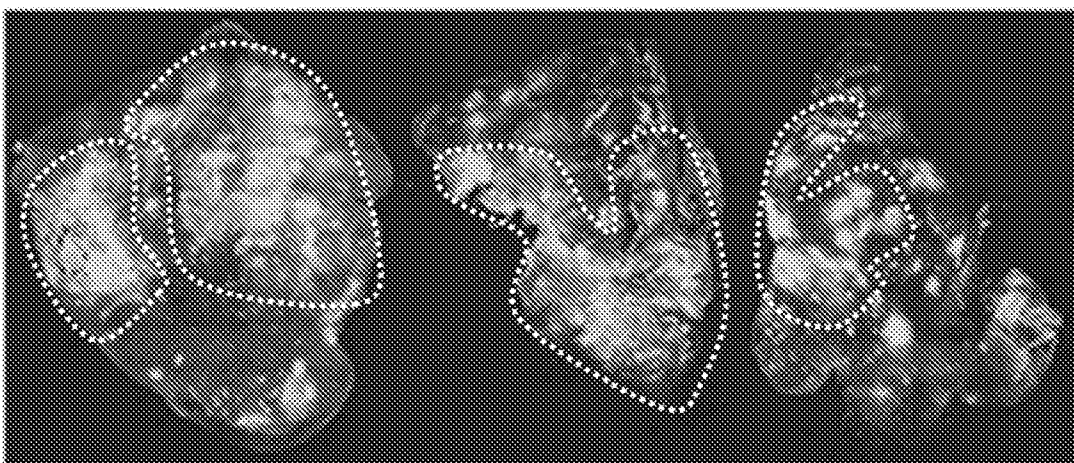
Uninduced**Induced**

FIG. 5D

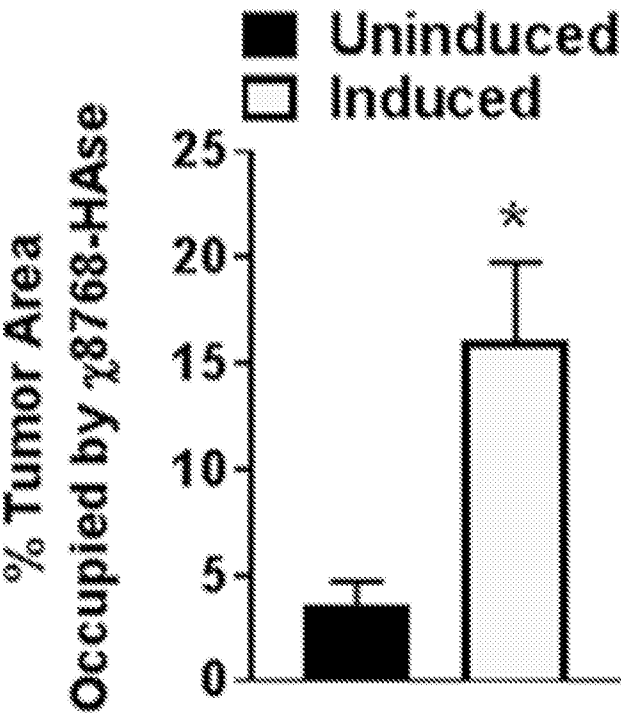


FIG. 6A

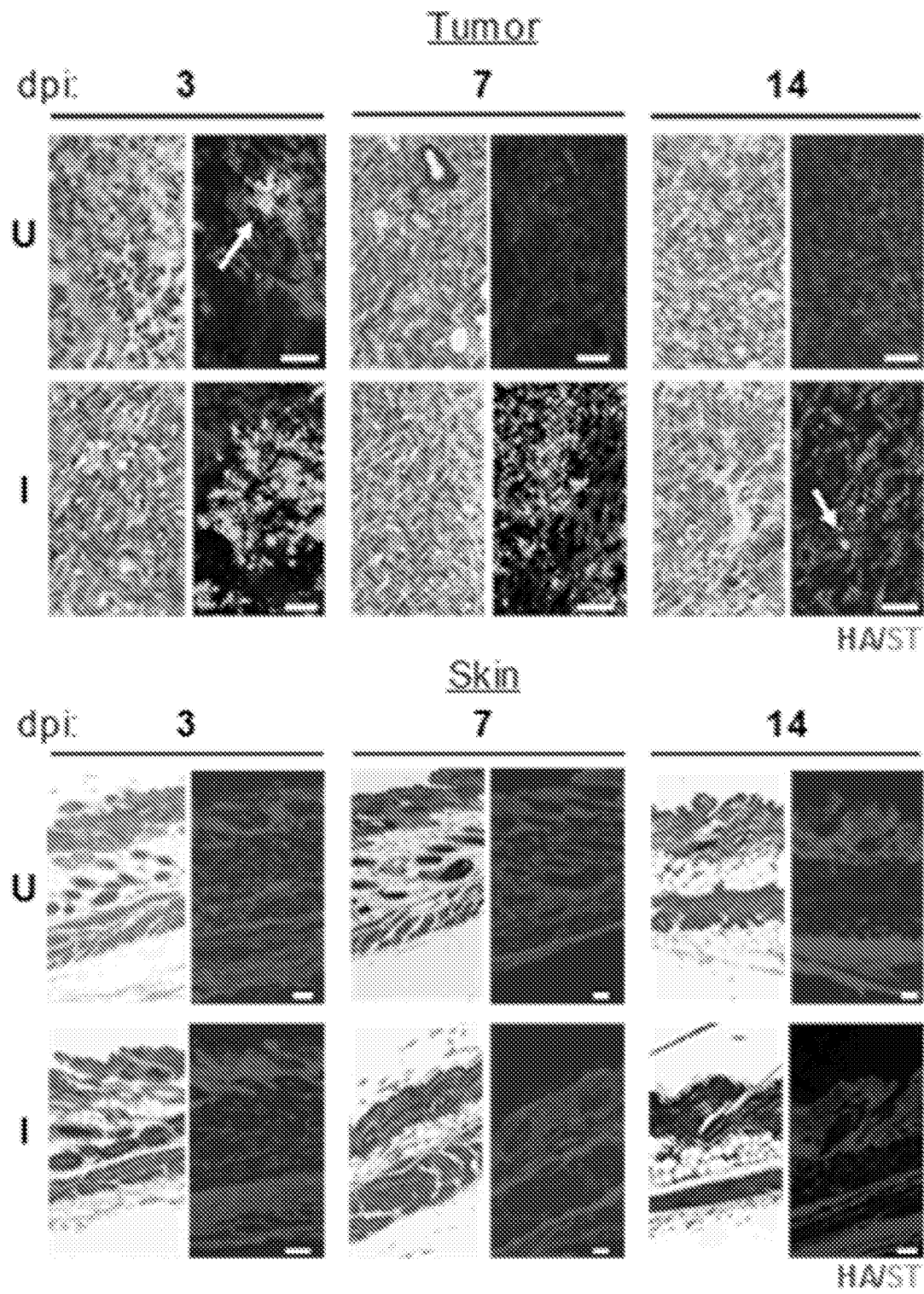


FIG. 6B

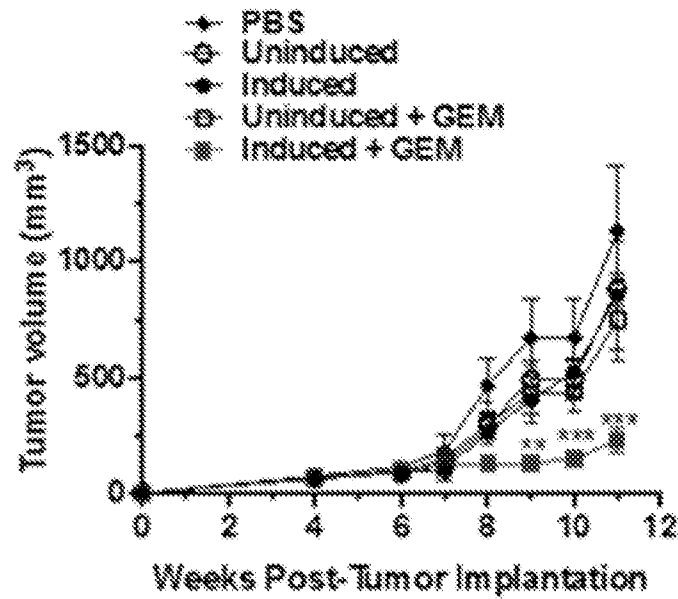


FIG. 6C

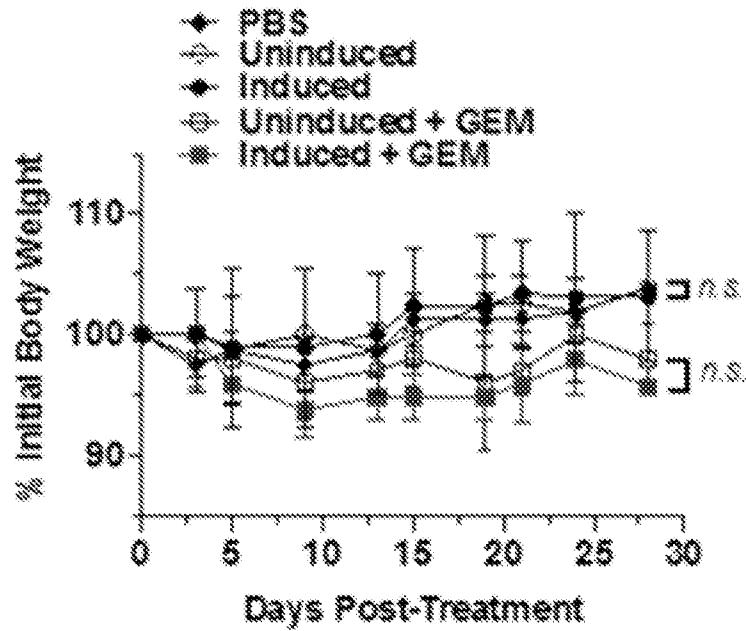


FIG. 7A

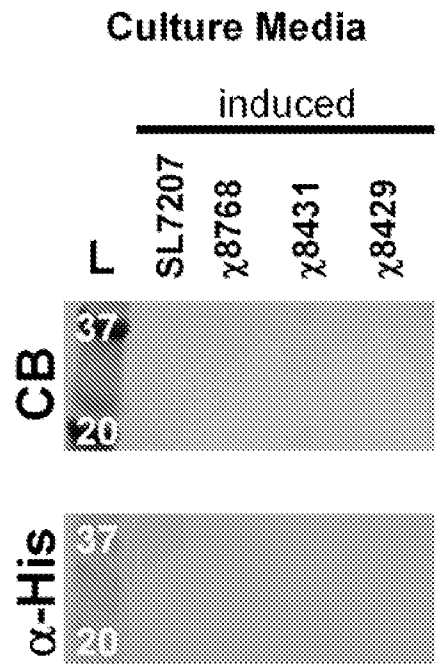


FIG. 7B

N-terminus					
seq	MPVARRLFLG	SFTAGAVTVA	TAAATGTASA	AGENGATTTF	DGPVAAERFS 50
pred	IiiiiiiHHHH	HHHHHHHHHH	HHHHHHHHHH	Hooooooooo	oooooo0000
seq	ADTTLEAAFL	KTTSETNHAA	TIYQAGTSGD	GAALNVISDN	PGTSAMYLSG 100
pred	0000000000	0000000000	0000000000	0000000000	0000000000
seq	TETARGTLKI	THRGYADGSD	KDAAALSDDL	RVAGTAAQGI	YVTATNGPTK 150
pred	0000000000	0000000000	0000000000	0000000000	0000000000
seq	GNLIALRNNT	GLDDFVVKGT	GRIGVGIDRA	ATPRAQVHIV	QRGDALAALL 200
pred	0000000000	0000000000	0000000000	0000000000	0000000000
seq	VEGSVRIGNA	ATVPTSVDSS	GGGALYASGG	ALLWRGSNGT	VTTIAPA 247
pred	0000000000	0000000000	0000000000	0000000000	00000000
C-terminus					

FIG. 7C

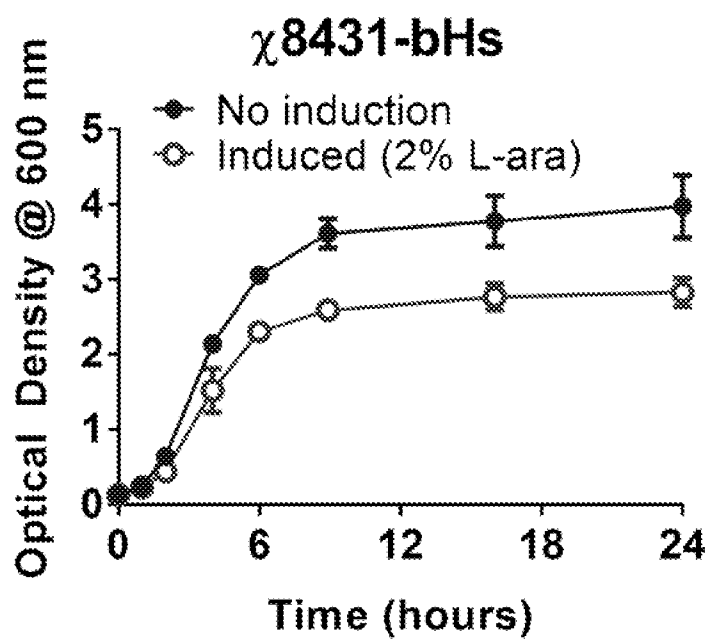


FIG. 7D

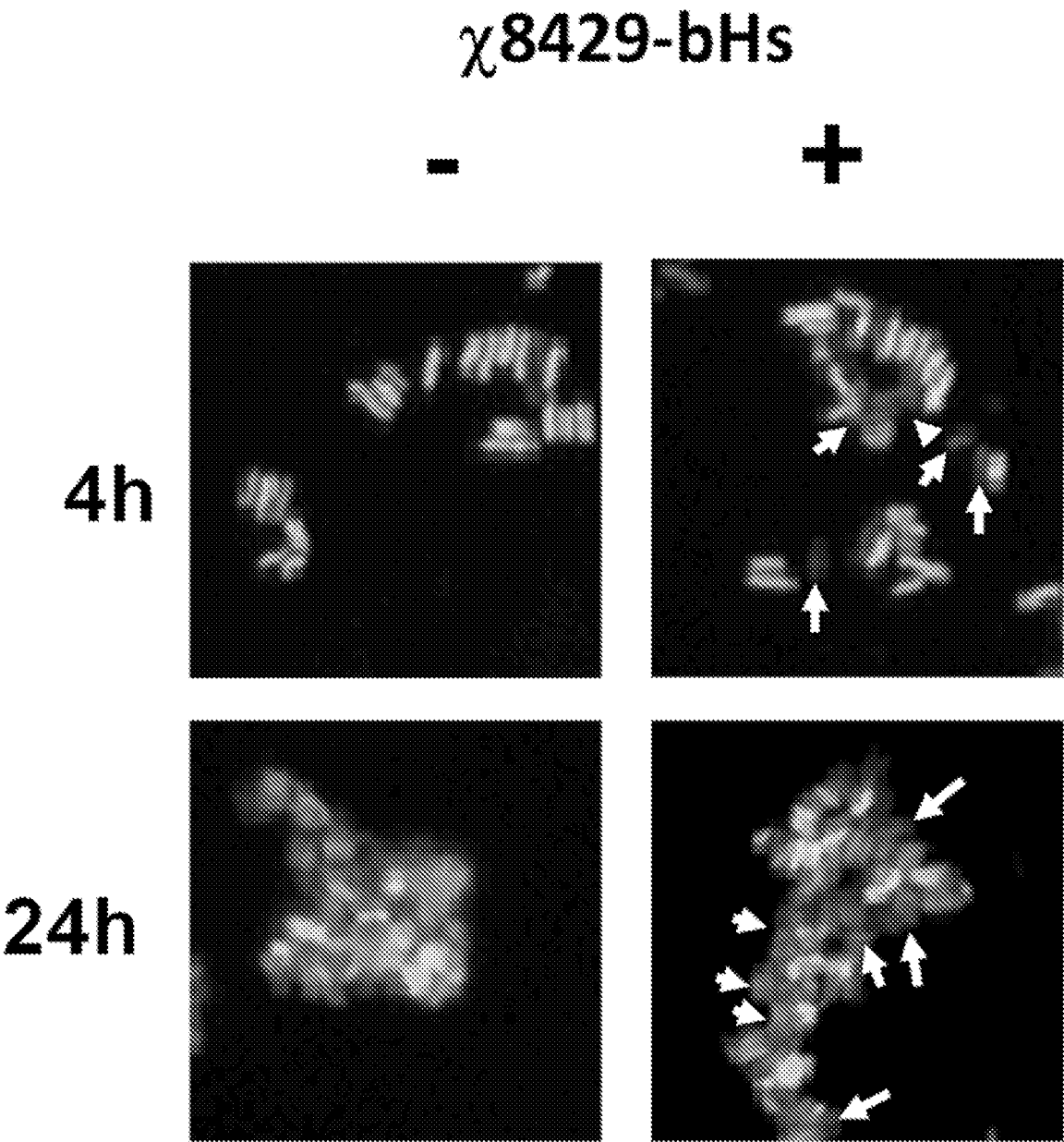
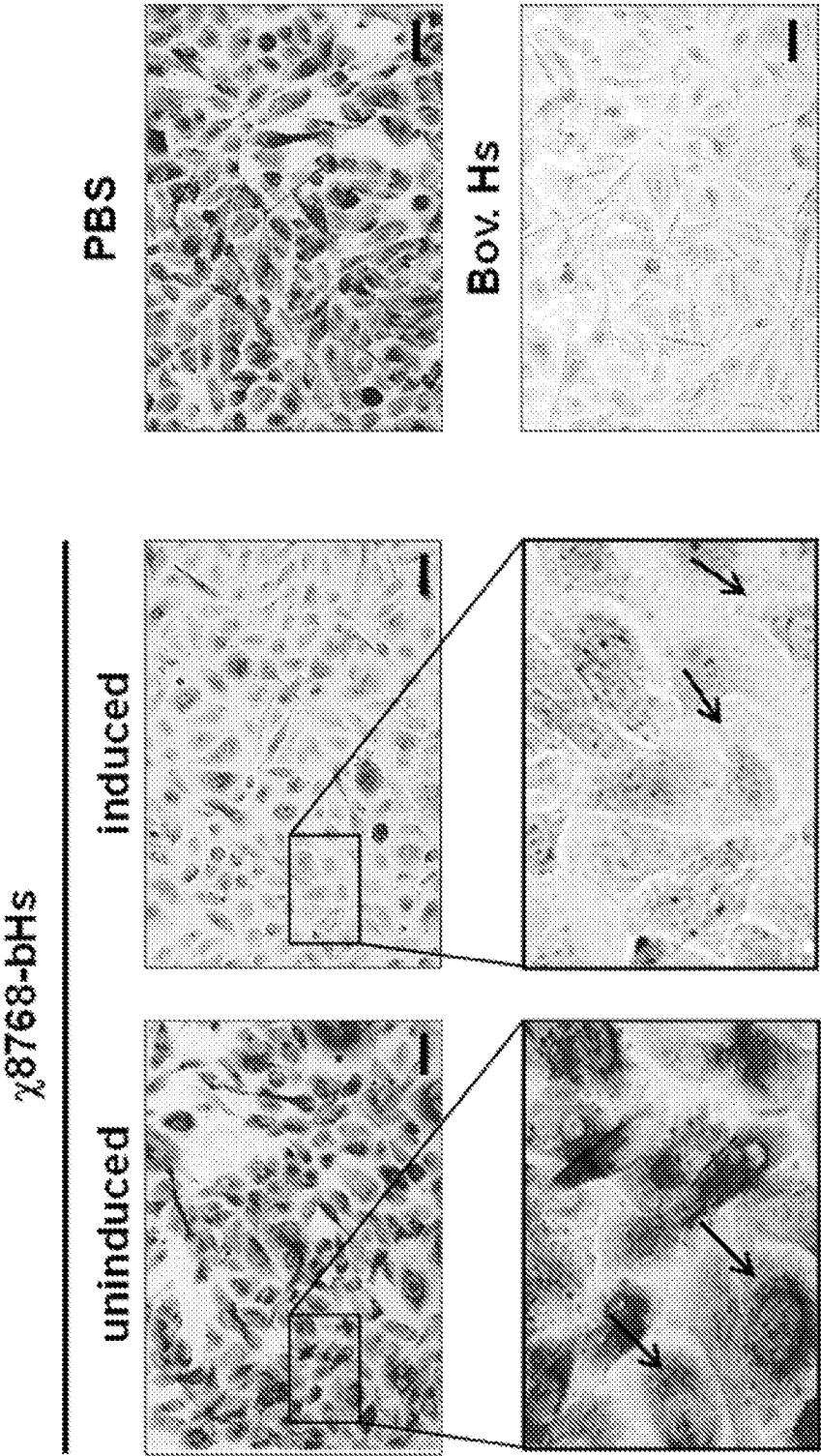


FIG. 8
HA^{high} human prostate cancer (PC3) cells



20 / 32

FIG. 9A

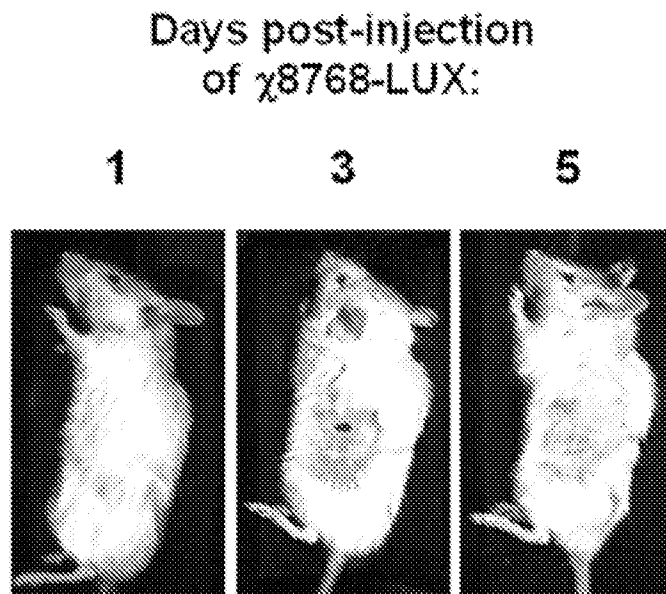


FIG. 9B

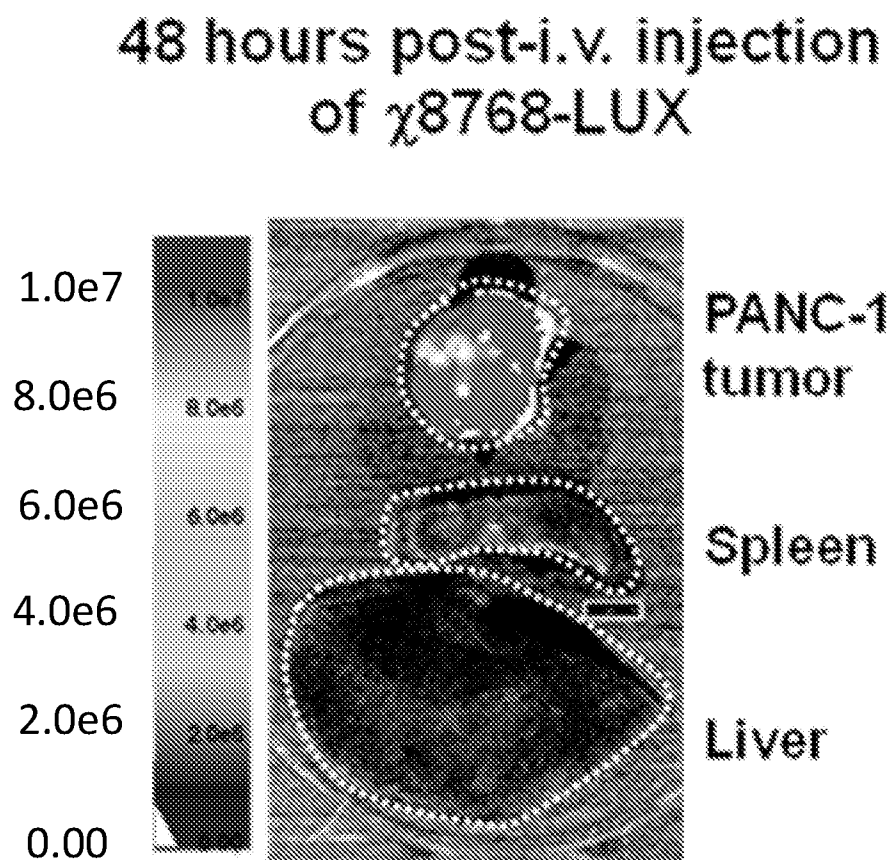
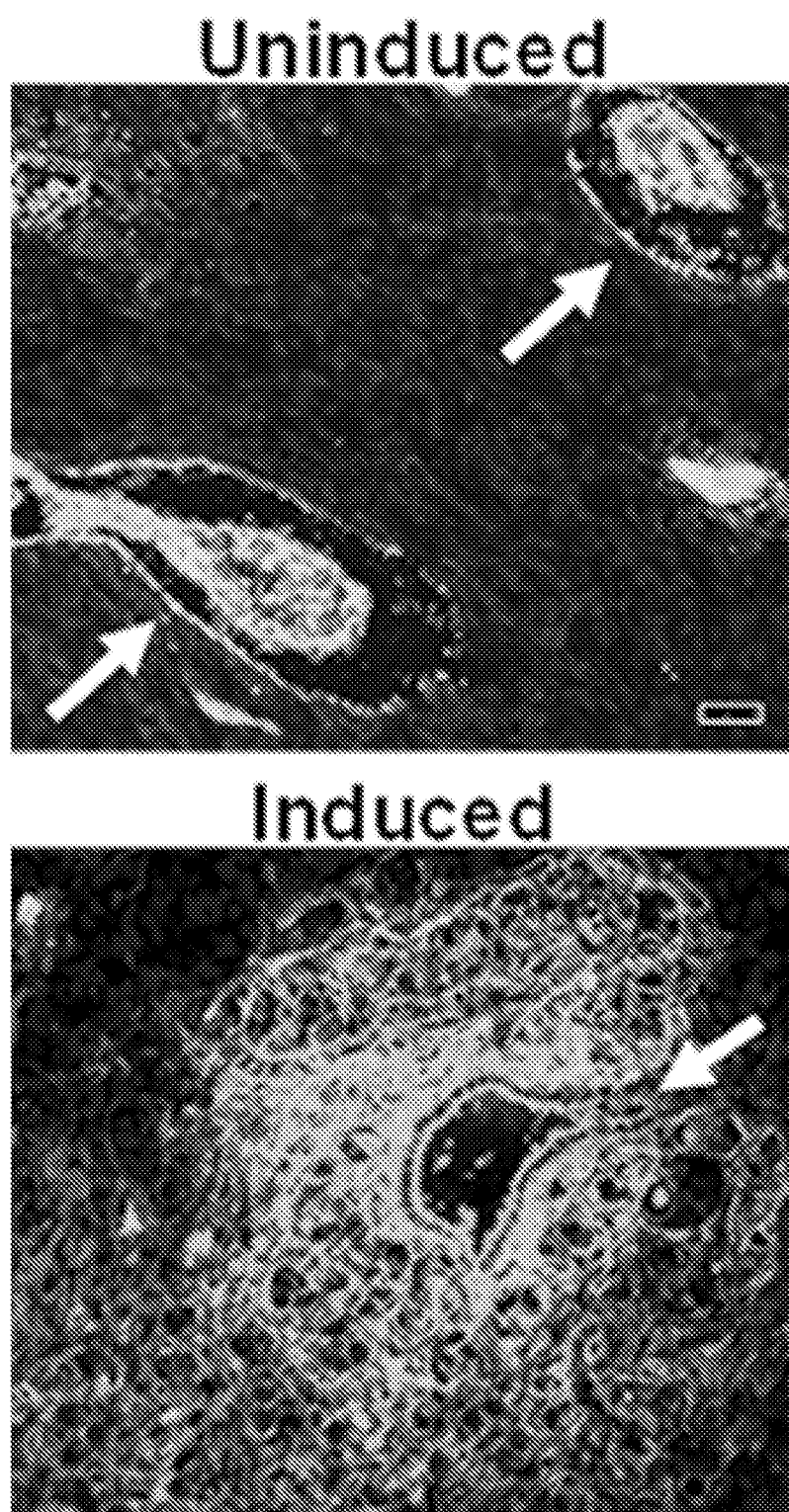


FIG. 9C



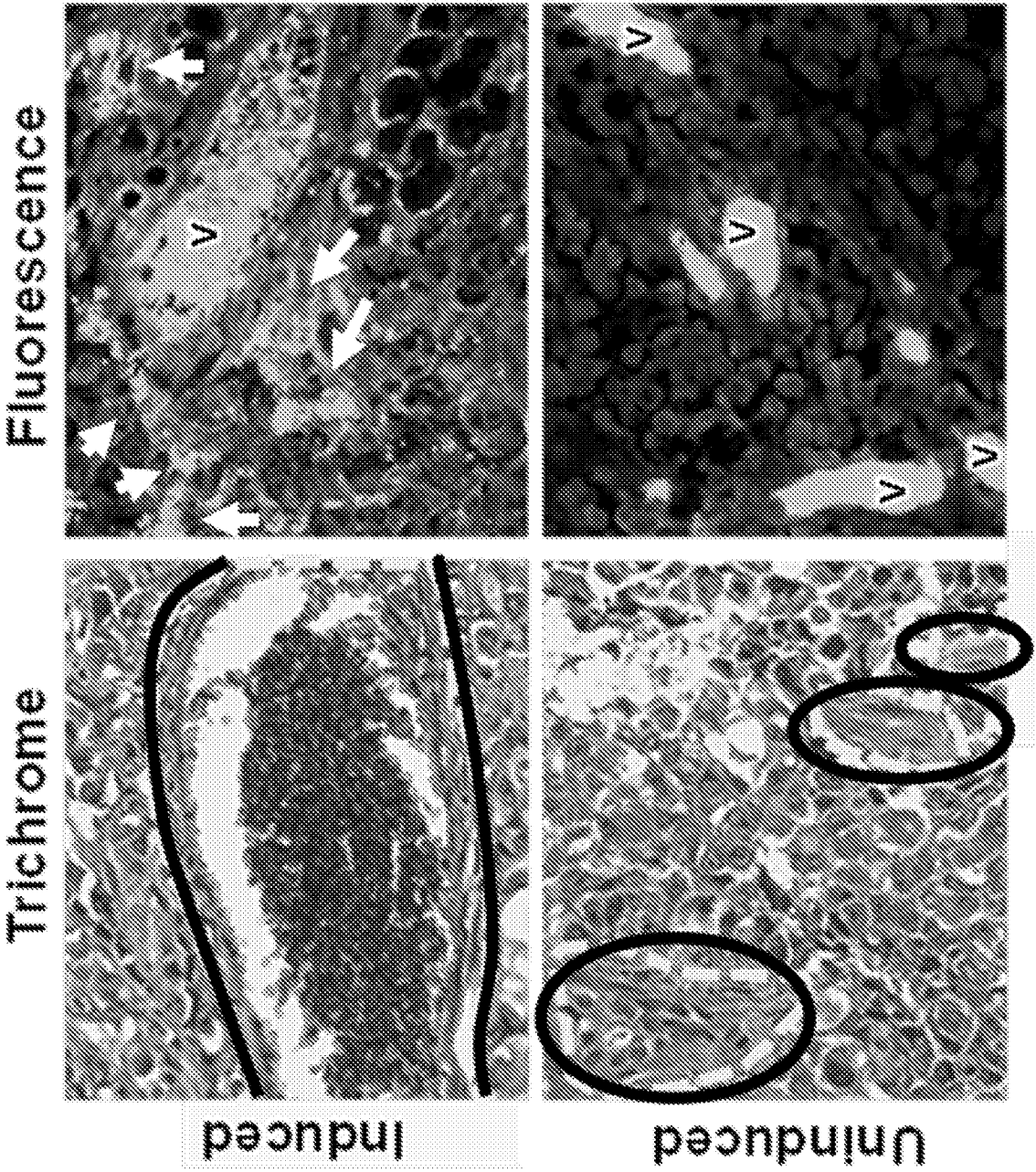


FIG. 9D

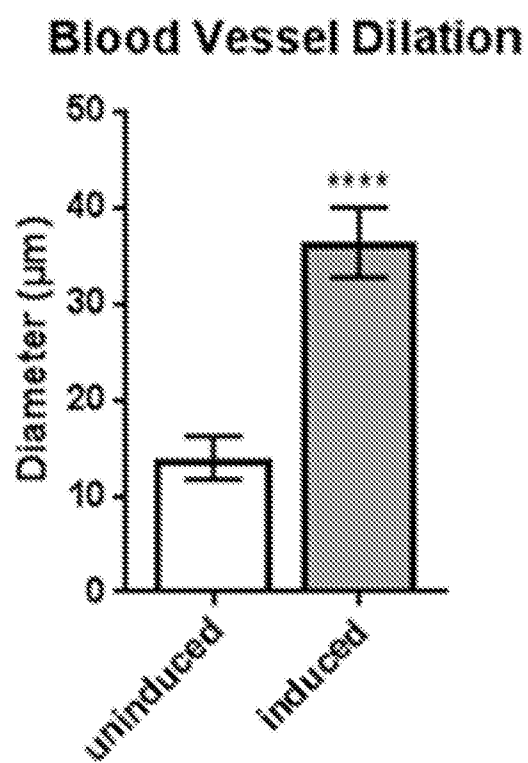
FIG. 9E

FIG. 10A

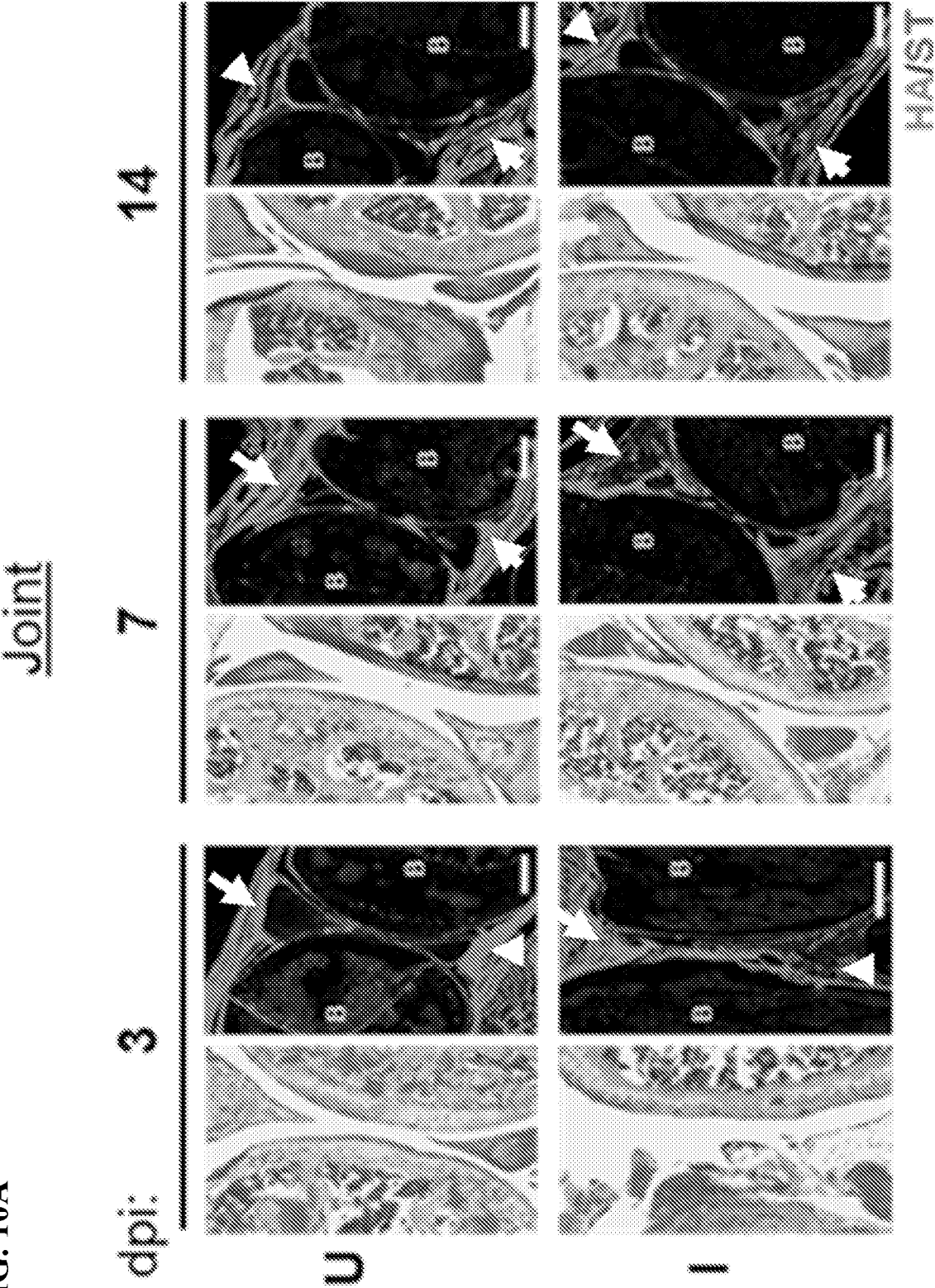


FIG. 10B

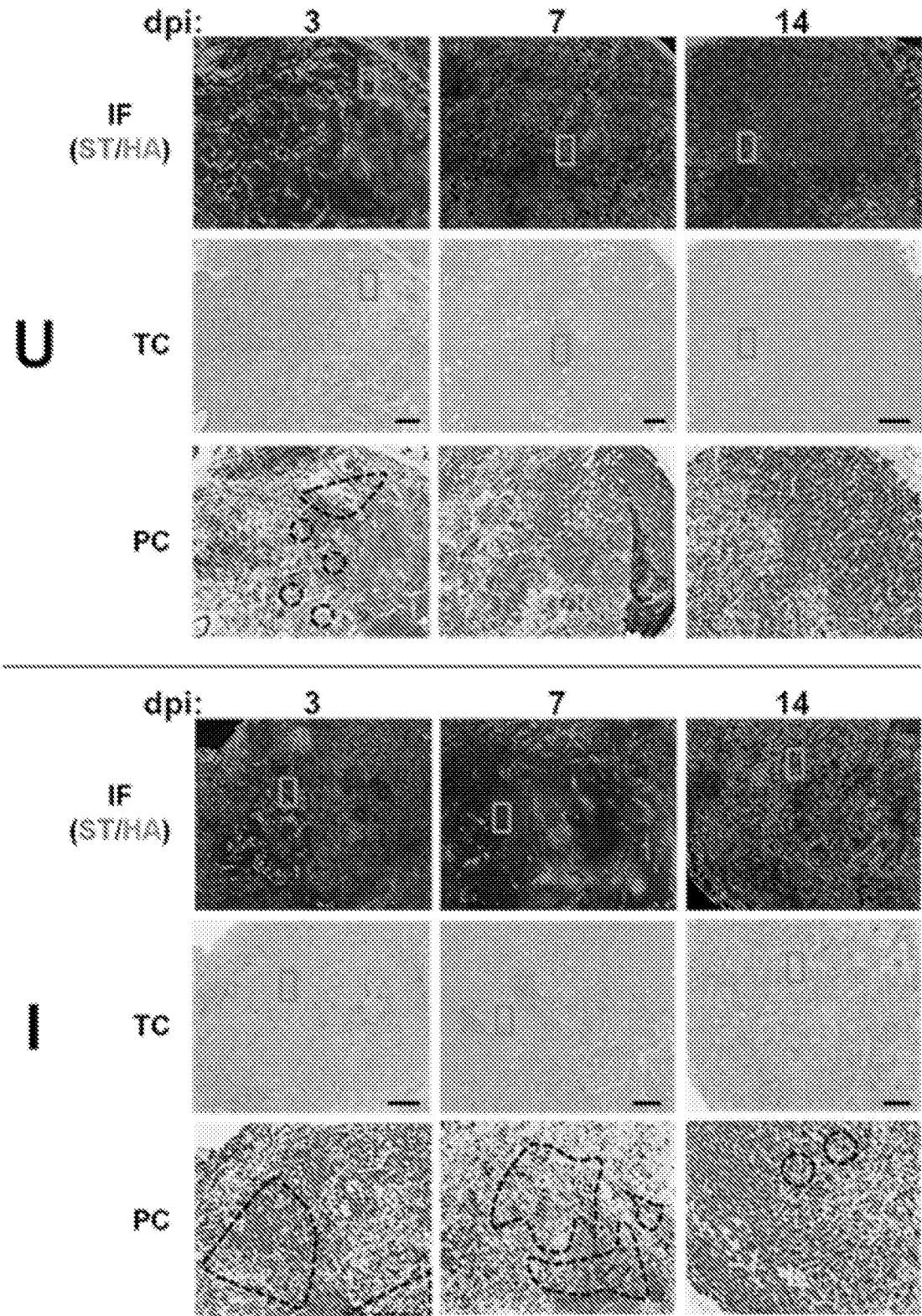


FIG. 10C

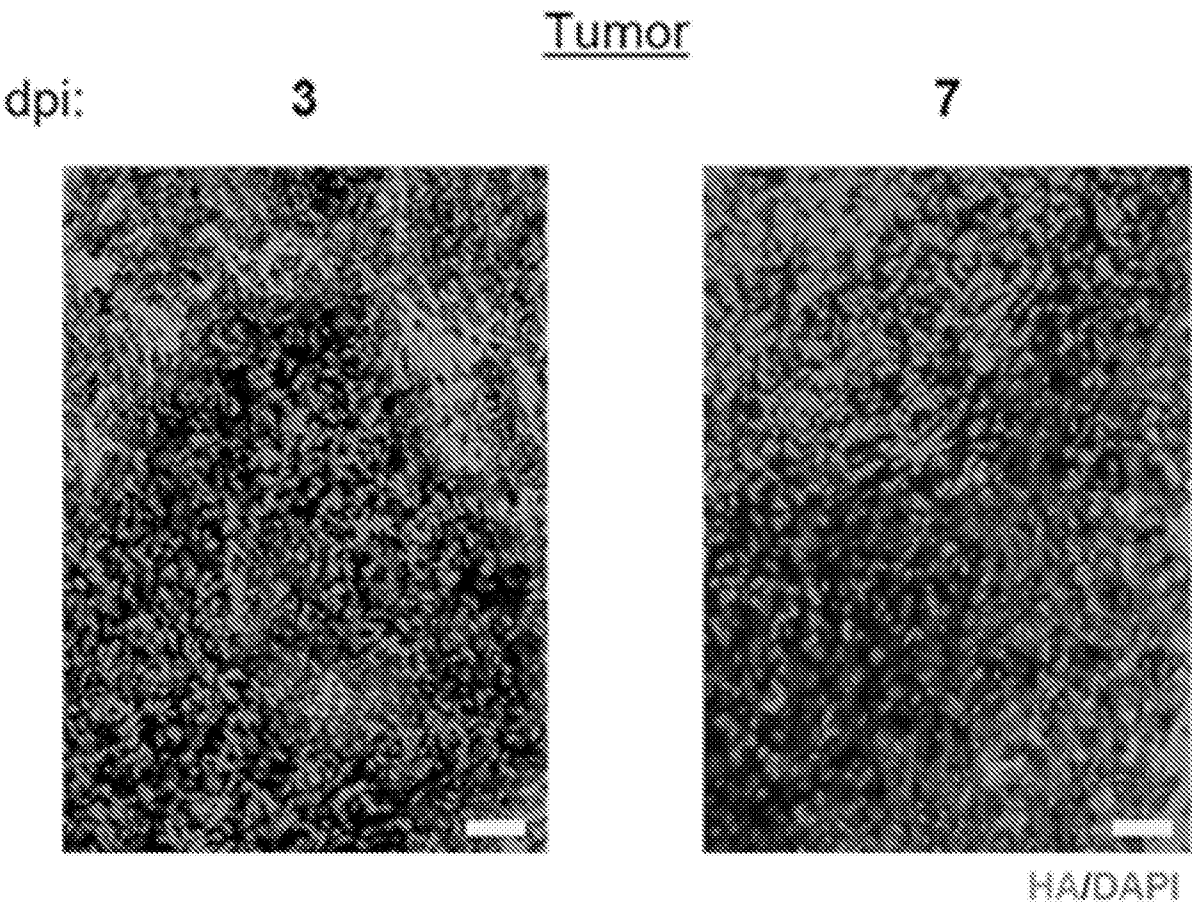


FIG. 11A

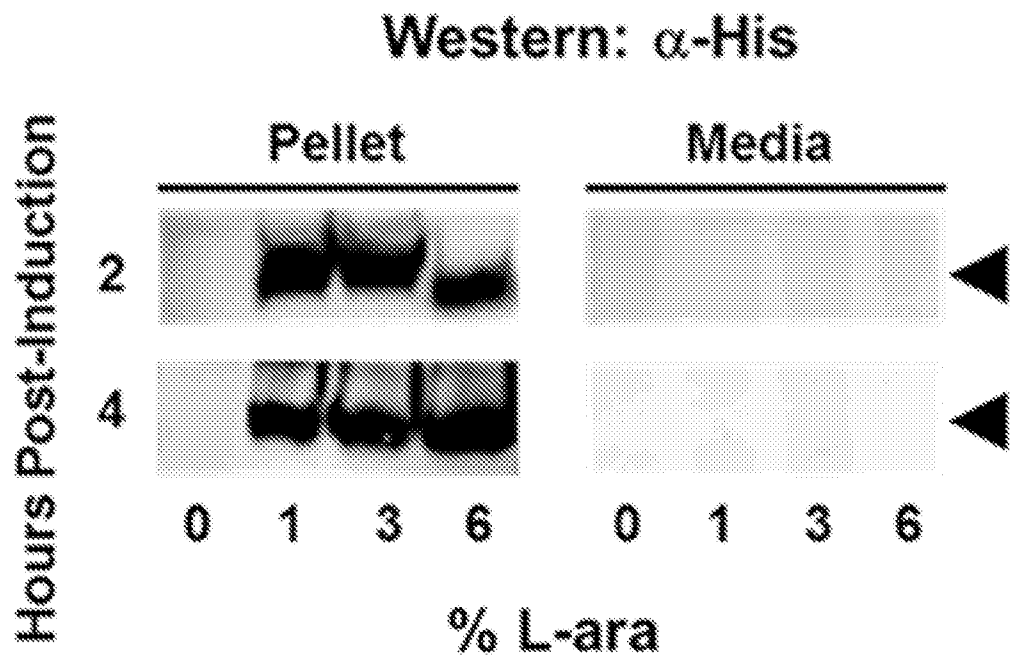


FIG. 11B

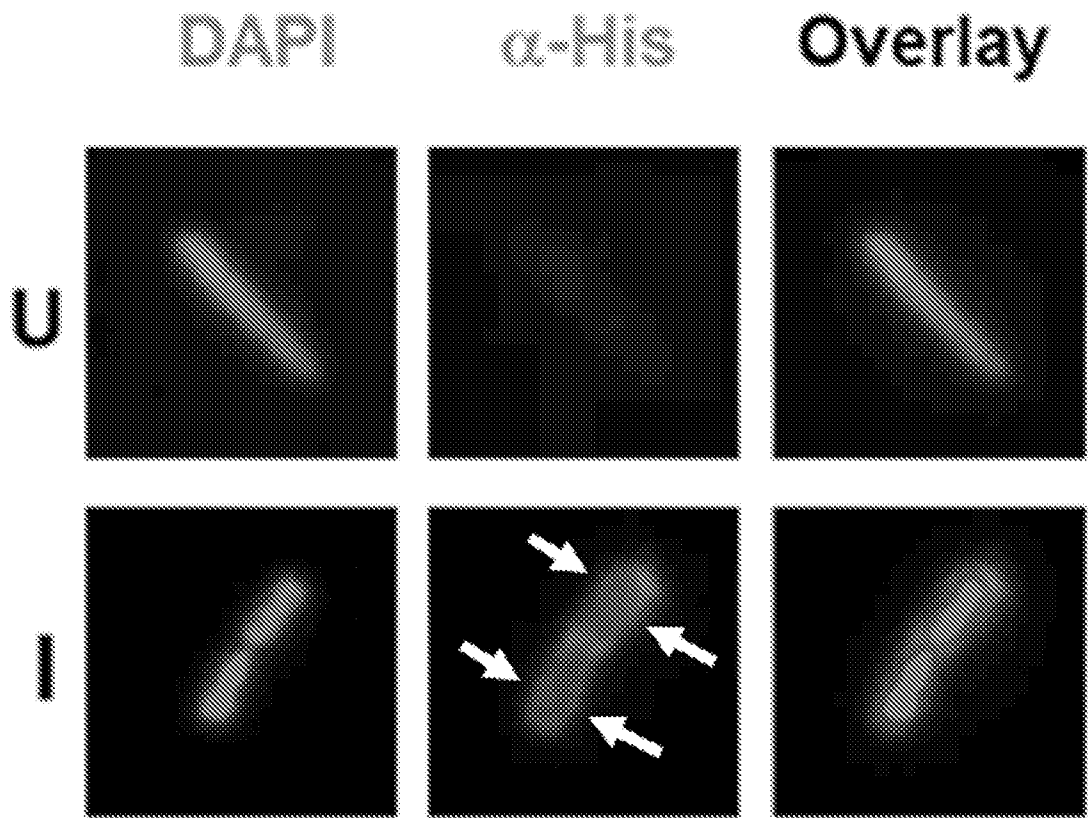


FIG. 11C

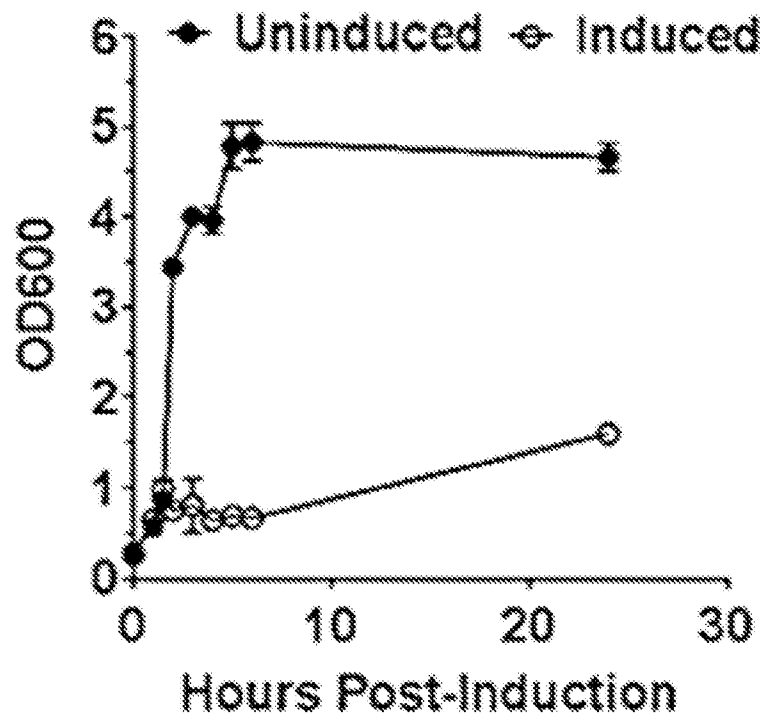
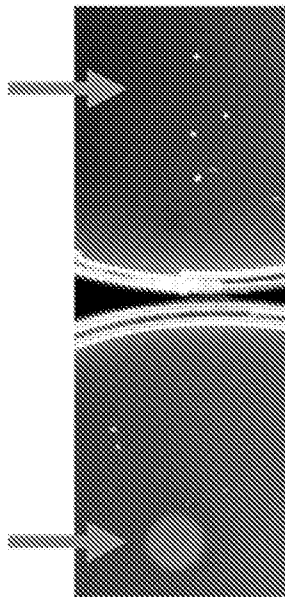


FIG. 12A

ST-CNase:

Uninduced

Induced



LB-Gelatin

FIG. 12B

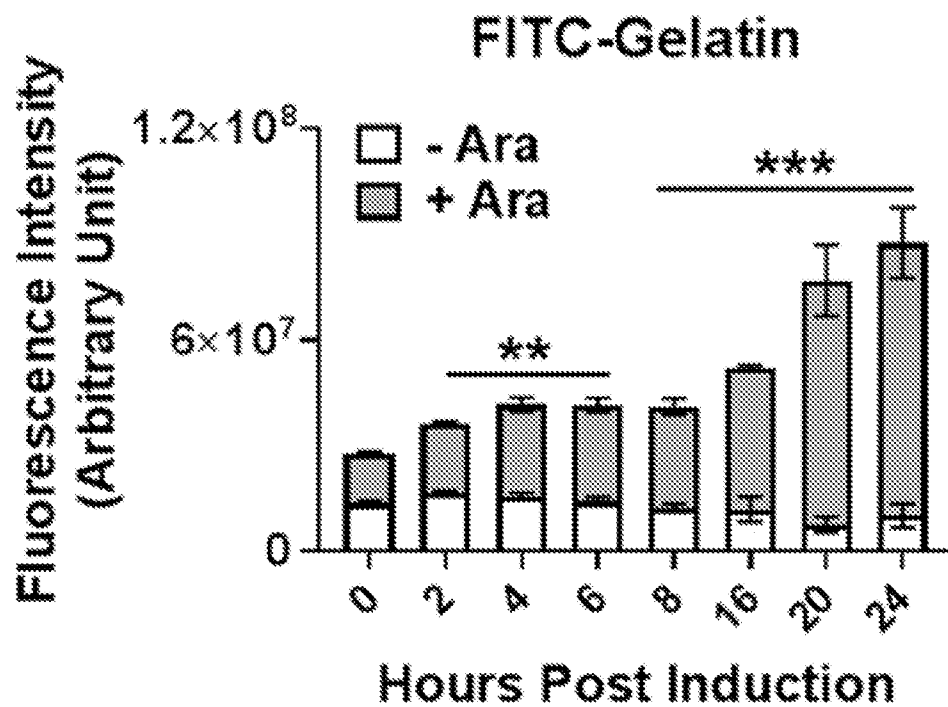


FIG. 12C

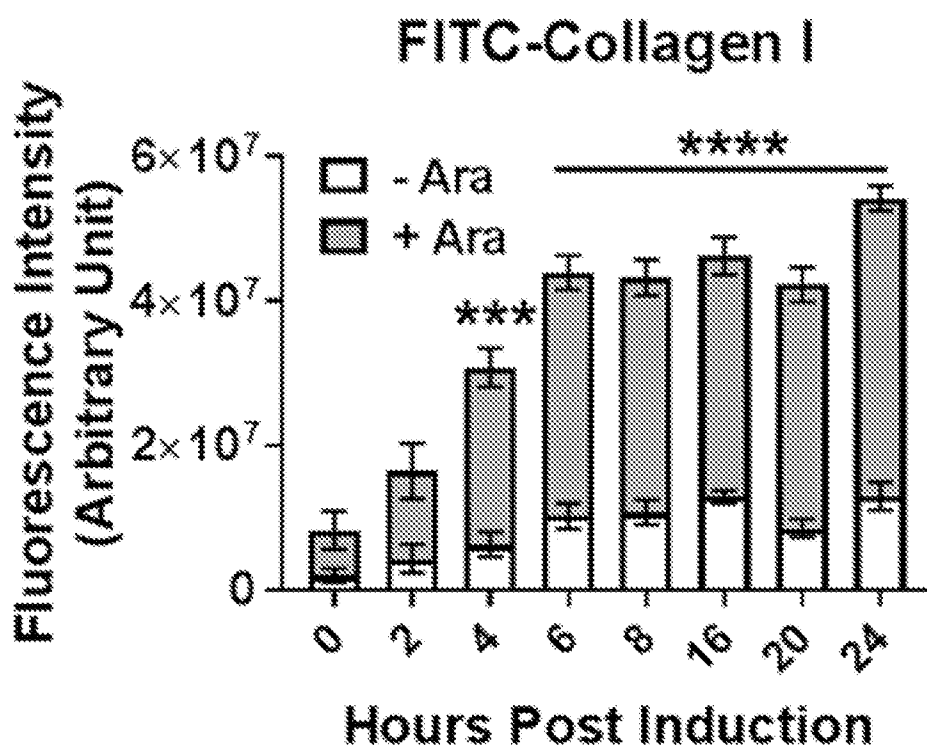
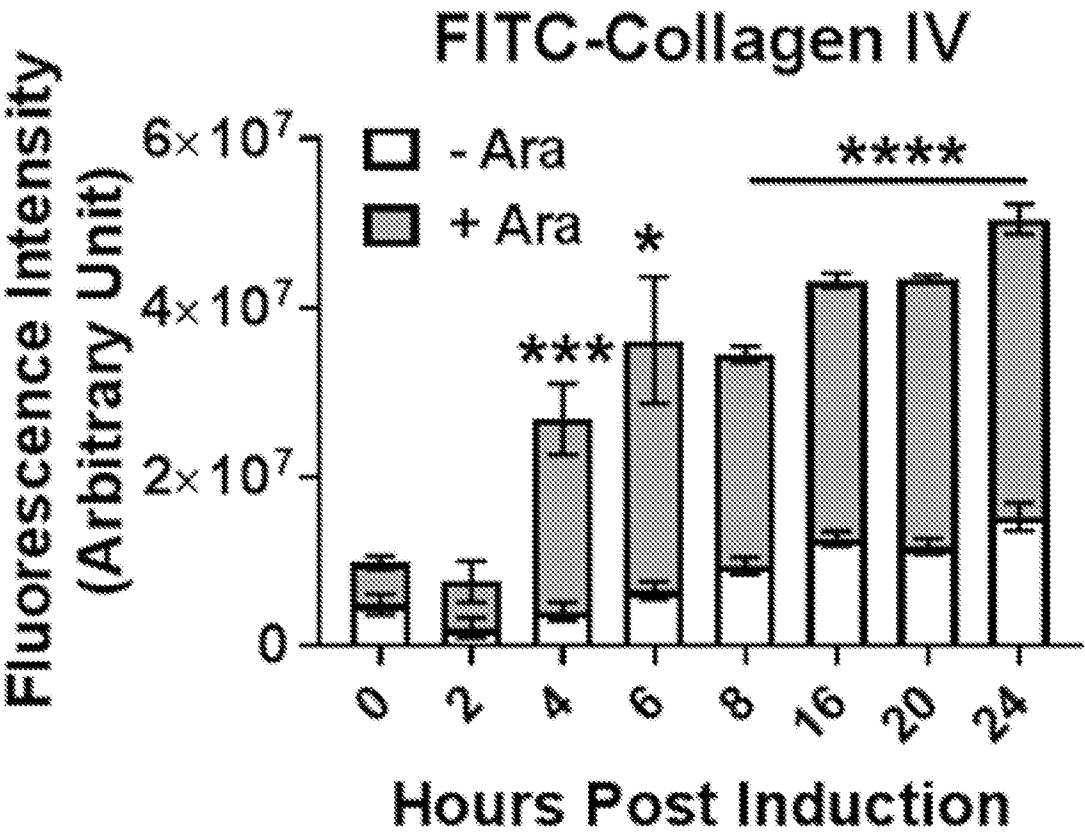


FIG. 12D



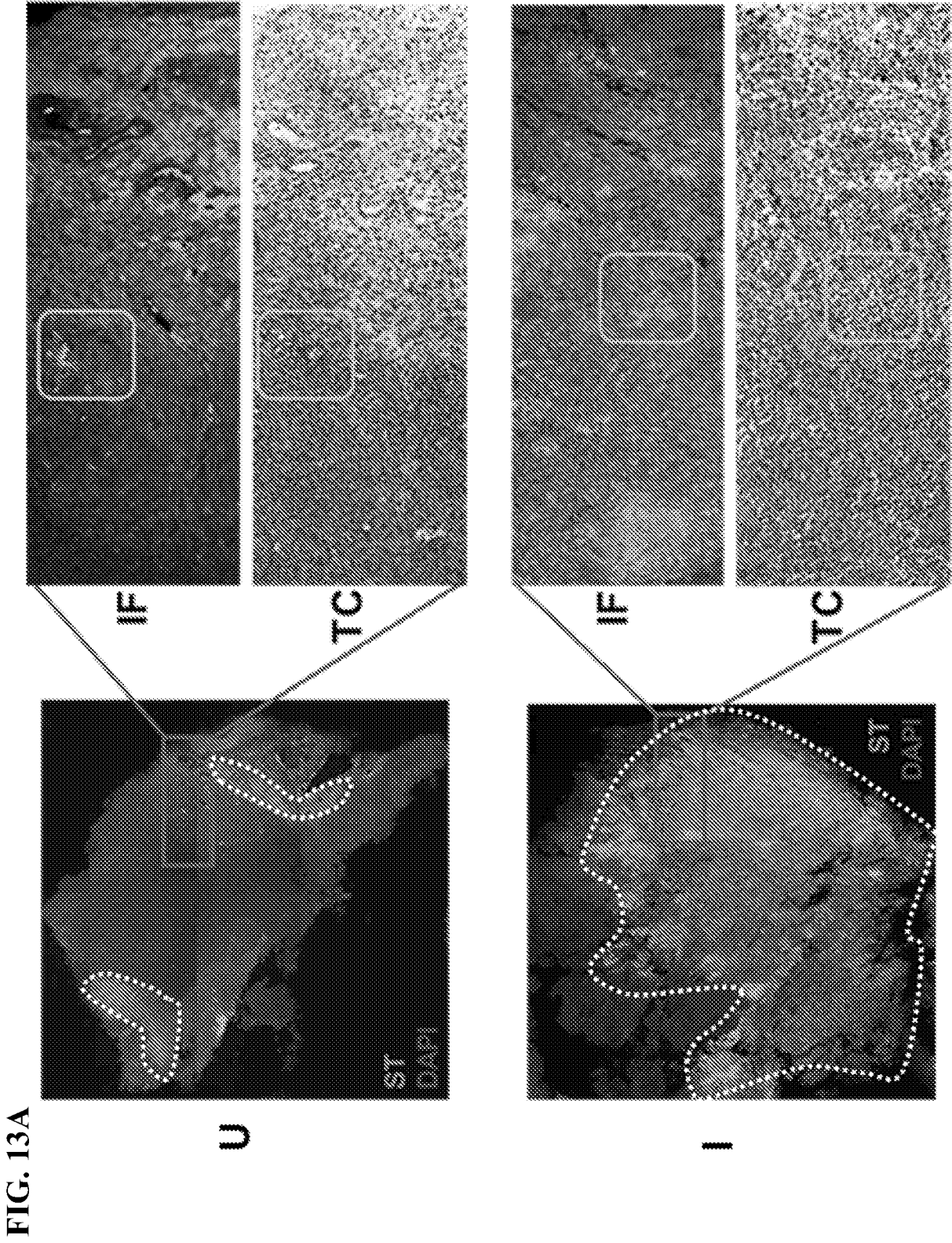


FIG. 13B

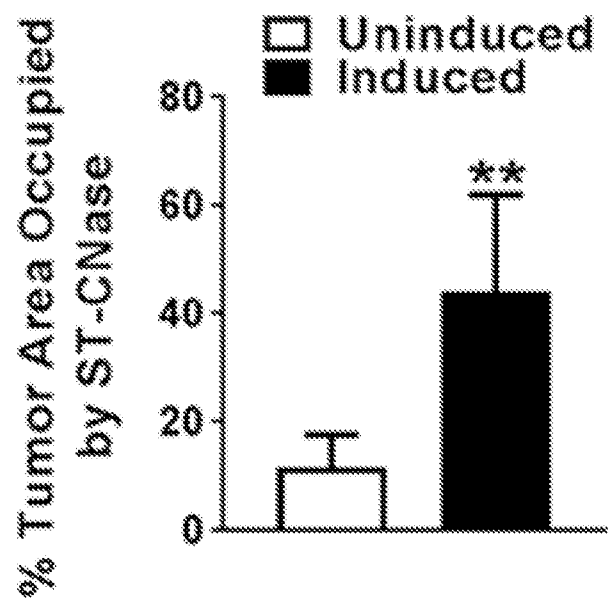
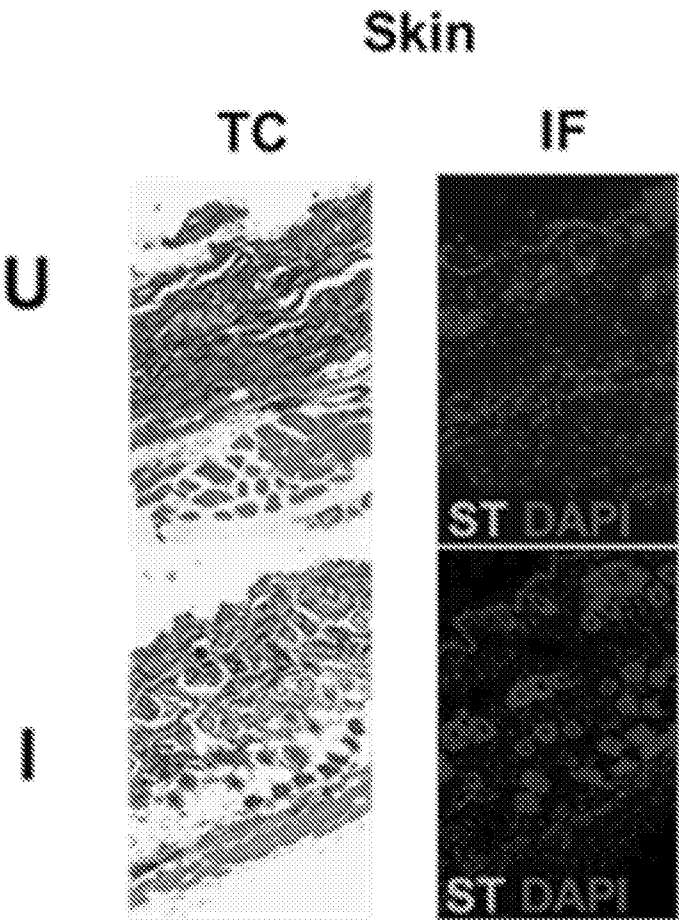


FIG. 13C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/033227

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 63/00; A61K 35/74; C12N 1/20; C12N 5/00; C12N 9/14; C12N 9/26; C12N 15/70 (2020.01)
 CPC - A61K 2039/51; A61K 2039/5152; A61K 2039/5156; A61K 2039/52; A61K 2039/521; A61K 2039/522; A61K 2039/523; C12N 9/00 (2020.05)

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	WO 2005/018332 A1 (THE GENERAL HOSPITAL CORPORATION et al) 03 March 2005 (03.03.2005) entire document	1-4, 17-19, 36-38
A	US 2016/0184456 A1 (CITY OF HOPE) 30 June 2016 (30.06.2016) entire document	1-4, 17-19, 36-38
A	US 10,195,259 B2 (NEWMAN) 05 February 2019 (05.02.2019) entire document	1-4, 17-19, 36-38
A	US 2006/0105423 A1 (RAPP et al) 18 May 2006 (18.05.2006) entire document	1-4, 17-19, 36-38
A	WO 2003/063593 A1 (VION PHARMACEUTICALS, INC. et al) 07 August 2003 (07.08.2003) entire document	1-4, 17-19, 36-38



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

"D" document cited by the applicant in the international application

"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

22 July 2020

Date of mailing of the international search report

14 AUG 2020

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Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/033227

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.: 5-16, 20-35, 39-54
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.