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(54) **METHODS FOR MODULATING INFLAMMASOME ACTIVITY AND INFLAMMATION IN THE LUNG**

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Publication Classification

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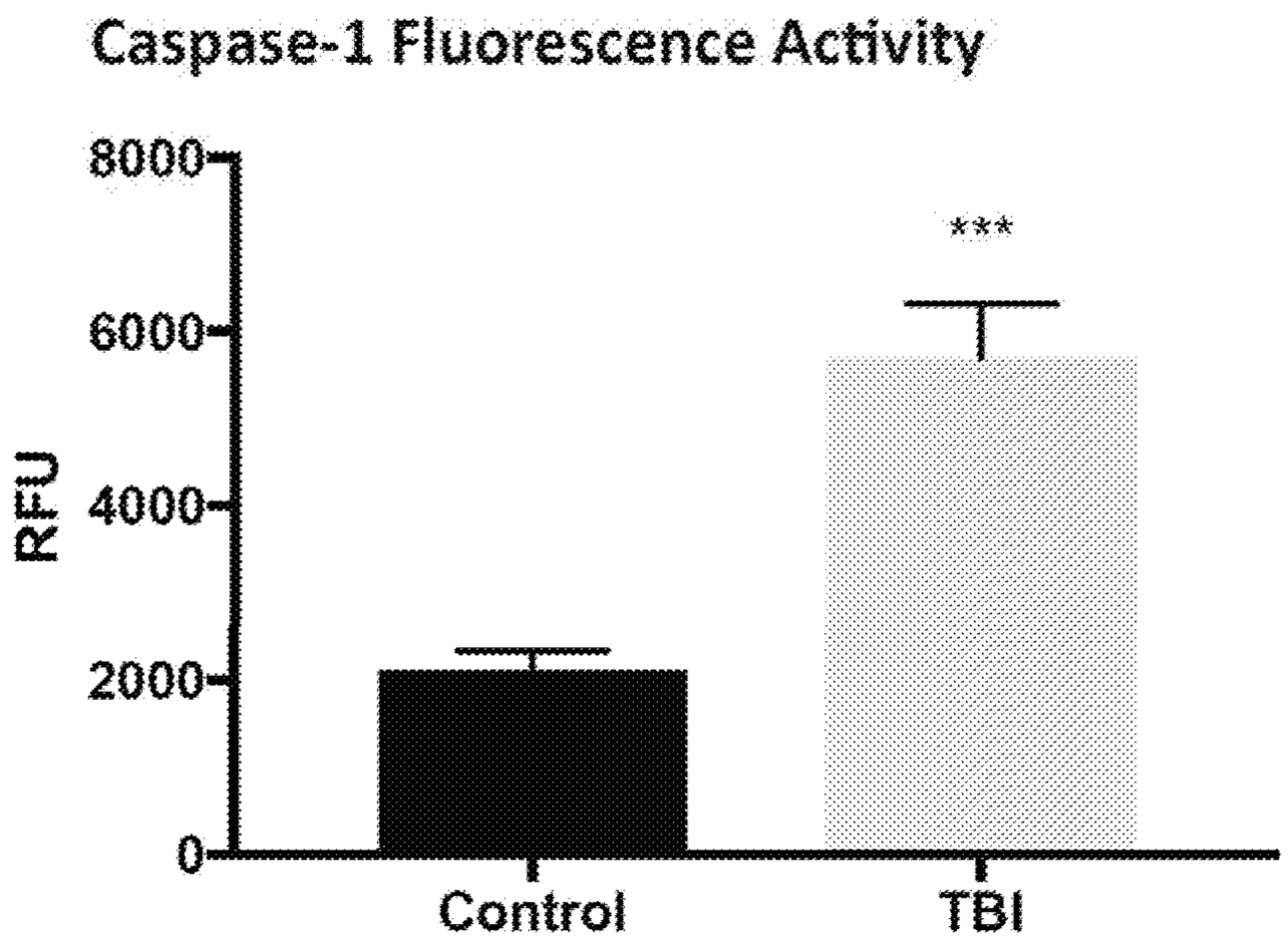
A61P 25/28	(2006.01)
A61P 37/06	(2006.01)
A61P 11/00	(2006.01)
A61P 25/00	(2006.01)
A61P 29/00	(2006.01)
A61K 31/727	(2006.01)
A61K 39/395	(2006.01)
C07K 16/18	(2006.01)
C07K 16/24	(2006.01)

(52) **U.S. Cl.**
CPC ***G11B 19/08*** (2013.01); ***A61K 31/727*** (2013.01); ***A61K 39/3955*** (2013.01); ***A61K 39/39541*** (2013.01); ***A61P 11/00*** (2018.01); ***A61P 25/00*** (2018.01); ***A61P 25/14*** (2018.01); ***A61P 25/28*** (2018.01); ***A61P 29/00*** (2018.01); ***A61P 37/06*** (2018.01); ***B61L 23/041*** (2013.01); ***B61L 29/30*** (2013.01); ***C07K 16/18*** (2013.01); ***C07K 16/24*** (2013.01); ***G11B 19/16*** (2013.01); *B61L 27/53* (2022.01)

(57) **ABSTRACT**

The present invention provides compositions and methods for reducing inflammation in the lungs of a mammal that is afflicted by a condition that leads to inflammation in the lungs. The compositions and methods described herein include agents that inhibit inflammasome signaling in the mammal such as antibodies directed against inflammasome components used alone or in combination with extracellular vesicle uptake inhibitor(s).

Specification includes a Sequence Listing.



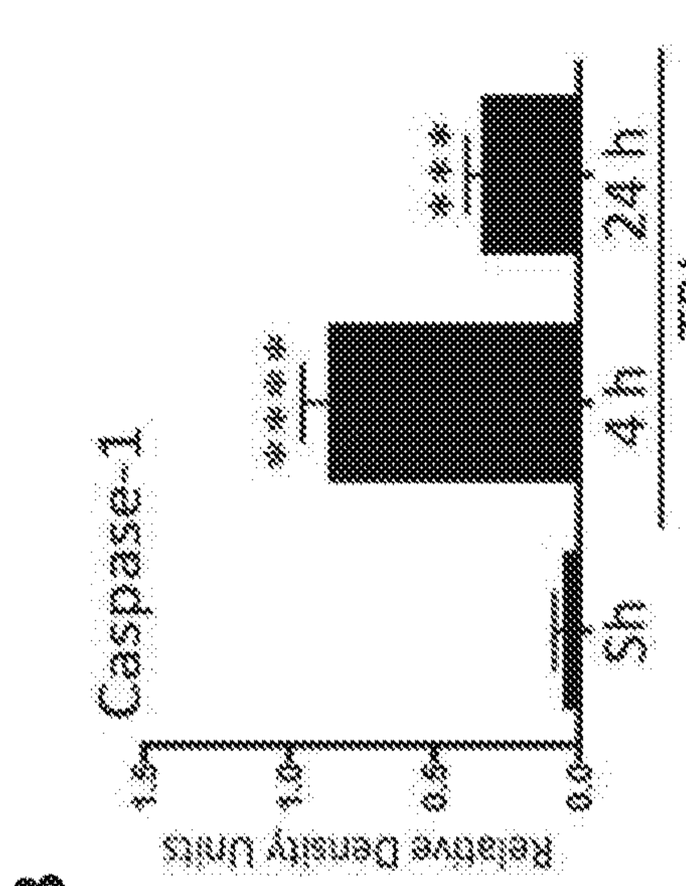


FIG. 1B

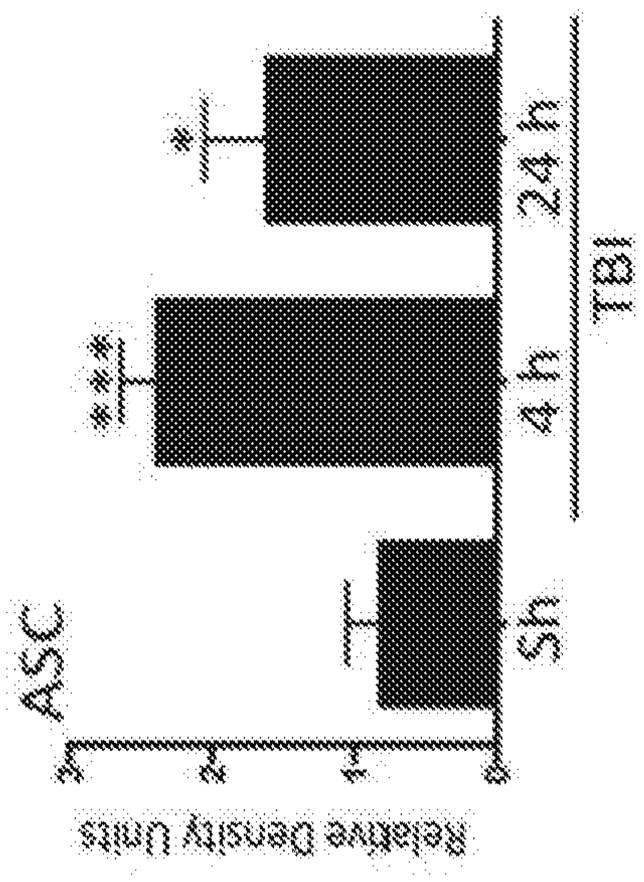
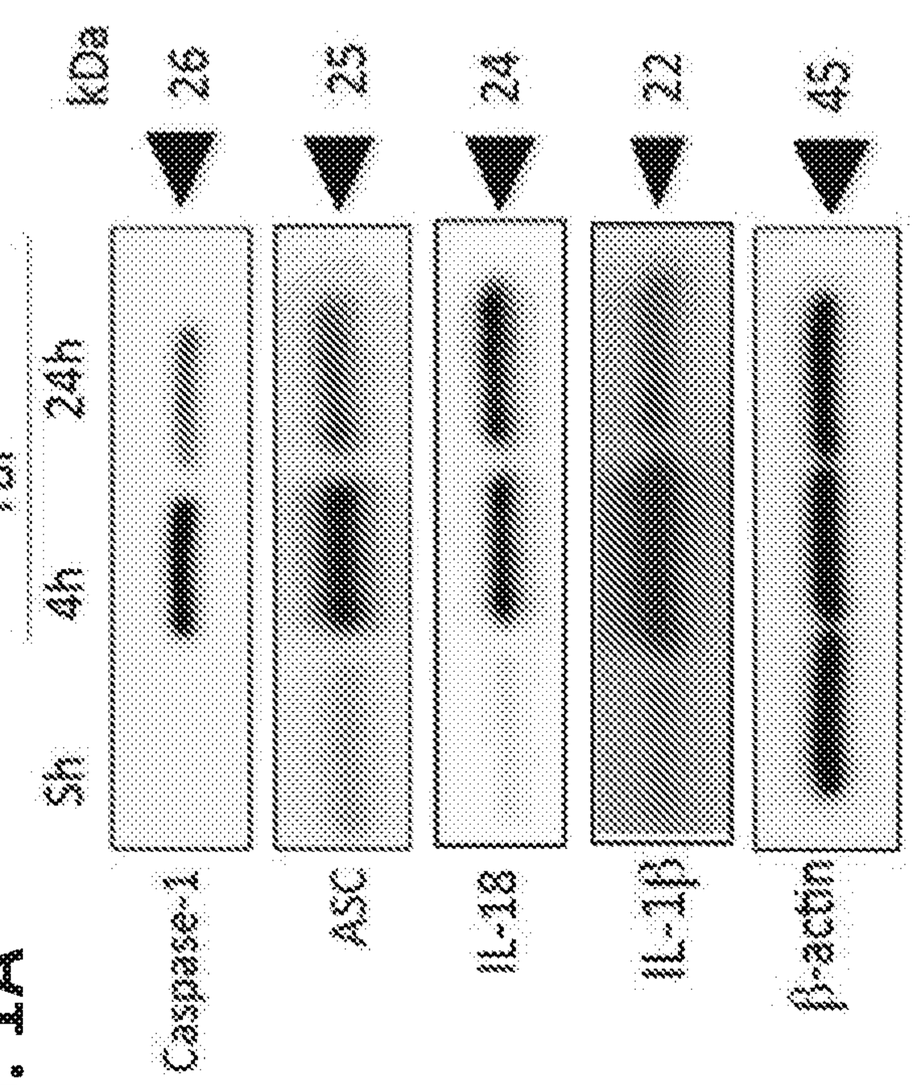


FIG. 1C



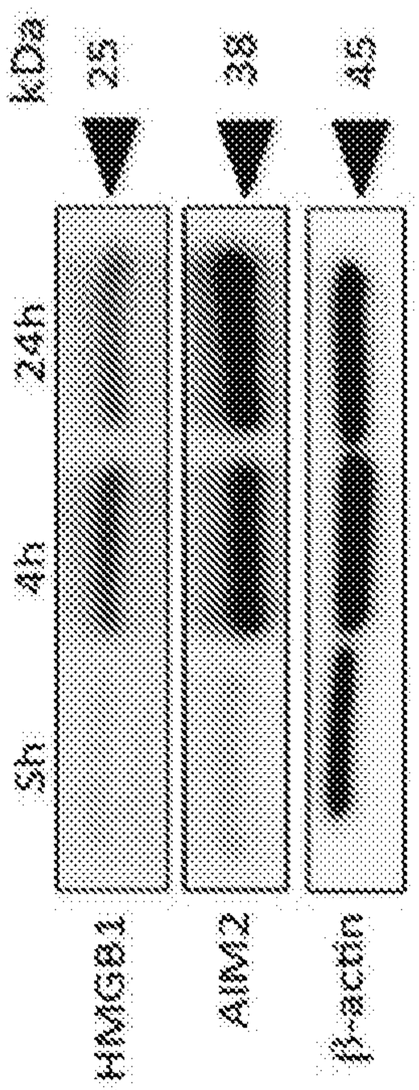


FIG. 1A

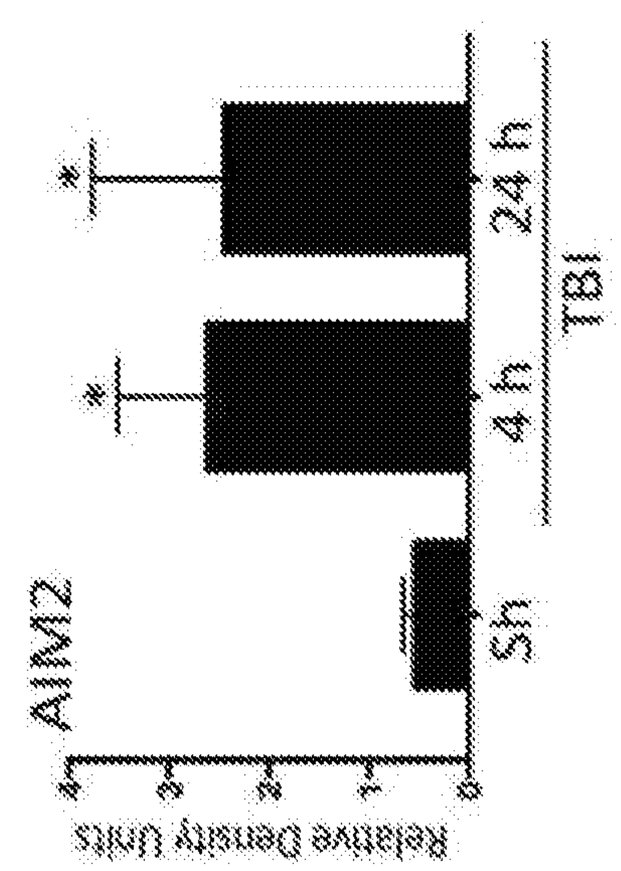


FIG. 1F

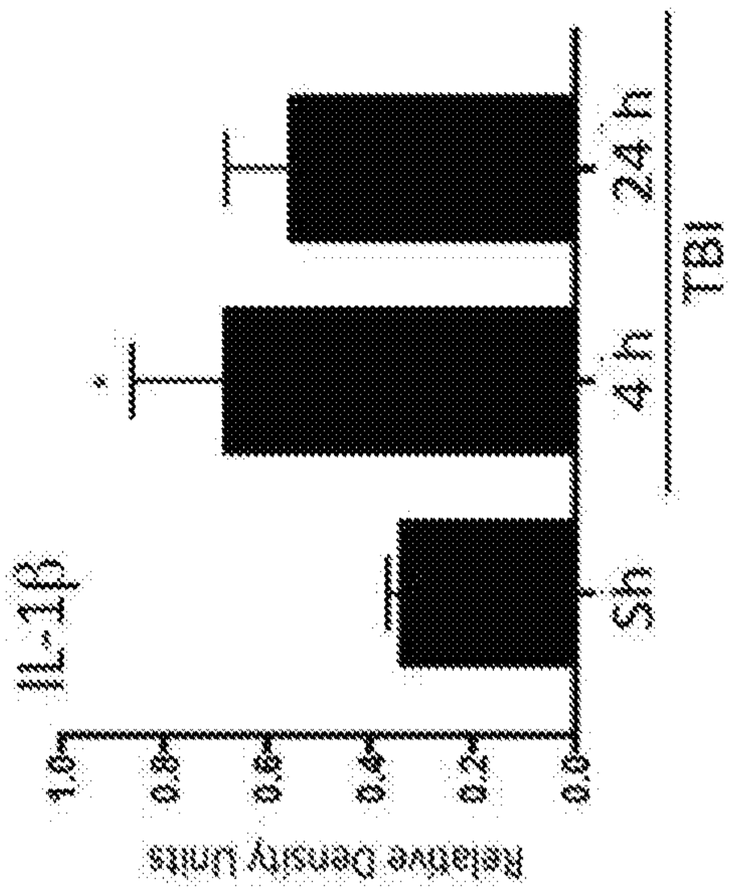


FIG. 1G

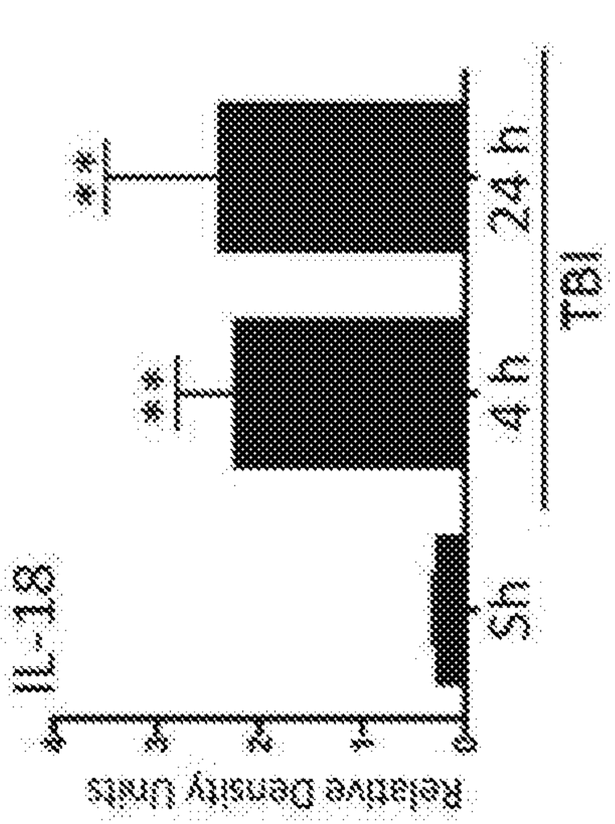


FIG. 1D

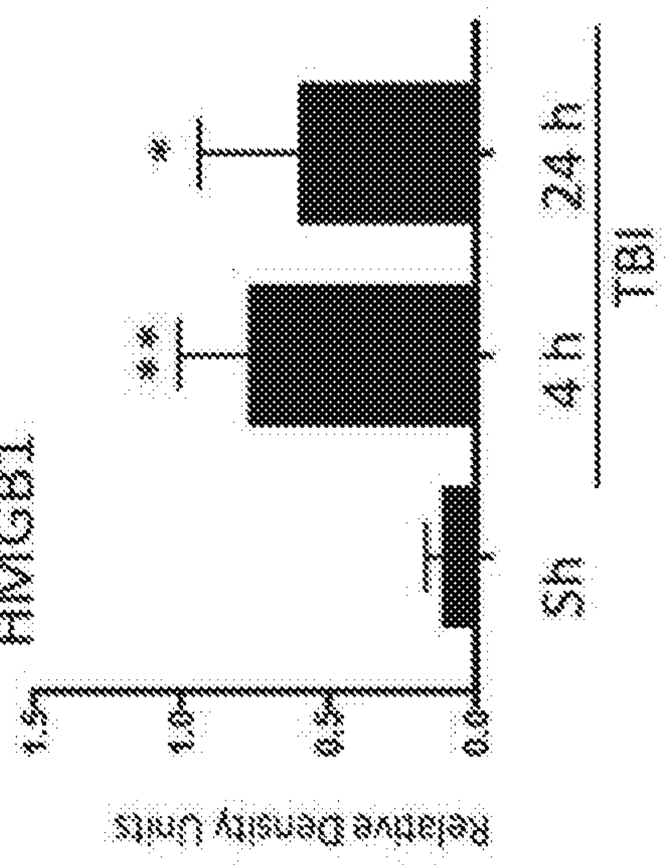


FIG. 1E

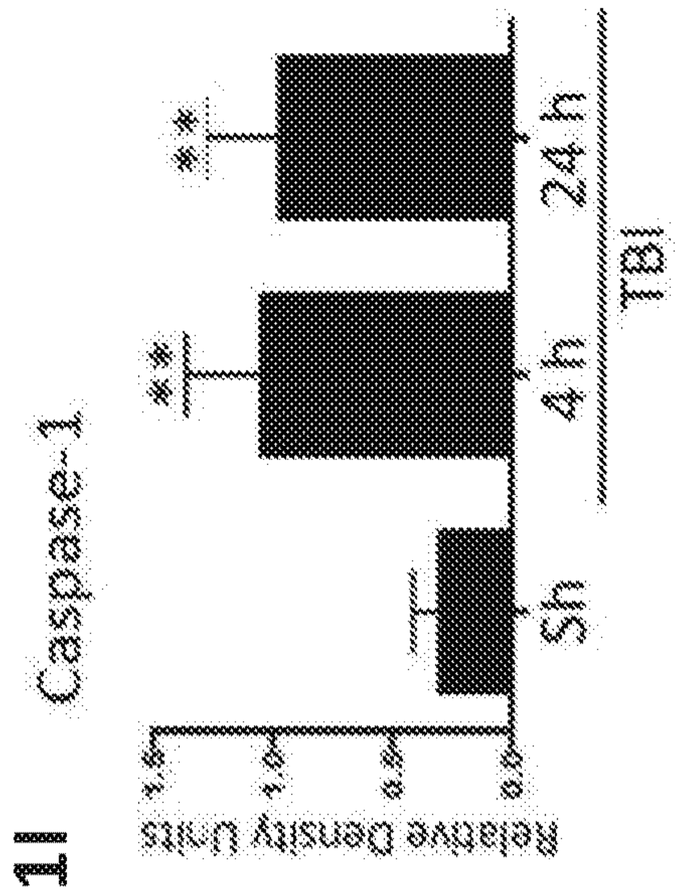


FIG. 1I

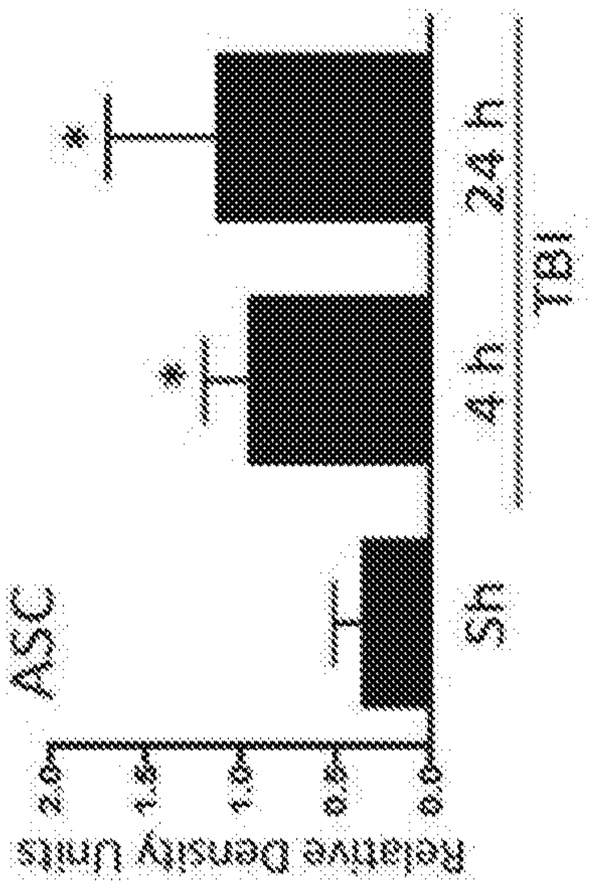


FIG. 1J

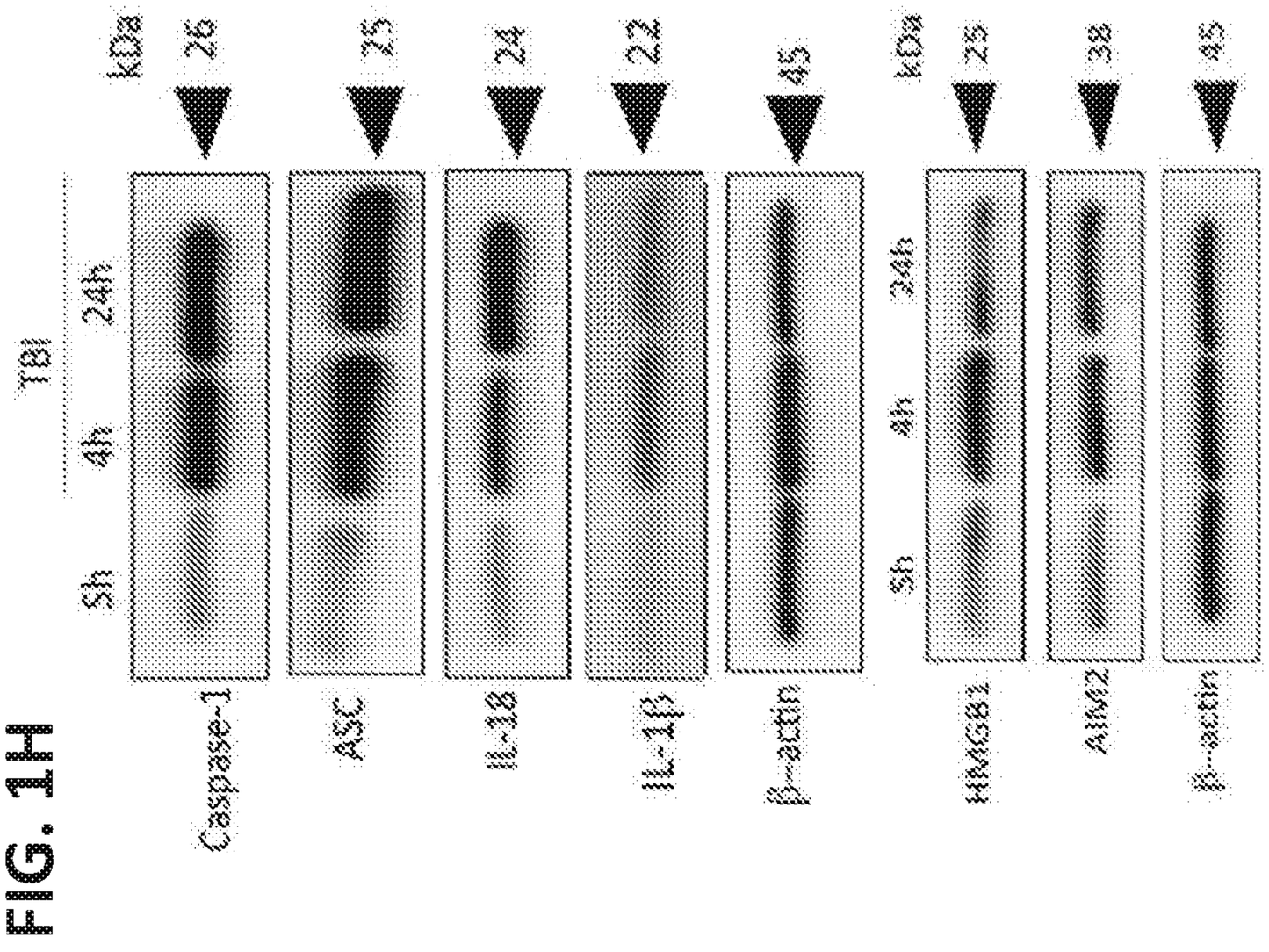


FIG. 1H

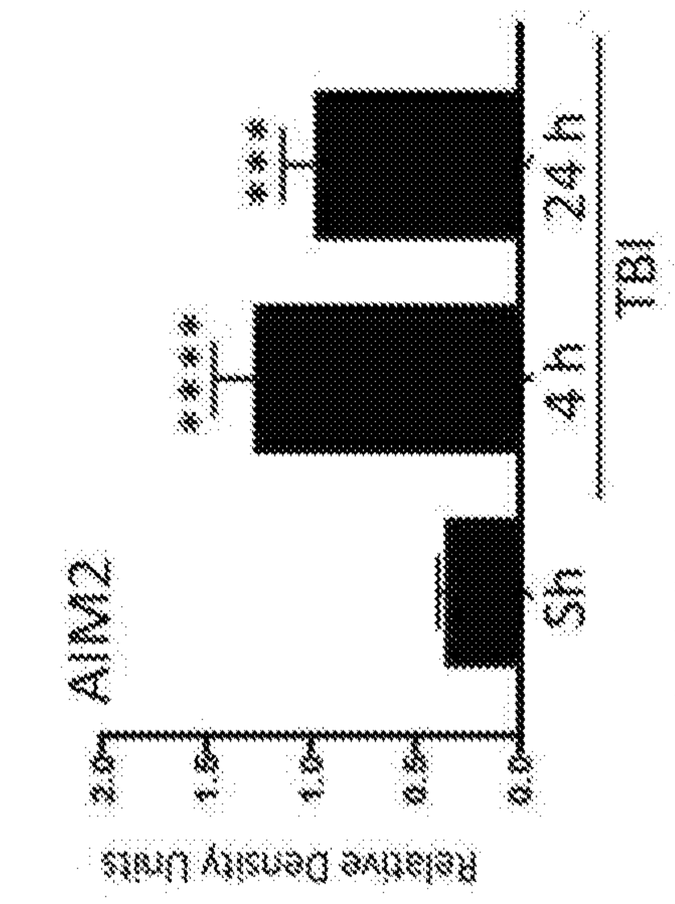


FIG. 1M

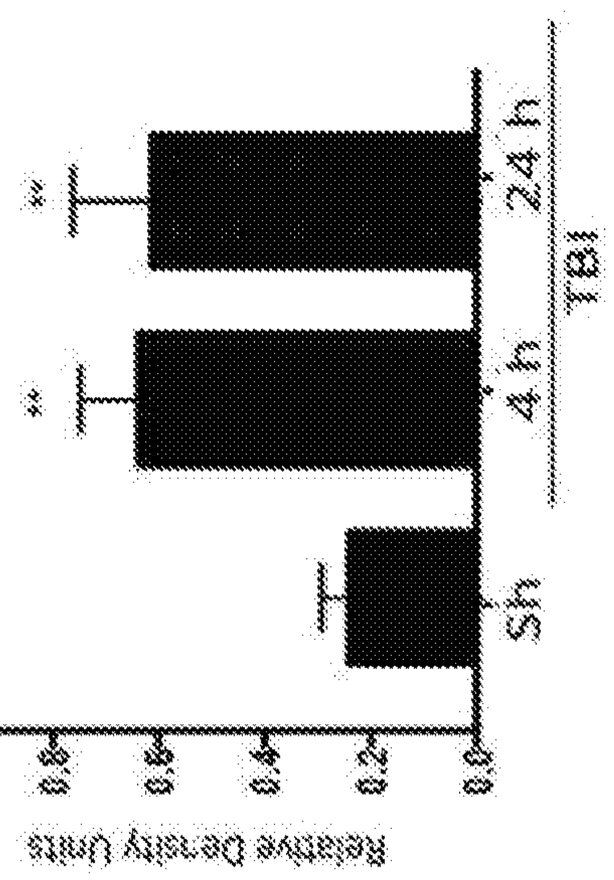


FIG. 1N

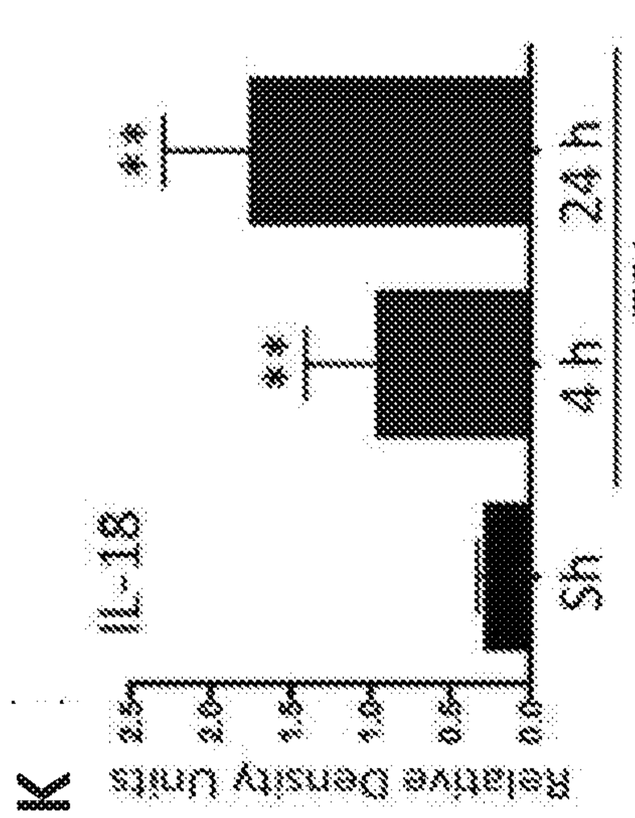


FIG. 1K

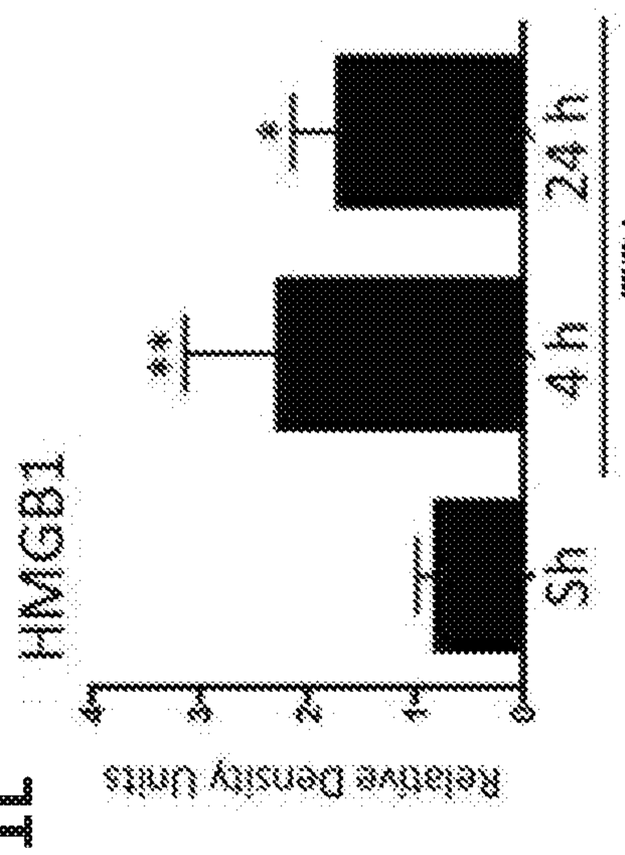


FIG. 1L

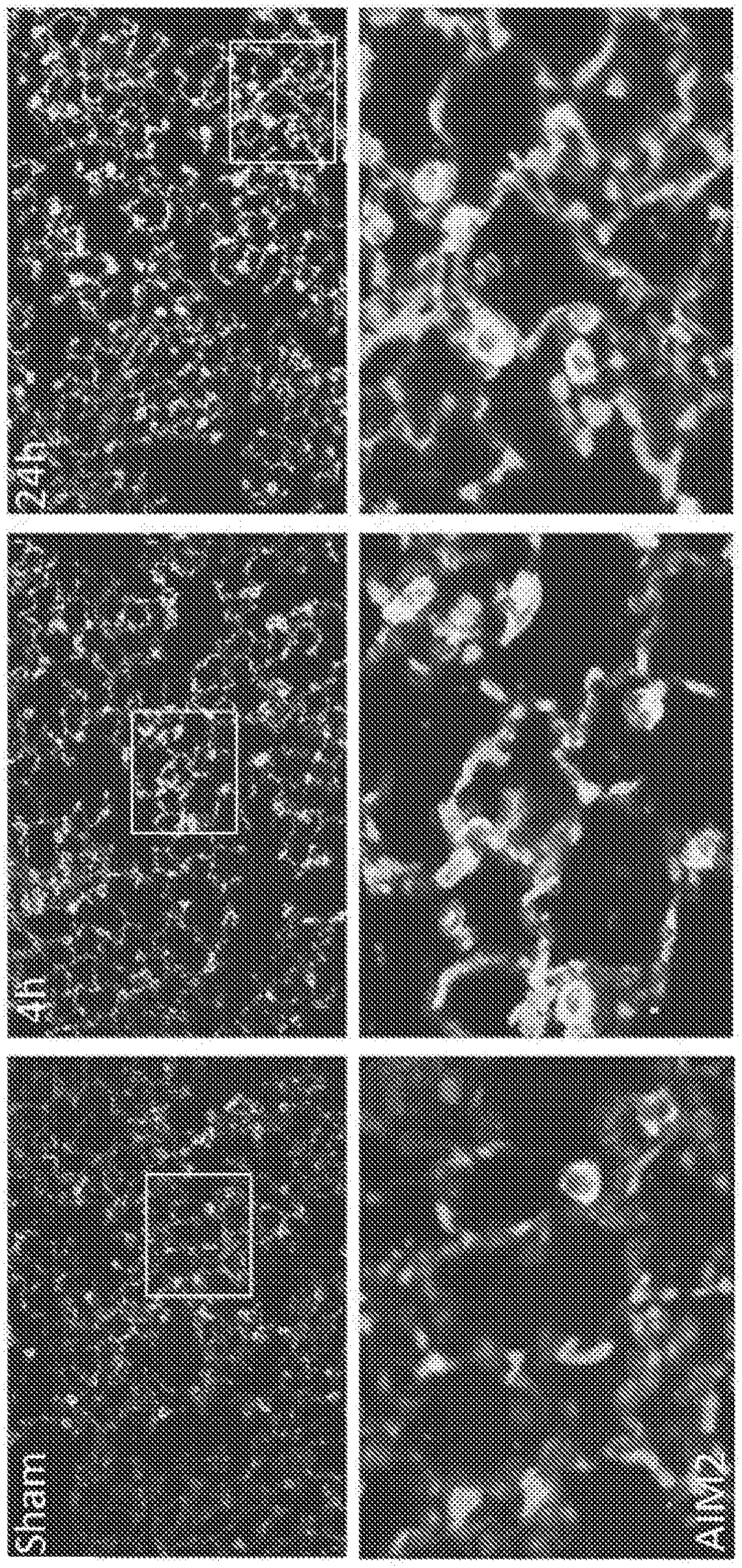


FIG. 2A

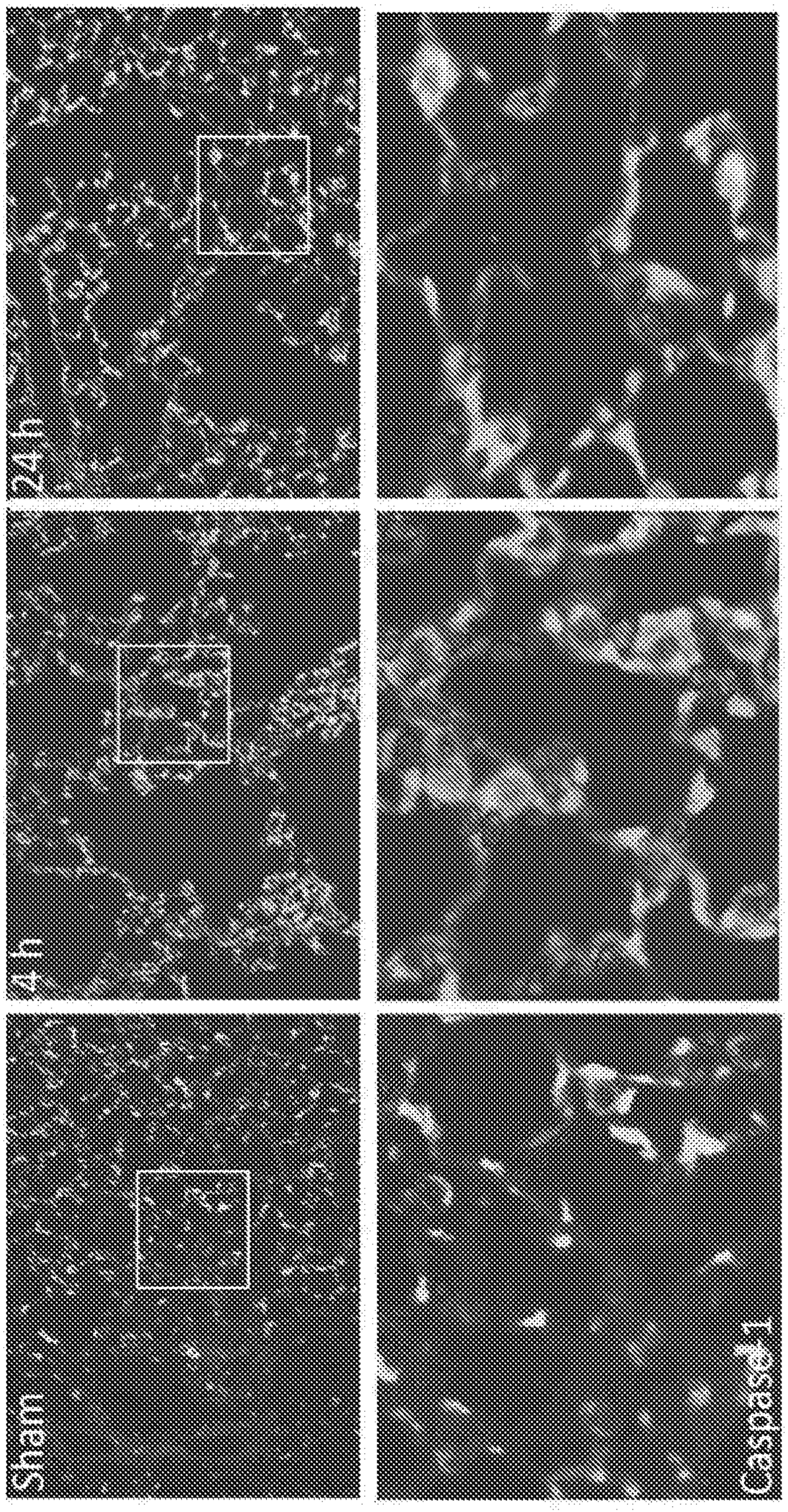


FIG. 2B

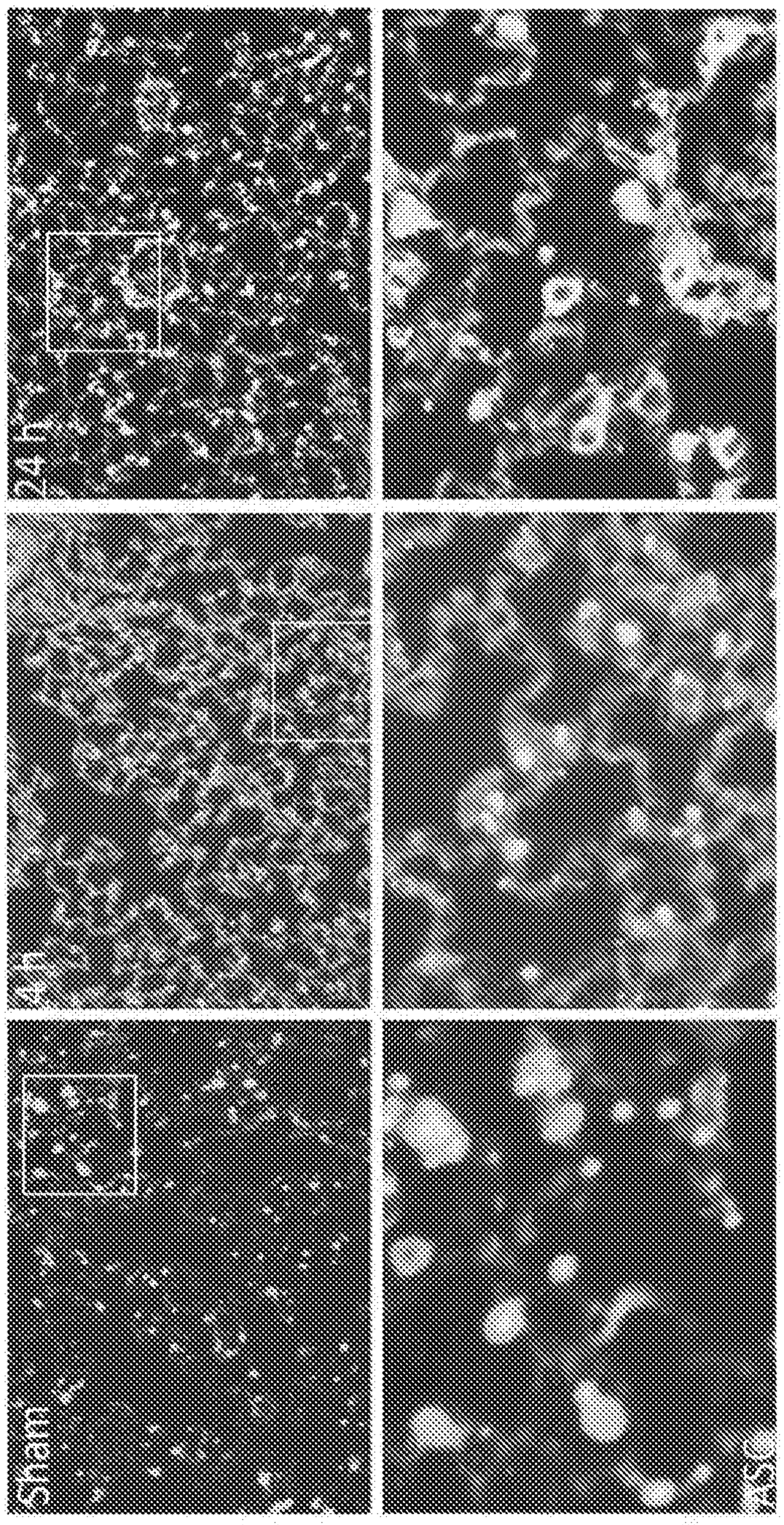


FIG. 2C

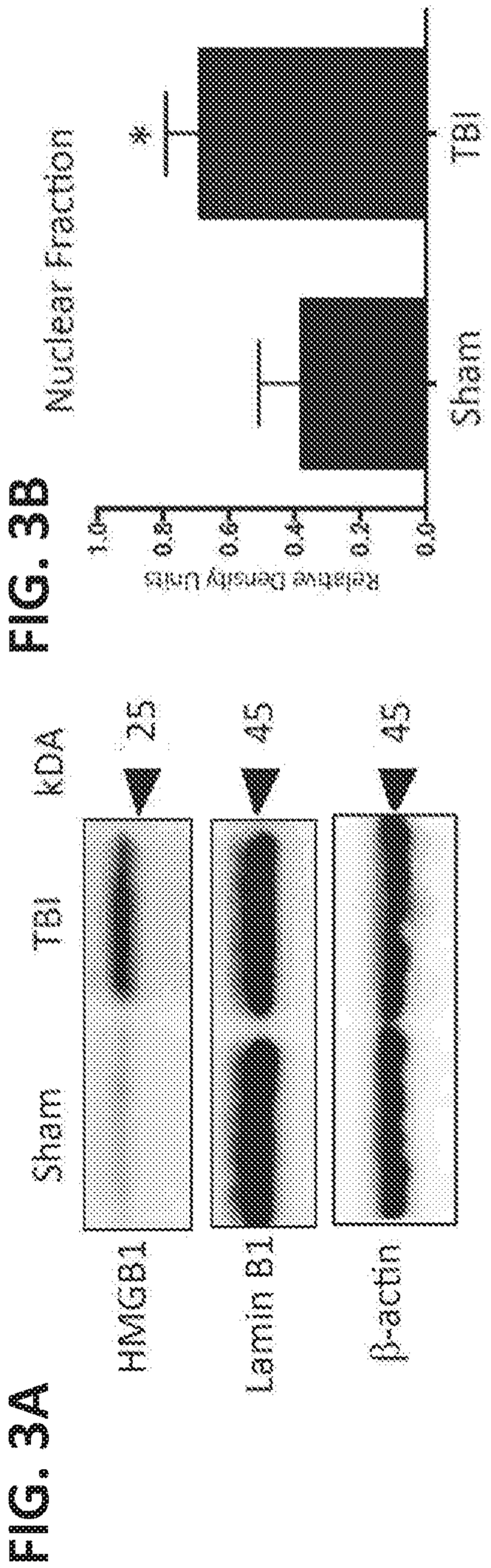


FIG. 3A

FIG. 3B

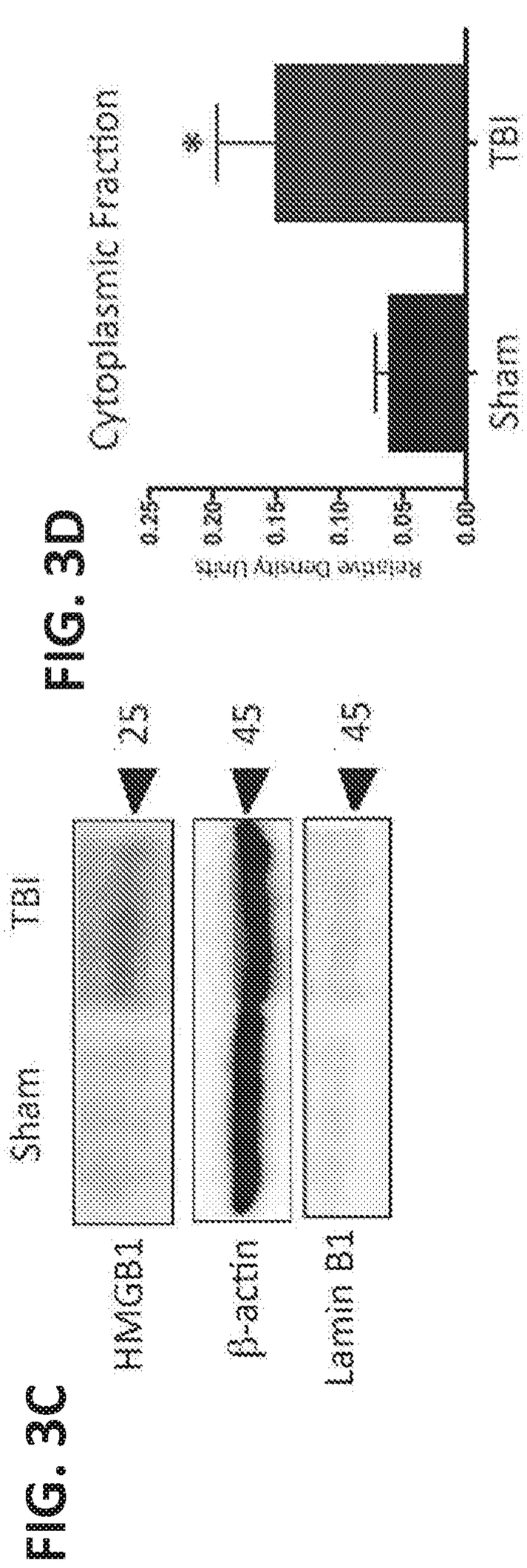


FIG. 3C

FIG. 3D

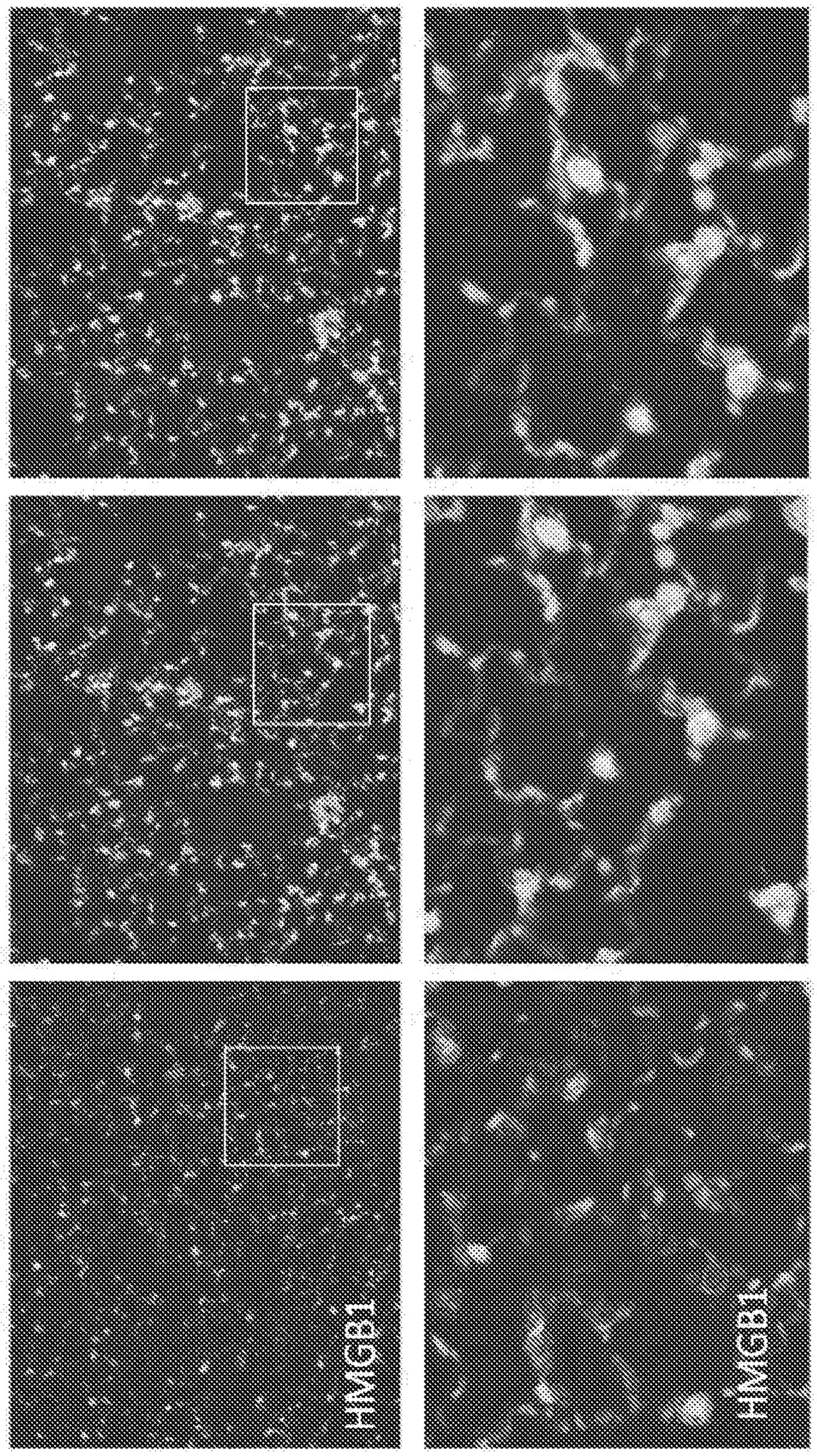


FIG. 3E

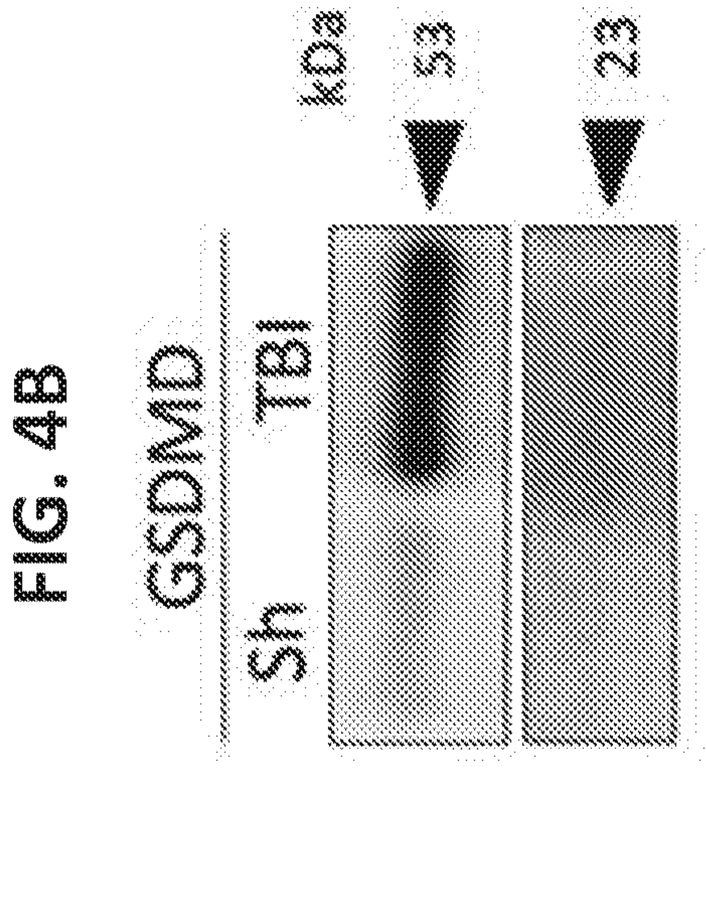


FIG. 4B

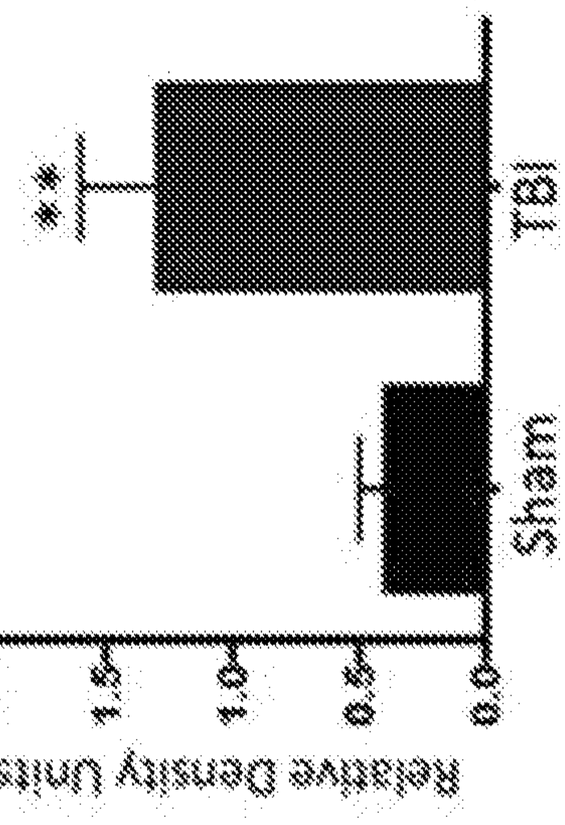


FIG. 4C

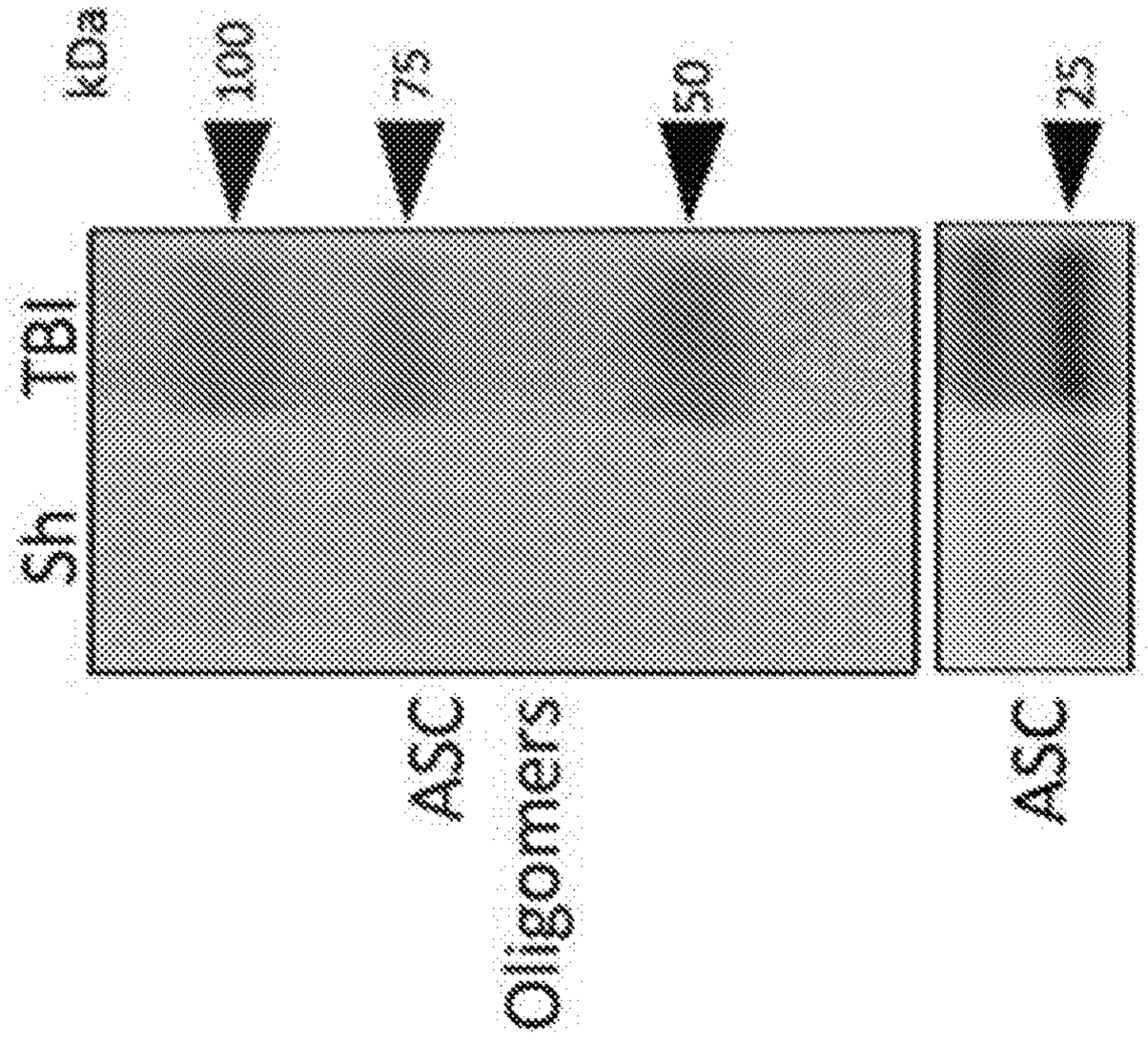


FIG. 4A

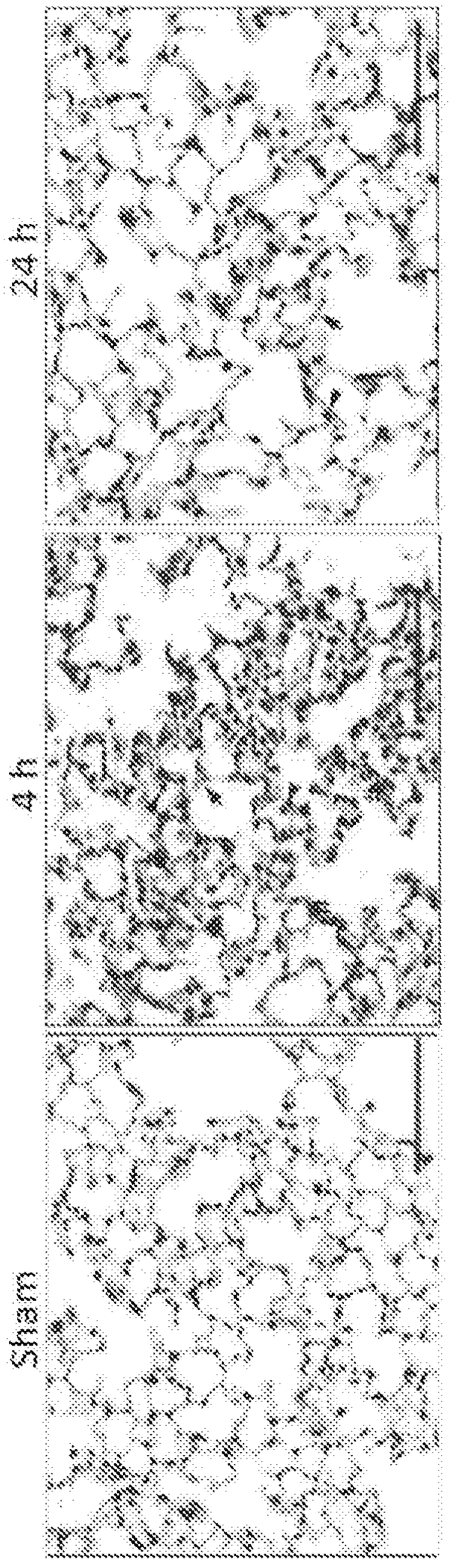


FIG. 5A

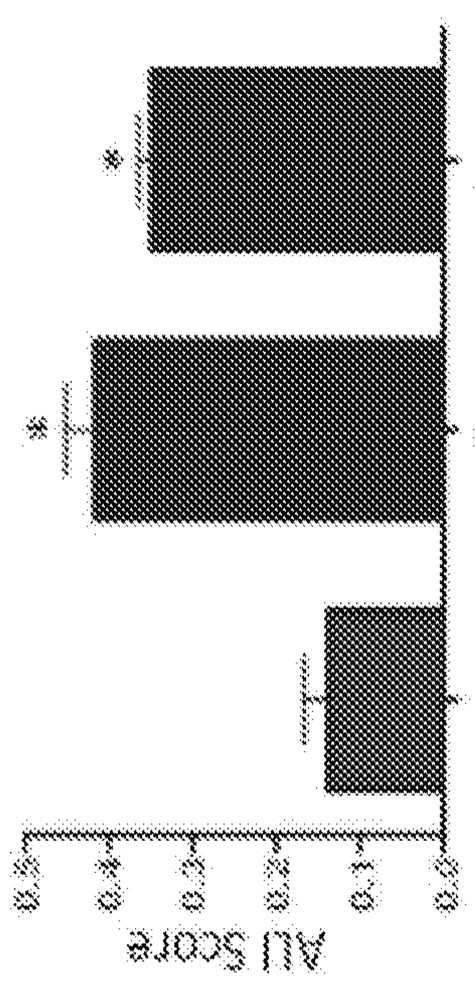
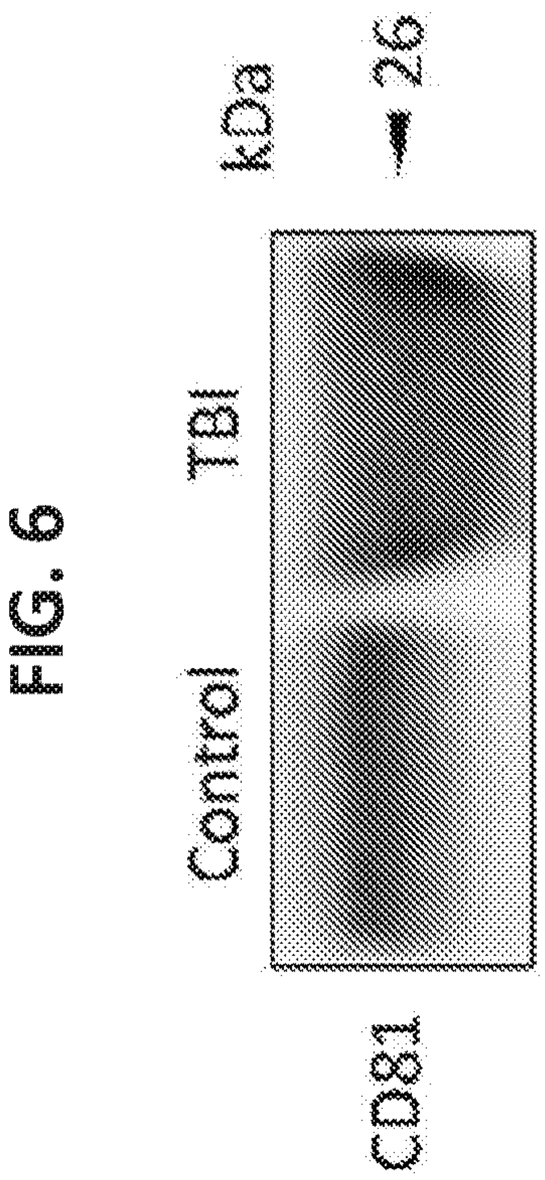


FIG. 5B

FIG. 6



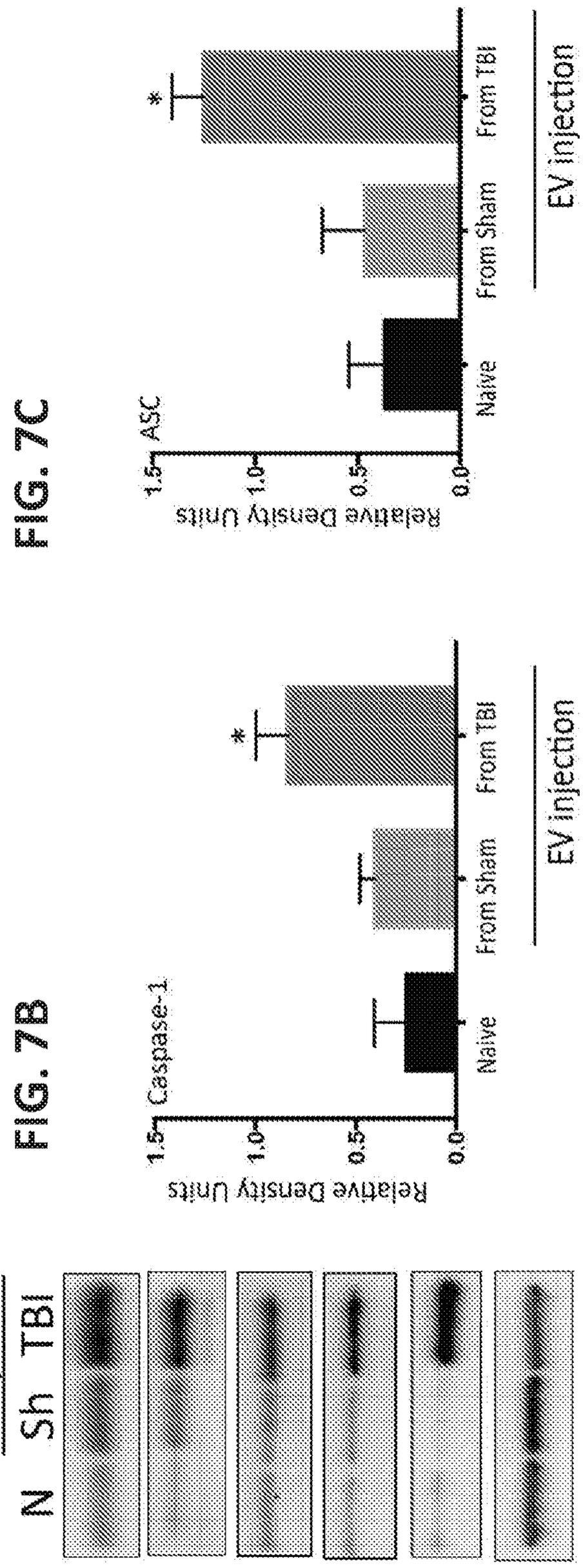


FIG. 7A
EV Injection
N Sh TBI
Caspase-1
ASC
IL-18
AIM 2
HMGB1
β-actin
Relative Density Units
FIG. 7B
Caspase-1
Relative Density Units
1.5
1.0
0.5
0.0
Naive
From Sham
From TBI
*
EV injection
FIG. 7C
ASC
Relative Density Units
1.5
1.0
0.5
0.0
Naive
From Sham
From TBI
*
EV injection

FIG. 7F

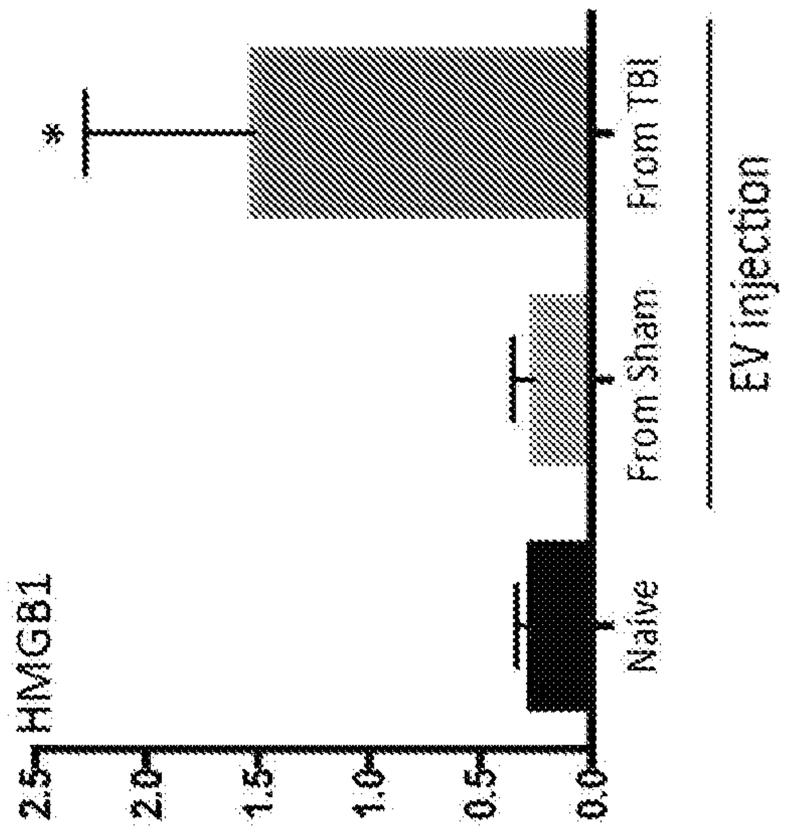


FIG. 7E

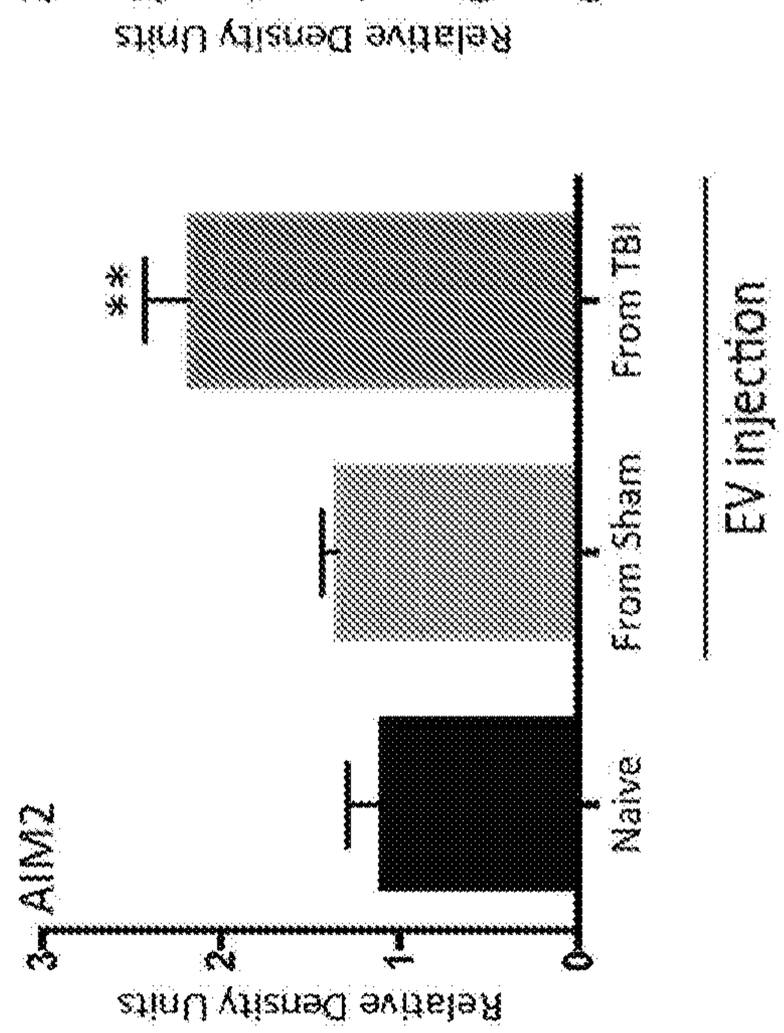
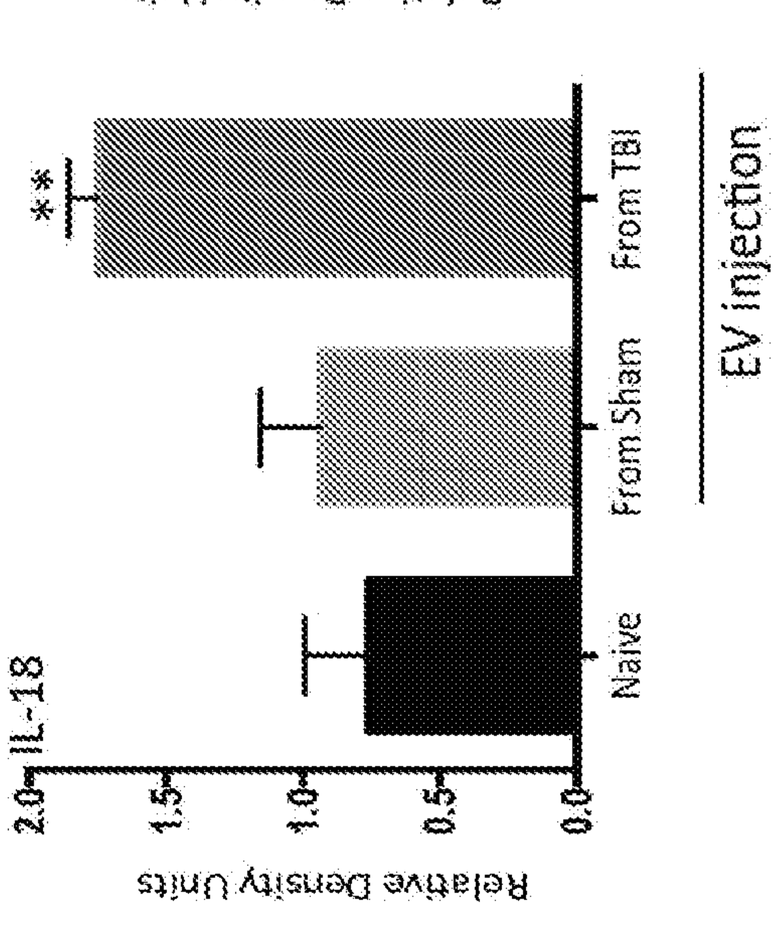


FIG. 7D



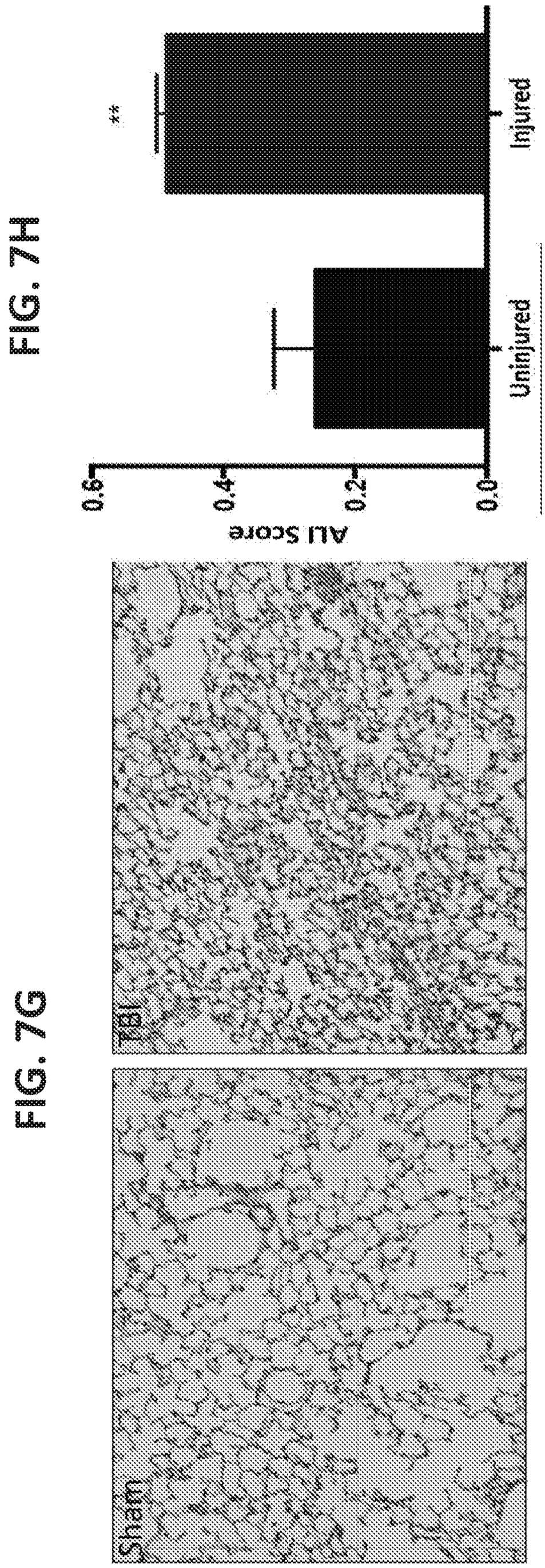


FIG. 7H
**
Injured
Uninjured
*
0.6
0.4
0.2
0.0
ALI Score
7G
TBI
FIG. 7I
Sham

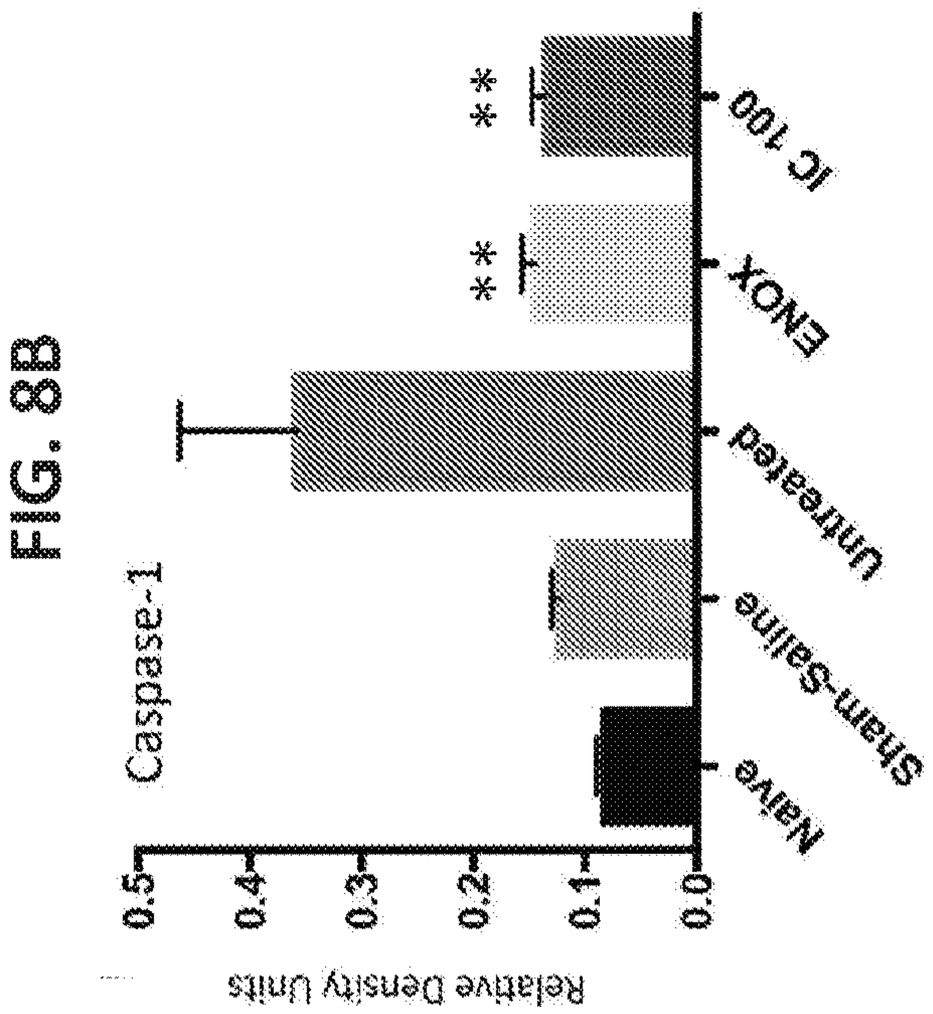


FIG. 8B
Caspase-1
Relative Density Units
0.5
0.4
0.3
0.2
0.1
0.0
Naive
Sham-Saline
Untreated
ENOX
IC 100
**
**

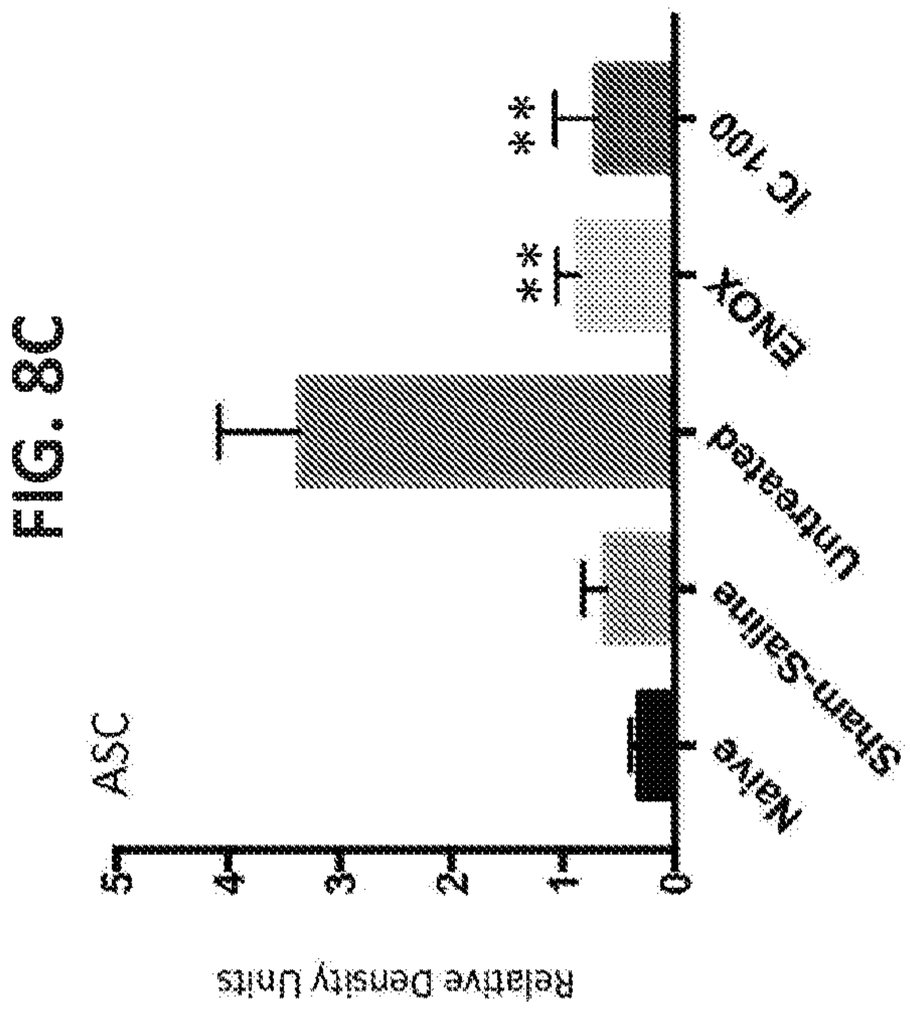


FIG. 8C
ASC
Relative Density Units
5
4
3
2
1
0
Naive
Sham-Saline
Untreated
ENOX
IC 100
**
**

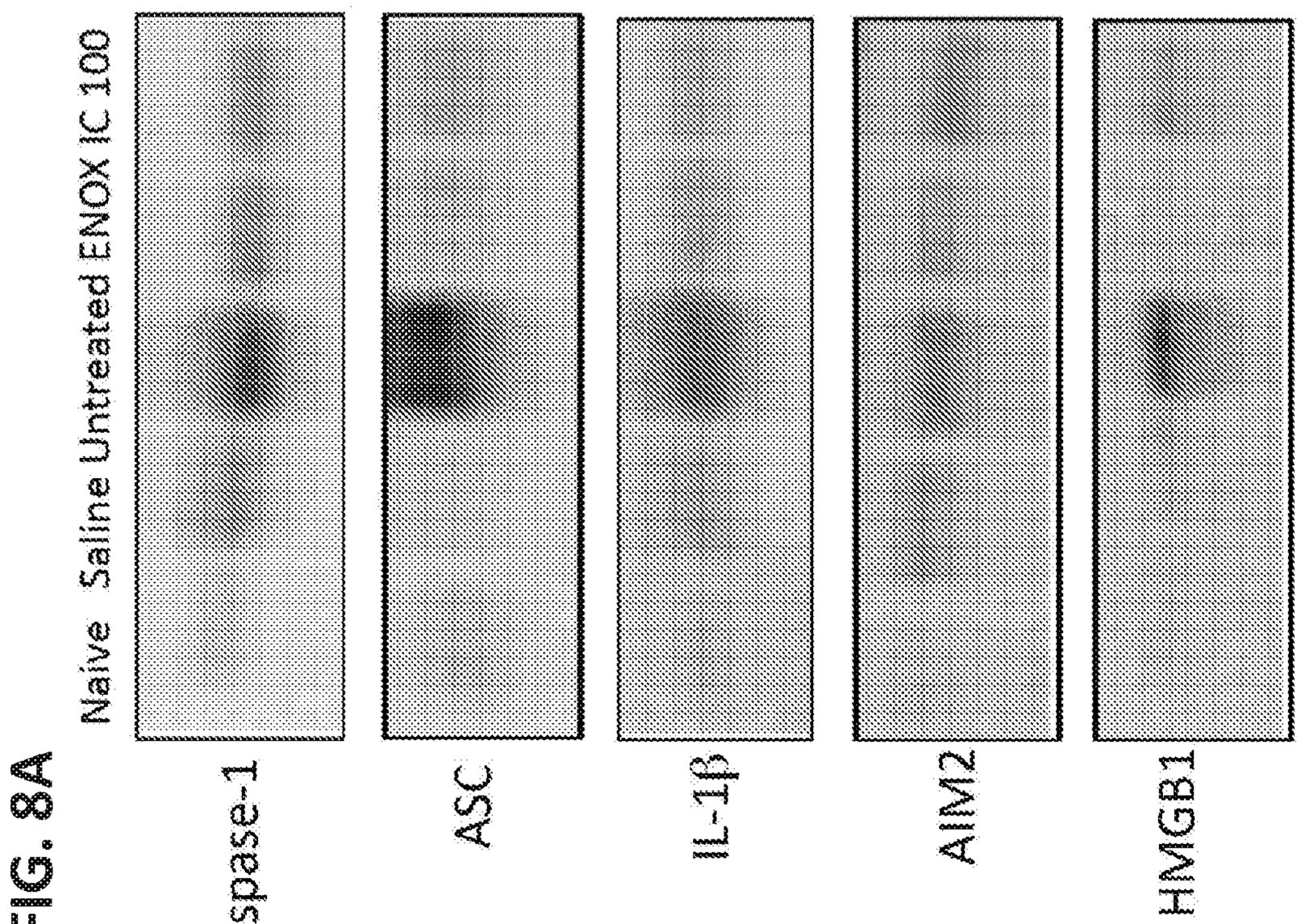


FIG. 8A
Naive Saline Untreated ENOX IC 100
Caspase-1
ASC
IL-1β
AIM2
HMGB1

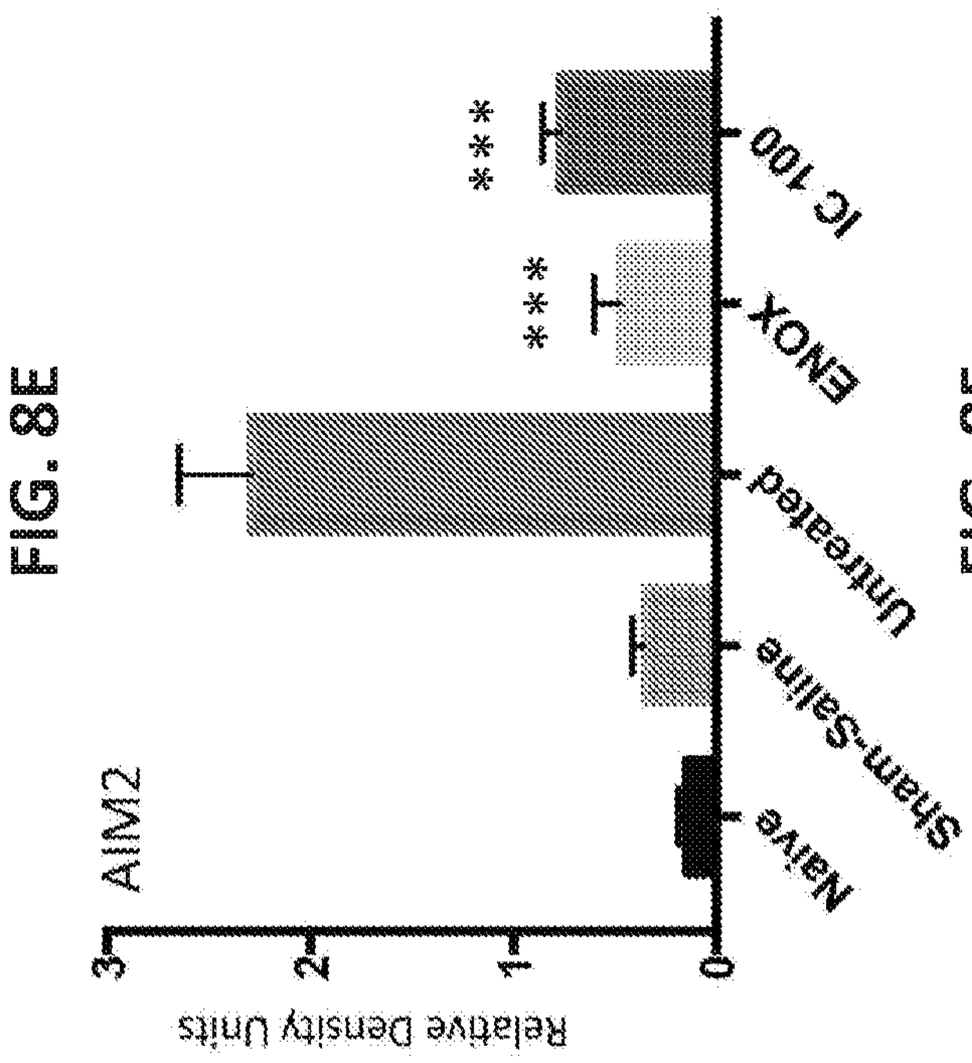


FIG. 8E

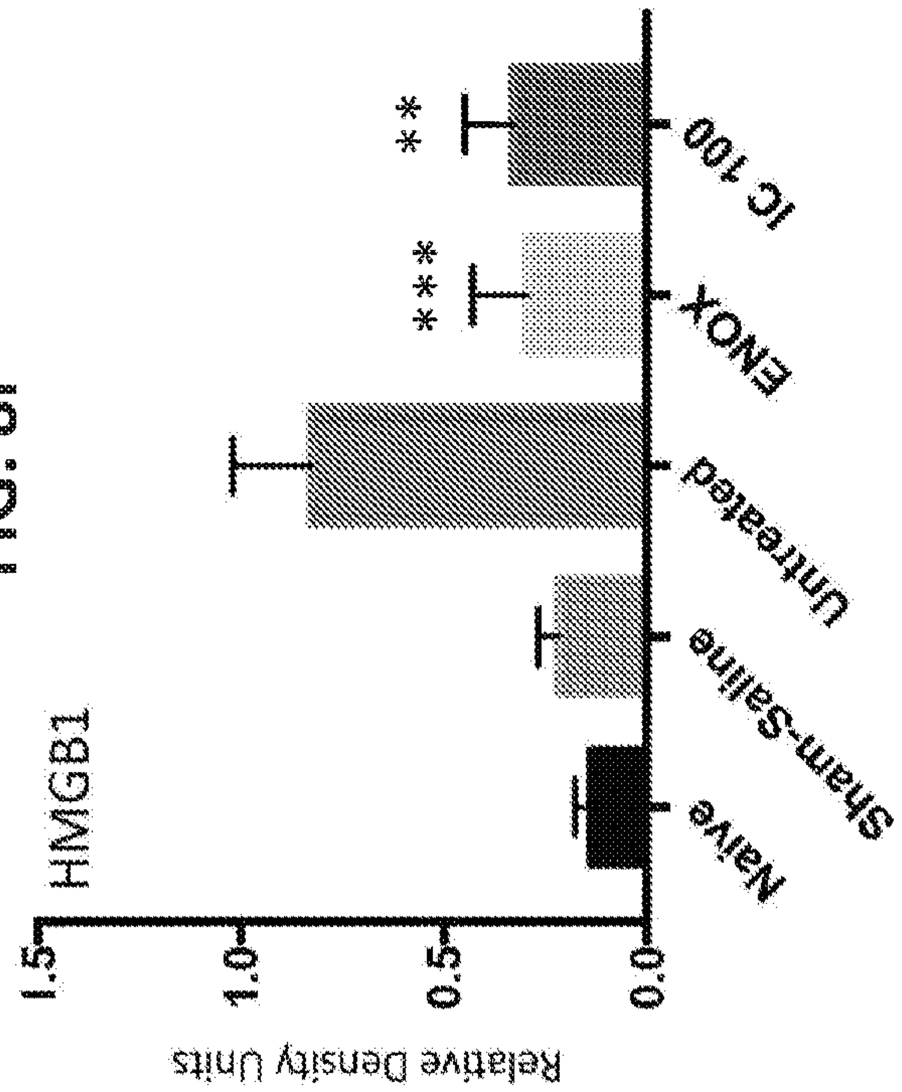


FIG. 8F

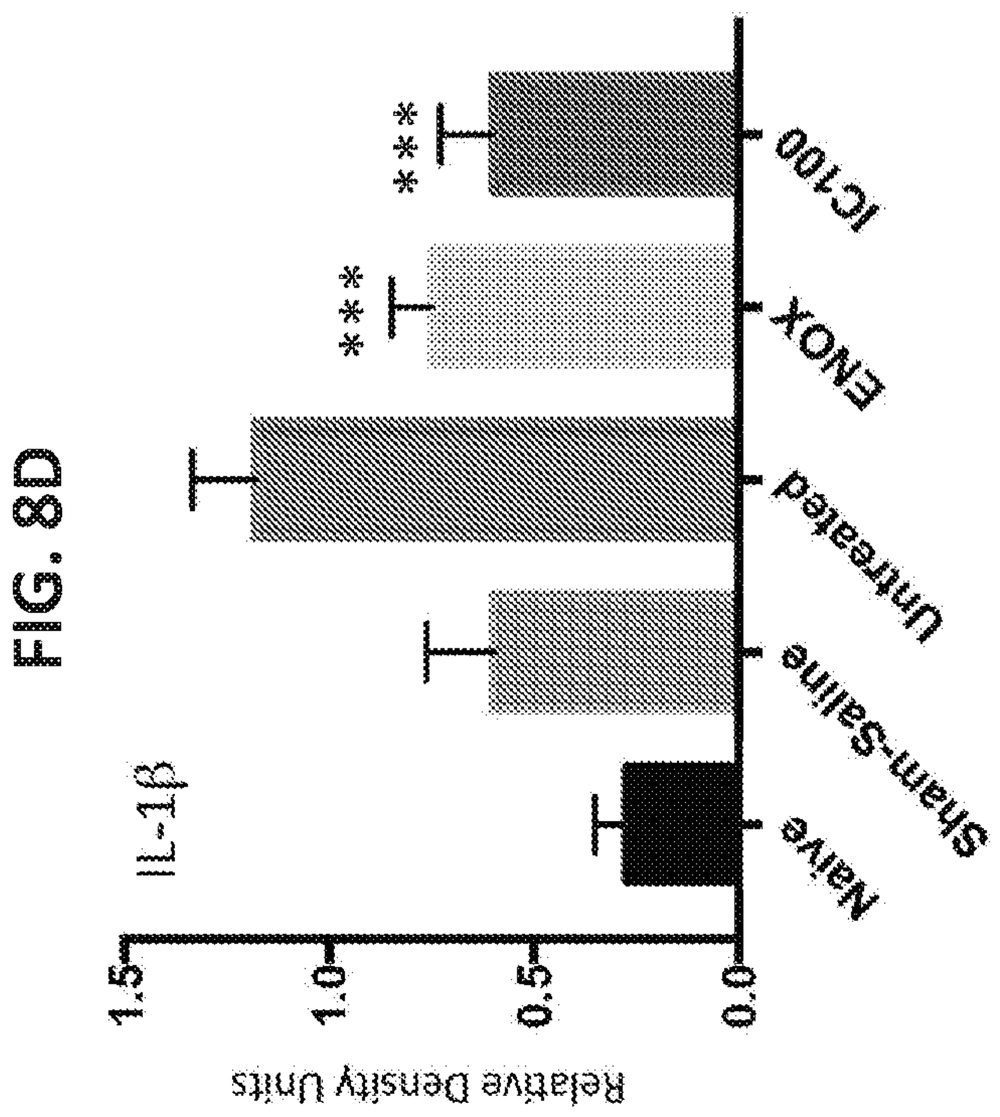


FIG. 8D

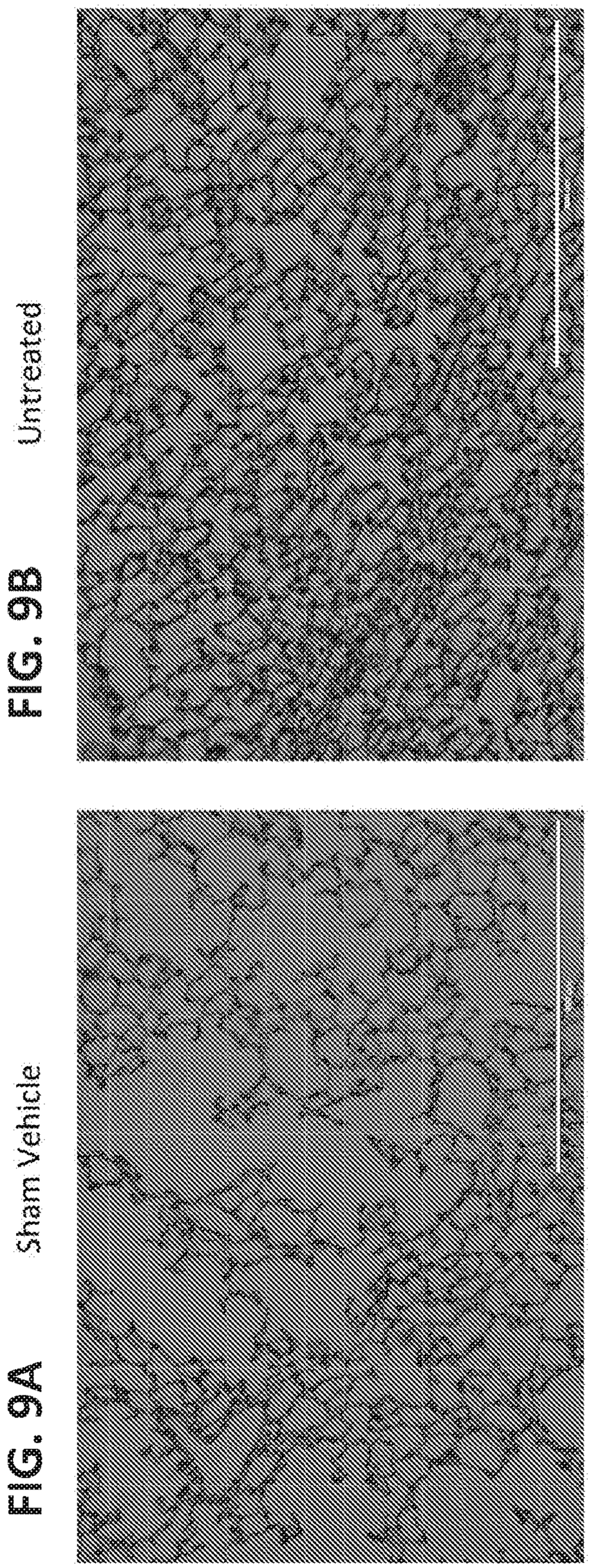


FIG. 9A

FIG. 9B

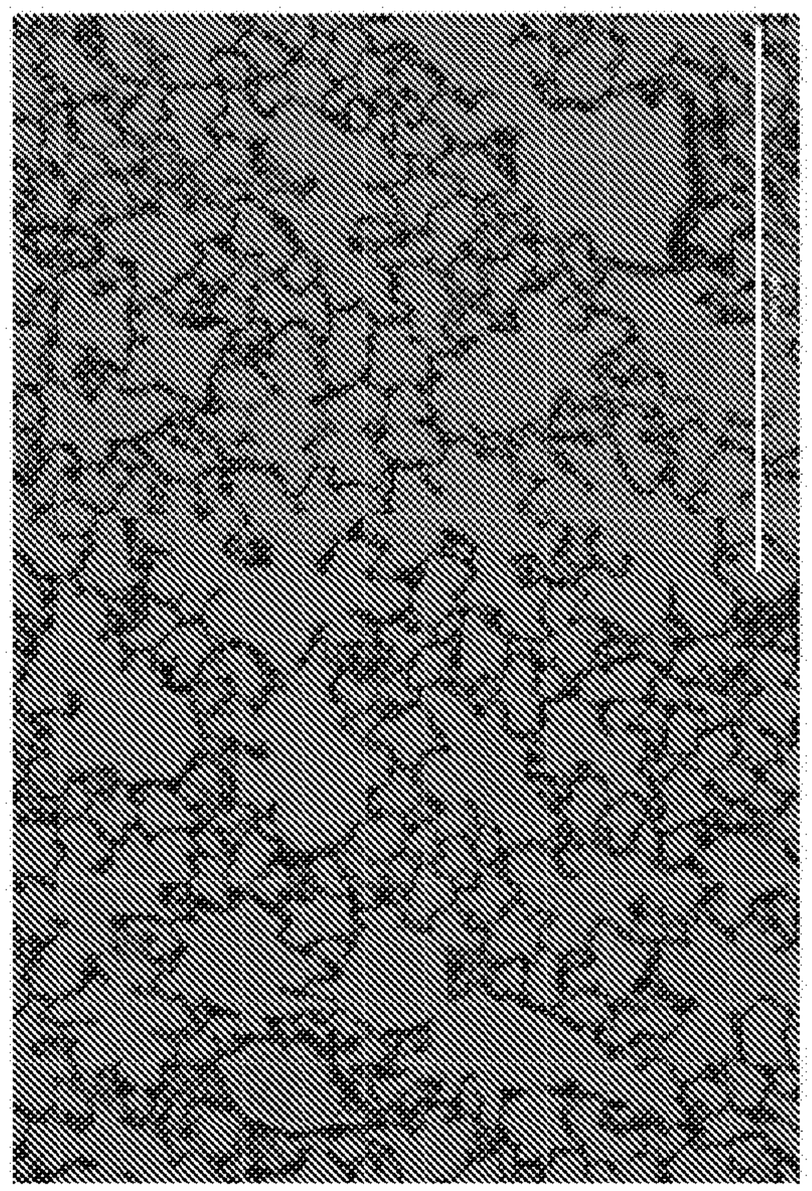


FIG. 9C

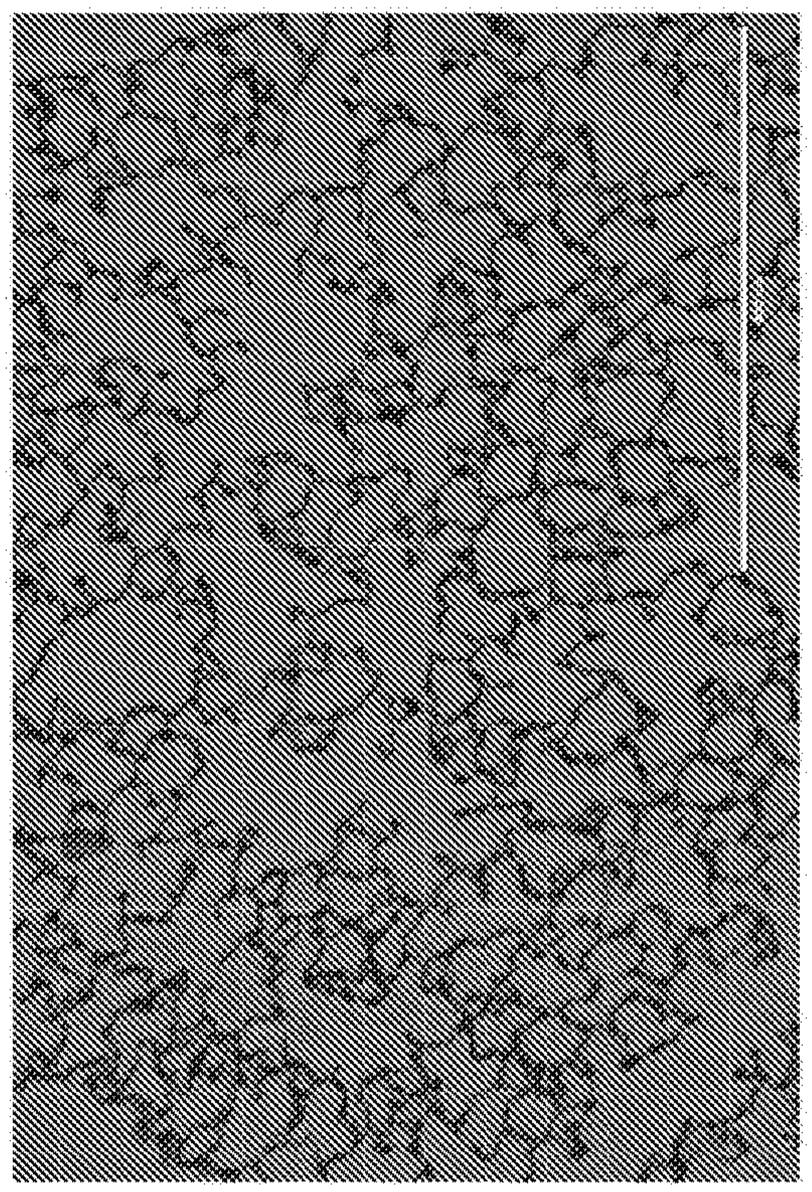


FIG. 9D

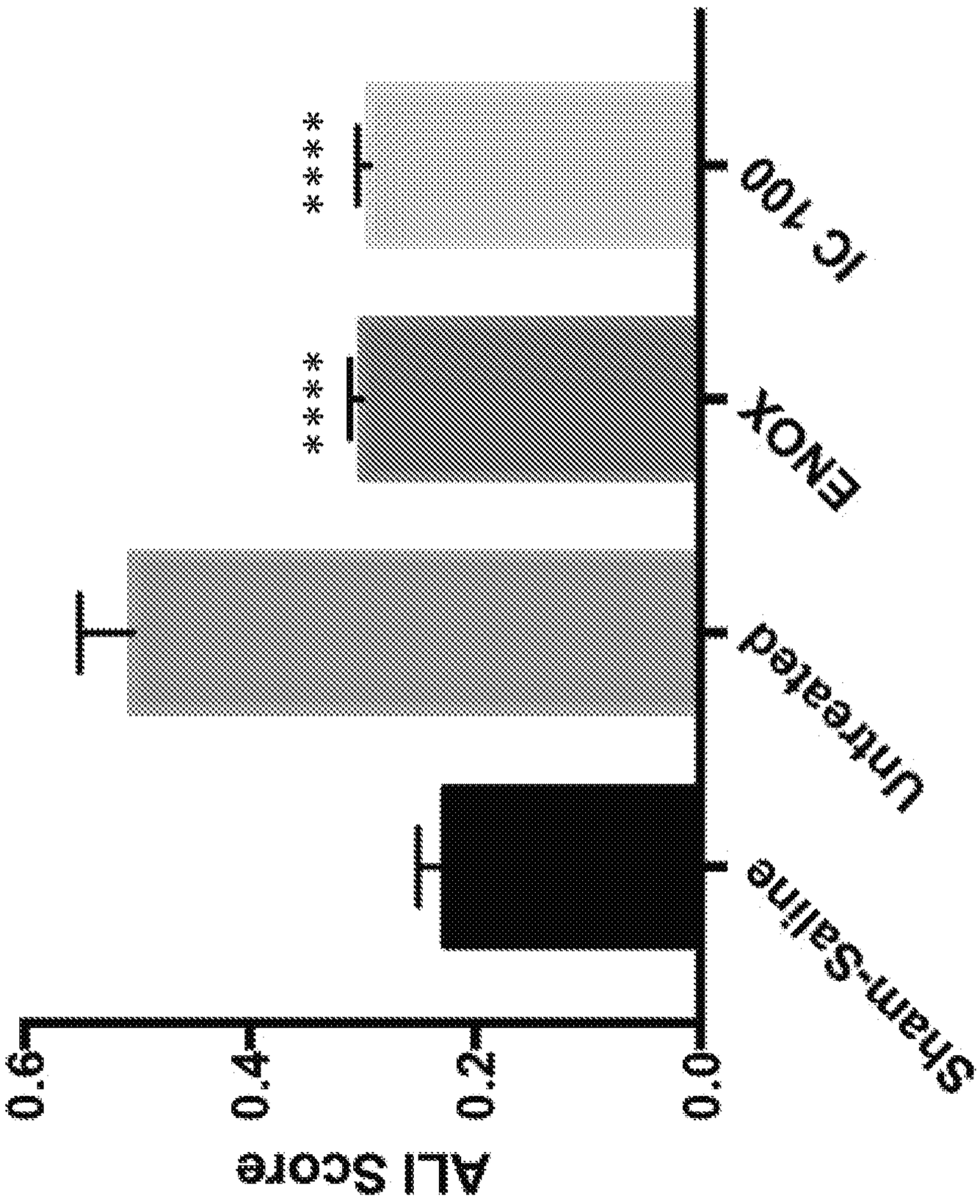


FIG. 9E

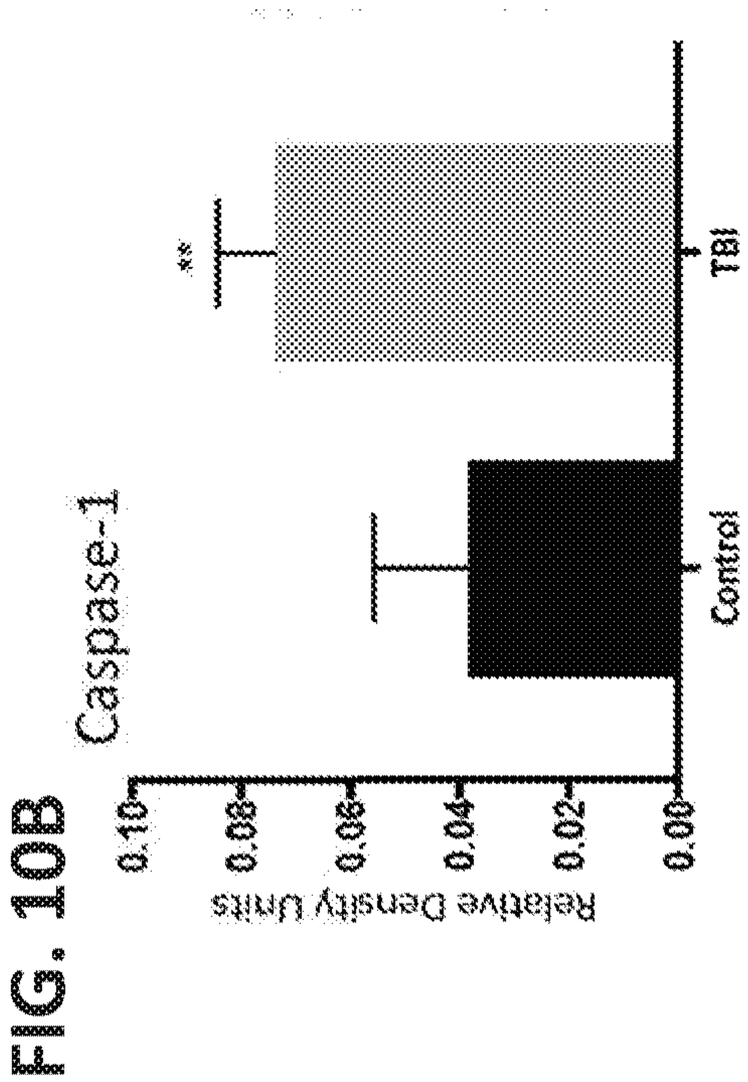


FIG. 10B

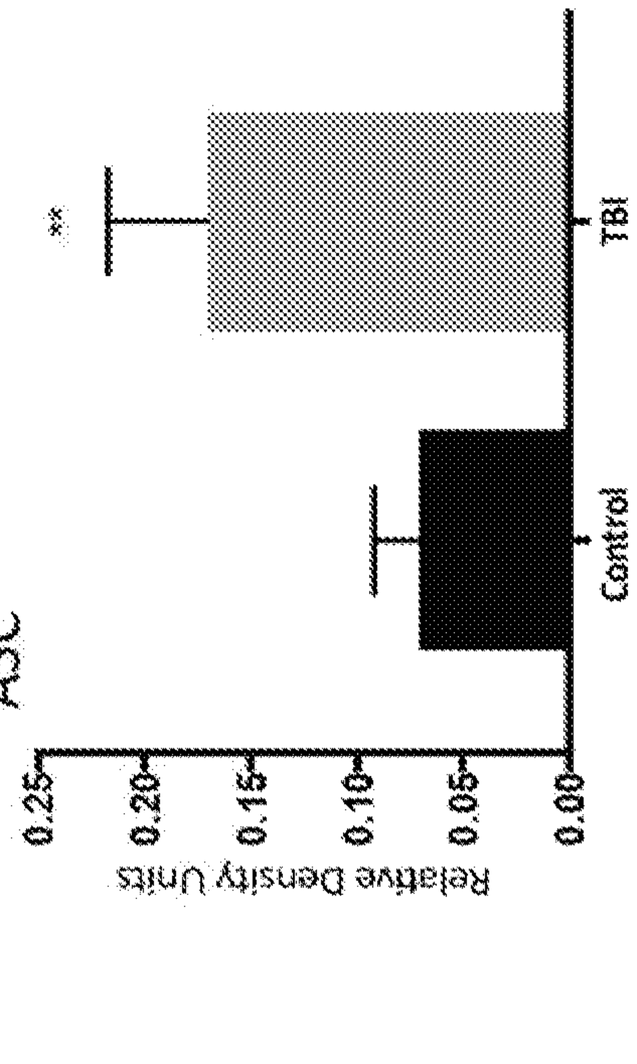


FIG. 10C

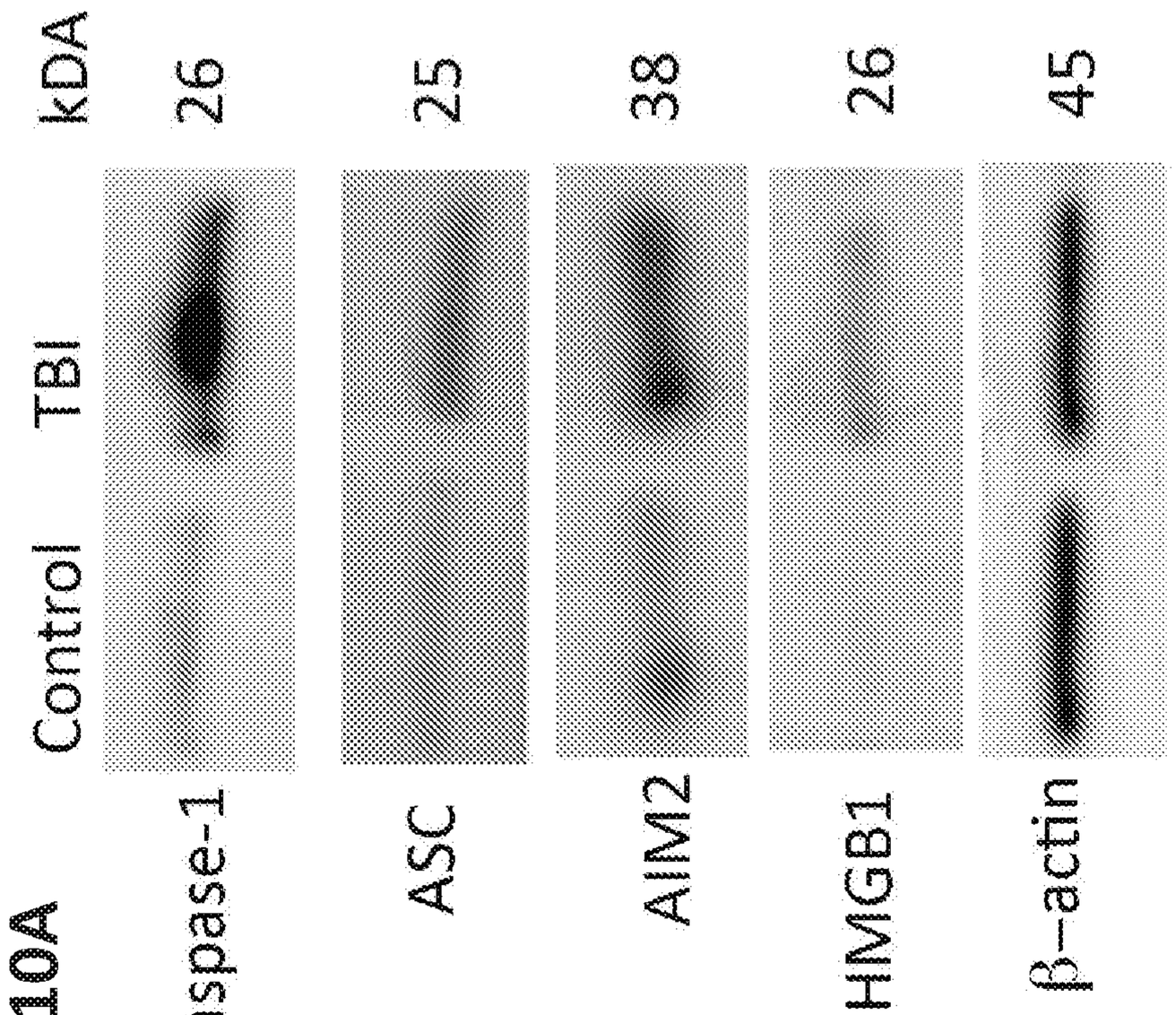


FIG. 10A

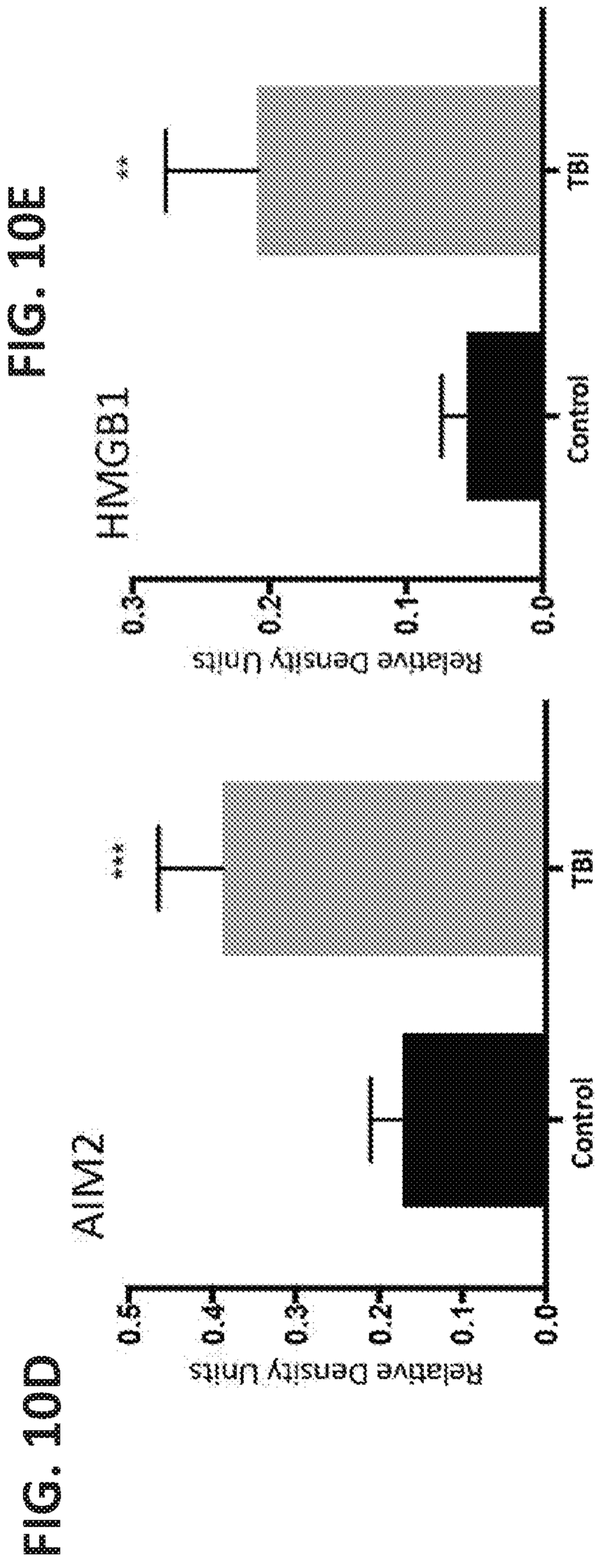


FIG. 10E

FIG. 10D

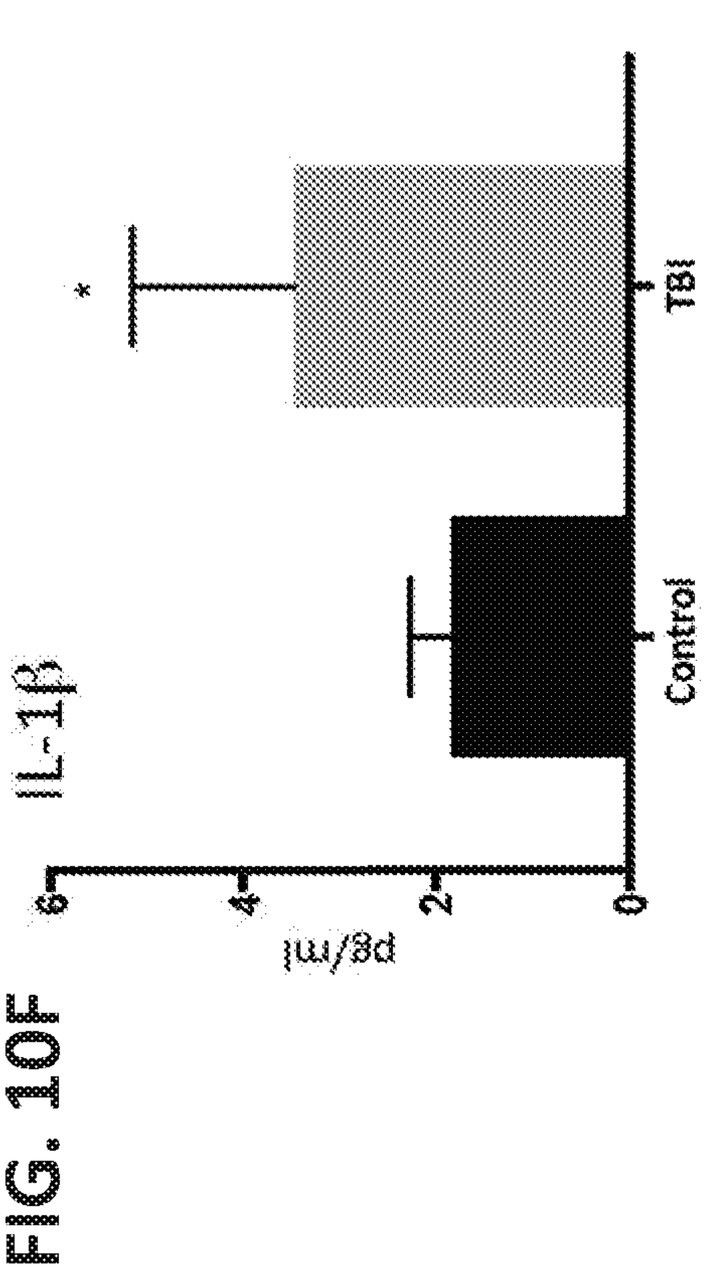


FIG. 10F

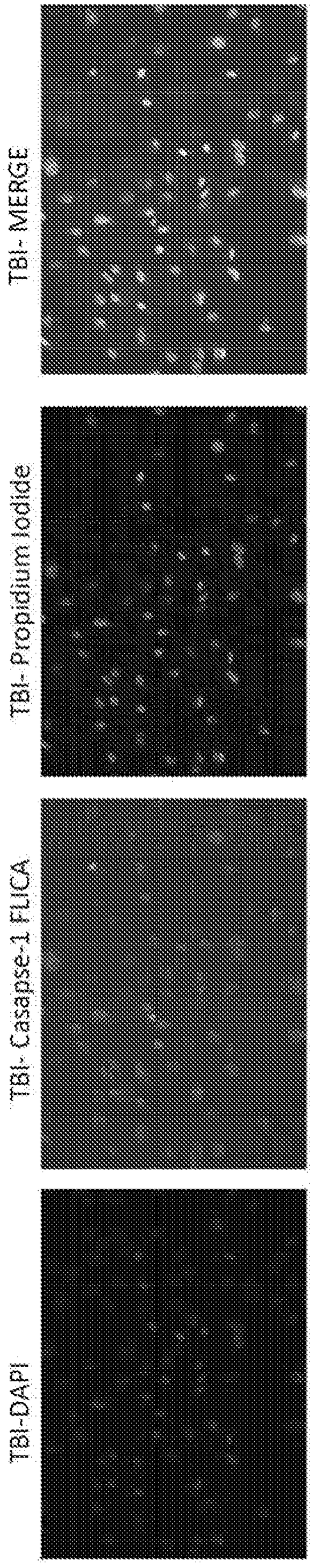


FIG. 11A

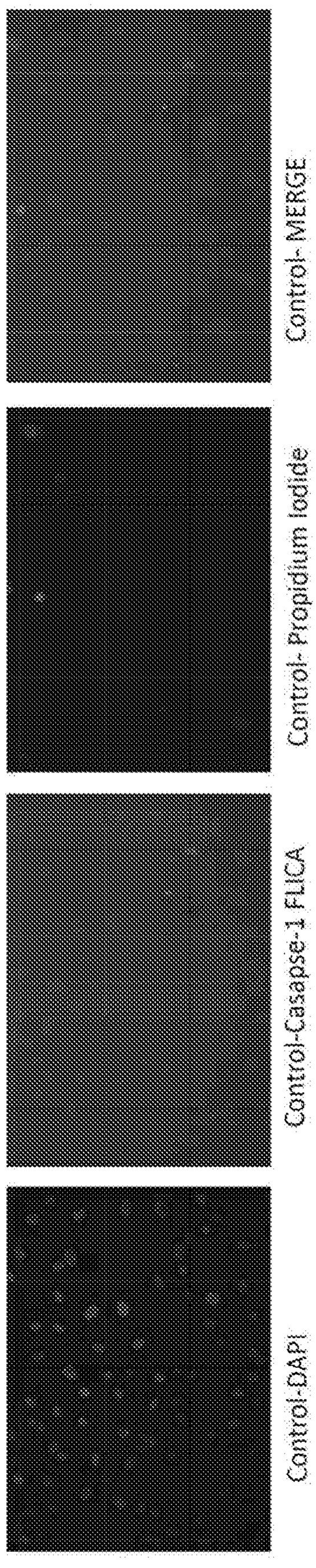
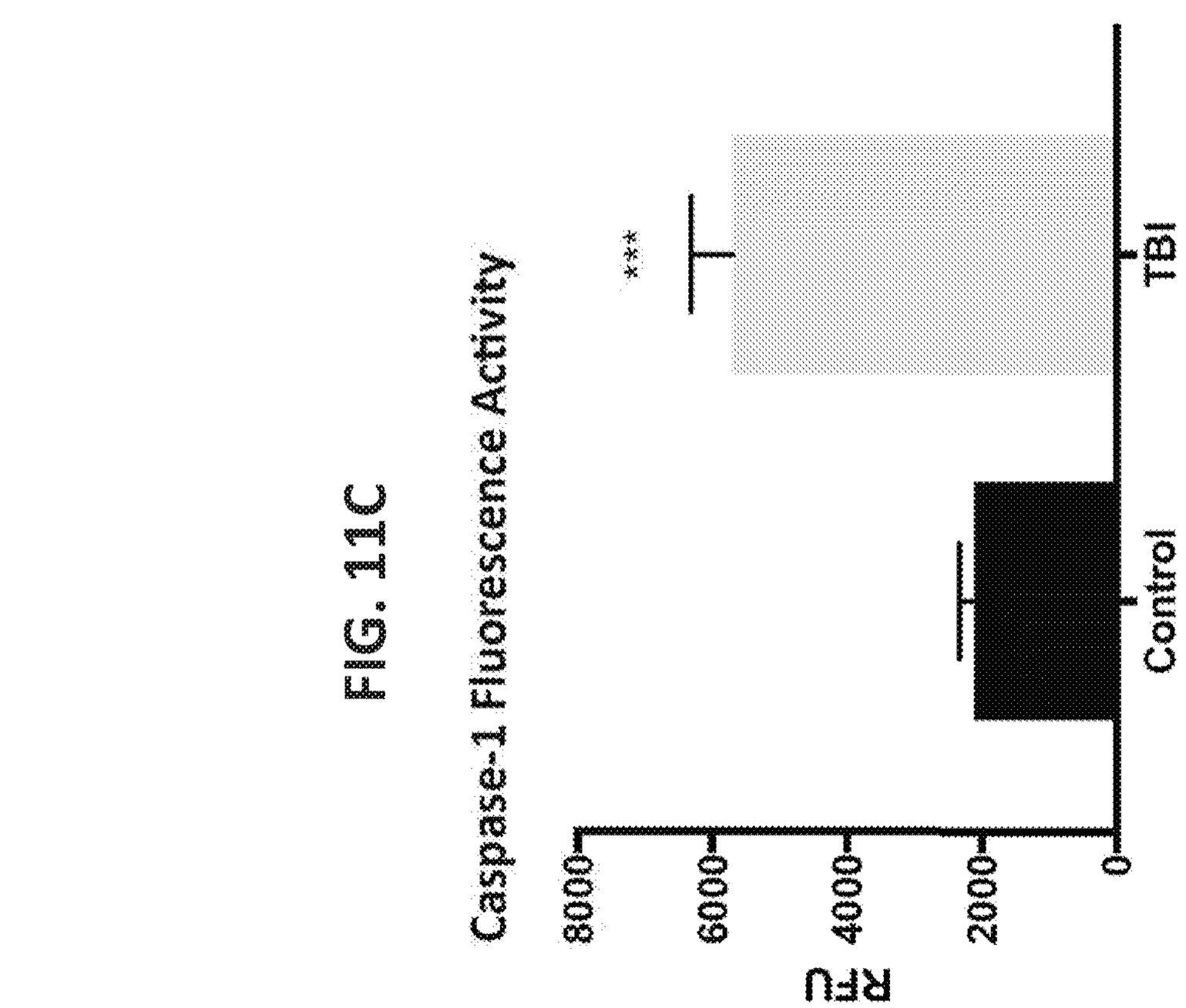


FIG. 11B

FIG. 11C


Caspase-1 Fluorescence Activity
RFU
8000
6000
4000
2000
0
***
Control
TBI

METHODS FOR MODULATING INFLAMMASOME ACTIVITY AND INFLAMMATION IN THE LUNG

[0001] This application claims priority from U.S. Provisional Application Serial No. 62/440,180, filed Dec. 29, 2016, which is herein incorporated by reference in its entirety for all purposes.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with U.S. government support under grant number 4R42BS086274-02 awarded by the National Institute of Neurological Disorders and Stroke (NINDS). The U.S. government has certain rights in the invention.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0003] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: UNMI_010_01WO_SeqList_ST25.txt, date recorded: Dec. 28, 2017, file size 2 kilobytes).

FIELD

[0004] The invention relates generally to the fields of immunology and medicine. More particularly, the invention relates to compositions and methods for modulating ASC (Apoptosis-associated Speck-like protein containing a Caspase Activating Recruitment Domain (CARD)) activity and Absent in Melanoma 2 (AIM2) inflammasome activity in the lungs of a mammal as treatments for reducing inflammation in response to conditions that produce inflammation in the lungs.

BACKGROUND

[0005] Severe Traumatic Brain Injury (TBI) is a major public health concern and is a leading cause of mortality and morbidity throughout the world (3). In addition to direct injury to the brain, TBI may lead to complications in other organs, such as the lungs. Acute Lung Injury (ALI; 2) is a common cardiopulmonary problem after trauma and is associated with a hospital mortality rate of up from 40% (4). TBI patients, in particular, are susceptible to develop ALI, with some studies reporting an incidence as high as 30% (5). Recent studies have shown that systemic inflammatory factors may lead to pulmonary dysfunction and lung injury after TBI (6), but the precise molecular mechanism underlying TBI-induced lung injury remain poorly defined.

[0006] A flood of secreted inflammatory mediators, including cytokines, chemokines, and damage-associated molecular patterns (DAMPs) released by injured cells contribute to brain inflammation and affect distal organs such as the lungs (5). One of the most widely studied DAMPs is the high mobility group box-1 (HMGB1), which can serve as an early mediator of inflammation in various pathogenic states, including TBI (7). A more recent study has shown that HMGB1 can be involved in the mechanism of TBI-induced pulmonary dysfunction (8). HMGB1 release can be regulated by the inflammasome (9), a multi-protein complex involved in the activation of caspase-1 and the processing of IL-1β and IL-18 after TBI (10).

[0007] A variety explanations have been put forth to explain pathomechanisms of pulmonary complications after TBI, including increased vascular permeability leading to capillary leakage and infiltration of proteinaceous debris (11). Extracellular vesicles (EV) are membrane-contained vesicles that play a role in cell-to-cell communication (12) and have been implicated to play a role in the development ALI in a LPS-induced murine model. Further, it has been shown that EV can carry bioactive cytokines such as IL-1β and inflammasome proteins (13) (14), and may trigger an immune response and amplify inflammation via its cargo to neighboring and surrounding cells. However, it is unknown if EV-mediated inflammasome signaling can contribute to the pathomechanism of TBI-induced ALI. Further, it is also unknown whether the pathomechanisms of TBI-induced ALI are shared by other conditions that produce lung inflammation. In addition, there is a scarcity of Federal Drug Administration (FDA) approved drugs to treat lung inflammation.

[0008] Accordingly, there is an urgent need not only for elucidating the pathomechanisms of lung inflammation caused by TBI as well as other conditions, but also the development of therapeutic compositions and uses thereof for treating and/or preventing lung inflammation.

SUMMARY

[0009] In one aspect, provided herein is a method of treating inflammation in lungs of a patient in need thereof, the method comprising: administering to the patient a composition comprising an agent that inhibits inflammasome signaling, whereby the inflammation in the lungs of the patient is treated. In some cases, the inflammation in the lungs is caused by a condition selected from a central nervous system (CNS) injury, a neurodegenerative disease, an autoimmune disease, asthma, chronic obstructive pulmonary disease, cystic fibrosis, interstitial lung disease and acute respiratory distress syndrome. In some cases, the CNS injury is selected from the group consisting of traumatic brain injury (TBI), stroke and spinal cord injury (SCI). In some cases, the neurodegenerative disease is selected from the group consisting of amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and Parkinson's disease (PD). In some cases, the administration of the composition results in inhibition of inflammasome activation in lung cells of the patient. In some cases, the administration of the composition results in a reduction of caspase-1, nucleotide-binding leucine-rich repeat pyrin domain containing protein 1 (NLRP1), nucleotide-binding leucine-rich repeat pyrin domain containing protein 2 (NLRP2), nucleotide-binding leucine-rich repeat pyrin domain containing protein 3 (NLRP3), NLR family CARD domain-containing protein 4 (NLRC4), caspase-11, X-linked inhibitor of apoptosis protein (XIAP), pannexin-1, Apoptosis-associated Spec-like protein containing a Caspase Activating Recruitment Domain (ASC), interleukin-18 (IL-18), high mobility group box 1 (HMGB1) or absent in melanoma 2 (AIM2) levels in lung cells of the patient as compared to a control, wherein the control is an untreated patient. In some cases, the lung cells are Type II alveolar cells. In some cases, the administration of the composition results in a reduction in acute lung injury (ALI) as compared to a control, wherein

the control is an untreated patient. In some cases, the reduction in ALI is evidenced by a reduction in neutrophil infiltration into alveolar and/or interstitial space, reduced or absent alveolar septal thickening or a combination thereof. In some cases, the agent is an extracellular vesicle (EV) uptake inhibitor, an antibody that binds to an inflammasome component or a combination thereof. In some cases, the EV uptake inhibitor is a compound or an antibody, wherein the antibody is selected from Table 1. In some cases, the agent is an EV uptake inhibitor in combination with an antibody that binds to an inflammasome component. In some cases, the EV uptake inhibitor is a heparin. In some cases, the heparin is Enoxaparin. In some cases, the antibody that binds to an inflammasome component is an antibody that specifically binds to a component of a mammalian AIM2, NLRP1, NLRP2, NLRP3 or NLRC4 inflammasome. In some cases, the inflammasome component is caspase-1, ASC or AIM2. In some cases, the inflammasome component is ASC. In some cases, the antibody binds to an N-terminal PYRIN-PAAD-DAPIN domain (PYD), C-terminal caspase-recruitment domain (CARD) domain or an epitope derived from the PYD or CARD domain of the ASC protein. In some cases, the antibody binds to a protein having at least 85% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2. In some cases, the antibody inhibits ASC activity in the lungs of the patient. In some cases, the composition is formulated with a pharmaceutically acceptable carrier or diluent. In some cases, the composition is administered intracerebroventricularly, intraperitoneally, intravenously or by inhalation.

[0010] In another aspect, provided herein is a method of treating inflammation in lungs of a patient that has been subjected to a central nervous system (CNS) injury, the method comprising: administering to the patient a composition comprising an agent that inhibits inflammasome signaling, whereby the inflammation in the lungs of the patient is treated. In some cases, the CNS injury is selected from the group consisting of traumatic brain injury (TBI), stroke and spinal cord injury (SCI). In some cases, the administration of the composition results in inhibition of inflammasome activation in lung cells of the patient. In some cases, the administration of the composition results in a reduction of caspase-1, NLRP1, NLRP2, NLRP3, NLRC4, caspase-11, XIAP, pannexin-1, Apoptosis-associated Spec-like protein containing a Caspase Activating Recruitment Domain (ASC), interleukin-18 (IL-18), high mobility group box 1 (HMGB1) or absent in melanoma 2 (AIM2) levels in lung cells of the patient as compared to a control, wherein the control is an untreated patient. In some cases, the lung cells are Type II alveolar cells. In some cases, the administration of the composition results in a reduction in acute lung injury (ALI) as compared to a control, wherein the control is an untreated patient. In some cases, the reduction in ALI is evidenced by a reduction in neutrophil infiltration into alveolar and/or interstitial space, reduced or absent alveolar septal thickening or a combination thereof. In some cases, the agent is an extracellular vesicle (EV) uptake inhibitor, an antibody that binds to an inflammasome component or a combination thereof. In some cases, the EV uptake inhibitor is a compound or an antibody, wherein the antibody is selected from Table 1. In some cases, the agent is an EV uptake inhibitor in combination with an antibody that binds to an inflammasome component. In some cases, the EV uptake inhibitor is a heparin. In some cases, the heparin is Enoxaparin. In some cases, the antibody that binds to an inflammasome component is an antibody that specifically binds to a component of a mammalian AIM2, NLRP1, NLRP2, NLRP3 or NLRC4 inflammasome. In some cases, the inflammasome component is caspase-1, ASC or AIM2. In some cases, the inflammasome component is ASC. In some cases, the antibody binds to the PYD, CARD domain or an epitope derived from the PYD or CARD domain of the ASC protein. In some cases, the antibody binds to a protein having at least 85% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2. In some cases, the antibody inhibits ASC activity in the lungs of the patient. In some cases, the composition is formulated with a pharmaceutically acceptable carrier or diluent. In some cases, the composition is administered intracerebroventricularly, intraperitoneally, intravenously or by inhalation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIGS. **1**A-**1**N illustrateinflammasome activation in C57/BL6 mouse cortical and lung tissue post-TBI. FIG. **1**A shows a representative immunoblot of active caspase-1, ASC, IL-18, IL-β, HMGB1, and AIM2 after TBI. Active caspase-1 (FIG. **1**B), ASC (FIG. **1**C), IL-18 (FIG. **1**D), HMGB1 (FIG. **1**E), AIM 2 (FIG. **1**F), and IL-β, (FIG. **1**G), are significantly elevated in cortical tissue at 4 and 24 h post-TBI. Data presented as mean+/- SEM; ****$p<0.001$, ***$p<0.01$, $p<0.05$ compared to sham. N=4-5 per group. FIG. **1**H shows a representative immunoblot of active caspase-1, ASC, IL-18, IL-β, HMGB1, and AIM2 in lung tissue. I, J, K, L, M, N) Active caspase-1 (FIG. **1**I), ASC (FIG. **1**J), IL-18 (FIG. **1**K), HMGB1 (FIG. **1**L), AIM 2 (FIG. **1**M), and IL-β, (FIG. **1**N) are significantly elevated in lung tissue 4 and 24 h after TBI. Data presented as mean +/-SEM. N= 4-5 per group, **$p<0.01$., *$p<0.05$ compared to sham.

[0012] FIGS. **2**A-**2**C illustrates Expression of inflammasome proteins in Type II alveolar epithelial cells. FIG. **2**A shows AIM2, FIG. **2**B shows active Caspase-1 and FIG. **2**C shows ASC immunoreactivity increases in lung tissue after CCI (4, 24 h) when compared to mice. Confocal images of AIM2, caspase-1, and ASC (green) and type II epithelial cells (surfactant protein C, red).

[0013] FIGS. **3**A-**3**E illustrates TBI increases nuclear and cytoplasmic HMGB1 expression in mice lung. FIG. **3**A shows representative immunoblot of nuclear HMGB1 after TBI. FIG. **3**B shows nuclear HMGB1 is significantly elevated in 4 hour injured animals compared to sham. FIG. **3**C shows representative immunoblot of cytoplasmic HMGB1 after TBI. FIG. **3**D shows cytoplasmic HMGB1 is significantly elevated in 4 hour injured animals compared to sham. Data presented as mean+/- SEM; ****$p<0.001$, ***$p<0.01$, *$p<0.05$ compared to sham. N=4-5 per group. FIG. **3**E shows HMGB1 immunoreactivity increased in lung tissue after CCI when compared to sham mice. Confocal images of HMGB1 and type II epithelial cells (surfactant protein C, red)

[0014] FIGS. **4**A-**4**C illustrates Pyroptosome formation in mice lungs 4 hours post-TBI. FIG. **4**A shows TBI induces laddering of ASC in lung tissue, indicating formation of the pyroptosome, an oligomerization of ASC dimers that leads to activation of caspase-1 and pyroptosis. FIG. **4**B shows

representative immunoblot and FIG. **4**C shows quantification of gasdermin. Gasdermin-D is significantly elevated in lung tissue post-TBI. Data presented as mean+/-SEM. N= 4-5per group, **p<0.01., *p<0.05 compared to sham.

[0015] FIGS. **5**A-**5**B illustrates TBI induces alveolar morphological changes and acute lung injury in mice. FIG. **5**A shows H&E staining of lung sections from sham and injured animals at 4 h and 24 h. Sections show evidence of neutrophil infiltration (arrow heads), changes in morphology of alveolar capillary membranes (asterisk, *), interstitial edema (short arrows), and evidence of thickening of the interstitium and the alveolar septum (pound, #). FIG. **5**B shows acute lung injury scoring is significantly increased in injured animals when compared to sham at 4 h and 24 h. Data presented as mean+/-SEM. N= 4-5 per group, **p<0.01., *p<0.05 compared to sham.

[0016] FIG. **6** illustrates expression of CD81 in serum-derived EV from control and TBI-injured mice. Representative immunoblot of CD81 in serum-derived EV from sham control and TBI-injured mice.

[0017] FIGS. **7**A-**7**H illustrates adoptive transfer of EV from TBI animals induce caspase-1 and ASC in the lungs of uninjured mice. FIG. **7**A illustrates a representative immunoblot showing that caspase-1 (FIG. **7**B), ASC (FIG. **7**C), IL-18 (FIG. **7**D), AIM2 (FIG. **7**E), HMGB1 (FIG. **7**F) are elevated in the lungs of animals that received EV isolated from TBI mice when compared to EV from sham animals. Data presented as mean+/- SEM; *p<.0.05 compared to sham. N=3 per group. EV from TBI mice induced alveolar morphological changes (decreased alveolar size) and infiltration of inflammatory cells as determined by H&E staining (FIG. **7**G). ALI score is significantly increased in EV delivered from injured mice compared to uninjured mice (FIG. **7**H). Data presented as mean+/- SEM; *p<.0.05 compared to uninjured group.

[0018] FIGS. **8**A-**8**F illustrates treatment with Enoxaparin (3 mg/kg) and IC 100 (5 mg/kg) reduces inflammasome expression in lungs of animals delivered EV from injured mice. FIG. **8**A illustrates a representative immunoblot showing that caspase-1 (FIG. **8**B), ASC (FIG. **8**C), IL-1β (FIG. **8**D), AIM2 (FIG. **8**E), HMGB1 (FIG. **8**F) are reduced in the lungs of animals that were treated with Enoxaparin and IC 100 when compared to untreated positive control animals. Data presented as mean+/- SEM; *p<.0.05 compared to sham. N=4 per group.

[0019] FIGS. **9**A-**9**E illustrates treatment with Enoxaparin (3 mg/kg) and IC 100 (5 mg/kg) reduces ALI score in lungs of animals delivered EV from injured mice. FIGS. **9**A-**9**D illustrates H&E staining of lung sections from saline (FIG. **9**A), untreated (FIG. **9**B), Enoxaparin (FIG. **9**C) and IC 100 (Anti-ASC; FIG. **9**D) treated mice lungs delivered EV from injured animals. Sections show evidence of neutrophil infiltration, changes in morphology of alveolar capillary membranes, interstitial edema, and evidence of thickening of the interstitium and the alveolar septum. FIG. **9**E illustrates that acute lung injury scoring is significantly decreased in animals treated with Enoxaparin, IC 100 when compared to untreated animals. Data presented as mean+/-SEM. N= 4 per group, **p<0.01., *p<0.05.

[0020] FIGS. **10**A-**10**F illustrates delivery of serum-derived EV from TBI patients increases inflammasome protein expression in pulmonary endothelial cells. FIG. **10**A shows western blot representation of caspase-1, ASC, AIM2, HMGB1 in PMVEC after incubation with TBI-EV and control-EV for 4 hours. FIGS. **10**B-**10**E) shows quantification of western blots, n=3 filters per group, n=6 patients, t-test, p<0.05. FIG. **10**F shows immunoassay results of a significant increase in IL-1β expression using Ella simple plex assay n=3 filters per group, n=6 patients, t-test, p<0.05.

[0021] FIGS. **11**A-**11**C illustrates delivery of TBI-EV to pulmonary endothelial cells increases immunoreactivity of active caspase-1 and cell death. FIG. **11**A shows co-localization of Caspase-1 FLICA and PI staining and PMVEC incubated with TBI-EV for 4 hours. FIG. **11**B shows caspsae-1 FLICA and PI staining in PMVEC incubated with control-EV for 4 hours. FIG. **11**C shows fluorescent plate reader analysis of PMVEC incubated with TBI and control-EV for 4 hours. n=6, p<0.05.

DETAILED DESCRIPTION

Definitions

[0022] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0023] As used herein, "protein" and "polypeptide" are used synonymously to mean any peptide-linked chain of amino acids, regardless of length or post-translational modification, e.g., glycosylation or phosphorylation.

[0024] As used herein, the term "antibody" refers generally and broadly to immunoglobulins, monoclonal antibodies, and polyclonal antibodies, as well as active fragments thereof. The fragment may be active in that it binds to the cognate antigen (e.g., ASC, NLRP1, AIM2, etc.), or it may be active in that it is biologically functional. The antibodies for use herein may be chimeric, humanized, or human, using techniques known in the art.

[0025] As used herein, the term "humanized antibody" refers to an antibody in which minimal portions of a non-human antibody are introduced into an otherwise human antibody.

[0026] As used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein is substantially non-immunogenic in humans, with only minor sequence changes or variations.

[0027] An antigen binding site can be generally formed by the heavy chain variable region (VH) and the light chain variable region (VL) immunoglobulin domains, with the antigen-binding interface formed by six surface polypeptide loops, termed complimentarity determining regions (CDRs). There are three CDRs each in VH (HCDR1, HCDR2, HCDR3) and VL (LCDR1, LCDR2, LCDR3), together with framework regions (FRs).

[0028] The term "CDR region" or "CDR" can be mean the hypervariable regions of the heavy or light chains of the immunoglobulin as defined by Kabat et al., 1991 (Kabat, E. A. et al., (1991) Sequences of Proteins of Immunological Interest, 5th Edition. US Department of Health and Human Services, Public Service, NIH, Washington), and later editions. An antibody typically contains 3 heavy chain CDRs and 3 light chain CDRs.

[0029] It has been shown that fragments of a whole antibody can also bind antigens. Examples of binding fragments include: (i) an Fab fragment consisting of VL, VH, CL and CH1 domains (Ward, E. S. et al., (1989) Nature 341, 544-546); (ii) an Fd fragment consisting of the VH and CH1 domains (McCafferty et al., (1990) Nature, 348, 552-554);

(iii) an Fv fragment consisting of the VL and VH domains of a single antibody (Holt et al., (2003) Trends in Biotechnology 21, 484-490); (iv) a dAb fragment (Ward, E. S. et al., Nature 341, 544-546 (1989), McCafferty, et al., (1990) Nature, 348, 552-554, Holt et al., (2003) Trends in Biotechnology 21, 484-490], which consists of a VH or a VL domain; (v) isolated CDR regions; (vi) $F(ab')_2$ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al., (1988) Science, 242, 423-426, Huston et al., (1988) PNAS USA, 85, 5879-5883); (viii) bispecific single chain Fv dimers (PCT/US92109965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; Holliger, P. (1993) et al., Proc. Natl. Acad. Sci. USA 90 6444-6448).

[0030] Fv, scFv or diabody molecules may be stabilized by incorporation of disulfide bridges linking the VH and VL domains (Reiter, Y. et al., Nature Biotech, 14, 1239-1245, 1996). Minibodies comprising a scFv joined to a CH3 domain may also be made (Hu, S. et al., (1996) Cancer Res., 56, 3055-3061). Other examples of binding fragments can be Fab', which differs from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain, including one or more cysteines from the antibody hinge region, and Fab'-SH, which is a Fab' fragment in which the cysteine residue(s) of the constant domains bear a free thiol group.

[0031] "Fv" when used herein can refer to the minimum fragment of an antibody that retains both antigen-recognition and antigen-binding sites. "Fab" when used herein can refer to a fragment of an antibody that comprises the constant domain of the light chain and the CH1 domain of the heavy chain. The term "mAb" refers to monoclonal antibody.

[0032] By the terms "Apoptosis-associated Speck-like protein containing a Caspase Activating Recruitment Domain (CARD)" and "ASC" is meant an expression product of an ASC gene or isoforms thereof, or a protein that shares at least 65% (but preferably 75, 80, 85, 90, 95, 96, 97, 98, or 99%) amino acid sequence identity with ASC (e.g., NP_037390 (Q9ULZ3-1), NP_660183 (Q9ULZ3-2) or Q9ULZ3-3 in human, NP 075747 in mouse or NP_758825 (BAC43754) in rat) and displays a functional activity of ASC. A "functional activity" of a protein is any activity associated with the physiological function of the protein. Functional activities of ASC include, for example, recruitment of proteins for activation of caspase-1 and initiation of cell death.

[0033] By the term "ASC gene," or "ASC nucleic acid" is meant a native ASC-encoding nucleic acid sequence, genomic sequences from which ASC cDNA can be transcribed, and/or allelic variants and homologues of the foregoing. The terms encompass double-stranded DNA, single-stranded DNA, and RNA.

[0034] As used herein, the term "inflammasome" means a multi-protein (e.g., at least two proteins) complex that activates caspase-1. Further, the term "inflammasome" can refer to a multi-protein complex that activates caspase-1 activity, which in turn regulates IL-1β, IL-18 and IL-33 processing and activation. See Arend et al. 2008; Li et al. 2008; and Martinon et al. 2002, each of which is incorporated by reference in their entireties. The terms "NLRP1 inflammasome", "NALP1 inflammasome", "NLRP2 inflammasome", "NALP2 inflammasome", "NLRP3 inflammasome", "NALP3 inflammasome", "NLRC4 inflammasome", "IPAF inflammasome" or "AIM2 inflammasome" mean a protein complex of at least caspase-1 and one adaptor protein, e.g., ASC. For example, the terms "NLRP1 inflammasome" and "NALP1 inflammasome" can mean a multiprotein complex containing NLRP1, ASC, caspase-1, caspase-11, XIAP, and pannexin-1 for activation of caspase-1 and processing of interleukin-1β, interleukin-18 and interleukin-33. The terms "NLRP2 inflammasome" and "NALP2 inflammasome" can mean a multiprotein complex containing NLRP2 (aka NALP2), ASC and caspase-1,while the terms "NLRP3 inflammasome" and "NALP3 inflammasome" can mean a multiprotein complex containing NLRP3 (aka NALP3), ASC and the terms "NLRC4 inflammasome" and "IPAF inflammasome" can mean a multiprotein complex containing NLRC4 (aka IPAF), ASC and caspase-1. Additionally, the term "AIM2 Inflammasome" can mean a multiprotein complex comprising AIM2, ASC and caspase-1.

[0035] As used herein, the phrase "sequence identity" means the percentage of identical subunits at corresponding positions in two sequences (e.g., nucleic acid sequences, amino acid sequences) when the two sequences are aligned to maximize subunit matching, i.e., taking into account gaps and insertions. Sequence identity can be measured using sequence analysis software (e.g., Sequence Analysis Software Package from Accelrys CGC, San Diego, CA).

[0036] By the phrases "therapeutically effective amount" and "effective dosage" is meant an amount sufficient to produce a therapeutically (e.g., clinically) desirable result; the exact nature of the result will vary depending on the nature of the disorder being treated. For example, where the disorder to be treated is SCI, the result can be an improvement in motor skills and locomotor function, a decreased spinal cord lesion, etc. The compositions described herein can be administered from one or more times per day to one or more times per week. The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the compositions of the invention can include a single treatment or a series of treatments.

[0037] As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent described herein, or identified by a method described herein, to a patient, or application or administration of the therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease, or the predisposition toward disease.

[0038] The terms "patient" "subject" and "individual" are used interchangeably herein, and mean a mammalian subject to be treated, with human patients being preferred. In some cases, the methods of the invention find use in experimental animals, in veterinary applications, and in the development of animal models for disease, including, but not limited to, rodents including mice, rats, and hamsters, as well as primates.

[0039] As interchangeably used herein, "Absent in Melanoma 2" and "AIM2" can mean an expression product of an AIM2 gene or isoforms; or a protein that shares at least 65% (but preferably 75, 80, 85, 90, 95, 96, 97, 98, or 99%) amino acid sequence identity with AIM2 (e.g., accession number(s) NX 014862, NP004824, XP016858337, XP005245673, AAB81613, BAF84731, AAH10940) and displays a functional activity of AIM2.

[0040] As interchangeably used herein, "NALP1" and "NLRP1" mean an expression product of an NALP1 or NLRP1 gene or isoforms; or a protein that shares at least 65% (but preferably 75, 80, 85, 90, 95, 96, 97, 98, or 99%) amino acid sequence identity with NALP1 (e.g., accession number(s) AAH51787, NP_001028225, NP_127500, NP_127499, NP_127497, NP055737) and displays a functional activity of NALP1.

[0041] As interchangeably used herein, "NALP2" and "NLRP2" mean an expression product of an NALP2 or NLRP2 gene or isoforms; or a protein that shares at least 65% (but preferably 75, 80, 85, 90, 95, 96, 97, 98, or 99%) amino acid sequence identity with NALP2 (e.g., accession number(s) NP_001167552, NP_001167553, NP_001167554 or NP_060322) and displays a functional activity of NALP2.

[0042] As interchangeably used herein, "NALP3" and "NLRP3" mean an expression product of an NALP3 or NLRP3 gene or isoforms; or a protein that shares at least 65% (but preferably 75, 80, 85, 90, 95, 96, 97, 98, or 99%) amino acid sequence identity with NALP3 (e.g., accession number(s) NP_001073289, NP_001120933, NP_001120934, NP_001230062, NP_004886, NP_899632, XP_011542350, XP_016855670, XP_016855671, XP_016855672 or XP_016855673) and displays a functional activity of NALP3.

[0043] As interchangeably used herein, "NLRC4" and "IPAF" mean an expression product of an NLRC4 or IPAF gene or isoforms; or a protein that shares at least 65% (but preferably 75, 80, 85, 90, 95, 96, 97, 98, or 99%) amino acid sequence identity with NLRC4 (e.g., accession number(s) NP_001186067, NP001186068, NP_001289433 or NP_067032) and displays a functional activity of NLRC4.

[0044] By the terms "stroke" and "ischemic stroke" is meant when blood flow is interrupted to part of the brain or spinal cord.

[0045] By "traumatic injury to the CNS" is meant any insult to the CNS from an external mechanical force, possibly leading to permanent or temporary impairments of CNS function.

[0046] The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, humanized antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments, regions or derivatives thereof, provided by any known technique, such as, but not limited to, enzymatic cleavage, peptide synthesis or recombinant techniques. Such anti-ASC and anti-NLHP1 antibodies of the present invention are capable of binding portions of ASC and NLRP1, respectively,that interfere with caspase-1 activation.

[0047] Methods involving conventional molecular biology techniques are described herein. Such techniques are generally known in the art and are described in detail in methodology treatises such as Molecular Cloning: A Laboratory Manual, 3rd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; and Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates). Immunology techniques are generally known in the art and are described in detail in methodology treatises such as Advances in Immunology, volume 93, ed. Frederick W. Alt, Academic Press, Burlington, MA, 2007; Making and Using Antibodies: A Practical Handbook, eds. Gary C. Howard and Matthew R. Kaser, CRC Press, Boca Raton, FL, 2006; Medical Immunology, 6th ed., edited by Gabriel Virella, Informa Healthcare Press, London, England, 2007; and Harlow and Lane ANTIBODIES: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988.

[0048] Although compositions and methods similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable compositions and methods are described below. All publications, patent applications, and patents mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. The particular embodiments discussed below are illustrative only and not intended to be limiting.

OVERVIEW

[0049] Provided herein are compositions and methods for reducing inflammation in the lungs of a mammal that has been subjected to or is afflicted by a condition that results in or causes lung inflammation. The compositions and methods described herein can include antibodies or active fragments thereof as provided herein that specifically bind to at least one component (e.g., ASC) of a mammalian inflammasome and/or compounds that modulate (e.g., inhibit or reduce) extracellular vesicle (EV) uptake and have use as treatments for lung inflammation in a mammal.

[0050] Described herein are methods for reducing inflammation in the lungs of a mammal having a condition that results in and/or causes an inflammatory response in the lungs. In one embodiment, the method of treating inflammation in the lungs of a mammal comprises administering to the mammal a composition comprising an agent that inhibits inflammasome signaling. The mammal can be a patient or subject as provided herein. Examples of conditions that can lead to inflammation in the lungs include a central nervous system (CNS) injury (e.g., spinal cord injury (SCI), traumatic brain injury (TBI) or stroke), a neurodegenerative disease, an autoimmune disease, asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, interstitial lung disease or acute respiratory distress syndrome. The composition can be administered in a therapeutically effective amount. The therapeutically effective amount can be a dose as provided herein. The agent can be an extracellular vesicle (EV) uptake inhibitor, an antibody or an active fragment thereof as provided herein that binds to a component of an inflammasome or a combination thereof. The composition can be administered by any suitable route, e.g., by inhalation, intravenously, intraperitoneally, or intracerebroventricularly. The composition can further include at least one pharmaceutically acceptable carrier or diluent.

[0051] In one embodiment, administration of the agent can result in a reduction in the activity and/or expression level of a component of a mammalian inflammasome in the lungs of the subject. The reduction can be in cells of

the lung such as, for example, Type II alveolar cells. The reduction can be in comparison to a control. The control can be the subject prior to administration of the agent. The control can be the activity and/or expression level of the inflammasome component(s) in a subject not administered the agent. In one embodiment, administration of the agent results in the reduction of caspase-1 activation in at least the lungs or lung cells of the subject. In one embodiment, administration of the agent results in the reduction of the expression level of one or more inflammasome components (e.g., ASC, AIM2, NALP1, NALP2, NALP2, NALP3 or NLRC4) in at least the lungs or lung cells of the subject.

[0052] In another embodiment, administration of the agent can result in a reduction in or elimination of acute lung injury (ALI). In one embodiment, the reduction in ALI is evidenced by a reduction in neutrophil infiltration into alveolar and/or interstitial space, reduced or absent alveolar septal thickening or a combination thereof. The reduction can be in comparison to a control. The control can be ALI in the subject prior to administration of the agent. The control can be ALI in a subject suffering from ALI not administered the agent.

[0053] In still another embodiment, administration of the agent can result in a reduction in or elimination of pyroptosis in the lungs of the subject. Pyroptosis is a proinflammatory form of cell death that involves activation of caspase-1. Pyroptosis can be triggered by the caspase-1 mediated cleavage of gasdermin D (GSDMD). In one embodiment, the reduction in pyroptosis is evidenced by a reduction in or lack of cleavage of GSDMD in the lungs or lung cells (e.g., Type II alveolar cells) of the subject. The reduction or elimination of pyroptosis can be in comparison to a control. The reduction in or lack of cleavage of GSDMD can be in comparison to a control. The control can be the level of pyroptosis in the subject prior to administration of the agent. The control can be the level of pyroptosis in a subject suffering from pyroptosis not administered the agent.

[0054] In one embodiment, the agent to be administered is an EV uptake inhibitor. The EV uptake inhibitor can be a compound, antisense RNA, siRNA, peptide, antibody or an active fragment thereof as provided herein or a combination thereof. The compound or peptide can be one or more compounds selected from heparin, α-difluoromethylornithine (DFMO), Enoxaparin, Asialofetuin, Human receptor-associated protein (RAP), RGD (Arg-Gly-Asp) peptide, Cytochalasin D, Cytochalasin B, Ethylenediaminetetra acetic acid (EDTA), Latrunculin A, Latrunculin B, NSC23766, Dynasore, Chlorpromazine, 5-(N-Ethyl-N-isopropyl)amiloride (EIPA), Amiloride, Bafilomycin A Monensin and Chloroquine, Annexin-V, Wortmannin, LY294002, Methyl-β-cyclodextrin (MβCD), Filipin, Simvastatin, Fumonisin B1 and N-butyldeoxynojirimycin hydrochloride, U0126 or a proton pump inhibitor. The EV uptake inhibitor antibody or an active fragment thereof as provided herein can be one or more antibodies or active fragments thereof directed against protein targets listed in Table 1. A composition for treating and/or reducing inflammation in the lungs of a mammal using an EV uptake inhibitor can further include at least one pharmaceutically acceptable carrier or diluent.

TABLE 1

Exemplary targets and corresponding antibodies for use in blocking EV uptake.

Gene Symbol	Gene Name	Exemplary Antibodies
ICAM-1	Intercellular Adhesion Molecule 1	Invitrogen ICAM-1 antibody (Life Technologies, 07-5403); CD54 (ICAM-1) Monoclonal Antibody (R6.5), eBioscience™
LFA-1	Lymphocyte function-associated antigen 1	Abbiotec LFA-1 antibody (Abbiotec, 250944); Developmental Studies Hybridoma Bank LFA-1 antibody (Developmental Studies Hybridoma Bank, MHM24)
TIM-4	T-cell membrane protein 4	BioLegend TIMD4 antibody (BioLegend, 354004); LifeSpan Biosciences TTMD4 antibody (Lifespan Biosciences, LS-B1413)
MFG-E8	Milk Fat Globule-EGF Factor 8 Protein	MBL International MFGE8 antibody (MBL, D199-3); Santa Cruz Biotechnology MFGE8 antibody (Santa Cruz, sc-8029); MBL International MFGE8 antibody (MBL, 18A2-G10)
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin	Invitrogen DC SIGN antibody (eBioscience, eB-h209, 17-2099-41); BD Biosciences DC SIGN antibody (BD, DCN46, 551186)
DEC205	cluster of differentiation 205	EMD Millipore LY75 antibody (Millipore, HD30); BioLegend LY75 antibody (BioLegend, 342203)
H-2Kb	MHC Class I (H-2Kd)	BioLegend H2-K1 antibody (BioLegend, 28-8-6, 114603); BioLegend H2-K1 antibody (BioLegend, 28-14-8, 14-5999-85)
Tspan8	Tetraspanin-8	R and D Systems TSPAN8 antibody (R&D Systems, MAB4734)
Tspan29	Tetraspanin-29	Santa Cruz Biotechnology CD9 antibody (Santa Cruz, sc-59140); Invitrogen CD9 antibody (eBioscience, eBioSN4; BD Biosciences CD9 antibody (BD Pharmingen, 555370)
ITGAL	Integrin subunit alpha L	TS1/22.1.1.13.3;M17/4.4.11.9
ITGAM	Integrin subunit alpha M	CD11b Monoclonal Antibody (VIM12)(CD11B00); BD Biosciences
		CD11b antibody (BD Pharmingen, ICRF44; 555385)
ITGAX	Integrin subunit alpha X	Anti-Integrin αX Antibody, clone N418 (MAB1399Z); BD Biosciences CD11c antibody (BD Bioscience, B-ly6; 560369)
CD44	Cluster of differentiation 44	Invitrogen CD44 antibody (eBioscience, VFF-7; MA1-82392); Invitrogen CD44 antibody (eBioscience, IM7; MA1-10225); Invitrogen CD44 antibody (eBioscience, 5F12; MA5-12394); BD Biosciences CD44 antibody (BD Biosciences, 515; 550990 OR 550988)
ITGA3	Integrin subunit alpha 3	EMD Millipore integrin alpha3 antibody (Millipore, P1B5; MAB1952Z OR MAB1952P)
ITGA4	Integrin subunit alpha 4	Bio X Cell ITGA4 antibody (BioXcell, PS/2) (BE0071-5MG); BD Biosciences ITGA4 antibody (BD Biosciences, 561892); BD

TABLE 1-continued

Exemplary targets and corresponding antibodies for use in blocking EV uptake.

Gene Symbol	Gene Name	Exemplary Antibodies
		Biosciences ITGA4 antibody (BD, 340976); EMD Millipore ITGA4 antibody (Millipore, P4C2; MAB1955)
ITGAV	Integrin subunit alpha V	Abcam integrin alpha v antibody (Abcam, ab77906); Abeam integrin alpha v antibody (Abcam, ab78289); Abeam integrin alpha v antibody (Abeam, ab16821); Invitrogen integrin alpha v antibody (Thermo Fisher Scientific, 272-17E6, MA1-91669); R & D Systems integrin alpha v antibody (R&D Systems, MAB2528)
ITGB3	Integrin subunit beta 3	Abeam integrin beta3 antibody (Abcam, ab78289); Abnova integrin beta3 antibody (Abnova, MHF4, MAB7098)
SELL	Selectin L	BioLegend CD62L antibody (Biolegend, 304804); BioLegend CD62L antibody (Biolegend, 304810)
CD81	CD81 molecule	BD Biosciences CD81 antibody (BD Pharmingen, 555675); R and D Systems CD81 antibody (R&D Systems, MAB4615)
LRP1	LDL receptor related protein 1	Invitrogen LRP1 antibody (Life Technologies, 37-7600); Invitrogen LRP1 antibody (Thermo Fisher, MA1-27198)
VCAM1	vascular cell adhesion molecule 1	Invitrogen VCAM-1 antibody (Caltag, IG11B1; MA5-16429); Immunotech anti-VCAM-1 antibody
CD151	CD151 molecule (Raph blood group)	BD Biosciences CD151 antibody (Becton Dickinson, 556056); Epitomics CD151 antibody (Epitomics, 5901-1)

[0055] In one embodiment, the agent to be administered is an antibody or an active fragment thereof as provided herein directed against a component of a mammalian inflammasome or an antigen or epitope derived therefrom. In another embodiment, the agent to be administered is an antisense RNA or siRNA directed against a component of a mammalian inflammasome. The inflammasome component can be a component of any inflammasome known in the art, such as, for example, the NAPL1, NALP2, NALP3, NLRC4 or AIM2 inflammasome. In a typical embodiment, the antibody specifically binds to ASC or an antigen or epitope derived therefrom. However, an antibody against any other component of a mammalian inflammasome (e.g., the NALP1, NALP2, NALP3, NLRC4 or AIM2 inflammasome) may be used.

[0056] An antibody as described herein can be a monoclonal or polyclonal antibody or active fragments thereof. Said antibodies or active fragments can be chimeric, human or humanized as described herein.

[0057] Any suitable antibody or an active fragment thereof as provided herein that specifically binds ASC can be used, e.g., an antibody that inhibits ASC activity in lung cells (e.g., Type II alveolar cells) of the subject. In a typical embodiment, the antibody specifically binds to an amino acid sequence having at least 85% sequence identity with amino acid sequence SEQ ID NO:1 or SEQ ID NO:2. Similarly, in another embodiment, the inflammasome is the NALP1 inflammasome, and the at least one component is NALP1 (i.e., NLRP1). In this embodiment, the antibody or an active fragment thereof as provided herein specifically binds to an amino acid sequence having at least 85% sequence identity with amino acid sequence SEQ ID NO: 3 or SEQ ID NO: 4.

[0058] In yet another embodiment, the agent is one or more EV uptake inhibitors in combination with one or more antibodies or active fragments thereof as provided herein that bind a component of an inflammasome. The EV uptake inhibitor can be any EV uptake inhibitor as provided herein. The antibody that binds a component of an inflammasome can any antibody that binds any inflammasome component as provided herein. In one embodiment, the agent administered to a subject suffering from lung inflammation comprises a heparin (e.g., Enoxaparin) in combination with an antibody that binds a component of the AIM2 inflammasome (e.g., ASC).

[0059] In one embodiment, the method comprises: providing a therapeutically effective amount of a composition including an antibody or an active fragment thereof as provided herein that specifically binds to at least one component (e.g., ASC) of a mammalian inflammasome (e.g., AIM2 inflammasome); and administering the composition to the mammal suffering from lung inflammation, wherein administering the composition to the mammal results in a reduction of caspase-1 activation in the lungs of the mammal. In another embodiment, the method comprises: providing a therapeutically effective amount of a composition including an antibody that specifically binds to at least one component (e.g., ASC) of a mammalian inflammasome (e.g., AIM2 inflammasome); and administering the composition to the mammal suffering from lung inflammation, wherein administering the composition to the mammal results in a reduction in the levels of one or more inflammasome components (e.g., ASC). In yet another embodiment, the method comprises: providing a therapeutically effective amount of a composition including an antibody that specifically binds to at least one component (e.g., ASC) of a mammalian inflammasome (e.g., AIM2 inflammasome); and administering the composition to the mammal suffering from lung inflammation, wherein administering the composition to the mammal results in a reduction ALI. The lung inflammation can be the result of a CNS injury (e.g., SCI or TBI), asthma, chronic obstructive pulmonary disorder (COPD), a neurodegenerative disease, or an autoimmune disease with an inflammatory component In one embodiment, the lung inflammation is caused by a CNS injury such as TBI or SCI.

[0060] In one embodiment, the methods provided herein further entail detecting a level or activity of one or more components of a mammalian inflammasome in a sample from a subject suspected of suffering from lung inflammation. The method of detecting the level or activity entails measuring the level of at least one inflammasome protein (e.g., ASC or AIM2) in the sample obtained from the subject; determining the presence or absence of an elevated level or activity of said at least one inflammasome protein (e.g., ASC or AIM2). The level or activity of said at least one inflammasome protein can be enhanced relative to the level of said at least one inflammasome protein in a control sample. The level or activity of said at least one inflammasome protein in the protein signature can be enhanced rela-

tive to a pre-determined reference value or range of reference values. The at least one inflammasome protein can be nucleotide-binding leucine-rich repeat pyrin domain containing protein 1 (NLRP1), NLRP2, NLRP3, NLRC4, AIM2, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1, or combinations thereof. The sample can be cerebrospinal fluid (CSF), saliva, blood, serum, plasma, urine or a lung aspirate.

Antibodies That Bind Specifically to At Least One Component of A Mammalian Inflammasome

[0061] The methods described herein for reducing inflammation in the lungs of a mammal include compositions including an antibody or an active fragment thereof as provided herein that specifically binds to at least one component (e.g., ASC, AIM2) of a mammalian inflammasome (e.g., the AIM2 inflammasome). A composition for treating and/or reducing inflammation in the lungs of a mammal can further include at least one pharmaceutically acceptable carrier or diluent. Exemplary antibodies directed against components of a mammalian inflammasome for use in the methods herein can be those found in US8685400, the contents of which are herein incorporated by reference in its entirety.

[0062] In one embodiment, a composition for treating and/or reducing inflammation in the lungs of a mammal includes an antibody or an active fragment thereof as provided herein that specifically binds to a domain or portion thereof of a mammalian ASC protein such as, for example, a human, mouse or rat ASC protein. Any suitable anti-ASC antibody can be used, and several are commercially available. Examples of anti-ASC antibodies for use in the methods herein can be those found in US8685400, the contents of which are herein incorporated by reference in its entirety. Examples of commercially available anti-ASC antibodies for use in the methods provided herein include, but are not limited to 04-147 Anti-ASC, clone 2EI-7 mouse monoclonal antibody from MilliporeSigma, AB3607 - Anti-ASC Antibody from Millipore Sigma, orb194021 Anti-ASC from Biorbyt, LS-C331318-50 Anti-ASC from LifeSpan Biosciences, AF3805 Anti-ASC from R & D Systems, NBP1-78977 Anti-ASC from Novus Biologicals, 600-401-Y67 Anti-ASC from Rockland Immunochemicals, D086-3 Anti-ASC from MBL International, AL177 anti-ASC from Adipogen, monoclonal anti-ASC (clone o93E9) antibody, anti-ASC antibody (F-9) from Santa Cruz Biotechnology, anti-ASC antibody (B-3) from Santa Cruz Biotechnology, ASC polyclonal antibody – ADI-905-173 from Enzo Life Sciences, or A161 Anti-Human ASC - Leinco Technologies. The human ASC protein can be accession number NP_037390.2 (Q9ULZ3-1), NP 660183 (Q9ULZ3-2) or Q9ULZ3-3. The rat ASC protein can be accession number NP 758825 (BAC43754). The mouse ASC protein can be accession number NP_075747.3. In one embodiment, the antibody binds to a PYRIN-PAAD-DAPIN domain (PYD) or a portion or fragment thereof of a mammalian ASC protein (e.g. human, mouse or rat ASC). In this embodiment, an antibody as described herein specifically binds to an amino acid sequence having at least 65% (e.g., 65, 70, 75, 80, 85%) sequence identity with a PYD domain or fragment thereof of human, mouse or rat ASC. In one embodiment, the antibody binds to a C-terminal caspase-recruitment domain (CARD) or a portion or fragment thereof of a mammalian ASC protein (e.g. human, mouse or rat ASC). In this embodiment, an antibody as described herein specifically binds to an amino acid sequence having at least 65% (e.g., 65, 70, 75, 80, 85%) sequence identity with a CARD domain or fragment thereof of human, mouse or rat ASC. In still another embodiment, the antibody binds to a portion or fragment thereof of a mammalian ASC protein sequence (e.g. human, mouse or rat ASC) located between the PYD and CARD domains. In another embodiment, a composition for treating and/or reducing inflammation in the lungs of a mammal includes an antibody that specifically binds to a region of rat ASC, e.g., amino acid sequence

ALRQTQPYLVTDLEQS (SEQ ID NO: 1)

(i.e., residues 178-193 of rat ASC, accession number BAC43754). In this embodiment, an antibody as described herein specifically binds to an amino acid sequence having at least 65% (e.g., 65, 70, 75, 80, 85%) sequence identity with amino acid sequence

ALRQTQPYLVTDLEQS (SEQ ID NO: 1)

of rat ASC. In another embodiment, a composition for treating and/or reducing inflammation in the CNS of a mammal includes an antibody that specifically binds to a region of human ASC, e.g., amino acid sequence

RESQSYLVEDLERS (SEQ ID NO:2).

In one embodiment, an antibody that binds to an ASC domain or fragment thereof as described herein inhibits ASC activity in lung cells, e.g., Type II alveolar cells of a mammal.

[0063] In another embodiment, a composition for reducing inflammation in the lungs of a mammal includes an antibody or an active fragment thereof as provided herein that specifically binds to NLRP1 (e.g., anti-NLRP1 chicken antibody) or a domain thereof. Any suitable anti-NLRP1 antibody can be used, and several are commercially available. Examples of anti-NLRP1 antibodies for use in the methods herein can be those found in US8685400, the contents of which are herein incorporated by reference in its entirety. Examples of commercially available anti-NLRP1 antibodies for use in the methods provided herein include, but are not limited to human NLRP1 polyclonal antibody AF6788 from R&D Systems, EMD Millipore rabbit polyclonal anti-NLRP1 ABF22, Novus Biologicals rabbit polyclonal anti-NLRP1 NB100-56148, Sigma-Aldrich mouse polyclonal anti-NLRP1 SAB1407151, Abcam rabbit polyclonal anti-NLRP1 ab3683, Biorbyt rabbit polyclonal anti-NLRP1 orb325922 mybiosource rabbit polyclonal anti-NLRP1 MBS7001225, R&D systems sheep polyclonal AF6788, Aviva Systems mouse monoclonal anti-NLRP1 oaed00344, Aviva Systems rabbit polyclonal anti-NLRP1 AR054478_P050, Origene rabbit polyclonal anti-NLRP1 APO7775PU-N, Antibodies online rabbit polyclonal anti-NLRP1 ABIN768983, Prosci rabbit polyclonal anti-NLRP1 3037, Proteintech rabbit polyclonal anti-NLRP1 12256-1-AP, Enzo mouse monoclonal anti-NLRP1 ALX-

804-803-C100, Invitrogen mouse monoclonal anti-NLRP1 MA1-25842, GeneTex mouse monoclonal anti-NLRP1 GTX16091, Rockland rabbit polyclonal anti-NLRP1 200-401-CX5, or Cell Signaling Technology rabbit polyclonal anti-NLRP1 4990. The human NLRP1 protein can be accession number AAH51787, NP_001028225, NP_055737, NP_127497, NP_127499, or NP_127500. In one embodiment, the antibody binds to a Pyrin, NACHT, LRR1-6, FIIND or CARD domain or a portion or fragment thereof of a mammalian NLRP1 protein (e.g. human NLRP1). In this embodiment, an antibody as described herein specifically binds to an amino acid sequence having at least 65% (e.g., 65, 70, 75, 80, 85%) sequence identity with a specific domain (e.g., Pyrin, NACHT, LRR1-6, FIIND or CARD) or fragment thereof of human NLRP1. In one embodiment, a chicken anti-NLRP1 polyclonal that was custom-designed and produced by Ayes Laboratories is used for reducing lung inflammation. This antibody can be directed against the following amino acid sequence in human NLRP1:

CEYYTEIREREREKSEKGR (SEQ ID NO:3).

In one embodiment, an antibody that binds to a NLRP1 domain or fragment thereof as described herein inhibits NLRP1 activity in lung cells, e.g., Type II alveolar cells of a mammal.

[0064] In yet another embodiment, a composition for reducing inflammation in the lungs of a mammal includes an antibody or an active fragment thereof as provided herein that specifically binds to AIM2 or a domain thereof. Any suitable anti-AIM2 antibody can be used, and several are commercially available. Examples of commercially available anti-AIM2 antibodies for use in the methods provided herein include, but are not limited to a rabbit polyclonal anti-AIM2 cat. Number 20590-1-AP from Proteintech,, Abcam anti-AIMS antibody (ab119791), rabbit polyclonal anti-AIM2 (N-terminal region) Cat. Number AP3851 from ECM biosciences, rabbit polyclonal anti-ASC Cat. Number E-AB-30449 from Elabsciences,, Anti-AIM2 mouse monoclonal antibody called AIM2 Antibody (3C4G11) with catalog number sc-293174 from Santa Cruz Biotechnology, mouse monoclonal AIM2 antibody with catalog number TA324972 from Origene, AIM2 monoclonal antibody (10M2B3) from Thermofisher Scientific, AIM2 rabbit polyclonal antibody ABIN928372 or ABIN760766 from Antibodies-online, Biomatix coat anti-AIM2 polyclonal antibody with cat. Number CAE02153. Anti-AIM2 polyclaonl antibody (OABF01632) from Aviva Systems Biology, rabbit polyclonal anti-AIM2 antibody LS-C354127 from LSBio-C354127, rabbit monoclonal anti-AIM2 antibody from Cell Signaling Technology, with cat number MA5-16259. Rabbit polyclonal anti-AIM2 monoclonal antibody from Fab Gennix International Incorporated, Cat. Number AIM2 201AP, MyBiosource rabbit polyclonal anti-AIM2 cat number MBS855320, Signalway rabbit polyclonal anti AIM2 cateaog number 36253, Novus Biological rabbit polyclonal anti-AIM2 catalog number 43900002, GeneTex rabbit polclonal anti-AIM2 GTX54910, Prosci, rabbit polyclonal anti-AIM2 26-540, Biorbyt mouse monoclonal anti-AIM2 orb333902, Abcam rabbit polyclonal anti-AIM2 ab93015), Abcam rabbit polyclonal anti-AIM2 ab76423, Signma Aldrich mouse polyclonal anti-AIM2 SAB1406827, or Biolegend anti-AIM2 3B10. The human AIM2 protein can be accession number NX_014862, NP004824, XP016858337, XP005245673, AAB81613, BAF84731 or AAH10940. In one embodiment, the antibody binds to a Pyrin or HIN-200 domain or a portion or fragment thereof of a mammalian AIM2 protein (e.g. human AIM2). In this embodiment, an antibody as described herein specifically binds to an amino acid sequence having at least 65% (e.g., 65, 70, 75, 80, 85%) sequence identity with a specific domain (e.g., Pyrin or HIN-200) or fragment thereof of human AIM2. In one embodiment, an antibody that binds to a AIM2 domain or fragment thereof as described herein inhibits AIM2 activity in lung cells, e.g., Type II alveolar cells of a mammal.

[0065] Anti-inflammasome (e.g., Anti-ASC, anti-NLRP1 or anti-AIM2) antibodies as described herein include polyclonal and monoclonal rodent antibodies, polyclonal and monoclonal human antibodies, or any portions thereof, having at least one antigen binding region of an immunoglobulin variable region, which antibody specifically binds to a component of a mammalian inflammasome (e.g., AIM2 inflammasome) such as, for example, ASC or AIM2. In some cases, the antibody is specific for ASC such that an antibody is specific for ASC if it is produced against an epitope of the polypeptide and binds to at least part of the natural or recombinant protein.

[0066] Methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988, Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, Meth. Enzymol. 92:589-601, 1983, which references are entirely incorporated herein by reference.

[0067] Anti-inflammasome (e.g., Anti-ASC and anti-AIM2) antibodies of the present invention can be routinely made according to methods such as, but not limited to inoculation of an appropriate animal with the polypeptide or an antigenic fragment, in vitro stimulation of lymphocyte populations, synthetic methods, hybridomas, and/or recombinant cells expressing nucleic acid encoding such anti-ASC or anti-NLR1 antibodies. Immunization of an animal using purified recombinant ASC or peptide fragments thereof, e.g., residues 178-193 (SEQ ID NO: 1) of rat ASC (e.g., accession number BAC43754) or SEQ ID NO:2 of human ASC, is an example of a method of preparing anti-ASC antibodies. Similarly, immunization of an animal using purified recombinant NLRP1 or peptide fragments thereof, e.g., residues MEE SQS KEE SNT EG-cys (SEQ ID NO:4) of rat NALP1 or SEQ ID NO:3 of human NALP1, is an example of a method of preparing anti-NLRP1 antibodies.

[0068] Monoclonal antibodies that specifically bind ASC or NLRP1 may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, Nature 256:495-497, 1975; U.S. Pat. No. 4,376,110; Ausubel et al., eds., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1987, 1992); Harlow and Lane ANTIBODIES: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988; Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), the contents of which are incorporated entirely herein by reference. Such antibodies may be

of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof A hybridoma producing a monoclonal antibody of the present invention may be cultivated in vitro, in situ or in vivo.

Administration of Compositions

[0069] The compositions of the invention may be administered to mammals (e.g., rodents, humans) in any suitable formulation. For example, anti-ASC antibodies may be formulated in pharmaceutically acceptable carriers or diluents such as physiological saline or a buffered salt solution. Suitable carriers and diluents can be selected on the basis of mode and route of administration and standard pharmaceutical practice. A description of exemplary pharmaceutically acceptable carriers and diluents, as well as pharmaceutical formulations, can be found in Remington's Pharmaceutical Sciences, a standard text in this field, and in USP/NF. Other substances may be added to the compositions to stabilize and/or preserve the compositions.

[0070] The compositions of the invention may be administered to mammals by any conventional technique. Typically, such administration will be by inhalation or parenteral (e.g., intravenous, subcutaneous, intratumoral, intramuscular, intraperitoneal, or intrathecal introduction). The compositions may also be administered directly to a target site by, for example, surgical delivery to an internal or external target site, or by catheter to a site accessible by a blood vessel. The compositions may be administered in a single bolus, multiple injections, or by continuous infusion (e.g., intravenously, by peritoneal dialysis, pump infusion). For parenteral administration, the compositions are preferably formulated in a sterilized pyrogen-free form.

Effective Doses

[0071] The compositions described above are preferably administered to a mammal (e.g., a rat, human) in an effective amount, that is, an amount capable of producing a desirable result in a treated mammal (e.g., reducing inflammation in the CNS of a mammal subjected to a traumatic injury to the CNS or stroke or having an autoimmune or CNS disease). Such a therapeutically effective amount can be determined as described below.

[0072] Toxicity and therapeutic efficacy of the compositions utilized in methods of the invention can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD_{50} (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Those compositions that exhibit large therapeutic indices are preferred. While those that exhibit toxic side effects may be used, care should be taken to design a delivery system that minimizes the potential damage of such side effects. The dosage of preferred compositions lies preferably within a range that includes an ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

[0073] As is well known in the medical and veterinary arts, dosage for any one subject depends on many factors, including the subject's size, body surface area, age, the particular composition to be administered, time and route of administration, general health, and other drugs being administered concurrently.

EXAMPLES

[0074] The present invention is further illustrated by the following specific examples. The examples are provided for illustration only and should not be construed as limiting the scope of the invention in any way.

Example 1: Role of EV Mediated Inflammasome Signaling in ALI Following TBI and Effects of its Neutralization

[0075] Pulmonary dysfunction often presents as a complication of Severe Traumatic Brain Injury (1). Approximately 20-25 percent of TBI subjects develop acute lung injury (ALI) (2), but the mechanisms mediating the pathology of TBI-induced ALI remain poorly defined. Previous literature has supported the idea that pulmonary dysfunction after TBI is due to the sympathetic response to increased intracranial pressure leading to cardiopulmonary dysfunction (42). More recent studies, however, have shown that a systemic inflammatory response also plays a key role in TBI-induced lung injury (43). Specifically, the HMGB1-RAGE ligand receptor pathway serves as central transduction mechanism for pulmonary dysfunction after TBI (8). In addition, HMGB1 induces AIM2 inflammasome activation (37). Furthermore previous literature reveals that pathogens secrete EV that carry DAMPs, such as HMGB1, and trigger inflammation (Buzas et al., 2014). Various studies have shown that the blood brain barrier (BBB) is permeable after TBI as early as 3-6 hours after injury resulting in damage to the protective barrier between the brain and the intravascular compartment and leads to leakage of proteins and fluid (44). Disruption of the BBB after injury results in the secretion of inflammatory mediators, such as DAMPs, which can further brain inflammation and damage distal organs (5). Several inflammatory mediators can act as clear markers for brain injury, however their validity is not widely accepted (45). Furthermore, there is currently no clinically approved treatment or biomarker for TBI-induced ALI. Recently, EV have become an area of interest in biomarker research for a several different types of diseases, including lung injury (46) and TBI (47). It has been previously shown that in EV isolated from the cerebrospinal fluid of patient with TBI, there is an increase of inflammasome proteins when compared to control samples (14). In this Example, the contribution of EV mediated inflammasome signaling in the etiology of TBI-induced ALI was examined.

Materials and Methods

Animals and Traumatic Brain Injury

[0076] All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Miami Miller School of Medicine (Animal Welfare Assurance A3224-01) and were done according to the NIH Guide for the Care and Use of Laboratory Animals. The ARRIVE guidelines were followed when conducting this study. All C57/BL6 mice were 8-12 weeks and 24 to 32 grams. Mice were prospectively randomized to experimental groups (sham, 4 h, 24) for TBI, experimental groups (naive, sham-saline, untreated, enoxaparin, anti-ASC) for

adoptive transfer and treatment.. For TBI experiment-groups, sham animals underwent surgical procedure but were not injured. For adoptive transfer treatment studies, the sham-saline group underwent surgical procedures and received saline as vehicle treatment. Naive animals underwent no surgical procedures. A sample size of 5 to 6 was used for each group based on power analysis (using G* power analysis, with an effect size F=0.85, α set a 0.05) and historical data [49, 50]. All mice were housed in the viral antigen free (VAF) animal facility at the Lois Pope Life Center at the University of Miami on 12-hour light/dark cycles and food and water were supplied ad libitum. The facility conducts husbandry procedures twice a week and checks on the conditions of the animals daily. Animals were observed post-op, where they were kept on a heating pad and body temperature was controlled with a rectal probe where it was maintained at 37° C., in our operation room and then transferred to the animal quarters.

[0077] Prior to surgery animals were anesthetized with ketamine and xylazine (intraperitoneal, i.p.). The anesthetized animals were then placed on a heating pad to ensure a body temperature of 37° C. TBI was performed using a Controlled Cortical Impact (CCI) model. A 5 mm craniotomy was made on the right cortex (-2.5 mm posterior, 2.0 mm lateral from Bregma). Injury was induced using the ECCI-6.3 device (Custom Design & Fabrication, Richmond, VA, USA) with a 3 mm impounder at 6 m/s velocity, 0.8 mm depth, and 150 ms impact duration (15). Following these procedures animals were returned to their cages and given food and water. Animals were sacrificed at 4 hours and 24 hours after TBI as described. Sham animals were anesthetized and subjected to the same pre-surgical incision as injured animals but did not undergo a craniotomy or contusion.

Tissue Collection

[0078] All animals were anesthetized with ketamine and xylazine, prior to perfusion. Animals then underwent tracheal perfusion. Lungs were infused with 4% paraformaldehyde (PFA) using a tracheal catheter at 20 cm H2O and then fixed in 4% PFA overnight at 4° C. Fixed lung tissues were paraffin embedded and 5 μm sections were processed (16). Right lung tissue was collected for protein isolation and molecular analyses. Animals then underwent decapitation and right cortical tissue was collected for protein isolation and molecular analyses.

Pyroptosome Isolation Assay

[0079] Mice lung tissue lysates were filtered through a 5 μm low-binding polyvinylidene difluoride (PVDF) membrane (Millipore). After filtration, the supernatant was centrifuged at 2,700 xg for 8 minutes. The pellet was resuspended in 40 μl of 3[(3-cholamidopropyl) dimethylammonio]-propanesulfonic acid (CHAPS) buffer (20 mmol/L HEPES-KOH, pH 7.5, 5 mmol/L MgCl2, 0.5 mmol/L EGTA, 0.1 mmol/L phenylmethylsulfonyl fluoride, protease inhibitor cocktail, and 0.1% CHAPS). The pyroptosome was pelleted by centrifugation at 2,700 xg for 8 minutes. The pellet was then resuspended and incubated in 27.8 μl of CHAPS buffer with 2.2 μl of disuccinimidyl substrate (9) for 30 minutes at room temperature to cross-link ASC dimers. Lastly, an equal amount of 2× Laemmli buffer was added and proteins were analyzed by immunoblotting using commercially available antibodies to ASC and Gasdermin D (GSD)..

Nuclear and Cytoplasmic Extraction

[0080] Nuclear and Cytoplasmic fractions were extracted using the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific) according to manufacturer instructions. Briefly, mice lung tissue samples were cut into 20-100 mg pieces and centrifuged at 500 × g for 5 minutes. Tissue pieces were the homogenized with the Cytoplasmic Extraction Reagent and centrifuged at 16,000 × g for 5 minutes. Then the supernatant (cellular extract) was removed and the pellet was centrifuged with Nuclear Extraction Reagent (Thermo Scientific) at 16,000 × g for 10 minutes. This supernatant corresponded to the nuclear fraction, which was removed and stored at -80° C.

Immunoblotting

[0081] Lung and brain tissue samples were snap frozen in liquid nitrogen and stored in -80° C. 2-mm sections of right lower lung and right cortical tissue were homogenized in extraction buffer containing protease and phosphatase inhibitor cocktail (Sigma, St Louis, MO, USA) and resolved in 4-20% Tris-TGX Criterion precasted gels (Bio-Rad, Hercules, CA, USA) as described in de Rivero Vaccari et al. 2015 (13) using antibodies to caspase-1 (Novus Biologicals), ASC (Santa Cruz), IL-1 β (Cell Signaling), IL-18 (Abcam) AIM2 (Santa Cruz) and HMGB1(Millipore). Quantification of band density was performed using Image Lab and all data were normalized to β-actin.

Immunohistochemistry

[0082] Tissue sections were deparaffinized in xylene and then rehydrated using ethanol and Tris buffer saline. Immunohistochemical procedures were then carried out for double staining as previously described (16). Sections were incubated overnight at 4° C. with antibodies against Caspase-1 and ASC (Millipore), AIM2 (Santa Cruz), HMGB1(Millipore) and SPC (Millipore). Immunostained lung sections of sham, 4 hour, and 24 hour mice were examined with a Zeiss laser scanning confocal microscope (Zeiss, Inc., Thornwood, NY, USA). Lung sections were analyzed by individuals who were blinded to the groups.

EV Isolation

[0083] EV were isolated from serum from TBI-injured mice and injury mice using the Total Exosome Isolation solution according to manufacturer's instructions (Invitrogen). Briefly, 100 μl of each sample were centrifuged at 2000 × g for 30 minutes. The supernatant was then incubated with 20 μl of Total Exosome Isolation (TEI) reagent for 30 minutes at 4° C. followed by centrifugation at 10,000 × g for 10 minutes at room temperature. Supernatants were discarded and the pellet was resuspended in 100 μl of PBS. EV were characterized by the expression of CD81 and by Nanosight tracking analysis (FIG. **6**).

Adoptive Transfer of EV

[0084] Serum-derived EV from C57BL-6 TBI and sham mice were injected into naïve C57BL-6 mice through the jugular vein at a dose of 1.0×10^{10} particles per gram/

body weight [48]. Particle count was measured by Nanosight Tracking analysis and samples were diluted accordingly. Prior to surgery animals were anesthetized with ketamine and xylene. A 1-2 cm incision was made between the jaw and the clavicle. The jugular vein was elevated and tied, followed by catheter placement. Serum-derived EV were transferred and lung and brain tissues were collected 24 hours after injection for analysis (n=5).

Enoxaparin and Anti-ASC Treatment

[0085] Serum-derived EV from TBI mice were injected into naïve C57-BL6 mice through a jugular vein injection. One hour later, Enoxaparin (3 mg/kg) (n=4) and Anti-ASC (5 mg/kg) (n=4) were administered to recipient animals. The following groups were used: 1) the naïve group received no treatment, 2) the sham saline group was used as a negative control and underwent jugular vein injection of only saline, 3) the untreated group received EV from TBI mice without any treatment and was used as a positive control, 4) the ENOX group received EV from TBI mice and Enoxaparin, and 5) the Anti-ASC group received EV from TBI mice and Anti-ASC. The order of treatment was randomized. Lung and brain tissues were collected 24 hours after injection for analysis.It should be noted that the anti-ASC antibody used in the treatment experiments was a humanized monoclonal antibody against ASC and recognizes murine, human and swine ASC.

Histology and Lung Injury Scoring

[0086] Lung tissue sections were stained by a standard hematoxylin and eosin method for histology, morphometry and ALI scoring. Lung sections were scored by a blinded pathologist using the Lung Injury Scoring System from the American Thoracic Society Workshop Report (17). Twenty random high power fields were chosen for scoring. Criteria for ALI scoring was based on number of neutrophils in the alveolar space, interstitial space, hyaline membranes, proteinaceous debris filling the airspaces and alveolar septal thickening. Based on these criteria a score between 0 (no injury) and 1 (severe injury) was given.

Statistical Analysis

[0087] Data were analyzed using a student's T-test for two groups and a one-way ANOVA followed by Tukey's multiple comparison tests, (GraphPad Prism version 7.0) for two or more groups. D'Agostino-Pearson test was used to test for normality. Data are expressed as mean +/-SEM. P values of significance used were * $p<0.05$.

Results

Severe TBI Increases AIM2 Inflammasome Proteins and HMGB1 Expression in the Brain Of Mice

[0088] Excessive levels of the proinflammatory cytokine IL-1β and IL-18, and inflammasome proteins are associated with secondary damage after fluid-percussion brain injury (18). To determine whether severe CCI induced processing of proinflammatory cytokines and alterations in levels of inflammasome proteins, cortical lysates were analyzed, however there is limited research on inflammasome activation in severe TBI. In this Example, following severe CCI, cortical lysates were examined for the levels of the caspase-1 (FIGS. **1**A, B) ($p<0.001$), ASC (FIGS. **1**A, C) ($p=0.003$), IL-18 (FIGS. **1**A, D) ($p=0.0042$), AIM2 (FIGS. **1**A, F) ($p=0.0197$) and IL-1β (FIGS. **1**A, G) ($p=0.0141$) at 4 and 24 hrs after injury.. Levels of caspase-1, ASC, AIM2, and IL-1β peaked at 4 hours after CCI and decreased by 24 hrs. The time course for maturation of inflammatory cytokines differed slightly but peaked by 24 hours after TBI. Since others have shown a role for the inflammasome DAMP HMGB1activating the AIM2 inflammasome, the levels of these proteins were also determined in cortical lysates. As shown in FIGS. **1**A, **1**E, CCI induced a significant increase in the levels of HMGB1 (FIGS. **1**A, **1**E) ($p=0.0121$) at 4 and 24 hrs after injury. These data indicate that following severe CCI in mice, the levels of the AIM2 inflammasome proteins were significantly elevated in the cortex following injury.

Severe TBI Increases AIM2 Inflammasome Protein and HMGB1 Expression on the Lungs Of Mice

[0089] To determine whether CCI induced inflammasome activation in the lungs, an immunoblot analysis of lung lysates was performed for caspase-1 (FIGS. **1**H, I) ($p=0.0026$), ASC (FIGS. **1**H, J) ($p=0.0427$), IL-18 (FIGS. **1**H, K) ($p=0.0025$), IL-1β (FIGS. **1**H,N) ($p=0.0012$) and AIM2 (FIGS. **1**H,M) ($p<0.001$), and NLRP3 ($p=0.0047$) (Supplemental FIG. **1**). Increased levels of caspase-1, ASC, IL-18 and AIM2 were significantly increased at 4 hrs and 24 hrs after injury as compared to the sham control. However the time course of the increase in protein expression differed slightly from that observed in brain in which they peaked at 24 hr after CCI. Since, the HMGB1-RAGE axis plays a role in the mechanism by which TBI induces lung dysfunction (8), lung lysates were analyzed for levels of HMGB1 protein expression. FIGS. **1**H, **1**L ($p=0.0158$) shows that HMGB1 expression increased at 4 and 24 hours after TBI, indicating that the AIM2 inflammasome and HMGB1 play a role in the inflammatory response in the lungs post-TBI.

TBI Induces Pyroptosis in the Lungs of Mice

[0090] As shown previously, activation of the AIM2 inflammasome in cortical neurons leads to pyroptotic cell death (19).To investigate whether TBI results in pyroptosis in mice lung tissue, the pyroptosome in lung tissue was isolated after TBI. TBI animals, sacrificed at 4 hours post-injury showed evidence of ASC oligomerization compared to sham animals (FIG. **4**A). ASC dimers, and trimers were seen in TBI animals (50, 75 kDA respectively). These results were indicative of pyroptosome formation, which can be characterized by the supramolecular assembly of ASC oligomers. In addition, gasdermin D (GSDMD), which is cleaved upon activation of caspase-1 and triggers pyroptosis and the release of IL-1β (20), was significantly increased in the lungs of TBI animals compared to sham (FIGS. **4**B and **4**C) ($p=0.0001$). These findings indicated that pyroptosis contributes to cell death in lung tissue after TBI.

TBI Increases Immunoreactivity of Inflammasome Proteins in Type II Alveolar Epithelial Cells

[0091] TBI may lead to capillary leak, resulting in increased vascular permeability and damage to specialized

alveolar epithelial cells, called type II pneumocytes (5). To examine the cellular effects of TBI on inflammasome expression in the lungs after injury, immunohistochemical analysis was performed in lung sections of sham, 4 hour, and 24 hour injured animals. Type II alveolar epithelial cells are known to be the main type of lung cells injured in ALI (17). Lung sections were stained with antibodies against AIM2, caspase-1, and ASC (green) and co-stained with Pro-surfactant protein C (Pro-SPC, red), a marker of type II epithelial cells, and DAPI nuclear staining (blue). As shown in FIGS. **2**A-**2**C, active caspase-1 (FIG. **2**A), ASC (FIG. **2**B), as well as AIM2 (FIG. **2**C) are present in SPC-positive cells (arrow). Immunoreactivity of these inflammasome proteins increased after TBI. These findings indicate that inflammasome proteins are expressed in type II alveolar epithelial cells and that TBI results in increased immunoreactivity in these cells.

TBI Increases Nuclear and Cytoplasmic HMGB1 Expression

[0092] In order to determine the cellular distribution of HMGB1 in lung cells after TBI, nuclear and cytoplasmic fractions from lung homogenates were isolated (FIGS. **3**A, **3**C) (p=0.0337). Immunoblotting indicated that both fractions had significant increases in HMGB1 expression at 4 hrs post-TBI (FIGS. **3**B, **3**D) (p=0.0345). Immunohistochemical analysis of HMGB1 was also performed in order to determine the changes in immunoreactivity in lung sections after TBI. Sections were co-stained for HMGB1 (green) and SPC (red) and DAPI nuclear staining (blue). Immunoreactivity of HMGB1 was increased at 4 hrs and 24 hrs when compared to sham. Weak immunoreactivity of HMGB1 was observed in SPC-positive cells (arrow) (FIG. **3**E); therefore, suggesting that HMGB1 changes in the injured lung tissue may be cytoplasmic.

TBI Induces Changes in Lung Morphology and Induces ALI

[0093] ALI can be characterized by inflammatory processes, which lead to alveolar and interstitial edema as well as infiltration of inflammatory cells into the alveolar space (23). Histopathological analysis of lung tissue (FIG. **5**A) indicate that severe TBI causes substantial changes in the lung architecture and morphology at 4 and 24 hours after injury. Sham animals showed a normal alveolar morphology, whereas injured animals showed acute changes in alveolar edema but decreased slightly by 24 hours after injury (long arrows). In addition, there was evidence of neutrophil infiltration (arrow heads) and changes in morphology of alveolar capillary membranes (*) at both time points. Injured animals showed signs of interstitial edema, which was more pronounced at 4 hours post-injury, but was still evident at 24 hours post injury (short arrows). Lastly, injured animals also showed evidence of thickening of the interstitial area and the alveolar septum (pound, #).

[0094] To confirm that severe injury induces ALI, histological sections were analyzed using the ALI scoring system defined by the American Thoracic Society (17). This system is based on evidence of neutrophil infiltration into the alveolar and interstitial spaces, hyaline membrane formation, proteinaceous debris filling the airspaces, and alveolar septal thickening.(17). These characteristics were significantly elevated in injured animals and ALI scores were higher overall in TBI animals compared to sham (FIG. **5**B) (p=0.0017).

Enoxaparin and Anti-ASC Antibody Treatment Significantly Reduces Inflammasome Expression and ALI After Adoptive Transfer of EV from TBI Mice

[0095] In order to provide evidence that EV and their cargo that can be released into the circulation after TBI may induce inflammasome activation in the lung, a classic adoptive transfer experiment was performed using serum-derived EV from severe CCI mice. EV preparations were validated using Western Blot for EV marker CD81 (FIG. **6**). Controls received EV isolated from sham or naive animals. As shown in FIGS. **7**A-**7**F, active caspase-1 (FIGS. **7**A, **7**B), ASC (FIGS. **7**A, **7**C), IL-18 (FIGS. **7**A, **7**D), AIM2 (FIGS. **7**A, **7**E) and HMGB1 (FIGS. **7**A, **7**F) were significantly elevated in the lungs of animals that received the EV from TBI injured animals when compared to the lungs of animals that receive EV from uninjured or naive mice or naive mice. Furthermore, infiltration of inflammatory cells (arrows) was apparent in lungs treated with EV from TBI mice (FIG. **7**G). Lastly, ALI score was also significantly higher in animals that received EV from injured mice (FIG. **7**H). These studies provided evidence for a neural-respiratory-inflammasome axis in which EV released into the circulation after TBI activate the inflammasome in lung target cells contributing to the pathogenesis of ALI.

[0096] Next, exosome uptake blockade was attempted by treatment with either Enoxaparin or a monoclonal antibody against ASC after adoptive transfer of EV from injured to naive mice. Negative control animals received saline and positive control animals received no treatment. As shown in FIGS. **8**A-**8**F, Caspase-1 (FIGS. **8**A, **8**B), ASC (FIGS. **8**A, **8**C), IL-1β (FIGS. **8**A, **8**D), AIM2 (FIGS. **8**A, **8**E), and HMGB1 (FIGS. **8**A, **8**F) were significantly reduced (p=<0001) as compared to untreated (positive control) group after treatment with Enoxaparin or a humanized monoclonal anti-ASC antibody (e.g. IC 100 antibody). In addition, H&E stained lung sections showed significantly less neutrophil infiltration into alveolar and interstitial space, as well as no signs of septal thickening (FIGS. **9**A-D). ALI scores for animals treated with Enoxaparin and anti-ASC antibody (IC 100) were significantly lower compared to untreated group (FIG. **9**E) (p=<0.0001). Thus, EV released into the circulation after TBI play a role in inflammasome activation in lung cells leading to ALI.

Conclusions

[0097] TBI can be associated with higher rates of certain medical complications, especially pulmonary and central nervous system dysfunction. In this Example, severe TBI was shown to increase HMGB1 and inflammasome expression (e.g., AIM2, caspase-1 and ASC expression) in cortical and lung tissue and induce changes in lung morphology consistent with ALI (e.g., infiltration of neutrophils into the alveolar and interstitial space, alveolar septal thickening, and alveolar edema and hemorrhage) and introduces the idea of a Neural Respiratory Inflammatory Axis. Importantly, TBI resulted in pyroptosis in lung tissue (e.g., presence of GSDMD cleavage and increased expression of inflammasome proteins in Type II alveolar epithelial cells. Additionally, adoptive transfer of EV from TBI mice activated the inflammasome and induced ALI, indicating that brain injury induces the release of EV containing a cargo of inflammasome proteins that are then carried to the result-

ing in ALI. Moreover, it was shown that by both inhibiting EV uptake (Enoxaparin) and inflammasome activation (anti-ASC antibody (IC 100) treatment), there is a reduction in inflammasome protein expression and in the development of ALI.

[0098] In summary, this Example showed that AIM2 inflammasome signaling plays a central role in the pathomechanism of lung injury after TBI and demonstrates a mechanism of TBI-induced ALI involving EV-mediated inflammasome signaling. These data provided evidence that EV-mediated inflammasome signaling can play a central role involving a Neuronal-Respiratory-Inflammatory Axis. Therefore, targeting this axis with antibodies against inflammasome proteins or drugs that block EV uptake may provide a therapeutic approach in Neurotrauma-induced ALI in all areas of critical care medicine. In light of these results, the disclosed therapeutic strategies may be useful for the treatment of inflammatory diseases of the lung in general.

INCORPORATION BY REFERENCE

[0099] The following references are incorporated by reference in their entireties for all purposes.

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Example 2: Role of EV Mediated Inflammasome Signaling in ALI Following TBI in Human Patients

[0150] As a follow up to the experiments on mice in Example 1, the role of EV isolated from human TBI patients on inflammasome signaling in human pulmonary endothelial cells was examined.

[0151] In a first experiment, serum-derived EV were isolated from TBI and control patients using Total Exosome Isolation kit (Thermofisher). Pulmonary Human Microvascular Endothelial Cells (HMVEC-Lonza) were cultured and plated on a 12-well plate. After confluency was reached, isolated EV from TBI and control patients were delivered (1.94 × 108 particles/ml) to cells for an incubation period of 4 hours. After incubation cells were harvested with 200 ul of lysis buffer and cell lysates were used for Western Blot analysis.

[0152] In a second experiment, serum-derived EV were isolated from TBI and control patients using Total Exosome Isolation kit (Thermofisher). Pulmonary Human Microvascular Endothelial Cells (HMVEC- Lonza) were cultured and plated on a 96-well plate. After confluency was reached, isolated EV from TBI and control patients were delivered (1.94 × 108 particles/ml) to cells for an incubation period of 3 hours and then 1 additional hour with caspase-1 FAM FLICA (Immunohistochemistry Technologies) with a 1:30 volume to volume ratio. After incubation, media was removed and cells were washed 3 times with apoptosis wash buffer (Immunohistochemistry Technologies). Cells were then co-stained with Hoechst for nuclear staining and Propidium Iodide for cell death. Images were taken using an EVOS microscope and then cells were read under a fluorescent plate reader at an excitation wavelength of 492 nm and an emission wavelength of 520 nm.

Results

[0153] As shown in FIGS. **10**A-**10**F, delivery of serum-derived EV from TBI patients increased inflammasome protein expression in pulmonary endothelial cells. FIGS. **10**A-**10**E showed that caspase-1, ASC, AIM2, and HMGB 1 were elevated in PMVEC incubated with TBI-EV for 4 hours as compared to PMVEC incubated with control-EV for 4 hours. Immunoassay results showed a significant increase in IL-1beta expression using Ella simple plex assay (FIG. **10**F).

[0154] As shown in FIGS. **11**A-**11**C, delivery of TBI-EV to pulmonary endothelial cells increased immunoreactivity of caspase-1 and cell death.

Conclusion

[0155] These studies provided further evidence for a neural-respiratory-inflammasome axis in which EV released into the circulation after TBI activate the inflammasome in lung target cells contributing to the pathogenesis of ALI.

[0156] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent application, foreign patents, foreign patent application and non-patent publications referred to in this specification are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, application and publications to provide yet further embodiments.

[0157] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

SEQUENCE LISTING

Sequence total quantity: 4

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organism = Rattus norvegicus
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SEQ ID NO: 2 moltype = AA length = 14
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source 1..14
mol_type = protein
organism = Homo sapiens
SEQ ID NO: 2
RESQSYLVED LERS 14

SEQ ID NO: 3 moltype = AA length = 19
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mol_type = protein
organism = Homo sapiens
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CEYYTEIRER EREKSEKGR 19

SEQ ID NO: 4 moltype = AA length = 15
FEATURE Location/Qualifiers
source 1..15
mol_type = protein
organism = Rattus norvegicus
SEQ ID NO: 4
MEESQSKEES NTEGC 15

What is claimed is:

1. A method of treating inflammation in lungs of a patient in need thereof, the method comprising: administering to the patient a composition comprising an agent that inhibits inflammasome signaling, whereby the inflammation in the lungs of the patient is treated.

2. The method of claim **1**, wherein the inflammation in the lungs is caused by a condition selected from a central nervous system (CNS) injury, a neurodegenerative disease, an autoimmune disease, asthma, chronic obstructive pulmonary disease, cystic fibrosis, interstitial lung disease and acute respiratory distress syndrome.

3. The method of claim **2**, wherein the CNS injury is selected from the group consisting of traumatic brain injury (TBI), stroke and spinal cord injury (SCI).

4. The method of **2**, wherein the neurodegenerative disease is selected from the group consisting of amyotrophic lateral

sclerosis (ALS), multiple sclerosis (MS) and Parkinson's disease (PD).

5. The method of any one of the above claims, wherein the administration of the composition results in inhibition of inflammasome activation in lung cells of the patient.

6. The method of any one of claims **1-4**, wherein the administration of the composition results in a reduction of caspase-1, nucleotide-binding leucine-rich repeat pyrin domain containing protein 1 (NLRP1), nucleotide-binding leucine-rich repeat pyrin domain containing protein 2 (NLRP2), nucleotide-binding leucine-rich repeat pyrin domain containing protein 3 (NLRP3), NLR family CARD domain-containing protein 4. (NLRC4), caspase-11, X-linked inhibitor of apoptosis protein (XIAP), pannexin-1, Apoptosis-associated Spec-like protein containing a Caspase Activating Recruitment Domain (ASC), interleukin-18 (IL-18), high mobility group box 1 (HMGB1) or absent in melanoma 2 (AIM2) levels in lung cells of the patient as compared to a control, wherein the control is an untreated patient.

7. The method of claim **5** or **6**, wherein the lung cells are Type II alveolar cells.

8. The method of any one of claims **1-5**, wherein the administration of the composition results in a reduction in acute lung injury (ALI) as compared to a control, wherein the control is an untreated patient.

9. The method of claim **8**, wherein the reduction in ALI is evidenced by a reduction in neutrophil infiltration into alveolar and/or interstitial space, reduced or absent alveolar septal thickening or a combination thereof.

10. The method of any one of the above claims, wherein the agent is an extracellular vesicle (EV) uptake inhibitor, an antibody that binds to an inflammasome component or a combination thereof.

11. The method of claim **10**, wherein the EV uptake inhibitor is a compound or an antibody, wherein the antibody is selected from Table 1.

12. The method of any of claims **10-11**, wherein the agent is an EV uptake inhibitor in combination with an antibody that binds to an inflammasome component.

13. The method of claim **12**, wherein the EV uptake inhibitor is a heparin.

14. The method of claim **13**, wherein the heparin is Enoxaparin.

15. The method of any of claims **10-14**, wherein the antibody that binds to an inflammasome component is an antibody that specifically binds to a component of a mammalian AIM2, NLRP1, NLRP2, NLRP3 or NLRC4 inflammasome.

16. The method of claim **10** or **15**, wherein the inflammasome component is caspase-1, ASC or AIM2.

17. The method of claim **16**, wherein the inflammasome component is ASC.

18. The method of claim **17**, wherein the antibody binds to an N-terminal PYRIN-PAAD-DAPIN domain (PYD), C-terminal caspase-recruitment domain (CARD) domain or an epitope derived from the PYD or CARD domain of the ASC protein.

19. The method of claim **17**, wherein the antibody binds to an amino acid having at least 85% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

20. The method of any of claims **17-19**, wherein the antibody inhibits ASC activity in the lungs of the patient.

21. The method of any one of the above claims, wherein the composition is formulated with a pharmaceutically acceptable carrier or diluent.

22. The method of any one of the above claims, wherein the composition is administered intracerebroventricularly, intraperitoneally, intravenously or by inhalation.

23. A method of treating inflammation in lungs of a patient that has been subjected to a central nervous system (CNS) injury, the method comprising: administering to the patient a composition comprising an agent that inhibits inflammasome signaling, whereby the inflammation in the lungs of the patient is treated.

24. The method of claim **23**, wherein the CNS injury is selected from the group consisting of traumatic brain injury (TBI), stroke and spinal cord injury (SCI).

25. The method of any one of claims **23-24**, wherein the administration of the composition results in inhibition of inflammasome activation in lung cells of the patient.

26. The method of any one of claims **23-24**, wherein the administration of the composition results in a reduction of caspase-1, NLRP1, NLRP2, NLRP3, NLRC4, caspase-11, XIAP, pannexin-1, Apoptosis-associated Spec-like protein containing a Caspase Activating Recruitment Domain (ASC), interleukin-18 (IL-18), high mobility group box 1 (HMGB1) or absent in melanoma 2 (AIM2) levels in lung cells of the patient as compared to a control, wherein the control is an untreated patient.

27. The method of claim **25** or **26**, wherein the lung cells are Type II alveolar cells.

28. The method of any one of claims **23-27**, wherein the administration of the composition results in a reduction in acute lung injury (ALI) as compared to a control, wherein the control is an untreated patient.

29. The method of claim **28**, wherein the reduction in ALI is evidenced by a reduction in neutrophil infiltration into alveolar and/or interstitial space, reduced or absent alveolar septal thickening or a combination thereof.

30. The method of any one of claims **23-29**, wherein the agent is an extracellular vesicle (EV) uptake inhibitor, an antibody that binds to an inflammasome component or a combination thereof.

31. The method of claim **30**, wherein the EV uptake inhibitor is a compound or an antibody, wherein the antibody is selected from Table 1.

32. The method of any of claims **30-31**, wherein the agent is an EV uptake inhibitor in combination with an antibody that binds to an inflammasome component.

33. The method of claim **32**, wherein the EV uptake inhibitor is a heparin.

34. The method of claim **33**, wherein the heparin is Enoxaparin.

35. The method of any of claims **30-34**, wherein the antibody that binds to an inflammasome component is an antibody that specifically binds to a component of a mammalian AIM2, NLRP1, NLRP2, NLRP3 or NLRC4 inflammasome.

36. The method of claim **30** or **35**, wherein the inflammasome component is caspase-1, ASC or AIM2.

37. The method of claim **36**, wherein the inflammasome component is ASC.

38. The method of claim **37**, wherein the antibody binds to the PYD, CARD domain or an epitope derived from the PYD or CARD domain of the ASC protein.

39. The method of claim **37**, wherein the antibody binds to an amino acid having at least 85% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

40. The method of any of claims **37-39**, wherein the antibody inhibits ASC activity in the lungs of the patient.

41. The method of any one of claims **23-40**, wherein the composition is formulated with a pharmaceutically acceptable carrier or diluent.

42. The method of any one of claims **23-41**, wherein the composition is administered intracerebroventricularly, intraperitoneally, intravenously or by inhalation.

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