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Office de la Propriété Intellectuelle du Canada

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Canadian Intellectual Property Office

CA 2974925 C 2022/03/22

(11)(21) 2 974 925

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2016/02/09
(87) Date publication PCT/PCT Publication Date: 2016/08/18
(45) Date de délivrance/Issue Date: 2022/03/22
(85) Entrée phase nationale/National Entry: 2017/07/25
(86) N° demande PCT/PCT Application No.: IL 2016/050156
(87) N° publication PCT/PCT Publication No.: 2016/128975
(30) Priorité/Priority: 2015/02/09 (US62/113,551)

(51) Cl.Int./Int.Cl. *A61K 39/395* (2006.01), *A61K 31/215* (2006.01), *A61K 31/7088* (2006.01), *A61P 31/00* (2006.01), *A61P 31/04* (2006.01), *A61P 31/16* (2006.01)

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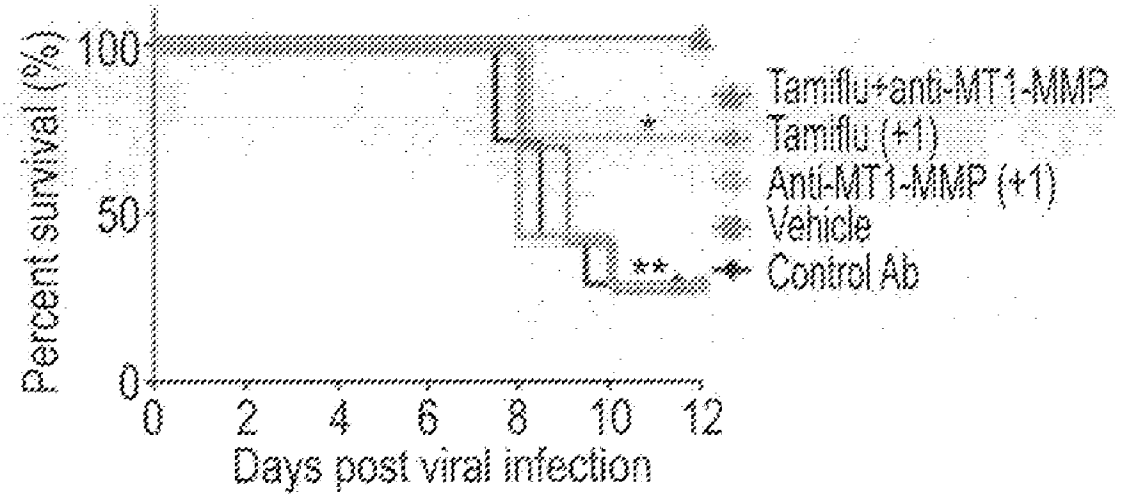
(54) Titre : PROCEDES POUR PREVENIR DES INFECTIONS SECONDAIRES
(54) Title: METHODS OF PREVENTING SECONDARY INFECTIONS



(57) Abrégé/Abstract:
A method of treating or preventing a disease associated with a secondary infection in a subject infected with a pathogen is provided. The method comprises administering to the subject a therapeutically effective amount of an anti-pathogenic agent directed towards the pathogen and a therapeutically effective amount of an agent which down-regulates at least one extracellular matrix-associated polypeptide.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 August 2016 (18.08.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/128975 A1

(51) International Patent Classification:
A61K 31/65 (2006.01) *A61K 39/395* (2006.01)
A61K 38/39 (2006.01) *A61P 31/16* (2006.01)

(21) International Application Number:
PCT/IL2016/050156

(22) International Filing Date:
9 February 2016 (09.02.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/113,551 9 February 2015 (09.02.2015) US

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

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

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

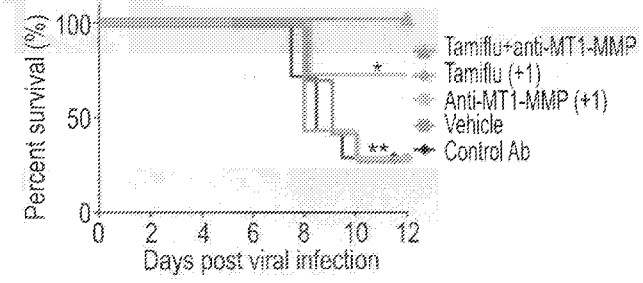
Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: METHODS OF PREVENTING SECONDARY INFECTIONS

FIG. 5E



(57) **Abstract**: A method of treating or preventing a disease associated with a secondary infection in a subject infected with a pathogen is provided. The method comprises administering to the subject a therapeutically effective amount of an anti-pathogenic agent directed towards the pathogen and a therapeutically effective amount of an agent which down-regulates at least one extracellular matrix-associated polypeptide.

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METHODS OF PREVENTING SECONDARY INFECTIONS

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to a method of preventing secondary infections in subjects infected with a pathogen using agents that down-regulate extracellular matrix remodeling.

Viral pandemics, such as influenza have caused millions of deaths worldwide. An extreme example is the 1918 pandemic which spread to six continents and infected ~500 million people reaching death toll of 50 million. Investigation of clinical cases and autopsy samples indicated that more than 95% of case fatalities were complicated by secondary bacterial infections, most commonly *Streptococcus pneumoniae* (*S. pneumoniae*). Immune cells recruited to the site of infection are critical for influenza clearance. However, growing evidence shows that infiltrating immune cells can also generate excessive inflammatory responses resulting in collateral tissue damage and disruption of the blood-air-barrier.

Tissue tolerance to pathogens is an important evolutionary trade-off, balancing the host immune response to pathogens while maintaining tissue function. However, tolerance capacity differs between various organs; lungs have a relatively low tissue tolerance capacity, and are more vulnerable to tissue damage. Accordingly, it has been argued that during respiratory viral infections uncontrolled host-derived immune responses, rather than viral titers, may be the leading cause of death. These responses are primarily associated with inflammatory monocytes, granulocytes, macrophages and dendritic cells. Accordingly, influenza-infected lungs are diffusely hemorrhagic, potentially linking the host response with tissue destruction. Tissue breaching may prime secondary bacterial invasion coupled with tissue disruption and, in extreme cases, may result in death. The interaction between influenza and secondary bacterial infections has long been studied, yet the molecular mechanisms by which influenza infection primes the tissue to secondary infections are not fully understood.

One of the host's tolerance components is the integrity of respiratory epithelial barriers anchored to the extracellular matrix (ECM). The ECM scaffold is produced by the cells in the tissue and is composed of two layers: I) the interstitial matrix, a three-dimensional gel of polysaccharides and fibrous proteins, and II) the basement

membrane, a mesh-like sheet formed at the base of epithelial tissues. ECM turnover is regulated by multiple proteolytic enzymes including matrix metalloproteinases (MMPs) that are responsible for the irreversible cleavage of a plethora of ECM molecules under normal and pathological conditions. Dysregulated proteolytic activity is often associated with inflammation, cancer, and infectious diseases. Accordingly, studies in pathological conditions have shown that dysregulated proteolysis of ECM molecules and related protein fibers have significant effects on tissue function. Specifically, MMPs were shown to play critical roles in lung organogenesis and many MMPs are involved in the acute and chronic phases of lung inflammatory diseases (Greenlee et al., 2007, Physiological reviews *87*, 69-98). Several substrates of MMPs have been identified during lung development, including ECM scaffold proteins, cell adhesion molecules, growth factors, cytokines, and chemokines (Greenlee et al., 2007, Physiological reviews *87*, 69-98).

Membrane type-I matrix metalloproteinase (MT1-MMP/MMP-14), a membrane tethered collagenase, is a key regulator in development and homeostasis of the lung as well as mediating wound healing, airway remodeling, and cell trafficking. Accordingly, it is expressed by multiple cell populations in the respiratory tract, including fibroblasts, endothelial cells and macrophages (Greenlee et al., 2007, Physiological reviews 87, 69-98). The functions of macrophage-derived proteases during inflammation are typically associated with tissue invasion or degradative events. In macrophages MT1-MMP serves not only as a protease acting on the ECM, but also regulates macrophage immune response. Recruited monocytes and macrophages up-regulate a broad spectrum of ECM remodelers including various MMPs. Depending on the conditions, macrophages express a spectrum of MMPs and their inhibitors: these have been associated with both physiological and pathological lung remodeling events. MMP-9 (gelatinase B) was shown to be beneficial for recovery from influenza infection by promoting migration of neutrophils to the infection site (Bradley et al., 2012, PLoS pathogens 8, e1002641). Despite these important findings, a systematic analysis of ECM proteolytic pathways during respiratory infections, including the trade-off between ECM integrity and immune protection, has never been completed.

Background art includes Cheung et al., Cardiovasc Pathol. 2006 Mar-Apr;15(2):63-74, Elkington et al., 2005 British Society for Immunology, Clinical and

Experimental Immunology, 142:12–20; Devy et al., Biochemistry Research International, Volume 2011, Article ID 191670, doi:10.1155/2011/191670; Renckens et al., J Immunol 2006; 176:3735-3741; Vanlaere et al., Clinical Microbiology Reviews, Apr. 2009,Vol 22, p. 224–239 and Udi et al., 2015, Structure 23, 1–12, January 6, 2015.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided a method of treating or preventing a disease associated with a secondary infection in a subject infected with a pathogen comprising administering to the subject a therapeutically effective amount of an anti-pathogenic agent directed towards the pathogen and a therapeutically effective amount of an agent which down-regulates at least one extracellular matrix-associated polypeptide, thereby treating or preventing the disease associated with a secondary infection in the subject.

According to an aspect of some embodiments of the present invention there is provided a method of treating a subject infected with a pathogen comprising administering to the subject a therapeutically effective amount of an anti-pathogenic agent directed towards the pathogen and a therapeutically effective amount of an agent which down-regulates at least one extracellular matrix-associated polypeptide, thereby treating the subject.

According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising an anti-pathogenic agent and an agent which down-regulates at least one extracellular matrix-associated polypeptide.

According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising an anti-pathogenic agent as a first active agent, an agent which down-regulates at least one extracellular matrix-associated polypeptide as a second active agent and a pharmaceutically acceptable carrier.

According to an aspect of some embodiments of the present invention there is provided a method of treating influenza in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an agent which down-regulates an extracellular matrix-associated polypeptide, thereby treating the influenza.

According to some embodiments of the invention, the extracellular matrix-associated polypeptide is set forth in Table 1.

According to some embodiments of the invention, the secondary infection is a bacterial infection, a viral infection or a fungal infection.

According to some embodiments of the invention, the secondary infection is a blood infection.

According to some embodiments of the invention, the disease is sepsis.

According to some embodiments of the invention, the administering comprises co-administering.

According to some embodiments of the invention, the pathogen is selected from the group consisting of a virus, a bacteria and a fungus.

According to some embodiments of the invention, the at least one polypeptide is a matrix metalloproteinase (MMP).

According to some embodiments of the invention, the matrix metalloproteinase is selected from the group consisting of membrane type 1-matrix metalloproteinase 1 (MT1-MMP1), MMP-9, MMP-8 and MMP-3.

According to some embodiments of the invention, the at least one polypeptide is membrane type 1-matrix metalloproteinase 1 (MT1-MMP1).

According to some embodiments of the invention, the infection is a respiratory infection.

According to some embodiments of the invention, the pathogen is a virus.

According to some embodiments of the invention, the virus is a respiratory virus.

According to some embodiments of the invention, the respiratory virus is influenza.

According to some embodiments of the invention, the anti-pathogenic agent is a neuraminidase inhibitor (NAI).

According to some embodiments of the invention, the neuraminidase inhibitor is selected from the group consisting of Laninamivir, Oseltamivir, Peramivir and Zanamivir.

According to some embodiments of the invention, the neuraminidase inhibitor is Oseltamivir.

According to some embodiments of the invention, the secondary infection is a bacterial infection.

According to some embodiments of the invention, the bacterial infection is *S. pneumoniae.*

According to some embodiments of the invention, the agent which down-regulates the at least one polypeptide is an antibody.

According to some embodiments of the invention, the agent which down-regulates the at least one polypeptide is a polynucleotide agent.

According to some embodiments of the invention, the extracellular matrix-associated polypeptide is set forth in Table 1.

According to some embodiments of the invention, the at least one polypeptide is a matrix metalloproteinase (MMP).

According to some embodiments of the invention, the matrix metalloproteinase is selected from the group consisting of membrane type 1-matrix metalloproteinase 1 (MT1-MMP1), MMP-9, MMP-8 and MMP-3.

According to some embodiments of the invention, the at least one polypeptide is membrane type 1-matrix metalloproteinase 1 (MT1-MMP1).

According to some embodiments of the invention, the anti-pathogenic agent is an antiviral agent.

According to some embodiments of the invention, the anti-viral agent is a neuraminidase inhibitor (NAI).

According to some embodiments of the invention, the neuraminidase inhibitor is selected from the group consisting of Laninamivir, Oseltamivir, Peramivir and Zanamivir.

According to some embodiments of the invention, the neuraminidase inhibitor is Oseltamivir.

According to some embodiments of the invention, the extracellular matrix-associated polypeptide is set forth in Table 1.

According to some embodiments of the invention, the extracellular matrix associated polypeptide is MT1-MMP1.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which

the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-D. Global analysis of extra cellular matrix gene circuits during influenza viral infection (A) K-means clustering (k = 20) of 3530 differentially expressed genes (Experimental Procedures) in lungs following influenza infections at 10 time points (n=4 for each time point). Dynamic range is scaled between -2 to 2 fold changes and color coded. 13.5 % (479) of the elevated genes are annotated as involved in ECM remodeling. Functional annotation was done using (cbl-gorilladotcsdottechniondotacdotil) clusters are annotated accordingly and colored. (B) Shown are a subset of gene ontologies (GO) enriched (p<10-4) in infected lungs. (C) Submatrix of gene expression dynamics following influenza infection of ECM remodeling genes. (D) Bar graph showing fold changes relative to T0 using qPCR measurement of MT1-MMP expression following influenza infection. Each sample was run in triplicates from 4 mice (2 biological repeats). Error bars represent standard deviation (SD) of the average number. The target genes were normalized to the endogenous reference gene GAPDH and relative to a non-infected control sample using $\Delta\Delta$ CT normalization method.

FIGs. 2A-C. MT1-MMP expression is mostly induced in myeloid cells following influenza infection. (A) FACS analysis from lung of influenza infected mice 74 hours post viral infection (Experimental procedures; n=25) compared to non-infected controls

(n=25). Gated are MT1-MMP expressing cells stained using anti-MT1-MMP antibody as well as CD45, CD11b and Epcam (Experimental procedures). (B) Histogram plots showing MT1-MMP, Epcam and CD11b mean fluorescence intensity before (grey) and 74 hours post influenza viral infection (red). (C) Bar graph showing qPCR measurement of MT1-MMP expression in sorted cell populations. Error bars represent SD of the average number. T-test ** $p<0.001$.

FIGs. 3A-I. Influenza infection induces changes in ECM morphology (A) Global mass spectrometry analysis of cell free ECM scaffolds (see experimental procedures). Quantitative protein abundance is presented by relative measurement (with reference to control uninfected tissue) using gray scale color code ranging from -1 to 1 white to black. Proteins depleted from the ECM post infection are annotated and colored white. Heatmap showing significant changes in protein quantification ($p<0.01$, t-test) in de-cellularized infected lung tissue as compared to non-infected control. Samples were analyzed in duplicates for 2 time points post infection (74, 122 hours post infection) with lethal dose of influenza infection using Mascot software (B) Representative scanning electron microscope imaging of infected versus control lungs. Arrows and arrow-heads point to orientation changes in collagen fibrils with D-banding patterns as quantified in sub figure (C) Directionality of fibers on the boundaries of alveoli are analyzed using Fiji package (Experimental procedures). (D-H) Representative immuno-staining images of ECM components during infection taken from (n=20) animals and screened in multiple tissue sections and slides imaged under the same exposure conditions. (E-I) Quantification of immunostaining using imageJ package (Experimental procedures), Error bars represent SD of the average number.

FIGs. 4A-K. Blocking MT1-MMP activity protects lung ECM components. (A) Cartoon showing experimental setup for influenza infection with various treatments. Mice were infected with sub-lethal dose of PR8 influenza strain (Experimental Procedures) (B-E) AirSEM imaging of alveolar and bronchial compartments 74 hours post infection using fixed tissue sections from whole lung, cut 300μm thick and stained for AirSEM (Experimental Procedures). Alveolar wall thickness and bronchial cell numbers were measured at different areas in multiple sections. Scale of main image- 50μm, inset scale- 20μm. Bar graph quantifies wall thickness and cell numbers in alveoli and bronchi, respectively. Error bars represent SD of the average number; field

of view (FOV). (F-G) Representative second harmonic generation (SHG) images originating from an unstained 50μm thick lung tissue sections. The detected SHG signal representing collagen is shown in red after reproduction of the z-stack using Imaris™ software package version 7.7.1. Bar graph is showing collagen volumes analyzed and quantified using Imaris™ package and tested for significance using t-test. Error bars represent SD of the average number (H-I) Lung immuno-staining for laminin. Bar graph shows laminin intensity analyzed using ImageJ package. (J-K) Collagen type I *in situ* zymography in lung tissue using fluorogenic substrate to detect collagenolytic activity in lung section with high sensitivity. Green signal and arrows point to active collagenase localization among bronchial epithelial lining cells or infiltrating immune cells. Scale- 50μm; Error bars represent SD of the average number; field of view (FOV).

FIGs. 5A-H. Combining anti-viral treatment with ECM protection supports survival and prevents systemic bacterial sepsis. (A, D) Cartoon showing experimental setup for influenza and S. pneumoniae co-infections in preventative and therapeutic modes. Mice were infected with sub-lethal doses of influenza followed by infection with *Strep. pneumoniae* (Experimental procedures). Treatment groups included: Tamiflu™, anti-MT1-MMP Fab, or the combinations of both. Administration was done using preventive mode, one day before infection (A-C) or as therapeutic mode one-day post infection (D-E). Vehicle-treated mice served as controls (Data are combined from three independent experiments with 7-10 mice in each group). (B, E) Survival curves (Kaplan-Meier) of co-infected mice receiving different treatments a day before (-1) or a day after (+1) the infections. Data is collected from 3 independent experiments of 5 mice in each group. * $P<0.01$; **$p< 0.001$ using Log-rank (Mantel-Cox) test. (C, F) Relative weight loss of co-infected mice at several time points post viral infection. Error bars represent SD from the mean. (G-H) *S. pneumonia* bacterial loads from spleen lysates of infected mice 6 days post viral infection (Experimental procedures).

FIGs. 6A-D. Gene and protein expression levels of ECM modulators during the course of influenza infection. (A) Bar graph showing qPCR measurements of ECM representative genes during different time points post infection in whole lung tissue (fold change relative to expression levels in T0) infection with lethal dose of influenza infection (experimental procedures). Each sample was run in triplicates from 4 mice (2

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biological repeats). Error bars represent standard deviation (SD) (B) Western blot analysis of several representative proteases during different time points of influenza infection using a reducing SDS-PAGE gel. (n=10). (C) Quantification of western blot results using ImageJ software. Average relative density of the protein of interest is relative to GAPDH internal control (D) Mean values of body weight (blue; left y axis) and viral titers (red; right y axis) during the course of influenza infection. Error bars represent standard deviations of body weight, calculated on 2-4 animals in each time point from 3 independent experiments. Viral burdens of whole lung homogenates in the lungs of mice using qPCR for genome copies of Matrix protein 2 (M2) followed by conversion into viral particle numbers using a calibration curve.

FIGs. 7A-D. Immune cells express active MT1-MMP during infection. (A) Immunostaining and bar graph quantification of MT1-MMP and F4/80 marker co-localization in infected lungs (74 hours PI) versus healthy controls. Arrows point to MT1-MMP stained cells. Representative images from multiple sections. (B) Bar graph quantifying panel A. Error bar represent SD, *P $\leq$ 0. 01, t-test. (C) Collagen type I *in situ* zymography combined with CD45 staining. Arrows point to cells expressing either marker at both control and infected sections (74 hours PI). Scale bar-50μm (D) Bar graph quantifying panel C. Error bar represent SD, *P $\leq$ 0. 01, t-test.

FIG. 8. Global expression analysis of MT1-MMP expressing cells. (A) K-means clustering (k = 6) of 2169 differentially expressed genes in $CD45^{pos}$ and $CD45^{neg}$ populations of cells sorted 74 hours post infection (n$\geq$5 mice included in each group-infected and non-infected control). Mice were infected with lethal dose of $4x10^3$ PFU of PR8 influenza (experimental procedures).

FIGs. 9A-D. Lung destructive phenotypes demonstrated using AirSEM imaging of whole lung or de-cellularized tissue. (A) Imaging of whole lung tissue. Arrows point to boundaries of alveolar openings with cells (non-infected control) or depleted of cells (infected) (B) Imaging of ECM scaffolds (after de-cellularization) of infected lungs compared to healthy controls. Arrows point to alveolar duct boundaries containing thick organized collagen bundles (non-infected control) or distorted fibrils (infected). (C) Lung cell counts in control and infected lungs scanning multiple lung sections, n=5. (D) Directionality imaging analysis was done by Fiji package. Graphs were plotted using

GraphPad Prism™ 6. The relative frequency of fiber spatial orientation was measured using the "Directionality" plugin analysis tool in Fiji package version 6.1.1.

FIGs. 10A-C. Calibration of single viral infection. (A) Weight loss of mice subjected to single viral infection at different dosages. Data set was analyzed from 5 mice at each time point. Error bar represent SD and analyzed using t-test. (B) Survival of mice exposed to single viral infection at different dosages. Error bar represent SD and analyzed using t-test ** $P \leq 0.001$. (C) Viral burdens of whole lung homogenates in the lungs 4 days post infection using standard PFU assay. Samples were run with 2 biological repeats 3 animals at each time point. X-axis represents the viral amounts used for the infection.

FIGs. 11A-F. MT1-MMP inhibition does not interfere with immune cell recruitment or cytokine induction. (A) FACS analysis of whole lung tissue subjected to influenza infection 74 hours post infection and treated either with anti-MT1-MMP inhibitor antibody or non-relevant GST control Ab. Mice were infected at sub-lethal influenza dose (experimental procedures). Data is gated on MT1-MMP expressing cells stained with MT1-MMP antibody as well as CD45, Ly6G, Ly6C, CD11b, NK46, TCRβ (Experimental procedures). Experiments were done twice using 3 mice per group. (B) Representative sections of lung tissue stained for macrophages using F4/80 marker taken at 74 hours post infection. Scale bars = 50μm. FOV indicates the entire field of view at magnification of x20. (C) Quantification of figure B using multiple tissue sections from at least 3 mice per group. Error bar represent SD, ***$P \leq 0.0001$, t-test. (D) Infiltrating immune cells in BALF from mice subjected to single viral infection and taken at 24, 48, 72, 122 hours PI. Mice were infected with sub-lethal dose of influenza. Samples were run with 2 biological repeats. Tested significant over non-infected mice using t-test * $P \leq 0.01$. (E-F) TNF-α and IL-1β levels in BALF of mice subjected to single viral infection and taken at 24, 48, 72, 96, 122 hours PI. Samples were run with 2 biological repeats. Error bar represent SD and analyzed using t-test * $P \leq 0.01$.

FIGs. 12A-F. Viral loads in the lung following Anti-MT1-MMP Ab treatment 74 Hours PI (A) Representative lung tissue sections stained for influenza virus using Tamiflu™, control Ab and anti-MT1-MMP Ab. Mice were infected sub-lethal dose of influenza (experimental procedures). (B-C) Bar graph quantification of influenza virus 24 and 48 hours PI. Error bar represent SD, **$P \leq 0.001$, t-test. FOV indicates the entire

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field of view at magnification of x20. Number of infected cells was normalized to DAPI using ImageJ. (D) PFU values of whole lung tissue 4 days and 7 days post viral infection (experimental procedures). Samples were run in triplicates of 2 biological repeats. Error bar represent SD. LEM -1; Tami-1; Tami+LEM-1 designate the different treatments, single or combined agents, given one day before the infection (Day-1). LEM+1; Tami+1; Tami+LEM+1 designate the different treatments, single or combined agents, given one day after the infection (Day+1). LEM refers to anti-MT1-MMP Ab (LEM2/15), GST refers to non-relevant Ab. (E) Viral burdens in the lungs 24, 48 and 96 hours post infection. Whole lung homogenates were used for PFU assay, testing 2 animals at each time point and running 2 biological replicates. Error bar represent SD, * $P \leq 0.01$ using 2-way ANOVA. (F) CFU values in the lungs of co-infected mice 2 days post bacterial infection. Error bars represent SD from the mean. Data are combined from two independent experiments with five mice in each group.

FIGs. 13A-D. ECM destruction is not perturbed by low viral titers. (A) AirSEM images of lungs 74 hours post infection from either Tamiflu™-treated, vehicle-treated or control mice. (B) Alveolar wall thickness measured using ImageJ (Experimental procedures), each mark represents a mean of measurements from a section-based region for an individual animal. (C) Bar graph represents viral counts in vehicle-treated and Tamiflu™-treated mice lungs using qPCR. Each column represents the mean of 3 mice. Bars indicate mean and SD from the average. X-axis represents hours post viral infection. (D) MT1-MMP expression levels in mice lungs infected with 90 PFU of PR8 influenza strain undergoing different treatments. Error bar represent SD and analyzed using t-test ** $P \leq 0.001$.

FIGs. 14A-B. Combined anti-viral and tissue protection therapy maintains lung structural features. (A) AirSEM imaging of lung sections representing changes in lung bronchi and alveoli during infection under several treatment modalities. Scale bar - 20μm. (B) Bar graph quantifying cell numbers in the different treatments taken from multiple sections.

FIG. 15. MT1-MMP expression in human respiratory epithelial cells upon influenza infection. Log2 relative expression levels of MT1-MMP correlating with the infection course (hours post infection) of human bronchial epithelial cells infected with

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H1N1 strain A/PR/8/34 (PR8). Error bars represent SD. Data analyzed from (Shapira SD, 2009).

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to a method of preventing secondary infections in subjects infected with a pathogen using agents that down-regulate extracellular matrix remodeling.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Infectious disease treatments have conventionally focused on pathogen elimination, either by administering antimicrobial drugs or by stimulating host immune responses using vaccination. The present inventors performed global genomics and proteomics analyses of an influenza mouse model and revealed an unexpected plethora of extracellular matrix (ECM)-related genes and proteins responsible for dysregulated ECM remodeling events during the course of infection (Figures 1A-D and 6A-D). MT1-MMP was the main collagenase leading to destruction of ECM scaffolds of alveoli and bronchi of infected mouse lungs. Electron microscopy of intact lungs, global mass spectrometry, two-photon and immune staining, and tissue zymography, revealed a multifaceted destruction of basement membrane components (Figures 3A-I and 9A-D). This unprecedented damage to lungs contributed to loss of blood-air barrier and resulted in systemic spread of secondary bacterial infection through leakage from lungs to internal organs causing sepsis and mortality. These devastating phenotypes and resulting deadly outcome were reversed by blocking the activity of MT1-MMP (Figures 4A-K), thus offering a new mode of therapeutic intervention through tissue support. As shown in Figures 5A-H, combining anti-viral treatment with ECM protection supports survival and prevents systemic bacterial sepsis.

The present inventors suggest this novel treatment opportunity for infection, designed to support tissue morphology and homeostasis while mitigating inappropriate host responses and collateral tissue damage.

Thus, according to a first aspect of the present invention there is provided a method of treating a subject infected with a pathogen comprising administering to the subject a therapeutically effective amount of an anti-pathogenic agent directed towards the pathogen and a therapeutically effective amount of an agent which down-regulates at least one extracellular matrix-associated polypeptide, herein below, thereby treating the subject.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

As used herein, the term "subject" refers to a mammalian subject – for example a human subject.

The subjects who are treated have pathogens which cause an infection.

As used herein, the term "pathogen" refers to a microbe or microorganism such as a virus, bacterium, prion or fungus that causes a disease (e.g. a respiratory disease).

According to a particular embodiment, the pathogen is a human pathogen.

Exemplary pathogenic viruses may belong to the following families: Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, Togaviridae. Particular pathogenic viruses contemplated by the present invention are those that cause smallpox, influenza, mumps, measles, chickenpox, ebola, or rubella.

According to a particular embodiment, the virus is one which brings about a respiratory infection (e.g. an upper respiratory tract infection and/or a lower respiratory tract infection).

Thus, according to a particular embodiment, the pathogenic virus is an influenza virus (e.g. influenza virus A - (e.g. H1N1, H2N2, H3N2, H5N1, H7N7, H1N2, H9N2, H7N2, H7N3, H10N7 and H7N9), influenza virus B or influenza virus C).

In another embodiment, the pathogenic virus is a parainfluenza virus (hPIV) including the human parainfluenza virus type 1 (hPIV-1) (causes croup); the human parainfluenza virus type 2 (hPIV-2) (causes croup and other upper and lower respiratory tract illnesses), the human parainfluenza virus type 3 (hPIV-3) (associated with bronchiolitis and pneumonia) and the human parainfluenza virus type 4 (hPIV-4).

In yet another embodiment, the pathogenic virus is a respiratory syncytial virus (RSV).

Exemplary pathogenic bacteria include *Mycobacterium tuberculosis* which causes *tuberculosis, Streptococcus* and *Pseudomonas* which cause *pneumonia*, and *Shigella*, *Campylobacter* and *Salmonella* which cause foodborne illnesses. Other exemplary pathogenic bacteria contemplated by the present invention are those that cause infections such as tetanus, typhoid fever, diphtheria, syphilis and Hansen's disease.

According to one embodiment, the pathogen causes an acute infection in the subject.

According to another embodiment, the pathogen causes a chronic infection in the subject.

The term "anti-pathogenic agent" refers to an antimicrobial agent and includes, but is not limited to antiviral agents, antibacterial agents, antiviral agents, anti-prion agents.

1. antiviral agents

Antiviral agents which can be used for combination therapy according to aspects of the present invention include CRX4 and CCR5 receptor inhibitors such as amantadine and rimantadine and pleconaril. Further antiviral agents that can be used in the combination therapy of this aspect of the present invention include agents which interfere with viral processes that synthesize virus components after a virus invades a cell. Representative agents include nucleotide and nucleoside analogues that look like the building blocks of RNA or DNA, but deactivate the enzymes that synthesize the RNA or DNA once the analogue is incorporated. Acyclovir is a nucleoside analogue, and is effective against herpes virus infections. Zidovudine (AZT), 3TC, FTC, and other

nucleoside reverse transcriptase inhibitors (NRTI), as well as non-nucleoside reverse transcriptase inhibitors (NNRTI), can also be used. Integrase inhibitors can also be used. Other antiviral agents include antisense oligonucleotides and ribozymes (directed against viral RNA or DNA at selected sites).

Some viruses, such as HIV, include protease enzymes, which cleave viral protein chains apart so they can be assembled into their final configuration. Protease inhibitors are another type of antiviral agent that can be used in the combination therapy described herein.

The final stage in the life cycle of a virus is the release of completed viruses from the host cell. Some active agents, such as zanamivir (Relenza™) and oseltamivir (Tamiflu™) treat influenza by preventing the release of viral particles by blocking a molecule named neuraminidase that is found on the surface of flu viruses.

Still other antiviral agents function by stimulating the patient's immune system. Interferons, including pegylated interferons, are representative compounds of this class. Interferon alpha is used, for example, to treat hepatitis B and C. Various antibodies, including monoclonal antibodies, can also be used to target viruses.

Anti-bacterial agents:

The antibacterial agent which can be used for combination therapy according to aspects of the present invention may be bactericidal or bacteriostatic.

In one embodiment, the antibacterial agent is an antibiotic.

As used herein, the term "antibiotic agent" refers to a group of chemical substances, isolated from natural sources or derived from antibiotic agents isolated from natural sources, having a capacity to inhibit growth of, or to destroy bacteria. Examples of antibiotic agents include, but are not limited to; Amikacin; Amoxicillin; Ampicillin; Azithromycin; Azlocillin; Aztreonam; Aztreonam; Carbenicillin; Cefaclor; Cefepime; Cefetamet; Cefinetazole; Cefixime; Cefonicid; Cefoperazone; Cefotaxime; Cefotetan; Cefoxitin; Cefpodoxime; Cefprozil; Cefsulodin; Ceftazidime; Ceftizoxime; Ceftriaxone; Cefuroxime; Cephalexin; Cephalothin; Cethromycin; Chloramphenicol; Cinoxacin; Ciprofloxacin; Clarithromycin; Clindamycin; Cloxacillin; Co-amoxiclavuanate; Dalbavancin; Daptomycin; Dicloxacillin; Doxycycline; Enoxacin; Erythromycin estolate; Erythromycin ethyl succinate; Erythromycin glucoheptonate; Erythromycin lactobionate; Erythromycin stearate; Erythromycin; Fidaxomicin; Fleroxacin;

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Gentamicin; Imipenem; Kanamycin; Lomefloxacin; Loracarbef; Methicillin; Metronidazole; Mezlocillin; Minocycline; Mupirocin; Nafcillin; Nalidixic acid; Netilmicin; Nitrofurantoin; Norfloxacin; Ofloxacin; Oxacillin; Penicillin G; Piperacillin; Retapamulin; Rifaxamin, Rifampin; Roxithromycin; Streptomycin; Sulfamethoxazole; Teicoplanin; Tetracycline; Ticarcillin; Tigecycline; Tobramycin; Trimethoprim; Vancomycin; combinations of Piperacillin and Tazobactam; and their various salts, acids, bases, and other derivatives. Anti-bacterial antibiotic agents include, but are not limited to, aminoglycosides, carbacephems, carbapenems, cephalosporins, cephamycins, fluoroquinolones, glycopeptides, lincosamides, macrolides, monobactams, penicillins, quinolones, sulfonamides, and tetracyclines.

Antibacterial agents also include antibacterial peptides. Examples include but are not limited to abaecin; andropin; apidaecins; bombinin; brevinins; buforin II; CAP18; cecropins; ceratotoxin; defensins; dermaseptin; dermcidin; drosomycin; esculentins; indolicidin; LL37; magainin; maximum H5; melittin; moricin; prophenin; protegrin; and or tachyplesins.

Anti-fungal agents:

The term "anti-fungal agent" refers to an agent or chemical that interferes with fungal infection through blocking spore germination, adhesion to substrates, or interfering with any metabolic process or step that is required for growth and development of the fungus or its spores.

Anti-Protozoal agent:

The term "anti-protozoal" as used herein refers to any chemical or agent that interferes with the parasitic or other life cycle features of a broad range of eukaryotic microbes and invertebrate worms. The agent or chemical might block protein synthesis, essential lipid production, respiratory processes or other metabolic events or growth control steps.

As mentioned herein above, the present invention contemplates administering both an agent directed against the pathogen (as detailed herein above) and an agent which down-regulates at least one extracellular matrix-associated polypeptide.

The term one extracellular matrix-associated polypeptide refers to a polypeptide that reduces the formation or enhances the degradation of the extracellular matrix or is comprised in the extracellular matrix.

According to a particular embodiment, the extracellular matrix-associated polypeptide is a fibrous protein such as collagen, elastin, fibronectin, and laminin.

According to another embodiment, the extracellular matrix-associated polypeptide is a protease such as a matrix metalloproteinase, an enzyme belonging to the class A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS) including ADAMTS1-17 and those belonging to the lysyl oxidase family such as Lysyl oxidase homolog 2 (LOXL).

In one embodiment, the extracellular matrix-associated polypeptide is set forth in Table 2B of the Examples section herein below.

Preferably, the extracellular matrix-associated polypeptide is set forth in Table 1, herein below. Exemplary cDNA sequences of each of the genes are provided therein.

Table 1

Symbol	*Gene (Human)*	*SEQ ID*	*Gene (mouse)*
TIMP1	NM_003254.2	1	NM_001044384
ADAMTS4	NM_005099.4	2	NM_172845
TNC	NM_002160.3	3	NM_011607
VCAN	NM_001126336.2	4	NM_001134475
THBS1	NM_003246.3	5	NM_011580
PLAU	NM_001145031.1	6	NM_008873
HAS1	NM_001297436.1	7	NM_008215
SERPINA3	NM_001085.4	8	NM_001033335 (3F), NM_009253 (3M), NM_009251 (3G) NM_009252 (3N)
SERPINE1	NM_000602	9	NM_008871
MMP3	NM_002422.3	10	NM_010809
ADAMTS15	NM_139055.2	11	NM_001024139
PRSS22	NM_022119.3	12	NM_133731
ITGA5	NM_002205.2	13	NM_010577
LGMN	NM_005606.6	14	NM_011175
MMP14	NM_004995.3	15	NM_008608
GZMB	NM_004131.4	16	NM_013542
MMP9	NM_004994.2	17	NM_013599
LCN2	NM_005564.3	18	NM_008491
MMP8	NM_001304441.1	19	NM_008611

LOXL3	NM_001289164.1	20	NM_013586
AIF1	NM_001623.3	21	NM_019467
LOXL2	NM_002318.2	22	NM_033325
TIMP3	NM_000362.4	23	NM_011595
LOXL1	NM_005576.3	24	NM_010729
ADAM8	NM_001109.4	25	NM_007403
SERPING1	NM_000062.2	26	NM_009776
SERINC3	NM_006811.2	27	NM_012032

Downregulation of ECM-associated polypeptides can be effected on the genomic and/or the transcript level using a variety of molecules which interfere with transcription and/or translation [e.g., RNA silencing agents (e.g., antisense, siRNA, shRNA, micro-RNA), Ribozyme and DNAzyme], or on the protein level using e.g., antagonists, enzymes that cleave the polypeptide and the like.

Following is a list of agents capable of downregulating expression level and/or activity of ECM-associated polypeptides.

One example, of an agent capable of ECM-associated polypeptides is an antibody or antibody fragment capable of specifically binding thereto and down-regulating activity thereof.

Preferably, the antibody binds with a Ki of less than 1000 nm, more preferably less than 100 nm and even more preferably less than 10 nm to its target polypeptide.

Preferably, the antibody specifically binds at least one epitope of the polypeptide. As used herein, the term "epitope" refers to any antigenic determinant on an antigen to which the paratope of an antibody binds.

Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or carbohydrate side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

According to one embodiment, the epitopic determinant is on the surface of the polypeptide.

According to another embodiment, when the polypeptide is a matrix metalloproteinase (MMP) such as MT1-MMP1, MMP-9, MMP-8 and MMP-3 the antibody binds to (and may optionally be generated by immunizing with) a hapten compound, [2-(2-minoethylcarbomoyl)-ethoxymethyl] -tris-[2-(N-(3-imidazol-1-yl-propyl))-ethoxymethyl]methane. This hapten molecule closely mimics the local

structure and conformation of the reactive zinc site inMMPs (see WO 2008/102359).

In one embodiment, the antibody is capable of specifically binding to the active form of the antibody and not to the proenzyme form.

Preferably, the antibody is specific to the particular matrix metalloproteinase (MMP) and binds with at least 5 times higher affinity to that particular MMP than a non relevant MMP.

According to a specific embodiment, the polypeptide is MT1-MMP1, also known as MMP-14.

Examples of antibodies that bind and down-regulate MMP-14 include those produced by the LEM-2/15 hybridoma cells as detailed in Udi et al., Structure 23, 1–12, January 6, 2015.

According to another embodiment, the antibody targets a surface epitope of MMP-14. Thus, for example the antibody may bind to the VB loop of MMP-14 (for example residues 160–173 and/or residues 218–233 of MMP-14). In another embodiment, the antibody is one which causes a conformational swiveling motion of the V-B loop of MMP-14.

An exemplary amino acid sequence of the V_H of a MMP-14 downregulating antibody is presented in SEQ ID NO: 54. An exemplary amino acid sequence of the V_L of a MMP-14 downregulating antibody is presented in SEQ ID NO: 55.

In yet another embodiment, the antibody is such that it down-regulates the collagenase activity of MMP-14, but does not affect the activation of pro-MMP-2.

Additional antibodies which down-regulate MMP-14 are disclosed in US patent No. 8,501,181 and Devy et al., Biochemistry Research International, Volume 2011, Article ID 191670, 11 pages, doi:10.1155/2011/191670.

The term "antibody" as used in this invention includes intact molecules as well as functional fragments thereof, such as Fab, F(ab')2, and Fv that are capable of binding to macrophages. These functional antibody fragments are defined as follows: (1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (2) Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin,

followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (3) (Fab')2, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')2 is a dimer of two Fab' fragments held together by two disulfide bonds; (4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (5) Single chain antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of producing polyclonal and monoclonal antibodies as well as fragments thereof are well known in the art (See for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York, 1988).

Antibody fragments according to some embodiments of the invention can be prepared by proteolytic hydrolysis of the antibody or by expression in E. coli or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')2. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. Nos. 4,036,945 and 4,331,647, and references contained therein. See also Porter, R. R. [Biochem. J. 73: 119-126 (1959)]. Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Fv fragments comprise an association of VH and VL chains. This association may be noncovalent, as described in Inbar et al. [Proc. Nat'l Acad. Sci. USA 69:2659-62 (19720]. Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise VH and VL chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the VH and VL domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by [Whitlow and Filpula, Methods 2: 97-105 (1991); Bird et al., Science 242:423-426 (1988); Pack et al., Bio/Technology 11:1271-77 (1993); and U.S. Pat. No. 4,946,778].

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick and Fry [Methods, 2: 106-10 (1991)].

Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab').sub.2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two,

variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introduction of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Pat. Nos.

5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology 10,: 779-783 (1992); Lonberg et al., Nature 368: 856-859 (1994); Morrison, Nature 368 812-13 (1994); Fishwild et al., Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13, 65-93 (1995).

Down-regulation of ECM-associated polypeptides can be also achieved by RNA silencing. As used herein, the phrase "RNA silencing" refers to a group of regulatory mechanisms [e.g. RNA interference (RNAi), transcriptional gene silencing (TGS), post-transcriptional gene silencing (PTGS), quelling, co-suppression, and translational repression] mediated by RNA molecules which result in the inhibition or "silencing" of the expression of a corresponding protein-coding gene. RNA silencing has been observed in many types of organisms, including plants, animals, and fungi.

As used herein, the term "RNA silencing agent" refers to an RNA which is capable of specifically inhibiting or "silencing" the expression of a target gene. In certain embodiments, the RNA silencing agent is capable of preventing complete processing (e.g, the full translation and/or expression) of an mRNA molecule through a post-transcriptional silencing mechanism. RNA silencing agents include noncoding RNA molecules, for example RNA duplexes comprising paired strands, as well as precursor RNAs from which such small non-coding RNAs can be generated. Exemplary RNA silencing agents include dsRNAs such as siRNAs, miRNAs and shRNAs. In one embodiment, the RNA silencing agent is capable of inducing RNA interference. In another embodiment, the RNA silencing agent is capable of mediating translational repression.

According to an embodiment of the invention, the RNA silencing agent is specific to the target RNA (e.g., MMP-14) and does not cross inhibit or silence a gene or a splice variant which exhibits 99% or less global homology to the target gene, e.g., less than 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81% global homology to the target gene.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of

post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla. Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex.

Accordingly, some embodiments of the invention contemplate use of dsRNA to down-regulate protein expression from mRNA.

According to one embodiment, the dsRNA is greater than 30 bp. The use of long dsRNAs (i.e. dsRNA greater than 30 bp) has been very limited owing to the belief that these longer regions of double stranded RNA will result in the induction of the interferon and PKR response. However, the use of long dsRNAs can provide numerous advantages in that the cell can select the optimal silencing sequence alleviating the need to test numerous siRNAs; long dsRNAs will allow for silencing libraries to have less complexity than would be necessary for siRNAs; and, perhaps most importantly, long dsRNA could prevent viral escape mutations when used as therapeutics.

Various studies demonstrate that long dsRNAs can be used to silence gene expression without inducing the stress response or causing significant off-target effects - see for example [Strat et al., Nucleic Acids Research, 2006, Vol. 34, No. 13 3803–3810; Bhargava A et al. Brain Res. Protoc. 2004;13:115–125; Diallo M., et al.,

Oligonucleotides. 2003;13:381–392; Paddison P.J., et al., Proc. Natl Acad. Sci. USA. 2002;99:1443–1448; Tran N., et al., FEBS Lett. 2004;573:127–134].

In particular, the invention according to some embodiments thereof contemplates introduction of long dsRNA (over 30 base transcripts) for gene silencing in cells where the interferon pathway is not activated (e.g. embryonic cells and oocytes) see for example Billy et al., PNAS 2001, Vol 98, pages 14428-14433. and Diallo et al, Oligonucleotides, October 1, 2003, 13(5): 381-392. doi:10.1089/154545703322617069.

The invention according to some embodiments thereof also contemplates introduction of long dsRNA specifically designed not to induce the interferon and PKR pathways for down-regulating gene expression. For example, Shinagwa and Ishii [*Genes & Dev.* 17 (11): 1340-1345, 2003] have developed a vector, named pDECAP, to express long double-strand RNA from an RNA polymerase II (Pol II) promoter. Because the transcripts from pDECAP lack both the 5'-cap structure and the 3'-poly(A) tail that facilitate ds-RNA export to the cytoplasm, long ds-RNA from pDECAP does not induce the interferon response.

Another method of evading the interferon and PKR pathways in mammalian systems is by introduction of small inhibitory RNAs (siRNAs) either via transfection or endogenous expression.

The term "siRNA" refers to small inhibitory RNA duplexes (generally between 18-30 basepairs) that induce the RNA interference (RNAi) pathway. Typically, siRNAs are chemically synthesized as 21mers with a central 19 bp duplex region and symmetric 2-base 3'-overhangs on the termini, although it has been recently described that chemically synthesized RNA duplexes of 25-30 base length can have as much as a 100-fold increase in potency compared with 21mers at the same location. The observed increased potency obtained using longer RNAs in triggering RNAi is theorized to result from providing Dicer with a substrate (27mer) instead of a product (21mer) and that this improves the rate or efficiency of entry of the siRNA duplex into RISC.

It has been found that position of the 3'-overhang influences potency of an siRNA and asymmetric duplexes having a 3'-overhang on the antisense strand are generally more potent than those with the 3'-overhang on the sense strand (Rose et al., 2005). This can be attributed to asymmetrical strand loading into RISC, as the opposite efficacy patterns are observed when targeting the antisense transcript.

The strands of a double-stranded interfering RNA (e.g., an siRNA) may be connected to form a hairpin or stem-loop structure (e.g., an shRNA). Thus, as mentioned the RNA silencing agent of some embodiments of the invention may also be a short hairpin RNA (shRNA).

The term "shRNA", as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The number of nucleotides in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11. Some of the nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. It will be recognized by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.

It will be appreciated that the RNA silencing agent of some embodiments of the invention need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides.

In some embodiments, the RNA silencing agent provided herein can be functionally associated with a cell-penetrating peptide." As used herein, a "cell-penetrating peptide" is a peptide that comprises a short (about 12-30 residues) amino acid sequence or functional motif that confers the energy-independent (i.e., non-endocytotic) translocation properties associated with transport of the membrane-permeable complex across the plasma and/or nuclear membranes of a cell. The cell-penetrating peptide used in the membrane-permeable complex of some embodiments of the invention preferably comprises at least one non-functional cysteine residue, which is either free or derivatized to form a disulfide link with a double-stranded ribonucleic acid that has been modified for such linkage. Representative amino acid motifs conferring such properties are listed in U.S. Pat. No. 6,348,185.

The cell-penetrating peptides of some embodiments of the invention preferably include, but are not limited to, penetratin, transportan, pIsl, TAT(48-60), pVEC, MTS, and MAP.

mRNAs to be targeted using RNA silencing agents include, but are not limited to, those whose expression is correlated with an undesired phenotypic trait. Exemplary mRNAs that may be targeted are those that encode truncated proteins i.e. comprise deletions. Accordingly the RNA silencing agent of some embodiments of the invention may be targeted to a bridging region on either side of the deletion. Introduction of such RNA silencing agents into a cell would cause a down-regulation of the mutated protein while leaving the non-mutated protein unaffected.

According to another embodiment the RNA silencing agent may be a miRNA or miRNA mimic.

The term "microRNA", "miRNA", and "miR" are synonymous and refer to a collection of non-coding single-stranded RNA molecules of about 19-28 nucleotides in length, which regulate gene expression. miRNAs are found in a wide range of organisms (viruses.fwdarw.humans) and have been shown to play a role in development, homeostasis, and disease etiology.

The term "microRNA mimic" refers to synthetic non-coding RNAs that are capable of entering the RNAi pathway and regulating gene expression. miRNA mimics imitate the function of endogenous microRNAs (miRNAs) and can be designed as mature, double stranded molecules or mimic precursors (e.g., or pre-miRNAs). miRNA mimics can be comprised of modified or unmodified RNA, DNA, RNA-DNA hybrids, or alternative nucleic acid chemistries (e.g., LNAs or 2'-O,4'-C-ethylene-bridged nucleic acids (ENA)). For mature, double stranded miRNA mimics, the length of the duplex region can vary between 13-33, 18-24 or 21-23 nucleotides. The miRNA may also comprise a total of at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 nucleotides. The sequence of the miRNA may be the first 13-33 nucleotides of the pre-miRNA. The sequence of the miRNA may also be the last 13-33 nucleotides of the pre-miRNA.

Another agent capable of downregulating ECM-associated polypeptides is a DNAzyme molecule capable of specifically cleaving an mRNA transcript or DNA sequence of the polypeptide.

Downregulation ECM-associated polypeptides can also be effected by using an antisense polynucleotide capable of specifically hybridizing with an mRNA transcript encoding same.

Another agent capable of downregulating ECM-associated polypeptides is a ribozyme molecule capable of specifically cleaving an mRNA transcript encoding same.

Another agent capable of downregulating ECM-associated polypeptides would be any molecule which binds to and/or cleaves the polypeptide. Such molecules can be antagonists, or inhibitory peptide.

For example, Zarrabi et al (J Biol Chem. 2011 Sep 23; 286(38): 33167–33177) discloses peptides that inhibit MMP-14.

It will be appreciated that a non-functional analogue of at least a catalytic or binding portion of any of the disclosed polypeptides can be also used as an agent which down-regulates ECM-associated polypeptides.

Another agent which can be used along with some embodiments of the invention to down-regulate the ECM-associated polypeptides is a molecule which prevents activation or substrate binding thereto.

Additional exemplary inhibitors of matrix metalloproteinases include the hydroxamate inhibitors, small peptide analogs of fibrillar collagens, which specifically interact in a bidentate manner via the hydroxyl and carbonyl oxygens of the hydroxamic group with the zinc ion in the catalytic site [Grams et al., (1995), Biochem. 34: 14012-14020; Bode et al., (1994), EMBO J., 13: 1263-1269].

Hydroxamate-based MMP inhibitors are usually composed of either a carbon back-bone (WO 95/29892, WO 97/24117, WO 97/49679 and EP 0780386), a peptidyl back-bone (WO 90/05719, WO 93/20047, WO 95/09841 and WO 96/06074) or a peptidomimetic back-bone [Schwartz et al., Progr. Med. Chem., 29: 271-334(1992); Rasmussen et al., Pharmacol. Ther., 75: 69-75 (1997); Denis et al., Invest. New Drugs, 15: 175-185 (1997)]. Alternatively, they contain a sulfonamido sulfonyl group which is bonded on one side to a phenyl ring and a sulfonamido nitrogen which is bonded to an hydroxamate group via a chain of one to four carbon atoms (EP 0757984 A1).

Other peptide-based MMP inhibitors are thiol amides which exhibit collagenase inhibition activity (U.S. Pat. No. 4,595,700), N-carboxyalkyl derivatives containing a biphenylethylglycine which inhibit MMP-3, MMP-2 and collagenase (Durette, et al., WO-9529689), lactam derivatives which inhibit MMPs, TNF-alpha and aggrecanase (see U.S. Pat. No. 6,495,699) and Tricyclic sulfonamide compounds (see U.S. Pat. No. 6,492,422).

Other MMP inhibitors are the chemically modified nonmicrobial tetracyclines (CMTs) that were shown to block expression of several MMPs in vitro. (Axisa et al., 2002, Stroke 33: 2858-2864).

Recently, a mechanism-based MMP inhibitor, SB-3CT, was designed according to the X-ray crystallographic information of the MMP active site (Brown et al., 2000). X-ray absorption studies revealed that binding of this molecule to the catalytic zinc reconstructs the conformational environment around the active site metal ion back to that of the pro-enzyme [Kleifeld et al., 2001, J Biol. Chem. 276: 17125-31].

In the context of a combination therapy, combination therapy compounds may be administered by the same route of administration (e.g. intrapulmonary, oral, enteral, etc.) that the described compounds are administered. In the alternative, the agents for use in combination therapy with the herein described agents may be administered by a different route of administration.

The agent which down-regulates ECM-associated polypeptides can be administered immediately prior to (or after) the anti-pathogenic agent, on the same day as, one day before (or after), one week before (or after), one month before (or after), or two months before (or after) the anti-pathogenic agent, and the like.

The agents which down-regulate ECM-associated polypeptides and the anti-pathogenic agent can be administered concomitantly, that is, where the administering for each of these agents can occur at time intervals that partially or fully overlap each other. The agents described herein can be administered during time intervals that do not overlap each other. For example, the first agent can be administered within the time frame of t=0 to 1 hours, while the second agent can be administered within the time frame of t=1 to 2 hours. Also, the first agent can be administered within the time frame of t=0 to 1 hours, while the second agent can be administered somewhere within the time frame of t=2-3 hours, t=3-4 hours, t=4-5 hours, t=5-6 hours, t=6-7 hours, t=7-8 hours, t=8-9 hours, t=9-10 hours, and the like. Moreover, the second agent can be administered somewhere in the time frame of t=minus 2-3 hours, t=minus 3-4 hours, t=minus 4-5 hours, t=5-6 minus hours, t=minus 6-7 hours, t=minus 7-8 hours, t=minus 8-9 hours, t=minus 9-10 hours.

The agents of the present invention are typically provided in combined amounts to treat the infection and/or to reduce symptoms or disease associated with a secondary

infection. This amount will evidently depend upon the particular agent selected for use, the nature and number of the other treatment modality, the condition(s) to be treated, prevented and/or palliated, the species, age, sex, weight, health and prognosis of the subject, the mode of administration, effectiveness of targeting, residence time, mode of clearance, type and severity of side effects of the agents and upon many other factors which will be evident to those of skill in the art.

The present inventors have shown that administration of an antibody which binds to and down-regulates MMP-14 prevents complications of a secondary infection. More specifically, the present inventors showed that administration of an MMP-14 antibody together with an antiviral agent reduced the symptoms in animals infected with the influenza virus (as the primary infection) and *S. pneumoniae* (as the secondary infection).

Thus, the present inventors propose that administration of agents which specifically down-regulate ECM-associated polypeptides and an antipathogenic agent may prevent (or reduce the symptoms of) a secondary infection.

Thus, according to another aspect of the present invention there is provided a method of treating or preventing a disease associated with a secondary infection in a subject infected with a pathogen comprising administering to the subject a therapeutically effective amount of an anti-pathogenic agent directed towards the pathogen and a therapeutically effective amount of an agent which down-regulates an ECM-associated polypeptide, thereby treating or preventing the disease associated with the secondary infection in the subject.

As used herein, the phrase "secondary infection" refers to an infection that occurs during or after treatment of another pre-existing infection. It may result from the treatment itself or from changes in the immune system.

The term "preventing" refers to inhibiting or arresting the development of the secondary infection and/or causing the prevention, reduction, remission, or regression of symptoms of the secondary infection.

According to a particular embodiment, the combination therapy proposed by the present invention reduces the complications or treats a disease (e.g. sepsis) associated with the secondary infection.

The secondary infection may be a bacterial infection, a viral infection or a fungal infection.

The primary and the secondary infections are typically infections of the same organ (e.g. lungs and/or respiratory tract).

In one embodiment, the primary infection is viral infection (e.g. *influenza*) and the secondary infection is a bacterial infection (e.g. *S. pneumoniae*).

In another embodiment, the primary infection is a bacterial infection and the secondary infection is a viral infection.

In yet another embodiment, the primary infection is viral infection and the secondary infection is a fungal infection.

In yet another embodiment, the primary infection is a bacterial infection and the secondary infection is a fungal infection.

According to yet another aspect of the present invention there is provided a method of treating influenza in a subject in need thereof comprising administering to the subject a therapeutically effective amount of an agent which down-regulates at least one ECM-associated polypeptide, thereby treating the influenza.

ECM-associated polypeptides have been described herein above. According to a particular embodiment, the ECM-associated polypeptide is set forth in Table 1 – for example MT1-MMP1.

According to a particular embodiment, treatment of influenza is effected by administering an antibody which down-regulates an amount of MT1-MMP1, such as those described herein above.

In order to prevent the collapse of the ECM, preferably, the agent is provided no more than 5 days after the start of symptoms of the influenza virus, no more than 4 days after the start of symptoms of the influenza virus, no more than 3 days after the start of symptoms of the influenza virus, no more than 2 days after the start of symptoms of the influenza virus, and even no more than 1 day after the start of symptoms of the influenza virus.

In any of the method and uses described herein, the agents can be used *per se* or in a pharmaceutical composition which further comprises a pharmaceutically (or cosmetically) acceptable carrier.

In one embodiment, the agents are co-formulated in the same pharmaceutical composition.

In another embodiment, the agents are formulated in separate pharmaceutical compositions. The separate pharmaceutical compositions may be comprised in a single article of manufacture, e.g. a kit.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to any of the agents described herein. It will be appreciated that the pharmaceutical compositions may comprise additional active agents known to be useful in treating a particular disease.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

According to a particular embodiment, the route of administration is via topical delivery.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions,

and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum Arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g.,

gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients (e.g. the compounds described herein) effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g., fibrotic or inflammatory disease) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated from animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in experimental animals. The data obtained from these animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide cell numbers sufficient to induce normoglycemia (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate

container, and labeled for treatment of an indicated condition, as if further detailed above.

It is expected that during the life of a patent maturing from this application many relevant antiviral/antibacterial agents will be developed and the scope of the term antiviral/antibacterial is intended to include all such new technologies *a priori*.

As used herein the term "about" refers to ± 10 %

The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein

interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th

Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996).

Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader.

MATERIALS AND METHODS

Influenza Virus and Streptococcus pneumoniae Bacterial Agent: Mouse-adapted PR8 virus, influenza A/Puerto Rico/8/34 (A/PR/ 8/34, H1N1) was persistently grown in hen egg amnion and influenza effective titers were quantified as previously described (Achdout et al., 2003). *Streptococcus pneumoniae* (*S. pneumoniae*) D39 type 2 encapsulated strain was grown in Todd-Hewitt broth (Difco Laboratories) For isolation and infection of mice, bacteria were grown overnight on tryptic soy agar (Hylab Laboratories) supplemented with 3% (vol/vol) sheep erythrocyte at 37 °C and were then harvested by centrifugation at4000 g for 20 min to pellet the bacteria and dilute it to the desired concentration.

Infection Procedures: Female C57BL/6J mice (4-5 weeks of age) were anesthetized with ketamine-xylazine and were intra-nasally inoculated with 50 µl of diluted virus. The same stock was used for all the experiments containing influenza

A/Puerto Rico/8/34 (A/PR/ 8/34, H1N1) strain, $9X10^7$ PFU/ml, HA 1:1,024. To study pathogenesis of Influenza infection, C57BL/6 mice were intra-nasally infected with $4x10^3$ PFU of influenza PR8 virus equivalent to lethal dose. Mice were sacrificed on 3, 7, 11, 26, 32, 49, 74, 98, 122, 148 hours post infection and the lungs were harvested and homogenized for RNA isolation. To study the effectivity of anti-MT1-MMP inhibitor, both in the single viral infection model and in the double infection model combining *S. pneumoniae*, a sub-lethal dose of 800 PFU was used, which was diluted accordingly and administered along the same route. *S. pneumoniae* was grown on tryptic soy agar (Hylab Laboratories) supplemented with 3% (vol/vol) sheep erythrocytes. The bacterium was diluted in sterile PBS and administered intra-nasally 4 days post viral infection at a dose of 30 CFU, in a volume of 50µl. The mice were anesthetized and held in an upright position while inoculated. Mice were weighted and monitored at least daily for illness and mortality. All animal procedures were performed according to IACUC guidelines and were approved by the committee of the Weizmann Institute of Science.

Treatment of Animals: Mice were treated in the single infection experiments as well as in the co-infection experiments with 3 mg/kg of LEM 2/15 Fab fragment at a total volume of 100µl per injection, given intra-peritoneally every day. GST-Fab, designated in text as control Fab, served as non-relevant control and was given the same dose as the LEM 2/15 treated group. PBS used as a vehicle control.

LEM 2/15 (Anti-MT1-MMP Ab) Purification: Hybridoma cells of LEM-2/15 were grown in DCCM (serum-free medium designed for hybridoma cell growth and monoclonal antibody production, purchased from Biological Industries). Cells were precipitated by centrifugation at 193 g, and the supernatant was collected. The supernatant was dialyzed against 20 mM phosphate buffer (pH 8). A 1 ml HiTrap™ protein A high-performance column was equilibrated with 100 mM phosphate buffer (pH 8), and the supernatant was loaded at 1 ml/min. The antibody was eluted with 100 mM citrate buffer (pH 6) and dialyzed against 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl.

Antibody Digestion with Papain: Papain was activated in 0.5 M Tris-HCl (pH 8), 10 mM EDTA, and 5mM dithiothreitol for 15 min at 370C. Active papain was added to a solution of intact LEM-2/15 at a ratio of 1:1,000, and the digestion process was

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carried out for 3 h at 370C. The digestion reaction was terminated with the addition of 20mM iodoacetamide in the dark at room temperature for 30 min. The Fab fragment was isolated from the Fc by a protein A column, and the Fab fragment was collected from the flow through and dialyzed against 50 mM Tris-HCl (pH 7.5) and 150 mM NaCl. The purity of the Fab fragment was estimated by 12% SDS-PAGE gel. Pure Fab fragment was filtered to assure sterility and kept at -80 °C conditions until use.

Glutathione S-transferases (GST)-Fab Fragment: Fab fragment from the whole GST antibody were produced as described in the upper section (Antibody Digestion with Papain).

Quantification of Viral and Bacterial Loads: Viral titers in the lungs were determined by titration of organ homogenate on MDCK cells and plaque forming units (PFUs) were quantified as described in (Okuda et al.,2001). Strep. pneumoniae levels were determined by plating titrated amounts of organ homogenate on tryptic soy agar plates supplemented with 3% sheep erythrocytes (Hylab Laboratories). Organs were homogenized using the GentleMACS™ 1ml of appropriate buffer for PFUs or 10ml of sterile water for Strep. pneumoniae for CFUs. Viral burdens were also quantified using qPCR, as described before for the detection of virus in patients (Hindiyeh et al., 2005). S. pneumoniae identification was done using qPCR as previously described (Ogunniyi et al., 2002). Serial dilutions of Influenza A (A/PR/8/34) virus titrated on Madin-Darby Canine Kidney (MDCK) cells were used as standards to determine the quantity of the influenza virus by quantitative real-time PCR (qRT-PCR) and convert the qPCR results into viral load numbers.

RNA Isolation: Lungs were removed and immediately transferred into RNA Latter solution (Invitrogen). For RNA isolation, the lung was cut into small pieces in the presence of QIAzol™, homogenized using SPEX CertiPrep™ homogenizer, and total RNA was extracted with a miRNeasy Mini Kit™ (Qiagen®). RNA integrity was determined (Tapestation™, Agilent Technologies) and concentration measured with a Qubit Fluorometric Quantitation device (LifeTechnologies).

Preparation of RNA Sequencing Libraries: For RNA-seq a derivation of MARS-seq technique was used as described in (Jaitin et al., 2014). In brief, total RNA was fragmented into fragments having an average size of 300 nucleotides by chemical heat (95°C) treatment for 4:30 min (NEBNext™ Magnesium RNA Fragmentation

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Module). The 3′ polyadenylated fragments were enriched by selection on poly dT beads (Dynabeads™ Invitrogen). Strand-specific cDNA was synthesized using a poly T-VN oligo (18 T) and Affinity Script RT™ enzyme (Agilent). Double-strand DNA was obtained using Second strand synthesis kit (NEB). DNA ends were repaired using T4 polynucleotide kinase and T4 polymerase (NEB-Next). After the addition of an adenine base residue to the 5′ end using Klenow enzyme (NEB-Next), a barcode Illumina compatible adaptor (IDT) was ligated to each fragment. The washed DNA fragment was amplified by PCR (12 cycles) using specific primers (IDT) to the ligated adaptors. The quality of each library was analyzed by TapeStation™ (Agilent).

Pre-Processing of RNA-Seq Data: RNA-seq was performed as described in Lavin et al., 2014. In brief, all reads, both from whole lung (Figure 1) and cell populations (Figure 8) were aligned to the mouse reference genome (NCBI 37, MM9) using the TopHat aligner. Normalized expression table was created using ESAT garberlabdotumassmeddotedu/software/esat/ based on the negative binomial distribution and a local regression model. Data manipulation-Discard genes from table that have values > 0 only once; Calculate 75 percentile of data result is 33 (set noise to 32); Find max of raw and discard if max < 32; log2 values as x+32; Average replicates; keep rows where max - min > 0.8 (notice not 2 fold but 1.75); K-Means in matlab for 20 clusters; manually ordered the clusters for visual purpose picture was done in GeneE.

***qPCR*:** Total RNA was reverse transcribed to cDNA using high capacity cDNA reverse transcription kit (Applied Biosystems). RT-PCR was performed with LightCycler® 480 SYBR green I master mix (Roche) in triplicate, using GAPDH and β-actin for normalization. Primer list is provided in Table 2A, herein below.

Table 2A

Gene	Direction	sequence	SEQ ID
MT1-MMP	Forward	5-AGCACTGGGTGTTTGACG-3	28
MT1-MMP	Reverse	5-GTCTTCCCATTGGGCATC-3	29
MMP-9	Forward	5-CAGACGTGGGTCGATTCC-3	30
MMP-9	Reverse	5-TCATCGATCATGTCTCGC-3	31
MMP-8	Forward	5-GCAGCGCTTCTTCAGCTT-3	32
MMP-8	Reverse	5-GTGTGTGTCCACTTGGGA-3	33
MMP-2	Forward	5-ACGATGATGACCGGAAGT-3	34
MMP-2	Reverse	5-GTGTAGATCGGGGCCATC-3	35
TIMP1-var2	Forward	5-GCAGTGATTTCCCCGCCA-3	36
TIMP1-var2	Reverse	5-GGGGGCCATCATGGTATC-3	37

MMP-3	Forward	5-AAGGAGGCAGCAGAGAAC-3	38
MMP-3	Reverse	5-GCACTGTCATGCAATGGG-3	39
LGMN	Forward	5-GCCTACCAGATCATCCAC-3	40
LGMN	Reverse	5-ACATCTGTGCCGTTAGGT-3	41
GZMB	Forward	5- ACAACACTCTTGACGCTG-3	42
GZMB	Reverse	5-CGAGAGTGGGGCTTGACT-3	43
LCN2	Forward	5-ACAACCAGTTCGCCATGG-3	44
LCN2	Reverse	5-AAGCGGGGTGAAACGTTCC-3	45
PR8 MATRIX A INF A-CDC	Forward	5-GACCRATCCTGTCACTGAC-3	46
PR8 MATRIX A INF A-CDC	Reverse	5-TGCAGTCCTCGCTCACTGGGCACG-3	47
16S rRNA	Forward	5-GGTGAGTAACGCGTAGGTAA-3	48
16S Rrna	Reverse	5-ACGATCCGAAAACCTTCTTC-3	49
TIMP-2	Forward	TCTAGGAGTCCCAGTCAGCC	50
TIMP-2	Reverse	CAACAAGGACTGCCAAGCAC	51
GAPDH	Forward	GCCCTTGAGCTAGGACTGGA	52
GAPDH	Reverse	TACGGCCAAATCCGTTCACA	53

In Gel Proteolysis and Mass Spectrometry Analysis: Lung samples were de-cellularized using 0.5% EDTA supplemented with 2% triton, shaking for 24 hours. Samples were then dehydrated using a Speedvac and weighted. Samples were then subjected to in-solution digestion using activated MMP-13, 500nM in TNC buffer in (50 mM Tris-HCl, 150 mM NaCl, 5 mM MgCl2, 5 mM CaCl2, pH 7.4) a volume of 120µl, for 24 hours shaking at 30°C. The volume and concentration of activated MMP-13 were adjusted according to the weight of each sample, and each sample was run in duplicates. Protein extract was loaded on SDS-PAGE for a short run. The proteins in the gel were reduced with 2.8 mM DTT (60 °C for 30 min), modified with 8.8 mM iodoacetamide in 100mM ammonium bicarbonate (in the dark, room temperature for 30 min) and digested in 10% acetonitrile and 10mM ammonium bicarbonate with modified trypsin (Promega) at a 1:10 enzyme-to-substrate ratio, overnight at 37 °C. An additional second trypsinization was done for 4 hours. The resulting tryptic peptides were resolved by reverse-phase chromatography on 0.075 X 200-mm fused silica capillaries (J&W) packed with Reprosil® reversed phase material (Dr Maisch GmbH, Germany). The peptides were eluted with linear 95 minutes gradients of 7 to 40% and 8 minutes at 95% acetonitrile with 0.1% formic acid in water at flow rates of 0.25 µl/min. Mass spectrometry was performed by an ion-trap mass spectrometer (Orbitrap™ XP,

Thermo) in a positive mode using repetitively full MS scan followed by collision induces dissociation (CID) of the 7 most dominant ion selected from the first MS scan.

The mass spectrometry data was analyzed using the MaxQuant™ 1.3.0.5 software searching against the mouse section of the Uniprot database with mass tolerance of 20 ppm for the precursor masses. Peptide- and protein-level false discovery rates (FDRs) were filtered to 1% using the target-decoy strategy. Protein table were filtered to eliminate the identifications from the reverse database, and common contaminants and single peptide identifications. The data was quantified by label free analysis using the same software, based on extracted ion currents (XICs) of peptides enabling quantitation from each LC/MS run for each peptide identified in any of experiments. To search for ECM related proteins, annotations were determined using the GORILLA Bioinformatics Resources.

Western Blot: Lung samples were homogenized using gentleMACS™ (Miltenyi Biotec) according to manufacturer instructions using 500µl rippa buffer containing protease inhibitor cocktail (Roche). Protein levels were then measured using BCA kit (Pierce Biotechnology) and run in duplicates on SDS-PAGE gel using a mini-electrophoresis apparatus (Bio-Rad Laboratories, Inc.). The resolved polypeptides were transferred onto a nitrocellulose membrane in Tris-glycine buffer containing 25% methanol. The membranes were blocked with 5% dried milk, and then incubated with goat anti-MMP-8 (SantaCruz), rabbit anti-MMP-9 (Abcam) or rabbit anti-MT1MMP (Abcam) antibodies. Rabbit anti-GAPDH (SantaCruz) was included in each procedure to avoid inter-assay variations. Nitrocellulose membranes were incubated with goat anti-rabbit HRP conjugated antibody (Abcam), or bovine anti-goat HRP (Sigma). Membranes were developed using EZ-ECL chemiluminescence detection kit (Biological industries). A molecular mass protein standard (PageRuler™ Prestained Protein ladder, Fermentas) was included in each assay.

***Lung Preparation for Imaging*:** Lungs were inflated by using PBS for in situ zymography or 4% PFA for other imaging purposes. This was done by exposing the trachea, inserting a cannula 22G, 0.8x25mm, (Cathy IV cannula, HMD Healthcare LTD) and injecting 5ml fluid. The cannula was ligated to the trachea to avoid spillage. Mouse lungs were harvested at different time points post infection, embedded in OCT and frozen in -80°C until analyzed.

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Two-Photon Microscopy and Second Harmonics Generation: Before imaging, lungs were cut 300 μm and immediately visualized using a two-photon microscope in the in-vivo imaging unit in the Weizmann institute (2PM:Zeiss LSM 510 META NLO; equipped with a broadband Mai Tai-HP-femtosecond single box tunable Ti-sapphire oscillator, with automated broadband wavelength tuning 700–1,020 nm from Spectraphysics, for two-photon excitation). For collagen second harmonic imaging a wavelength of 800 nm was used (detection at 400nm).

AirSEM and SEM Imaging of Intact Lung Tissues and Lung ECM Scaffolds: Fixed lungs were sectioned into 300μm sections. Sections were washed three times in a large volume of PBS to remove OCT remnants, followed by three DDW washes. For tissue imaging, the slices were gently placed on a SuperFrost Plus glass slides and stained as previously described. Briefly, sections were washed with DDW and stained with 0.1% ruthenium red (EM grade, Sigma-Aldrich) in a 0.1 M sodium cacodylate buffer pH 7.4 (analytical standard, Sigma-Aldrich) for 15 min. The sections were then thoroughly washed with DDW and stained with a 2% uranyl acetate solution for 10 min. The samples were then washed with DDW and allowed to dry in the air at room temperature for 5–7 min before airSEM™. ECM scaffolds were first de-cellularized using 0.5% EDTA supplemented with 2% triton for 24 hours. Staining was done as previously described. For conventional scanning electron microscopy (SEM) samples were further dehydrated through an ethanol series increasing in concentration to 100% ethanol, were dried in a critical point dryer and coated by Au/Palladium according to standard sample preparation procedure for SEM imaging with an Ultra 55 Feg Zeiss SEM operating at 2 kV.

Immunohistochemistry: Immunohistochemistry was performed using standard techniques on 10μm cryo-sections. Sections were fixed with 4% PFA, blocked with 3% BSA, incubated overnight with primary rabbit anti-laminin (Sigma), or rabbit anti-lumican (abcam), or rabbit anti-collagen IV or rat antibody for F4/80 and CD45 cell surface protein (abcam). LEM2/15 was conjugated to Alexa Fluor® 555 Protein by Labeling Kit (Molecular Probes), according to manufacturer's instructions. Sections were then washed with PBS, incubated with a goat anti-rabbit HRP conjugated (Jackson). Fluorescein or Cy3 conjugated anti-HRP kit was used (Perkin Ehlmer)

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respectively, followed by DAPI staining (Sigma) and mounting with immune-mount (Thermo Scientific). Samples were imaged using Nikon 80i eclipse microscope.

Collagen Type I in Situ Zymography: Non-fixed lungs samples were cut into 10μm sections, gently washed to remove OCT. After washing a 1 mg/ml DQ collagen type I (Molecular Probes) was diluted to 40 mg/ml with developing buffer (50 mM Tris (pH 7.5), 100 mM NaCl, 5 mM $CaCl_2$). Samples were incubated for 4 hours at 37 ºC. the reaction was stopped with 4% paraformaldehyde and followed by the desired immune-staining, mounted with immune-mount (Thermo Scientific) and imaged using Nikon 80i eclipse microscope.

Relative Frequency of Fiber Orientation Analysis: Imaging analysis was done by Fiji package, Directionality analysis. Graphs were plotted using GraphPad Prism™ 6. The relative frequency of fiber spatial orientation was measured using the "Directionality" plugin analysis tool in Fiji package version 6.1.1.

***Flow Cytometry*:** Lungs from infected and control uninfected C57BL/6J mice were immersed in cold PBS, cut into small pieces in 5 ml DMEM containing 10% bovine fetal serum (FACS buffer). Cell suspensions were grinded using 1-ml syringe cup on a 70- lm cell strainers (BD Falcon). Cells were washed with ice-cold PBS. Remaining red blood cells were lysed using ammonium chloride solution (Sigma). Cells were harvested and immersed 1 ml FACS buffer [PBS+2% FCS, 1 mM EDTA]. Lung cells were stained with antibodies against multiple surface antigens: PE-conjugated LEM 2/15 (anti-mouse MT1-MMP ab), PerCP/cy5.5-conjugated anti-mouse CD45 (clone –F11) or Pacific blue- anti-mouse CD45, APC-Cy7-conjugated EPCAM, APC-conjugated anti-CD11b, PerCP/cy5.5- anti-mouse Ly6C, FITC- anti-mouse Ly6G (clone 1A8), FITC-anti-mouse NKp46, FITC-anti-mouse-TCR-β. Flow cytometry was performed using FACSAria™ III Flow Cytometer (BD biosciences), and data was analyzed using Flowjo™ V 10.0.8 software. Sorted cells from non- infected control and infected mice treated with PBS were further subjected to RNA extraction, as previously mentioned in RNA extraction section, and were sequenced using RNA-Seq profiling and qPCR.

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EXAMPLE 1

Extra Cellular Matrix Genes are Induced During Influenza Infection

In order to systematically describe the effect of influenza inflection on host ECM circuits, genome-wide RNA-seq was used to measure the temporal transcriptional response of whole lung tissue during a seven-day course of influenza (Altboum et al., 2014). Gene expression was measured at ten time points following infection. C57BL/6 mice were infected by intranasal inoculation of mouse-adapted PR8 influenza A H1N1 virus using either lethal or sub-lethal dosages. PR8 infection is widely used as a influenza infection model (Morens et al., 2008; Tate et al., 2011; Taubenberger and Morens, 2006; Watanabe et al., 2013) consisting of rigorous alveolar spread, acute pulmonary hemorrhage and intensive host responses. Along with the disease progression, the symptoms and loss of body weight are initiated 24-48 hours post infection with an increase in viral load in the lungs (Figure 6D). As expected, influenza infection resulted in induction of genes that are involved in inflammation chemotaxis (CXCL1, CXCL10, Il1b, Il1r,), defense against viral infection (ISG15, IFNB1, IRF7, IFIT1 and IFIT3) and various chemokines (CCL2, CCL3, CCL4, CXCL2) as well as down-regulation of genes related to lung homeostasis (secretoglobins and relevant transcription factors (e.g. NKx2.1)) (Figure 1A). Furthermore, many genes which were down-regulated following infection belong to oxygen-reduction processes, surfactant homeostasis (SFTPA1, SFTPC), cell-cell adhesion molecules such as integrins, cadherin, claudin (CLDN18, ITGB2, CDH5) and lipid metabolism (APOE, APOC1, APOA1BP). Additional genes that were statistically significantly up-regulated are set forth in Table 2B herein below.

Table 2B

Symbols	*Genes (mouse)*
1190002H23RIK	NM_025427
1500012F01RIK	NM_001081005
2010001M09RIK	NM_027222
AA467197	NM_001004174
Acta1	NM_009606
Actb	NM_007393
Adam15	NM_001037722

Adam8	NM_007403
Adamts15	NM_001024139
Adamts4	NM_172845
Aif1	NM_019467
Alpl	NM_007431
Angptl4	NM_020581
Apod	NM_007470
Apol6	NM_028010
Apol9a	NM_001162883
Apol9b	NM_173743
Arrb2	NM_145429
Atf3	NM_007498
AW112010	NM_001177351
B2m	NM_009735
B4galt1	NM_022305
Bak1	NM_007523
Batf2	NM_028967
Bcl3	NM_033601
Bdkrb1	NM_007539
Bgn	NM_007542
Bst2	NM_198095
C1qa	NM_007572
C1qb	NM_009777
C1qc	NM_007574
C1qtnf6	NM_028331
C3ar1	NM_009779
Casp4	NM_007609
Ccl2	NM_011333
Ccl20	NM_001159738
Ccl4	NM_013652
Ccl5	NM_013653
Ccl7	NM_013654
Cd274	NM_021893
Cd300lf	NM_001169153
Cd3d	NM_013487
Cd72	NM_001110322
Cd8a	NM_009857
Cd8b1	NM_009858
Cdca3	NM_013538
Cdkn1a	NM_007669
Cebpd	NM_007679
Cfb	NM_001142706
Cidea	NM_007702

Ckap4	NM_175451
Ckm	NM_007710
Cldn4	NM_009903
Cmklr1	NM_008153
Cmpk2	NM_020557
Col1a1	NM_007742
Col1a2	NM_007743
Col3a1	NM_009930
Cox7a1	NM_009944
Cpxm1	NM_019696
Csrnp1	NM_153287
Ctgf	NM_010217
Ctps	NM_016748
Ctss	NM_021281
Ctsz	NM_022325
Cxcl1	NM_008176
Cxcl10	NM_021274
Cxcl12	NM_021704
Cxcl13	NM_018866
Cxcl16	NM_023158
Cxcl2	NM_009140
Cxcl5	NM_009141
Cxcl9	NM_008599
Cyp4f18	NM_024444
Cyr61	NM_010516
Daxx	NM_001199733
Dbp	NM_016974
Ddit4	NM_029083
Ddx58	NM_172689
Dhx58	NM_030150
Dntt	NM_009345
Dtx3l	NM_001013371
Ecm1	NM_007899
Edem1	NM_138677
Eif2ak2	NM_011163
Eln	NM_007925
Epha2	NM_010139
Epsti1	NM_029495
F3	NM_010171
Fam26f	NM_175449
Fbn1	NM_007993
Fcer1g	NM_010185
Fcgr1	NM_010186

Fcgr4	NM_144559
Fgfr1	NM_001079908
Fkbp5	NM_010220
Flnb	NM_134080
Fn1	NM_010233
Fscn1	NM_007984
Fst	NM_008046
Fxyd5	NM_001111073
Gadd45g	NM_011817
Gbp10	NM_001039646
Gbp2	NM_010260
Gbp3	NM_018734
Gbp4	NM_008620
Gbp5	NM_153564
Gbp6	NM_194336
Gbp9	NM_172777
Glycam1	NM_008134
Gm12250	NM_001135115
Gm13889	NM_001145034
Gm14446	NM_001101605
Gm4841	NM_001034859
Gm4951	NM_001033767
Gpd1	NM_010271
Gpx3	NM_008161
Grn	NM_008175
Gvin1	NM_001039160
Gzmb	NM_013542
H2-Q7	NM_010394
H2-Q9	NM_001201460
H2-T10	NM_010395
H2-T22	NM_010397
H2-T23	NM_010398
H2-T9	NM_010399
Has1	NM_008215
Hcls1	NM_008225
Helz2	NM_183162
Hmga1	NM_001166537
Hmga1-rs1	NM_001166477
Hspa8	NM_031165
I830012O16Rik	NM_001005858
Ier5	NM_010500
Ifi203	NM_001045481
Ifi204	NM_008329

Ifi205	NM_172648
Ifi27l2a	NM_029803
Ifi35	NM_027320
Ifi44	NM_133871
Ifi47	NM_008330
Ifih1	NM_027835
Ifit1	NM_008331
Ifit2	NM_008332
Ifit3	NM_010501
Ifitm3	NM_025378
Igtp	NM_018738
Iigp1	NM_001146275
Il10ra	NM_008348
Il18bp	NM_010531
Il1b	NM_008361
Il1rn	NM_001039701
Il21r	NM_021887
Irf1	NM_001159396
Irf5	NM_012057
Irf7	NM_016850
Irf8	NM_008320
Irg1	NM_008392
Irgm1	NM_008326
Irgm2	NM_019440
Isg15	NM_015783
Isg20	NM_020583
Itga5	NM_010577
Junb	NM_008416
Kcnn4	NM_001163510
Krt13	NM_010662
Krt4	NM_008475
Laptm5	NM_010686
Lars2	NM_153168
Lck	NM_001162433
Lcn2	NM_008491
Lgals3bp	NM_011150
Lgals9	NM_001159301
Lgmn	NM_011175
Lilrb4	NM_013532
Lox	NM_010728
Loxl1	NM_010729
Loxl2	NM_033325
Loxl3	NM_013586

Ly6a	NM_010738
Ly6c1	NM_010741
Ly6c2	NM_001099217
Ly6i	NM_020498
Lyve1	NM_053247
Mmp14	NM_008608
Mmp3	NM_010809
Mmp8	NM_008611
Mnda	NM_001033450
Mndal	NM_001170853
Mpeg1	NM_010821
Ms4a4b	NM_021718
Ms4a4c	NM_029499
Ms4a6b	NM_027209
Ms4a6c	NM_028595
Ms4a6d	NM_026835
Mt1	NM_013602
Mt2	NM_008630
Mx1	NM_010846
Mx2	NR_003508
Mxd1	NM_010751
Myh1	NM_030679
Myh8	NM_177369
Mylpf	NM_016754
Nampt	NM_021524
Nfkbia	NM_010907
Nlrc5	NM_001033207
Nppa	NM_008725
Nt5c3	NM_026004
Oas1a	NM_145211
Oas1g	NM_011852
Oas2	NM_145227
Oasl1	NM_145209
Oasl2	NM_011854
Ogfr	NM_031373
Parp12	NM_172893
Parp14	NM_001039530
Parp9	NM_030253
Per1	NM_001159367
Pfkfb3	NM_001177757
Phf11b	NM_001164327
Phf11d	NM_199015
Pirb	NM_011095

Pla2g7	NM_013737
Plac8	NM_139198
Plat	NM_008872
Plau	NM_008873
Pld4	NM_178911
Pnp	NM_013632
Pnp2	NM_001123371
Pou2af1	NM_011136
Ppa1	NM_026438
Prmt1	NM_019830
Prss22	NM_133731
Psmb10	NM_013640
Psmb8	NM_010724
Psmc2	NM_011190
Pstpip1	NM_011193
Ptafr	NM_001081211
Ptk7	NM_175168
Ptx3	NM_008987
Pycard	NM_023258
Pyhin1	NM_175026
Qsox1	NM_001024945
Relb	NM_009046
Retnla	NM_020509
Rhox8	NM_001004193
Rpsa	NM_011029
Rsad2	NM_021384
Rtp4	NM_023386
S100a14	NM_001163526
S100a4	NM_011311
S100a6	NM_011313
S100a8	NM_013650
S100a9	NM_009114
Saa1	NM_009117
Saa3	NM_011315
Samd9l	NM_010156
Samhd1	NM_001139520
Sbno2	NM_183426
Sdc3	NM_011520
Sell	NM_001164059
Sema7a	NM_011352
Serinc3	NM_012032
Serpina3f	NM_001033335
Serpina3g	NM_009251

Serpina3m	NM_009253
Serpina3n	NM_009252
Serpine1	NM_008871
Serping1	NM_009776
Sfn	NM_018754
Sh3pxd2b	NM_177364
Slc15a3	NM_023044
Slc25a37	NM_026331
Slc7a5	NM_011404
Slfn1	NM_011407
Slfn2	NM_011408
Slfn4	NM_011410
Slfn5	NM_183201
Slfn8	NM_001167743
Slfn9	NM_172796
Snx32	NM_001024560
Socs1	NM_009896
Socs3	NM_007707
Sparcl1	NM_010097
Sphk1	NM_011451
Spp1	NM_009263
Sprr1a	NM_009264
Stat1	NM_009283
Stat2	NM_019963
Tap1	NM_013683
Tap2	NM_011530
Tapbp	NM_001025313
Tcf7	NM_009331
Tgfbi	NM_009369
Tgm2	NM_009373
Tgtp1	NM_011579
Tgtp2	NM_001145164
Thbs1	NM_011580
Themis2	NM_001033308
Thrsp	NM_009381
Thy1	NM_009382
Timp1	NM_001044384
Tinagl1	NM_001168333
Tnc	NM_011607
Tnfaip2	NM_009396
Tnfrsf12a	NM_013749
Tnni2	NM_009405
Tor3a	NM_023141

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Tpm2	NM_009416
Trafd1	NM_172275
Trex1	NM_011637
Trib1	NM_144549
Trim25	NM_009546
Trim30a	NM_009099
Tuba1c	NM_009448
Tubb5	NM_011655
Tubb6	NM_026473
Ubc	NM_019639
Ucp1	NM_009463
Usp18	NM_011909
Vcan	NM_001134475
Wars	NM_001164488
Xaf1	NM_001037713
Xdh	NM_011723
Zbp1	NM_021394
Znfx1	NM_001033196

Of special notice was a large group of genes involved in ECM remodeling including macromolecule metabolism and protease synthesis (Figure 1A). Remarkably, this group of genes was highly over-represented throughout infection, exhibiting a wide panel of pathways involved in multiple ECM remodeling events (Figure 1A-C). An enrichment of functional categories relating to extra cellular modulators involved in proteolysis, collagen remodeling and catabolism, fibrinolysis, wound healing, homeostasis and cell migration ($p \leq 10^{-4}$) was uncovered, peaking 74 hours post infection (Figure 1B). All together, 479 out of 3530 differentially expressed genes (13.6%) are related to ECM remodeling. These included serine proteases, lysyl oxidases, cathepsins, disintegrins (ADAMs), metalloproteinases (MMPs) and their natural inhibitors (TIMPs), which serve as ECM modifiers that determine the turnover of different ECM components. Within this group of genes, robust induction of MT1-MMP at both the RNA (400 fold change; Figure 1D) and protein levels 48 hours post infection (Figure 6A-B) was found. Using quantitative real time PCR, the temporal changes in MT1-MMP expression was corroborated as well as other representative genes belonging to the MMP family (MMP-3, 8 and 9) and modulating the ECM (Figure 6A, B, C).

EXAMPLE 2

MT1-MMP Expression is Induced in Myeloid Cells Post Influenza Infection

In order to identify the cell population acting as the source of MT1-MMP during infection course by flow cytometry was performed. Of the population of cells expressing MT1-MMP in the non-infected lung, the overwhelming majority was non-hematopoietic cell population ($CD45^-$, 89.5%), while only 10.5% were of hematopoietic origin ($CD45^+$) (Figure 2A). Post infection, the $CD45^+$ MT1-MMP expressing population increased four-fold (40.9%). Specifically, the $CD11b^+$, MT1-MMP^+ portion of the immune cells increased from 32.9% to 64.9%, while the non- hematopoietic ($CD45^-$) MT1-MMP-expressing cells decreased by two-fold (Figure 2A). Histogram plots (Figure 2B) further show that the overall increase in MT1-MMP expression post infection (Figure 2B) can be associated with increased expression of MT1-MMP in CD11b+ cells (Figure 2B), rather than lung epithelial cells, in which MT1-MMP was reduced post infection (Figure 2B). MT1-MMP expression in $CD45^+$ versus $CD45^-$ sorted populations before and during infection was further validated at the RNA level using qPCR (Figure 2C). Immunostaining for MT1-MMP as well as F4/80 markers in influenza-infected lungs confirm our observation that MT1-MMP expressing cells largely co-localize with F4/80 positive cells at 74 hours post infection. Since macrophages are both $CD11b^+$ and F4/80+ immune cells, these findings suggest that macrophages are a significant source of MT1-MMP following infection (Figure 7A arrow heads, 7B). In order to monitor the collagenase activity of influenza-infected lungs, *in situ* zymography was used. It was found that following infection, collagenolytic activity is mostly associated with $CD45^+$ cells, as well as $CD45^-$ cells lining the bronchi of infected lungs (Figure 7C arrows, D).

In order to further characterize the MT1-MMP-expressing populations before and after (74 hours) infection, RNA-seq analysis was performed on sorted MT1-MMP-expressing $CD45^+$ and $CD45^-$ subpopulations. In total, 2169 genes were found to be differentially expressed in cells post-infection as compared to un-infected cells in both $CD45^+$ and $CD45^-$ populations (Figure 8). Consistent with the analysis from whole lung RNA-seq, an increase in activation of inflammatory signaling pathways and cytokine production in both immune ($CD45^+$) and stromal ($CD45^-$) cells expressing MT1-MMP following infection was observed (Figure 8). The immune cells, in particular, exhibited

significant up-regulation of cytokines (CCL2, CCL3, CCL4, CXCL2, IL1b) and anti-viral response genes (e.g. SLFN4, IFIT1 and IFIT2), while the stromal cells exhibited down-regulation of genes associated with lung homeostatic functions such as surfactant production (e.g. SFTPB, SFTPC). The immune population showed increase in expression of monocyte/macrophage/DC markers (e.g. CD11b) and decrease of multiple B cell markers (e.g. CD19, CD37, CD79). Thus, MT1-MMP expression post-infection is associated with activated immune cells from the myeloid compartment (Figure 8).

EXAMPLE 3

Influenza Infection Induces Destruction of ECM Morphology and Composition

MT1-MMP plays a major role in cancer-associated invasion processes through degradation of fibrillar collagen, laminin and other ECM components. To evaluate the functional role of MT1-MMP in influenza infection, mass spectrometry analysis (Figure 3A) as well as scanning electron microscopy (SEM) imaging of lung tissues devoid of its cellular compartment (de-cellularized) before and after infection was performed (Figures 9A-B). SEM analysis of influenza-infected lungs showed massive distortion of ECM morphology (Figure 3B-C) as well as rearrangement of collagen fibers, specifically in the alveolar walls. At 74 hours post-infection, collagen bundles on the boundaries of alveolar sacs displayed unraveled fiber ends and dispersed orientation angles (Figure 3B). This was further confirmed by measuring the orientation of the fibrils composing the alveolar walls (Figure 3C). In addition, the alveolar space and septa were distorted in the infected lungs (Figure 3B). To validate these results and further analyze the integrity of the whole tissue – including the cells and ECM in their native environment – during infection, a novel form of electron microscopy imaging, AirSEM (Solomonov et al., 2014) was used. AirSEM enables visualization of native hydrated tissues in ambient conditions, thus avoiding potential artifacts associated with sample preparation for SEM. Imaging of virally-infected lung tissues exhibited tissue destruction characterized by both alveolar and bronchial cell depletion as well as distortion of alveolar sacs and ducts followed by alveolar wall thinning (Figure 9A-B). Finally, AirSEM imaging of fresh lung ECM scaffolds (de-cellularized tissues) showed similar alveolar collagen degradation and distortion patterns as observed in conventional SEM analysis (Figure 9A-B).

EXAMPLE 4

Global Proteomics Analysis Identifies Degradation of ECM Scaffolds During Influenza Infection

In order to globally examine the proteolytic implications on ECM remodeling during influenza infection, a tandem mass spectrometry (LC-MS-MS) approach was used (Figure 3A, Table 3, herein below). Analysis of the de-cellularized lung tissue 74 and 120 hours post infection compared with non-infected tissue showed compositional changes that could be connected to structural changes observed in the architecture of collagen fibrils lining the alveolar wall (Figure 3B-C). Specifically, the proteomic data analysis of the influenza-infected lungs identified modifications in the molecular composition of the ECM, including gradual depletion of collagen and subtypes of laminin molecules (Figure 3A). In addition, multiple basement-membrane-associated components as well as basal-cell-adhesion molecules (e.g. Nidogen, Decorin, Collagen types IV, XII, XIV and Fibrillin; Figure 3A) were depleted from infected lung tissue indicating massive transformation of ECM integrity and molecular composition. The loss of representative ECM molecules (Collagen IV, Decorin and Lumican) was confirmed by staining the ECM scaffolds of infected and non-infected lungs (Figure 3D-I). Importantly, several of the components depleted during infection, such as type I collagen, laminin nidogen, lumican, mimecan, fibrillin and decorin, are known MT1-MMP-substrates (Koziol et al., 2012; McQuibban et al., 2000; Noel et al., 2012; Overall, 2002; Shimizu-Hirota et al., 2012; Stegemann et al., 2013). Since multiple MT1-MMP substrates are degraded during the course of influenza infection, it was hypothesized that lung ECM proteolysis and host mortality can be protected by specifically blocking the collagenase activity of MT1-MMP.

Table 3

Protein annotation	*Normalized control*	*Normalized T72*	*Normalized T120*
ADP/ATP translocase 1 OS=Mus musculus GN=Slc25a4 PE=1 SV=4 - [ADT1_MOUSE]	6.153575947	5.977276302	0
ADP/ATP translocase 2 OS=Mus musculus GN=Slc25a5 PE=1 SV=3 - [ADT2_MOUSE]	6.223198168	5.887684499	0
Advanced glycosylation end product-specific receptor OS=Mus musculus GN=Ager PE=2 SV=1 - [C5H3H4_MOUSE]	6.473725601	0	0

Agrin OS=Mus musculus GN=Agrn PE=4 SV=1 - [M0QWP1_MOUSE]	6.30817 5158	6.5930 93331	6.51159 9322
Alpha-amylase 1 OS=Mus musculus GN=Amy1 PE=1 SV=2 - [AMY1_MOUSE]	0	5.6598 6227	7.46071 1241
Annexin A1 OS=Mus musculus GN=Anxa1 PE=1 SV=2 - [ANXA1_MOUSE]	0	5.8247 02042	0
Annexin A2 (Fragment) OS=Mus musculus GN=Anxa2 PE=2 SV=1 - [B0V2N8_MOUSE]	5.67946 5046	5.9576 16046	6.19540 5289
Aquaporin-5 OS=Mus musculus GN=Aqp5 PE=2 SV=1 - [AQP5_MOUSE]	6.16271 6299	5.9619 71735	0
Basal cell adhesion molecule OS=Mus musculus GN=Bcam PE=2 SV=1 - [BCAM_MOUSE]	6.22791 2096	5.9999 52109	0
Basement membrane-specific heparan sulfate proteoglycan core protein OS=Mus musculus GN=Hspg2 PE=2 SV=1 - [E9PZ16_MOUSE]	7.41537 7503	7.3222 87706	7.23077 0907
Beta-actin-like protein 2 OS=Mus musculus GN=Actbl2 PE=1 SV=1 - [ACTBL_MOUSE]	6.38613 8757	6.7857 14287	7.05208 4571
Beta-globin OS=Mus musculus GN=Hbb-b1 PE=2 SV=1 - [A8DUK4_MOUSE]	6.44700 7246	6.9642 98502	6.93812 6292
Carboxylesterase 1D OS=Mus musculus GN=Ces1d PE=1 SV=1 - [CES1D_MOUSE]	5.52786 9285	5.6346 53635	0
Caveolin (Fragment) OS=Mus musculus GN=Cav1 PE=2 SV=2 - [D3Z148_MOUSE]	6.49595 5194	6.2124 14678	0
Chitinase-3-like protein 4 OS=Mus musculus GN=Chi3l4 PE=1 SV=2 - [CH3L4_MOUSE]	5.78250 122	0	0
Collagen alpha-1(I) chain OS=Mus musculus GN=Col1a1 PE=1 SV=4 - [CO1A1_MOUSE]	7.07580 5627	7.0284 73097	6.75321 0524
Collagen alpha-1(III) chain OS=Mus musculus GN=Col3a1 PE=2 SV=4 - [CO3A1_MOUSE]	6.93420 7535	7.0603 66511	7.23163 175
Collagen alpha-1(IV) chain OS=Mus musculus GN=Col4a1 PE=2 SV=4 - [CO4A1_MOUSE]	7.69502 0525	7.6945 67589	7.53629 0851
Collagen alpha-1(VI) chain OS=Mus musculus GN=Col6a1 PE=2 SV=1 - [CO6A1_MOUSE]	6.86809 465	6.5512 79889	6.15880 609
Collagen alpha-1(XII) chain OS=Mus musculus GN=Col12a1 PE=4 SV=1 - [J3KMS9_MOUSE]	5.71461 1383	0	0
Collagen alpha-1(XIV) chain OS=Mus musculus GN=Col14a1 PE=2 SV=1 - [B7ZNH7_MOUSE]	6.12501 6434	0	0
Collagen alpha-2(I) chain OS=Mus musculus GN=Col1a2 PE=2 SV=2 - [CO1A2_MOUSE]	6.54328 0973	6.8139 95104	7.02359 1569
Collagen alpha-2(IV) chain OS=Mus musculus GN=Col4a2 PE=2 SV=4 - [CO4A2_MOUSE]	7.55082 7668	7.5309 63433	7.33268 6213
Collagen alpha-2(VI) chain OS=Mus musculus GN=Col6a2 PE=2 SV=3 - [CO6A2_MOUSE]	6.94736 9979	6.7924 65562	6.63568 8357
Collagen alpha-3(IV) chain OS=Mus musculus GN=Col4a3 PE=1 SV=2 - [CO4A3_MOUSE]	7.21593 568	7.6501 27224	7.41634 2179
Collagenase 3 OS=Mus musculus GN=Mmp13 PE=1 SV=1 - [MMP13_MOUSE]	7.72568 074	7.1590 2446	7.28630 4725
Decorin OS=Mus musculus GN=Dcn PE=2 SV=1 - [PGS2_MOUSE]	6.22520 272	0	0
Desmoglein-1-alpha OS=Mus musculus GN=Dsg1a PE=2 SV=2 - [DSG1A_MOUSE]	5.54874 7085	5.8527 24683	6.62085 048

Desmoplakin OS=Mus musculus GN=Dsp PE=2 SV=1 - [DESP_MOUSE]	6.16230 4582	6.3829 26331	6.99294 3381
Dimethylaniline monooxygenase [N-oxide-forming] 2 OS=Mus musculus GN=Fmo2 PE=1 SV=3 - [FMO2_MOUSE]	5.71466 1158	5.3119 17766	0
Elongation factor 1-alpha 1 OS=Mus musculus GN=Eef1a1 PE=1 SV=3 - [EF1A1_MOUSE]	5.59895 5869	5.7641 82366	0
EMILIN-1 OS=Mus musculus GN=Emilin1 PE=1 SV=1 - [EMIL1_MOUSE]	0	0	6.39283 6508
Fibrillin-1 OS=Mus musculus GN=Fbn1 PE=4 SV=1 - [A2AQ53_MOUSE]	5.87776 4759	5.7047 77353	0
Fibrinogen beta chain OS=Mus musculus GN=Fgb PE=2 SV=1 - [FIBB_MOUSE]	5.85849 0424	6.6872 89698	0
Fibrinogen gamma chain OS=Mus musculus GN=Fgg PE=2 SV=1 - [FIBG_MOUSE]	5.83914 522	7.0408 81119	6.62371 4799
Fibrinogen, alpha polypeptide OS=Mus musculus GN=Fga PE=2 SV=1 - [Q99K47_MOUSE]	6.05632 5883	7.2614 85562	6.90940 505
Fibronectin OS=Mus musculus GN=Fn1 PE=1 SV=4 - [FINC_MOUSE]	6.81868 8119	6.8807 40949	7.35353 9538
Filamin, alpha (Fragment) OS=Mus musculus GN=Flna PE=4 SV=1 - [B7FAV1_MOUSE]	7.00850 5708	6.6284 36178	6.82798 4047
Gelsolin OS=Mus musculus GN=Gsn PE=1 SV=3 - [GELS_MOUSE]	5.72935 6743	5.7633 26472	0
Haptoglobin OS=Mus musculus GN=Hp PE=1 SV=1 - [HPT_MOUSE]	0	6.1097 91413	0
Hemoglobin subunit alpha OS=Mus musculus GN=Hba PE=1 SV=2 - [HBA_MOUSE]	0	6.1079 0139	6.39890 4972
Hemopexin OS=Mus musculus GN=Hpx PE=1 SV=2 - [HEMO_MOUSE]	0	6.0102 80907	0
Histone H2A OS=Mus musculus GN=Hist1h2al PE=2 SV=1 - [F8WIX8_MOUSE]	7.39213 8835	6.9170 80096	7.01366 0548
Histone H2A type 1-H OS=Mus musculus GN=Hist1h2ah PE=1 SV=3 - [H2A1H_MOUSE]	7.39213 8835	6.9268 79953	6.85700 2872
Histone H2B type 1-F/J/L OS=Mus musculus GN=Hist1h2bf PE=1 SV=2 - [H2B1F_MOUSE]	7.49139 0907	7.1245 77253	7.00540 3699
Histone H3 (Fragment) OS=Mus musculus GN=H3f3a PE=2 SV=1 - [E0CZ27_MOUSE]	7.10148 4514	6.9597 92034	7.04992 2513
Histone H4 OS=Mus musculus GN=Hist1h4a PE=1 SV=2 - [H4_MOUSE]	7.67612 5661	7.5807 81002	7.45816 7442
Ig gamma-1 chain C region secreted form OS=Mus musculus GN=Ighg1 PE=1 SV=1 - [IGHG1_MOUSE]	5.96145 5553	0	0
Ig kappa chain V-II region 26-10 OS=Mus musculus PE=1 SV=1 - [KV2A7_MOUSE]	6.41901 4568	5.8368 03643	0
Ig mu chain C region secreted form OS=Mus musculus GN=Igh-6 PE=1 SV=2 - [IGHM_MOUSE]	6.20128 6175	6.4343 77902	6.52631 4192
Indolethylamine N-methyltransferase OS=Mus musculus GN=Inmt PE=1 SV=1 - [INMT_MOUSE]	5.63786 4703	0	0
Junction plakoglobin OS=Mus musculus GN=Jup PE=1 SV=3 - [PLAK_MOUSE]	6.26479 1243	6.4654 10008	7.01760 1329
Lactotransferrin OS=Mus musculus GN=Ltf PE=2 SV=4 - [TRFL_MOUSE]	0	5.7626 88986	0

Laminin subunit alpha-2 OS=Mus musculus GN=Lama2 PE=2 SV=1 - [F8VQ43_MOUSE]	5.93776 6596	0	0
Laminin subunit alpha-3 OS=Mus musculus GN=Lama3 PE=4 SV=1 - [E9PUR4_MOUSE]	6.67499 0286	6.2605 28414	0
Laminin subunit alpha-4 OS=Mus musculus GN=Lama4 PE=1 SV=2 - [LAMA4_MOUSE]	6.51138 5176	6.0999 45813	0
Laminin subunit alpha-5 OS=Mus musculus GN=Lama5 PE=1 SV=4 - [LAMA5_MOUSE]	6.60731 5399	6.5470 91642	6.32705 3775
Laminin subunit beta-1 OS=Mus musculus GN=Lamb1 PE=1 SV=3 - [LAMB1_MOUSE]	5.95133 5808	0	0
Laminin subunit beta-2 OS=Mus musculus GN=Lamb2 PE=2 SV=2 - [LAMB2_MOUSE]	6.61025 6604	6.2639 76054	0
Laminin subunit beta-3 OS=Mus musculus GN=Lamb3 PE=2 SV=2 - [LAMB3_MOUSE]	6.68721 5625	6.3794 47773	0
Laminin subunit gamma-1 OS=Mus musculus GN=Lamc1 PE=2 SV=1 - [F8VQJ3_MOUSE]	6.70043 806	6.2094 42052	6.04884 3786
Laminin subunit gamma-2 OS=Mus musculus GN=Lamc2 PE=4 SV=1 - [G5E874_MOUSE]	6.21890 5096	6.1420 10192	0
Lumican OS=Mus musculus GN=Lum PE=1 SV=2 - [LUM_MOUSE]	6.18972 3414	0	0
Lysozyme C-2 OS=Mus musculus GN=Lyz2 PE=1 SV=2 - [LYZ2_MOUSE]	5.83391 544	6.3067 2877	0
MCG1050941 OS=Mus musculus GN=Gm5414 PE=2 SV=1 - [Q6IFZ8_MOUSE]	6.89961 9386	7.1358 1107	7.36932 4207
MCG16555 OS=Mus musculus GN=Vdac3-ps1 PE=4 SV=1 - [J3QPE8_MOUSE]	5.51622 4232	0	0
Microfibril-associated glycoprotein 4 OS=Mus musculus GN=Mfap4 PE=1 SV=1 - [MFAP4_MOUSE]	6.34290 4739	6.4346 3342	0
Mimecan OS=Mus musculus GN=Ogn PE=2 SV=1 - [MIME_MOUSE]	0	5.7010 58917	0
Myelin proteolipid protein OS=Mus musculus GN=Plp1 PE=1 SV=2 - [MYPR_MOUSE]	6.98709 8666	6.4326 34236	6.58636 9313
Myeloid bactenecin (F1) OS=Mus musculus GN=Ngp PE=2 SV=1 - [O08692_MOUSE]	5.44115 199	6.1421 01018	0
Myeloperoxidase OS=Mus musculus GN=Mpo PE=2 SV=2 - [PERM_MOUSE]	0	6.3827 61391	0
Myosin-10 OS=Mus musculus GN=Myh10 PE=1 SV=2 - [MYH10_MOUSE]	6.10724 4043	0	0
Myosin-11 OS=Mus musculus GN=Myh11 PE=4 SV=1 - [E9QPE7_MOUSE]	6.54913 8497	6.0236 21662	6.48685 2262
Myosin-9 OS=Mus musculus GN=Myh9 PE=1 SV=4 - [MYH9_MOUSE]	6.53407 7311	5.8757 75823	6.34610 934
Neurofilament heavy polypeptide OS=Mus musculus GN=Nefh PE=1 SV=3 - [NFH_MOUSE]	6.13890 2554	6.9786 4523	6.97512 0413
Nidogen-1 OS=Mus musculus GN=Nid1 PE=1 SV=2 - [NID1_MOUSE]	7.12837 0461	7.1132 90014	6.73053 3502
Nidogen-2 OS=Mus musculus GN=Nid2 PE=1 SV=2 - [NID2_MOUSE]	6.24140 8448	5.9835 93362	0
Peptidyl-prolyl cis-trans isomerase B OS=Mus musculus GN=Ppib PE=2 SV=2 - [PPIB_MOUSE]	6.01287 4469	0	0
Periostin OS=Mus musculus GN=Postn PE=1 SV=2 - [POSTN_MOUSE]	6.72572 5241	6.2516 65549	6.20505 634

Peroxiredoxin-1 (Fragment) OS=Mus musculus GN=Prdx1 PE=2 SV=1 - [B1AXW5_MOUSE]	5.98103 7111	5.7530 60397	6.14260 1977
Phosphate carrier protein, mitochondrial OS=Mus musculus GN=Slc25a3 PE=1 SV=1 - [MPCP_MOUSE]	5.91683 531	0	0
Platelet glycoprotein 4 OS=Mus musculus GN=Cd36 PE=1 SV=2 - [CD36_MOUSE]	6.16384 265	5.9502 72926	0
Polyubiquitin-C (Fragment) OS=Mus musculus GN=Ubc PE=2 SV=1 - [E9Q5F6_MOUSE]	6.96891 1307	6.8488 9605	6.75618 1153
Prelamin-A/C OS=Mus musculus GN=Lmna PE=1 SV=2 - [LMNA_MOUSE]	5.74909 2722	0	0
Protein 4732456N10Rik OS=Mus musculus GN=4732456N10Rik PE=3 SV=1 - [E9Q1Z0_MOUSE]	7.25265 58	7.3998 52941	7.72608 2657
Protein Col4a5 (Fragment) OS=Mus musculus GN=Col4a5 PE=4 SV=1 - [F7CK55_MOUSE]	7.14155 8716	7.1129 9109	7.24588 5041
Protein Col4a6 OS=Mus musculus GN=Col4a6 PE=2 SV=1 - [B1AVK5_MOUSE]	6.66766 3539	7.0414 05713	0
Protein Col6a3 OS=Mus musculus GN=Col6a3 PE=4 SV=1 - [J3QQ16_MOUSE]	7.18277 2976	6.8878 60833	6.65193 8048
Protein Krt78 OS=Mus musculus GN=Krt78 PE=2 SV=1 - [E9Q0F0_MOUSE]	7.99164 9161	8.1841 26545	8.64001 7022
Protein-glutamine gamma-glutamyltransferase 2 OS=Mus musculus GN=Tgm2 PE=1 SV=4 - [TGM2_MOUSE]	6.60662 3968	6.3496 0286	6.82860 7255
Serotransferrin OS=Mus musculus GN=Tf PE=1 SV=1 - [TRFE_MOUSE]	5.56990 3604	5.9831 04216	0
Serum albumin OS=Mus musculus GN=Alb PE=1 SV=3 - [ALBU_MOUSE]	6.76985 6217	7.2117 12096	6.55564 8421
Spectrin alpha chain, non-erythrocytic 1 OS=Mus musculus GN=Sptan1 PE=2 SV=1 - [A3KGU5_MOUSE]	6.27396 2184	0	0
Spectrin beta chain, non-erythrocytic 1 OS=Mus musculus GN=Sptbn1 PE=1 SV=2 - [SPTB2_MOUSE]	5.99351 5702	0	0
Tenascin GRCm38.p3 [GCF_000001635.23]	7.12532 211	6.1124 90357	6.73051 2501
Titin OS=Mus musculus GN=Ttn PE=2 SV=1 - [E9Q8K5_MOUSE]	5.74329 1257	6.2951 32427	0
Tubulin alpha-1C chain OS=Mus musculus GN=Tuba1c PE=1 SV=1 - [TBA1C_MOUSE]	6.33172 549	5.9827 44883	6.39914 0301
Tubulointerstitial nephritis antigen-like OS=Mus musculus GN=Tinagl1 PE=2 SV=1 - [H3BJ97_MOUSE]	5.83145 8484	0	0
Voltage-dependent anion-selective channel protein 1 OS=Mus musculus GN=Vdac1 PE=1 SV=3 - [VDAC1_MOUSE]	5.66683 3354	5.4053 15445	0
von Willebrand factor OS=Mus musculus GN=Vwf PE=1 SV=2 - [VWF_MOUSE]	5.75776 8857	0	0

EXAMPLE 5

Inhibition of MT1-MMP Protects from Tissue Destruction without Modulating the Immune Response

MT1-MMP knockout mice suffer from multiple abnormalities and die within 3-5 weeks after birth (Holmbeck et al., 1999); hence, the role of MT1-MMP was

evaluated using a selective allosteric inhibitory antibody of MT1-MMP (LEM 2/15) effective at nano-molar concentrations (Udi et al., 2015). Importantly, this antibody has been shown to selectively interact with MT1-MMP expressed on the cell surface and inhibit its collagenase activity without significantly interfering with proMMP-2 activation and enzyme dimerization on the cell surface (Udi et al., 2015). In order to evaluate the role of MT1-MMP during influenza infection, mice were infected with sub-lethal doses of influenza virus and treated with either vehicle (PBS), control antibody or anti-MT1-MMP antibody during the course of the disease (26, 49, 74 hours; Figures 4A-K). Representative images are shown from tissue sections at 74 hours post infection in Figures 4A-K. AirSEM imaging of hydrated de-cellularized tissues demonstrated that blocking MT1-MMP collagenolytic activity protected tissue integrity (both alveolar and bronchi structures), ECM morphology, and collagen structure as well as the molecular composition of ECM scaffolds (Figure 4B-E). 3D analysis of two-photon microscopy in second harmonic generation showed that collagen type I fibers were more abundant and maintained a continuous alveolar sac boundaries when infected mice were treated with an anti-MT1-MMP inhibitor (Figure 4F-G). Furthermore, blocking MT1-MMP proteolytic activity also protected laminin, a major basement-membrane constituent, from degradation in the infected lungs (Figure 4H-J). Finally, functional *in situ* zymography indicated significant attenuation of proteolysis following treatment (Figure 4J-K), suggesting that MT1-MMP is a major driver of tissue destruction in the infection setting.

In order to better understand whether MT1-MMP is involved in immune modulation, the abundance of macrophages, neutrophils, lymphocytes and natural killer (NK) cells was analyzed at 74 hours post-infection using either anti-MT1-MMP inhibitor or a control antibody. The results show that there is enhanced recruitment of both macrophages and neutrophils to infected lungs with no detectable differences between anti-MT1-MMP and control-treated animals (Figure 11A). In addition, in lung tissue sections stained for F4/80 marker, similar macrophage infiltration was observed upon anti-MT1-MMP treatment (Figure 11B-C). Additionally, broncho-alveolar lavage fluid (BALF) was collected from both anti-MT1-MMP treated as well as control treated mice in order to evaluate cytokine level of major immune-modulating cytokines (IL-1β and TNF-α), which have been shown to play a major role in the infection process

(Aldridge et al., 2009; Glaccum et al., 1997; Goldbach-Mansky and Kastner, 2009). Both IL-1β and TNF-α were strongly induced in influenza-infected mice regardless of MT1-MMP activity (Figure 11D-F), suggesting the immune response was unaffected.

To evaluate whether MT1-MMP activity modulates viral loads during influenza infection, the plaque forming unit (PFU) assay was carried out (Figure 12A) and also the viral RNA was quantified using PFU assay (Figure 12B). In addition, lung tissue sections were stained for viral abundance at both 24 and 74 hours post infection (Figure 12D-F). Overall, these analyses showed minimal effect of MT1-MMP inhibition on viral burden which was limited to the early phases of infection (24 hours); at later stages, no effect was discernible. These results demonstrate that MT1-MMP is not significantly involved in immune modulation or regulation of viral loads; thus, the major MT1-MMP influence on influenza infection is the massive ECM fibrillary protein degradation and tissue damage stemming from its collagenase activity.

EXAMPLE 6

Tissue Damage Results from Host Proteolytic Activity Rather than Viral Cytopathology

The present inventors then investigated whether the destructive phenotypes in the lung tissue are a direct consequence of viral cytopathology or rather a result of a host-associated immune response driving the dysregulated ECM proteolysis. The conventional influenza treatment, Oseltamivir phosphate (Tamiflu™), a selective inhibitor of influenza A and B viral neuraminidase was analyzed (Figure 13A-D). Virus titers from whole-lung homogenates of vehicle-treated and Tamiflu™-treated mice were quantified using qPCR (Figure 13C) and compared to topography of lung structural features visualized in lung tissue sections using AirSEM (Figure 9A-B). Tamiflu™ dramatically reduces the viral burden (10-100 fold), meaning the tissue is exposed to low but persistent viral presence (Figure 13C). In spite of the lower viral titers, the same destructive lung tissue and ECM phenotypes were observed, including multifocal alveolar wall thinning and a substantial loss of alveolar cells, in both vehicle-treated and Tamiflu™-treated mice (Figure 13A, B). Such irreversible destruction may be the main cause for loss of barrier integrity, and thus provides a window of opportunity for bacterial invasion. These results prompted the present inventors to test whether

protecting ECM integrity via blocking MT1-MMP proteolysis can improve the lethal outcome of influenza-bacteria co-infections.

EXAMPLE 7

Blocking MT1-MMP in Infected Mice Promotes Tissue Maintenance and Prevents Sepsis

As noted above, the viral infection of influenza results in overwhelming damage to the ECM molecular composition and lung structure even with current first-line influenza medication targeting the virus. To further support the physiological consequences of influenza-induced ECM proteolysis and to mimic the frequent conditions that endanger influenza-infected hospitalized patients, an established protocol of influenza secondary bacterial infection using *S. pneumoniae* was used (McCullers and Herman, 2001; McCullers, 2003; McCullers, 2004). 10 groups of mice (10 mice per group) were infected twice within a range of 96 hours combining PR8 influenza virus followed by *S. pneumoniae,* with both at sub-lethal doses (Figure 5), and the different groups were treated with either vehicle, irrelevant control antibody, Tamiflu™, anti-MT1-MMP, or a combined therapy of both agents. To mimic potential treatment modes, mice were treated in two different protocols; prophylactic (before the infection) or therapeutic (post infection) (Figure 5A-B). The group of mice that was treated with Tamiflu™ as a preventive agent (Tamiflu™-1) exihibited the same survival rates and clinical scores as the group that received anti-MT1-MMP (Figure 5B-C), suggesting that anti-viral or ECM protection are equally effective in preventing co-infection induced mortality when administered as a preventive treatment.

In line with reports (Jefferson et al., 2014) demonstrating that Tamiflu™ is not effective in reducing hospitalozation duration or influenza symptoms when administered post-infection, co-infected mice treated with Tamiflu™ (+1 group) did not exhibit an improved response when compared with the vehicle group (20% survival) (Figure 5E-F). In these same therapeutic settings (Figure 5D), treatment with the anti-MT1-MMP inhibitor was significantly better, indicating a pronounced theraputic effect (70% survival). Importantly, combined application of Tamiflu™ and anti-MT1-MMP treatment resulted in 100% survival rates when used preventatively or therapeutically (Figure 5B-E). This was further supported by AirSEM images demonstrating the destructive phenotype of alveolar and bronchial structures of the Tamiflu™ (-1) group,

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which suggest that even as a prophylactic measure, Tamiflu™ is ineffective in preventing collateral damage in the lung ECM (Figure 14). To extend these observations of ECM fibrillar protein degradation, destruction of basement membrane constituents and disruption of the air-blood barrier in influenza infection, the present inventors further tested for bacterial dissemination into the blood stream (sepsis) and infections in distant organs of co-infected mice. It was found that vehicle-treated mice developed bacteremia and show dissemination of *S. pneumoniae* into the spleen 2 days post bacterial infection, while mice receiving anti-MT1-MMP inhibitor did not develop systemic bacterial dissemination and maintained a local and confined lung infection (Figure 5G-H).

DISCUSSION

The present examples suggest that therapeutic strategies to fight influenza infections should be aimed not only at the viral infectivity but also with the purpose of increasing tissue tolerance. This is especially true when taking into consideration that secondary bacterial infections involving *S. pneumoniae, Staphylococcus aureus, and Haemophilus influenzae* among others that are the main risk factors for reduced survival among high-risk populations (McCullers and Herman, 2001; McCullers, 2014; Morens et al., 2008). Co-infections, in many cases, involve severe lung inflammation driven by robust responses of the immune system to the viral insult. These responses dramatically impair tissue homeostasis, including disruption of the respiratory epithelium, ECM and basement membrane elements. The host response to viral infections includes tissue damage associated with oxidative stress (Avissar et al., 1996; Bozinovski et al., 2012; Campbell et al., 1987; Suliman et al., 2001; Yatmaz et al., 2013). On the one hand, MMP activity is required for the normal immune response to infection. On the other hand, host-derived MMPs may also cause infection-related immunopathology. This paradox results from the delicate balance between normal MMP function and destructive MMP-related host tissue damage (Elkington et al., 2005). Since MMPs play a crucial role in irreversible remodeling of the ECM, these robust proteases are tightly controlled and regulated (Gaffney J, 2015; Lopez-Otin and Matrisian, 2007; Turk, 2006). Moreover, maintaining tissue homeostasis during infection can be challenging when immune cells expressing active proteases are recruited towards respiratory pathogens.

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Using genome-wide transcriptional profiling of influenza infected lungs, an extraordinarily large number of genes engaged in extracellular matrix turnover and protein catabolism were observed at various time points during the infection course. Previous studies have assessed the transcriptional signatures during influenza infection by comparing *in vitro* host responses to different influenza viral strains using human respiratory bronchial and epithelial cell lines as well as *in vivo* experiments in mice and ferrets (Bortz et al., 2011; Brandes M, 2013; Chevrier et al., 2011; Elkington et al., 2005; Hartmann et al., 2015; Josset et al., 2014; Kash et al., 2004; Leon et al., 2013; Ljungberg et al., 2012; Peng et al., 2014; Shapira et al., 2009). To evaluate the relevance of MT1-MMP to human infections, data from primary human bronchial epithelial cells infected *in vitro* with a H1N1 influenza strain A/PR/8/34 was analyzed. While human macrophages would be a better model, this data show that MT1-MMP is up-regulated also in a primary human cell model (Figure 15) (Shapira et al., 2009) suggesting the relevance of our findings to the human ECM remodeling response to influenza infection.

Expanding on these studies, the present inventors focused a systematic analysis on ECM remodeling and, specifically, the activity of MT1-MMP during influenza infection. Noticeably, it was found that MT1-MMP is expressed almost entirely by stromal cells in the healthy lung during homeostasis. In contrast, following infection, MT1-MMP expression is primarily observed in the immune compartment and was accompanied by increased collagenolytic activity. Analysis of MT1-MMP-expressing cell populations showed a robust relationship with cytokine, chemokines and anti-viral response genes confirming that MT1-MMP is an inherent circuit of the host anti-viral response program. It was also found that multiple known substrates of MT1-MMP (Koziol A1, 2012; Stegemann et al., 2013), including fibrillary and basement membrane collagens (colIV, colXII, colXIV) as well as proteoglycans, are irreversibly cleaved and lost from the lungs of influenza-infected mice. These compositional changes were accompanied by ECM scaffold degradation and depletion of epithelial cells, thus contributing to a destructive phenotype that included loss of alveolar space, thinning of the alveolar wall and distortion of airway structures. Together, it has been shown that influenza infections induce expression and activity of MT1-MMP, which contributes to

uncontrolled degradation of the structural ECM components that are required for maintaining the lung integrity and function.

Elevated MT1-MMP expression levels have been described in the context of cancer cell metastasis implicating MT1-MMP as an invasive marker associated with advanced stages and poor prognosis (Zarrabi et al., 2011). This property was attributed to the collagenolytic activity of MT1-MMP, degrading the peri-cellular environment and endothelial barriers to make a path for metastatic cells. However, the role of MT1-MMP in influenza infection has not been previously reported. Accordingly, elevated expression levels of tissue inhibitors of metalloproteinases (TIMPs), such as TIMP-1 and TIMP-3, the endogenous inhibitors of most metalloproteinases were noted. Nevertheless, expression levels of TIMP-2 the endogenous inhibitor of MT1-MMP were not significantly changed. Other ECM enzymes have been shown to play a role in influenza infection as well (Bradley et al., 2012). A significant increase in MMP-8 at the protein level was also noted (Figures 6A-D).

Previous studies have sought to disentangle viral cytopathic effects from inflammatory collateral damage during influenza infection (Boon et al., 2011; Kash et al., 2004; Kobasa et al., 2007; Tate et al., 2009). It has now been shown that even in low viral titers, lung ECM destruction is significant. This suggests that the main damage to the ECM during infection is a parallel pathway driven by proteolytic events associated with host response irrespective of direct viral loads (Jamieson et al., 2013; Medzhitov et al., 2012; Schneider and Ayres, 2008). Under these conditions, it has now been shown that ECM damage can be almost completely rescued by selectively modulating MT1-MMP proteolytic activity. Using an anti-MT1-MMP inhibitory Fab fragment, it was possible to maintain tissue structure and improve the outcome of influenza infections irrespective of viral replication. It is noteworthy that this MT1-MMP antibody is highly selective in targeting the collagenase activity and does not interfere with enzyme dimerization and maturation of pro-MMP-2 required for tissue homeostasis (Udi et al., 2015). The present study shows that MT1-MMP is not critically involved in immune cell recruitment or IL-1β and TNF-α production. This is in line with previous studies showing that macrophage-derived MT1-MMP regulated subjacent cellular proteolysis rather than directly being involved in migration or cell trafficking through host tissues (Shimizu-Hirota et al., 2012).

In order to mimic the natural disease progression, co-infection settings of influenza and *S. pneumoniae* were used to show that targeting the virus with Tamiflu™ alone is ineffective in controlling ECM damage and does not predict successful management of the disease following bacterial infection. Importantly, inhibition of MT1-MMP activity, which protected tissue architecture and composition without significantly affecting the viral loads, exhibited improved disease management when administrated as either a prophylactic or therapeutic agent. In agreement, mice treated with Tamiflu™ developed sepsis due to dissemination of bacteria from the lungs to the systemic circulation, while those treated with the MT1-MMP inhibitory antibody exhibited reduced spread of bacteria through blood-air disruption. This further suggests that the maintenance of tissue homeostasis is a parallel process that, at least in our influenza model, is as important therapeutically as controlling the viral load. Importantly, the combination of the two treatments achieved complete survival rates both in the prophylactic and therapeutic modes. This further supports the present findings that the combination of the two strategies, targeting viral replication as well as maintaining host barrier homeostasis and preventing tissue destruction, greatly increases the survival outcome.

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Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

Citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. Use of an anti-viral agent directed towards a virus which causes a primary infection, wherein said primary infection is a respiratory infection, and an inhibitory antibody which specifically binds to membrane type 1-matrix metalloproteinase (MT1-MMP1) for preparation of a medicament for the prevention of a disease associated with a secondary infection in a subject, wherein said disease is due to dissemination of a pathogenic agent from the lungs of the subject, wherein the subject has a respiratory infection caused by said virus.

2. Use of an anti-viral agent directed towards a virus which causes a respiratory infection and an inhibitory antibody which specifically binds to MT1-MMP1 for preparation of a medicament for the treatment of a respiratory infection caused by said virus.

3. The use of claim 1, wherein said secondary infection is a blood infection.

4. The use of claim 1, wherein said disease is sepsis.

5. The use of claim 1 or claim 2, wherein said virus is influenza.

6. The use of claim 1 or claim 2, wherein said anti-viral agent is a neuraminidase inhibitor (NAI).

7. The use of claim 1, wherein said secondary infection is a bacterial infection.

8. The use of claim 7, wherein said bacterial infection is *S. pneumoniae.*

FIG. 1A

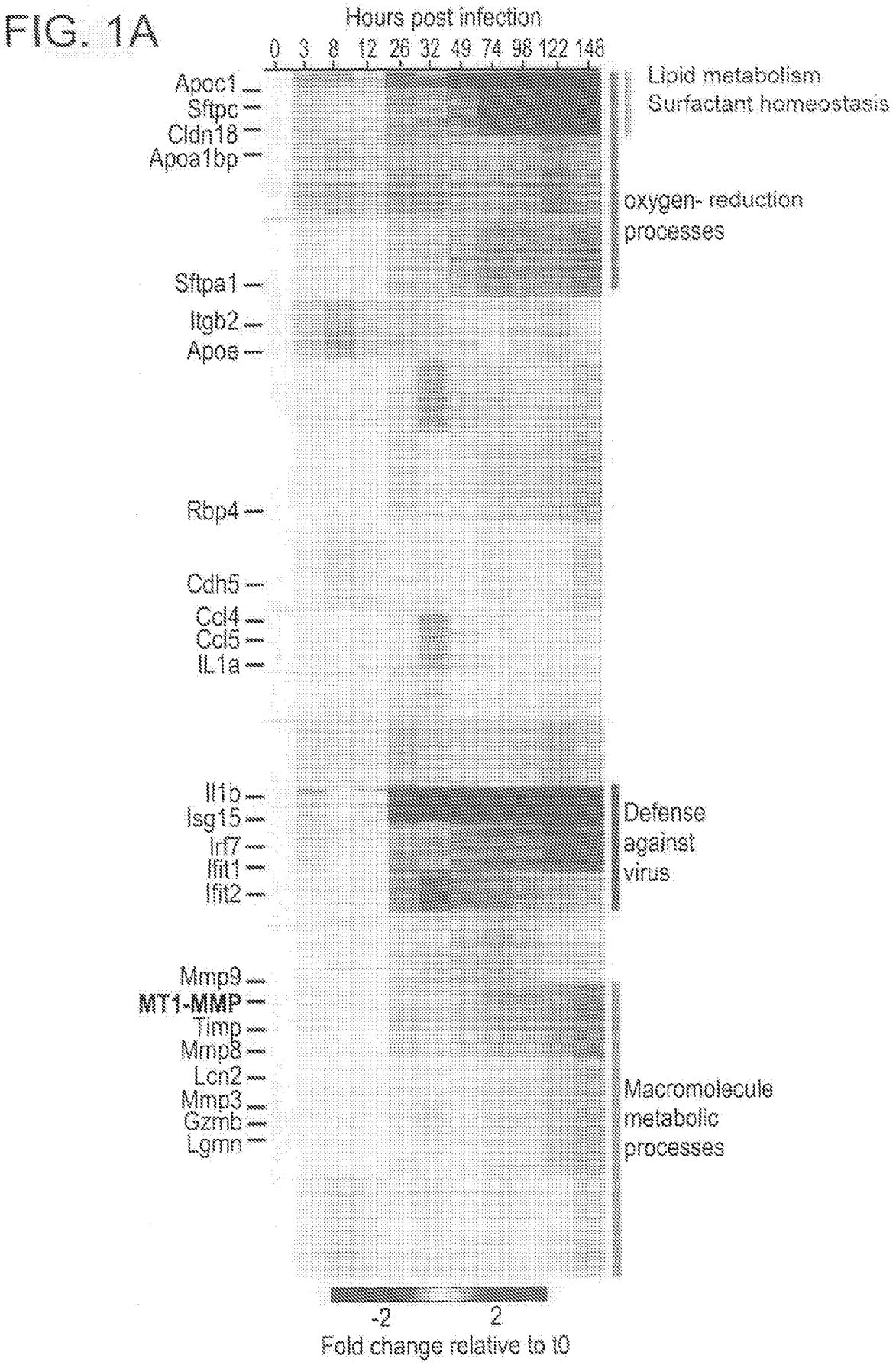

Hours post infection
0 3 8 12 26 32 49 74 98 122 148
Apoc1
Sftpc
Cldn18
Apoa1bp
Sftpa1
Itgb2
Apoe
Rbp4
Cdh5
Ccl4
Ccl5
IL1a
Il1b
Isg15
Irf7
Ifit1
Ifit2
Mmp9
MT1-MMP
Timp
Mmp8
Lcn2
Mmp3
Gzmb
Lgmn
Lipid metabolism
Surfactant homeostasis
oxygen- reduction processes
Defense against virus
Macromolecule metabolic processes
-2
2
Fold change relative to t0

FIG. 1B

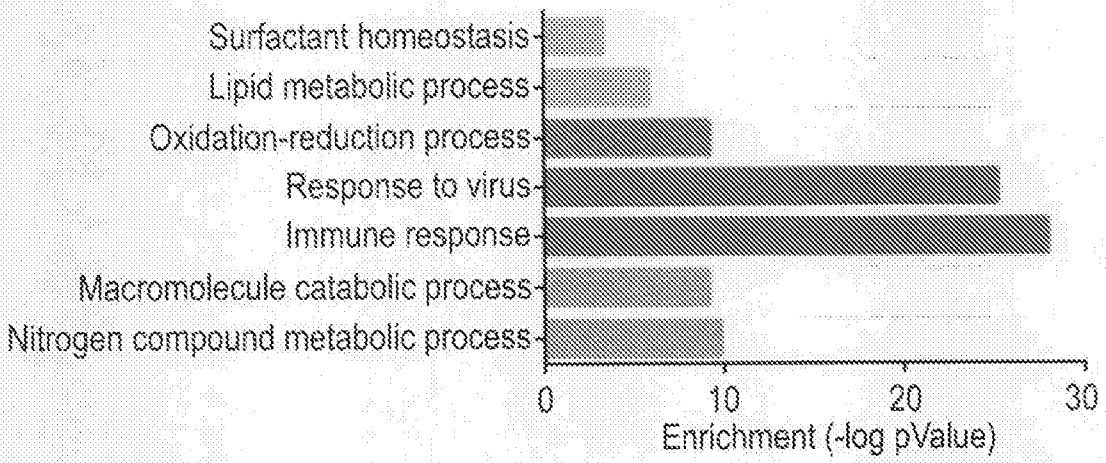


FIG. 1C

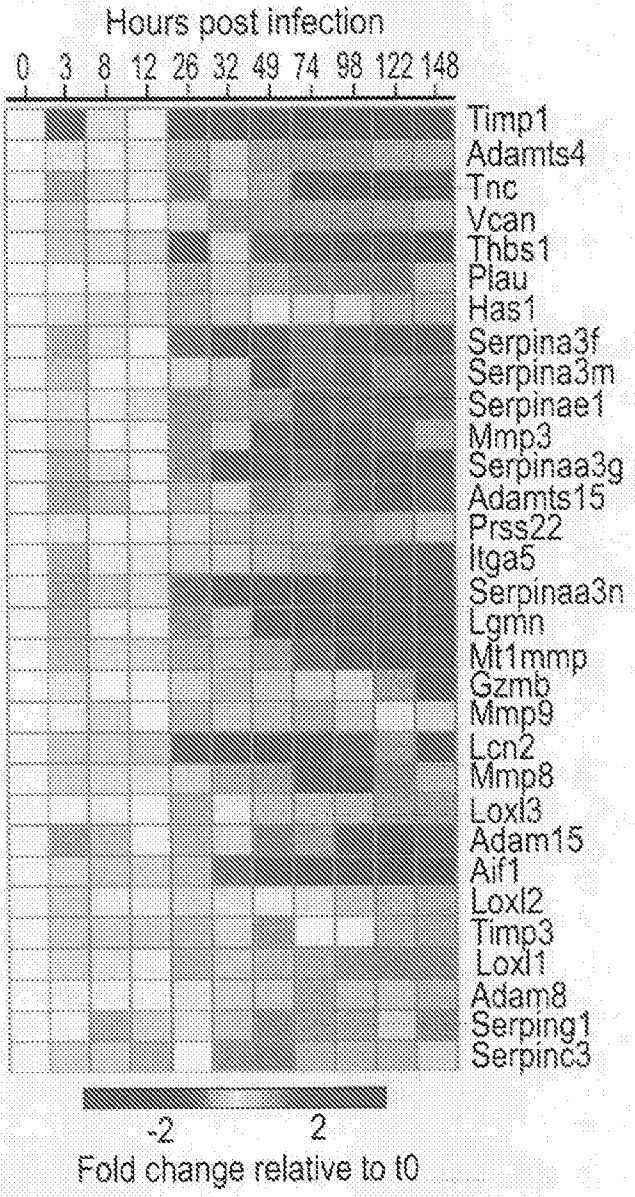


FIG. 1D

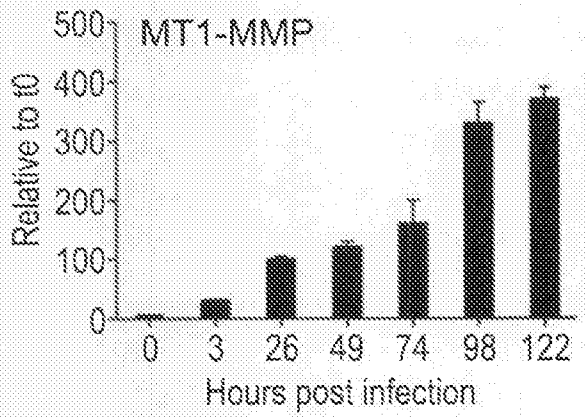


FIG. 2A

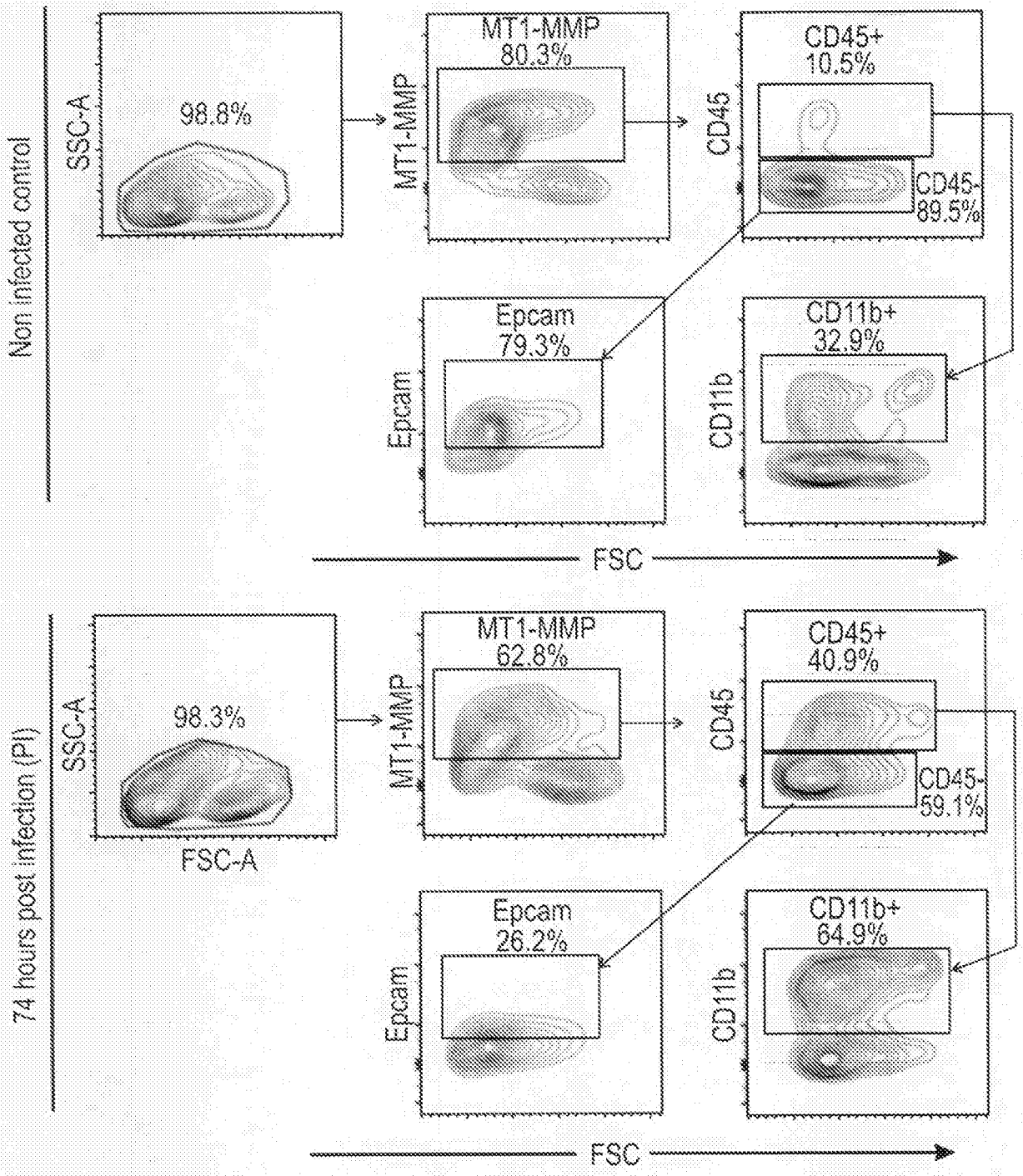

Non infected control
SSC-A
98.8%
MT1-MMP
MT1-MMP
80.3%
CD45
CD45+
10.5%
CD45-
89.5%
Epcam
Epcam
79.3%
CD11b
CD11b+
32.9%
FSC
74 hours post infection (PI)
SSC-A
98.3%
FSC-A
MT1-MMP
MT1-MMP
62.8%
CD45
CD45+
40.9%
CD45-
59.1%
Epcam
Epcam
26.2%
CD11b
CD11b+
64.9%
FSC

FIG. 2B

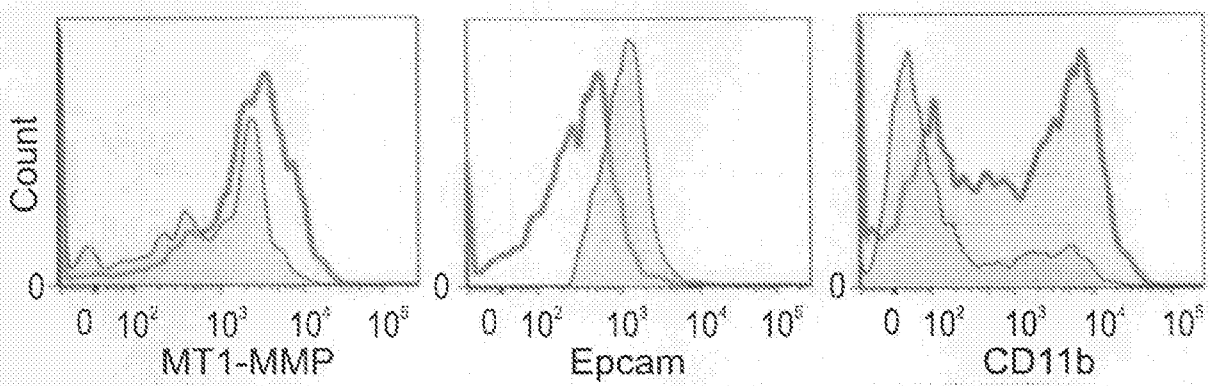

Count
0
0 10² 10³ 10⁴ 10⁵
MT1-MMP
Epcam
CD11b

FIG. 2C

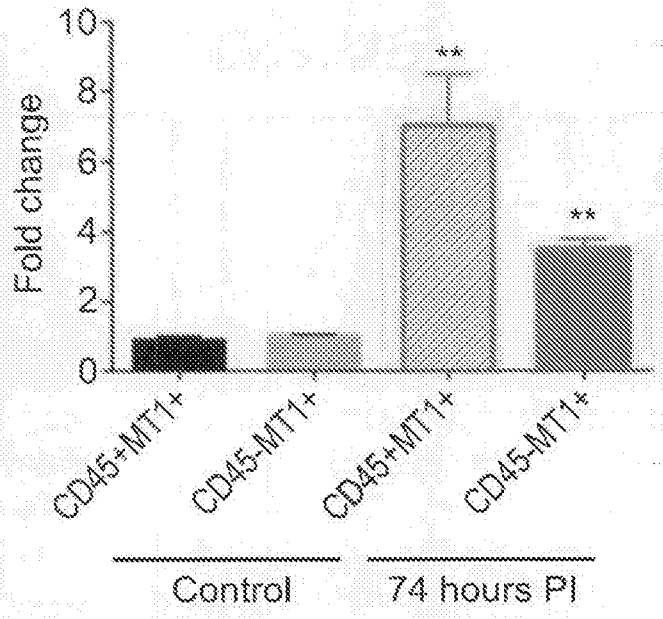

Fold change
10
8
6
4
2
0
**
**
CD45+MT1+
CD45-MT1+
CD45+MT1+
CD45-MT1+
Control
74 hours PI

FIG. 3A

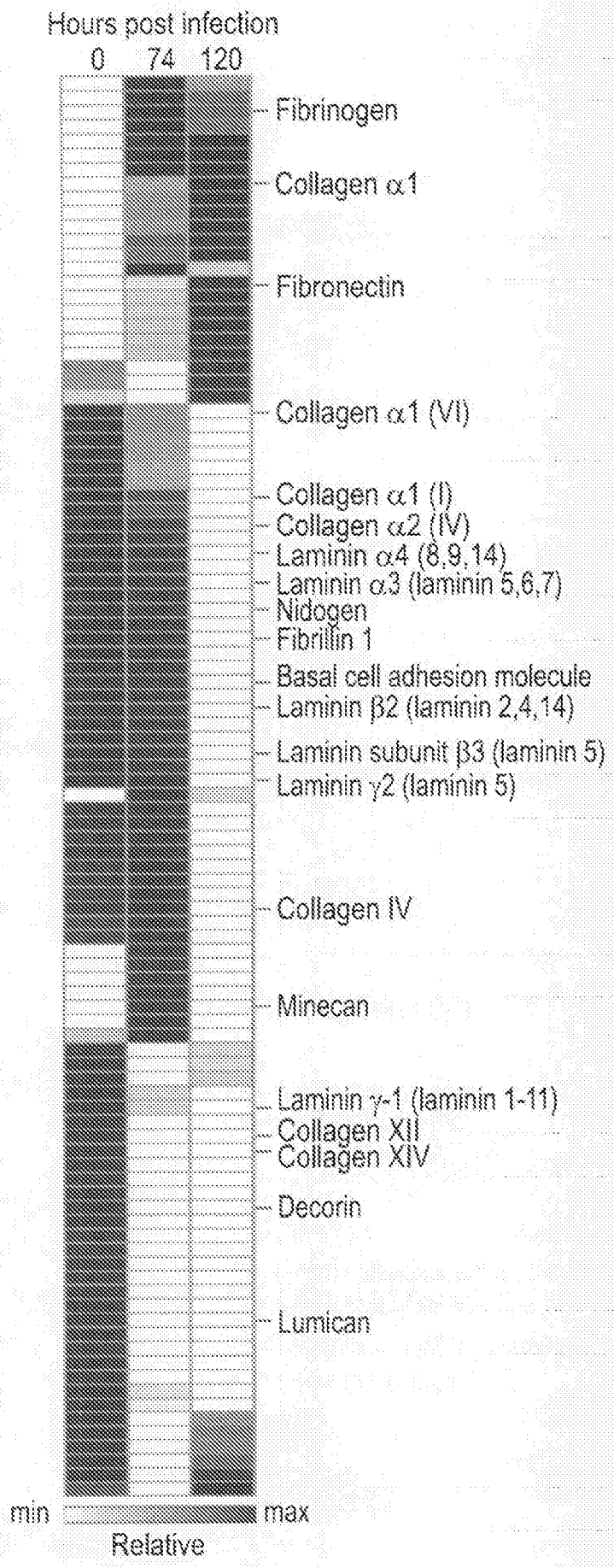

Hours post infection
0
74
120
Fibrinogen
Collagen α1
Fibronectin
Collagen α1 (VI)
Collagen α1 (I)
Collagen α2 (IV)
Laminin α4 (8,9,14)
Laminin α3 (laminin 5,6,7)
Nidogen
Fibrillin 1
Basal cell adhesion molecule
Laminin β2 (laminin 2,4,14)
Laminin subunit β3 (laminin 5)
Laminin γ2 (laminin 5)
Collagen IV
Minecan
Laminin γ-1 (laminin 1-11)
Collagen XII
Collagen XIV
Decorin
Lumican
min
max
Relative

FIG. 3B

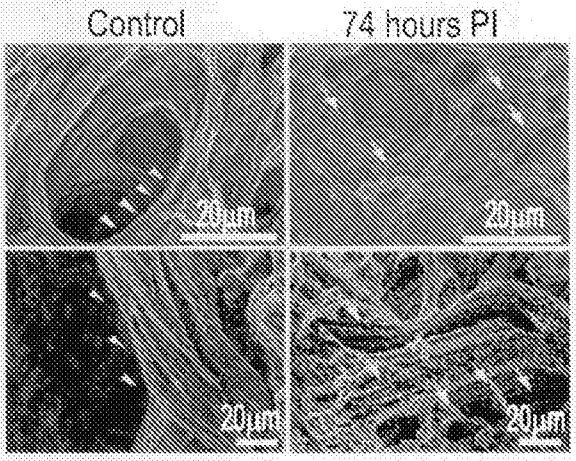


FIG. 3C

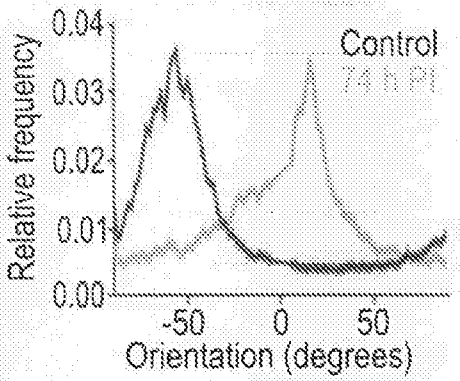


FIG. 3D

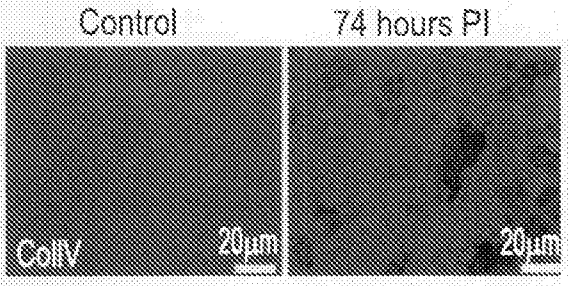


FIG. 3E

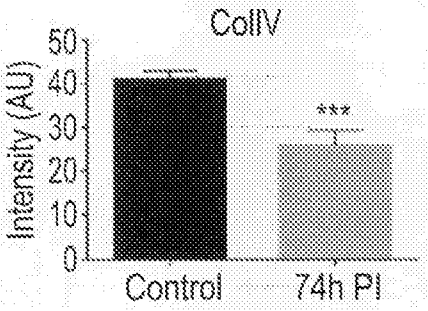


FIG. 3F

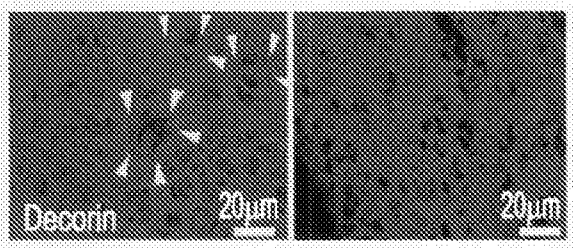


FIG. 3G

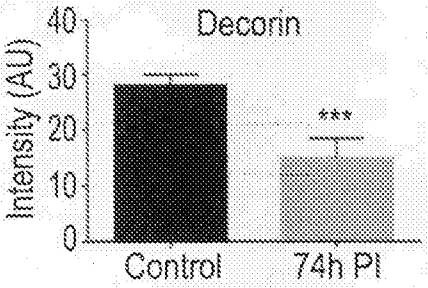


FIG. 3H

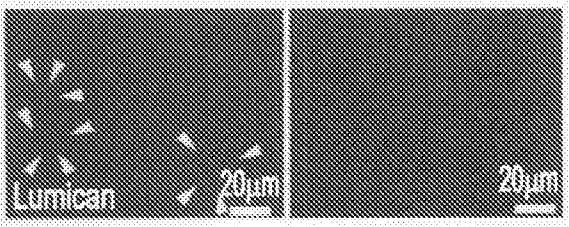
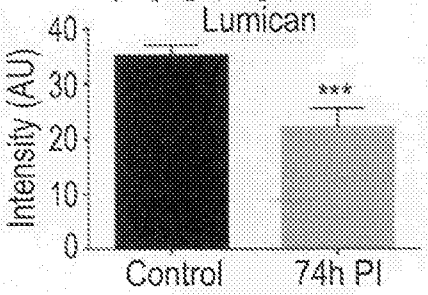


FIG. 3I



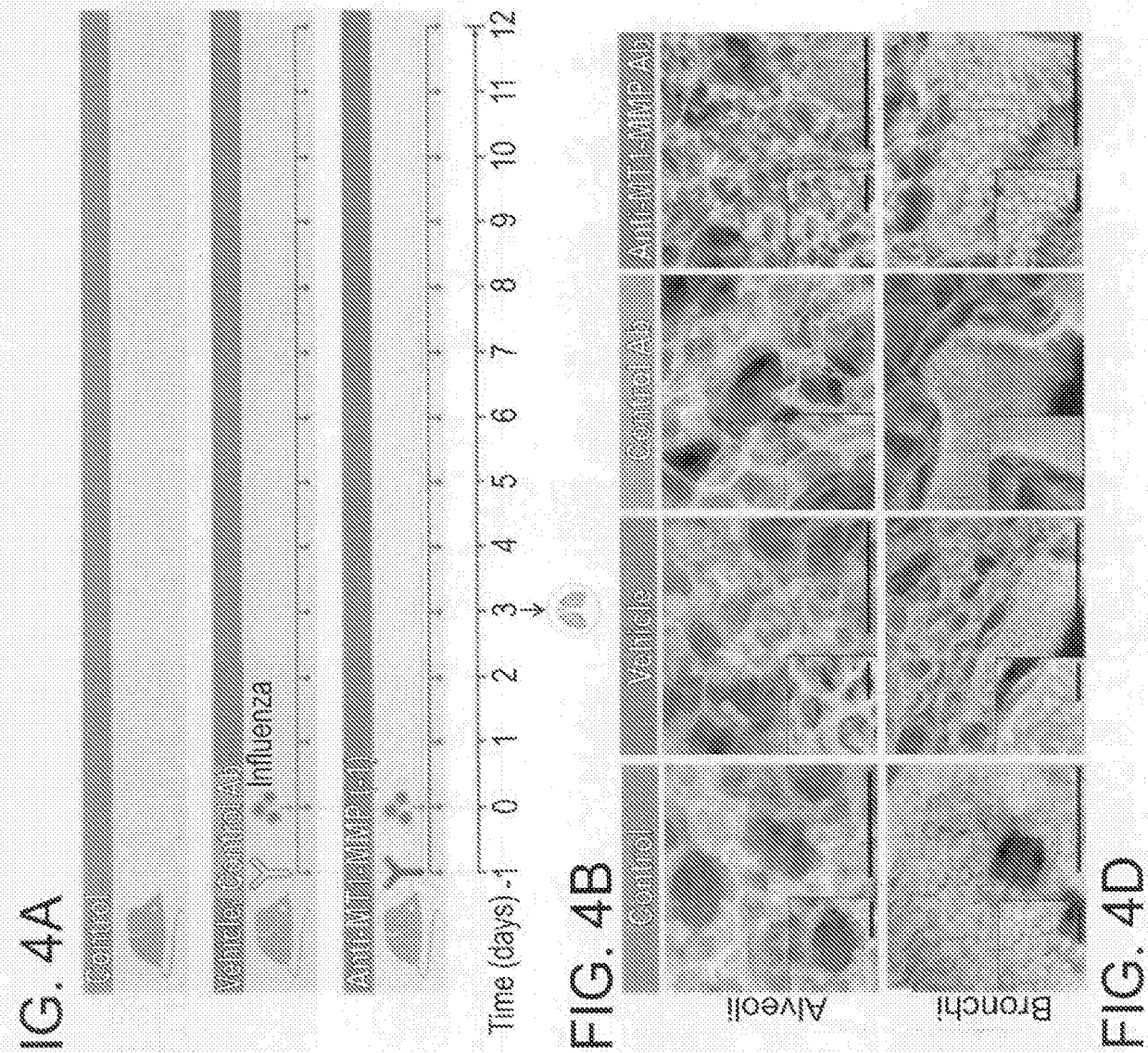


FIG. 4A

FIG. 4B

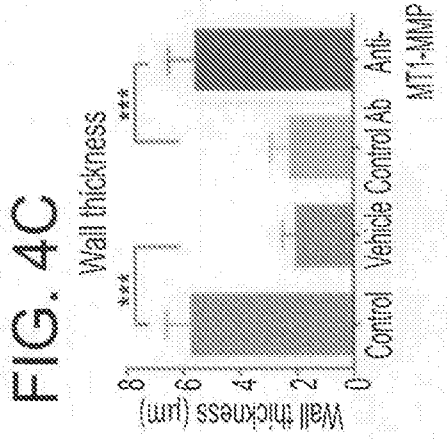


FIG. 4C

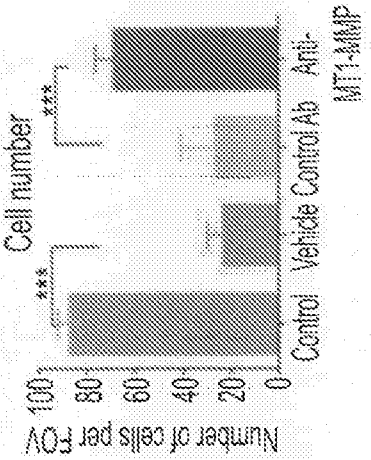


FIG. 4E

FIG. 4D

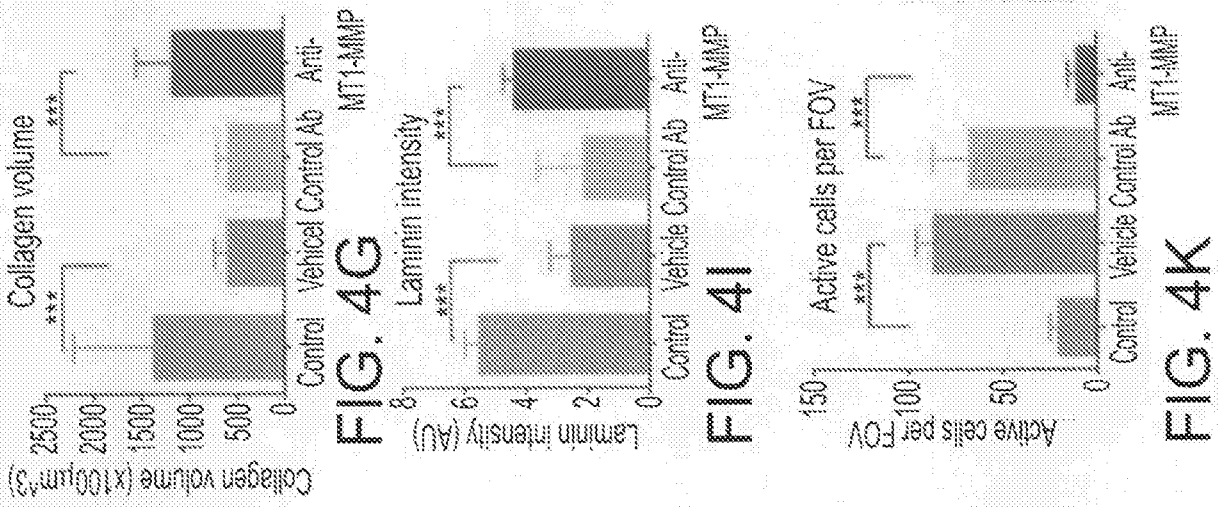


FIG. 4G

FIG. 4I

FIG. 4K

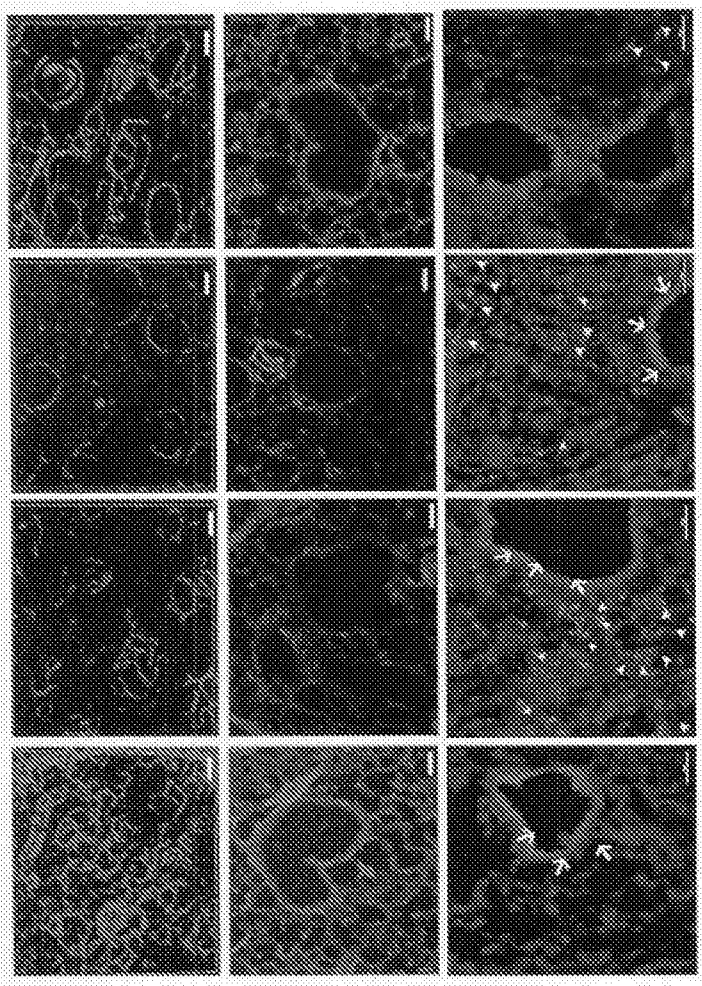


FIG. 4F

FIG. 4H

FIG. 4J

FIG. 5A

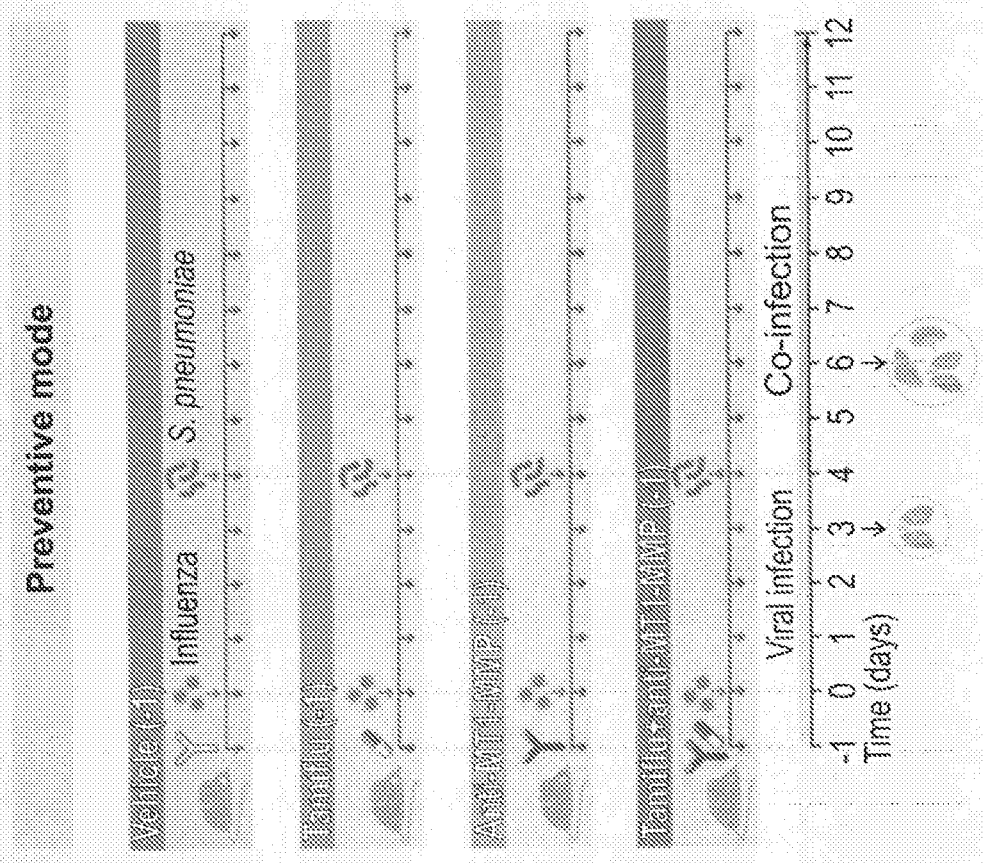
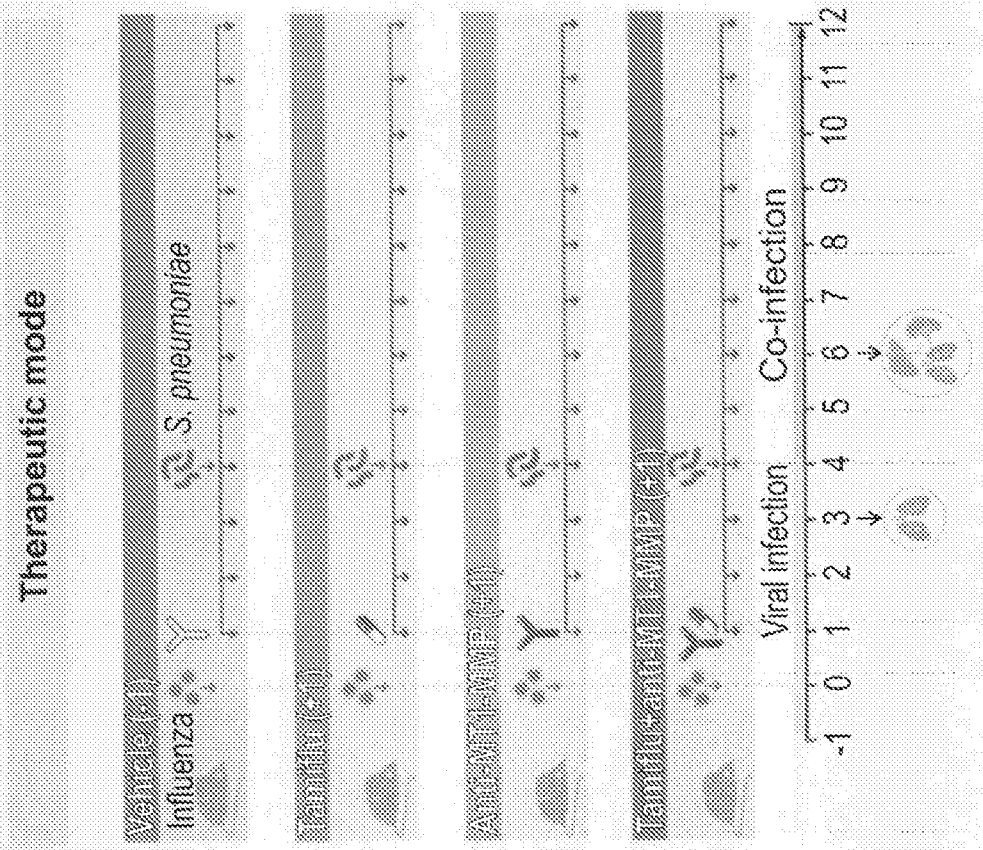

Preventive mode
Influenza
S. pneumoniae
Viral infection
Co-infection
-1 0 1 2 3 4 5 6 7 8 9 10 11 12
Time (days)

FIG. 5D


Therapeutic mode
Influenza
S. pneumoniae
Viral infection
Co-infection
-1 0 1 2 3 4 5 6 7 8 9 10 11 12

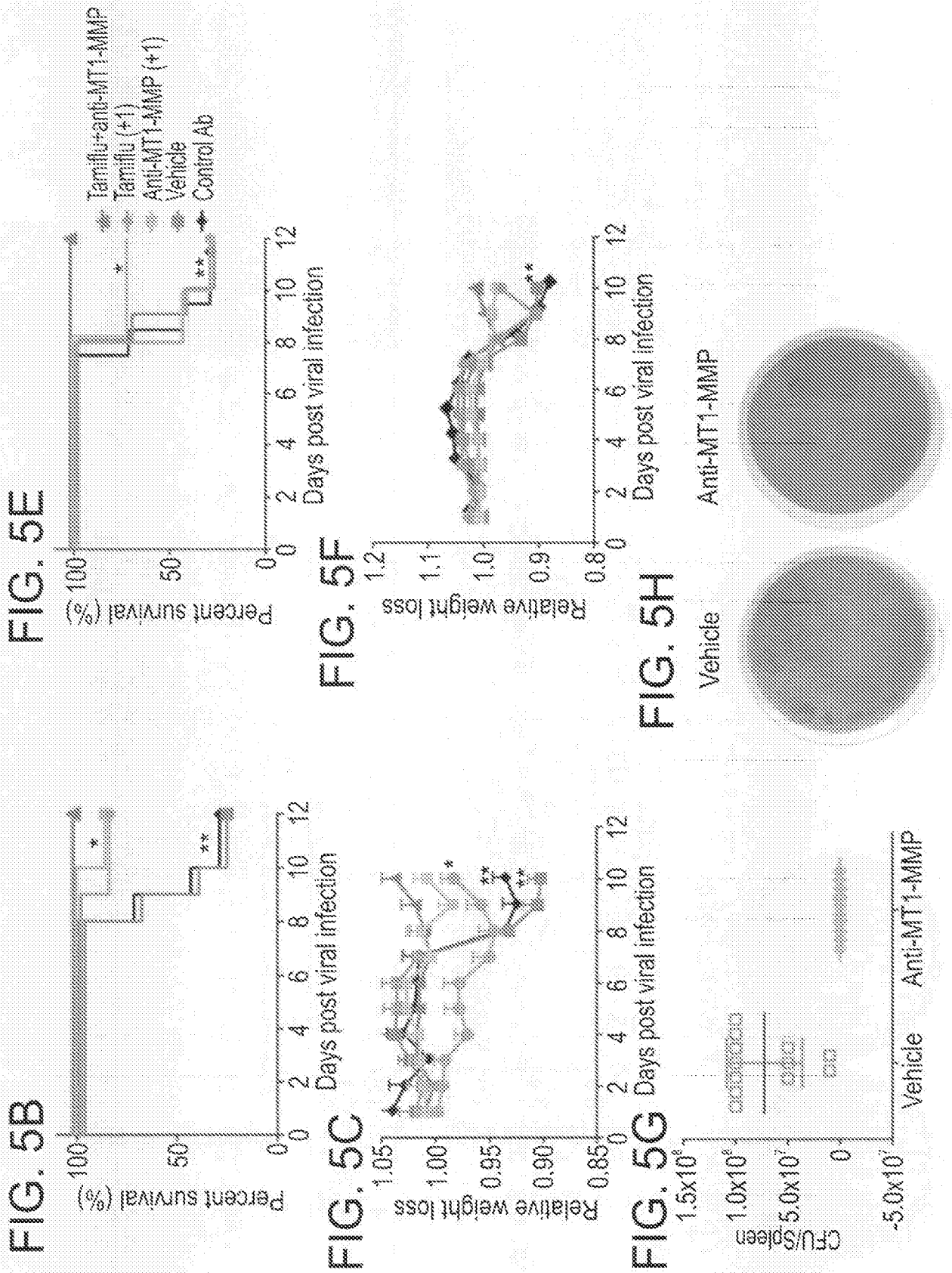


FIG. 5B
Percent survival (%)
Days post viral infection
FIG. 5C
Relative weight loss
Days post viral infection
FIG. 5E
Percent survival (%)
Tamiflu+anti-MT1-MMP
Tamiflu (+1)
Anti-MT1-MMP (+1)
Vehicle
Control Ab
Days post viral infection
FIG. 5F
Relative weight loss
Days post viral infection
FIG. 5G
CFU/Spleen
Vehicle
Anti-MT1-MMP
FIG. 5H
Vehicle
Anti-MT1-MMP

FIG. 6A

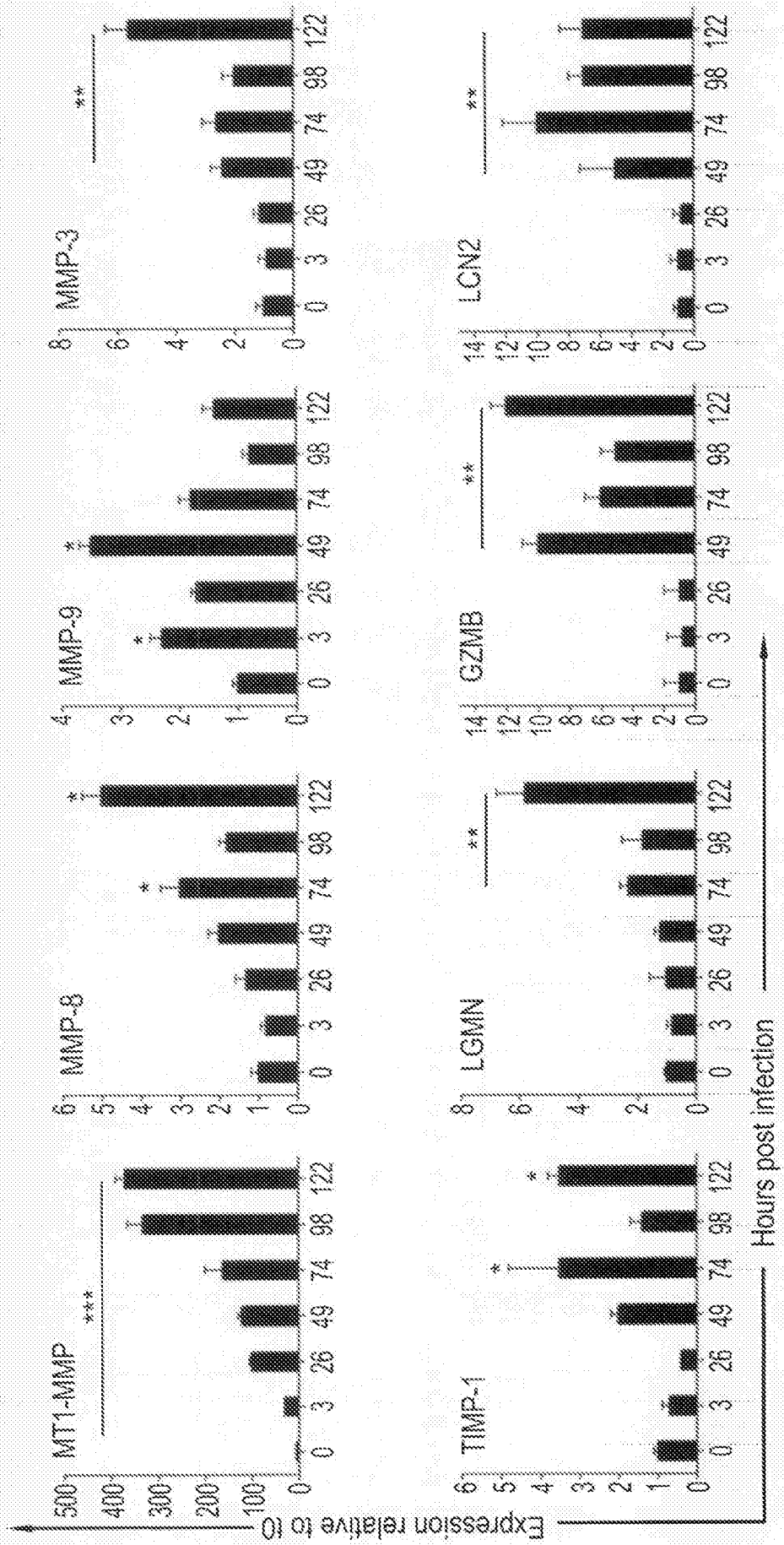

MT1-MMP
MMP-8
MMP-9
MMP-3
LGMN
GZMB
LCN2
TIMP-1
Expression relative to t0
Hours post infection
0 3 26 49 74 98 122

FIG. 6B

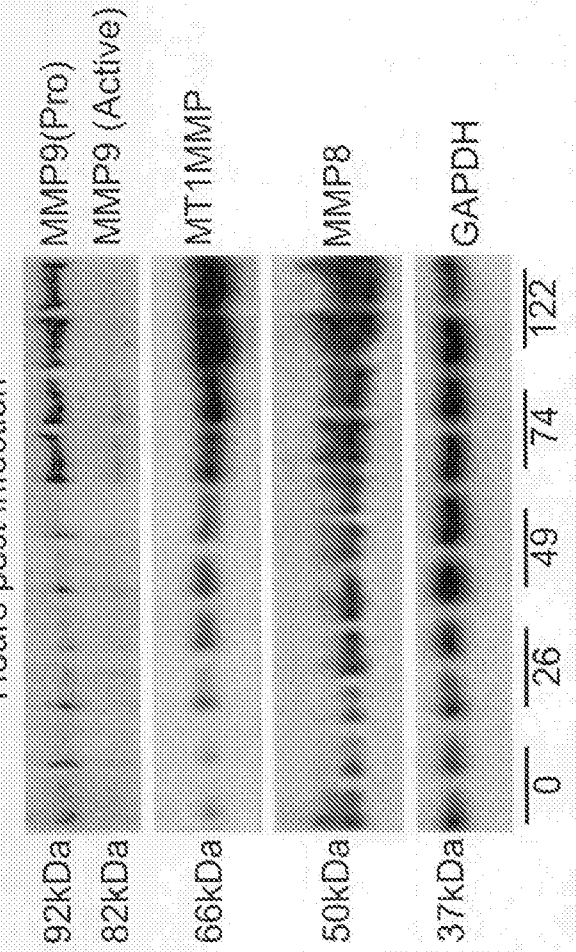


FIG. 6C

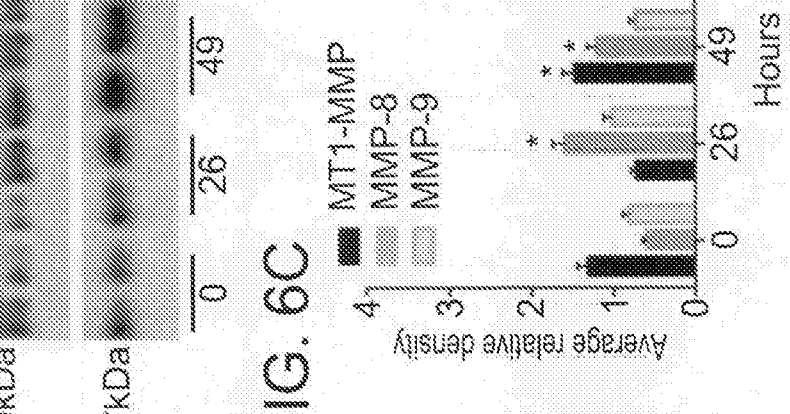


FIG. 6D

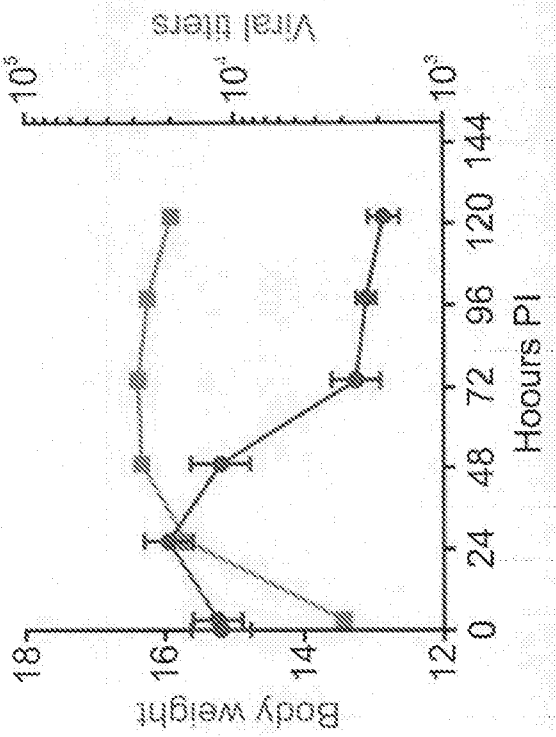


FIG. 7A

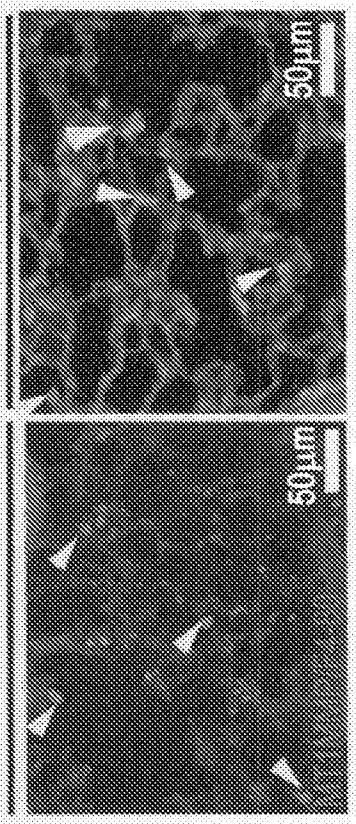


FIG. 7B

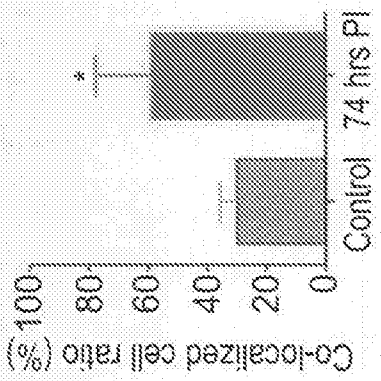


FIG. 7C

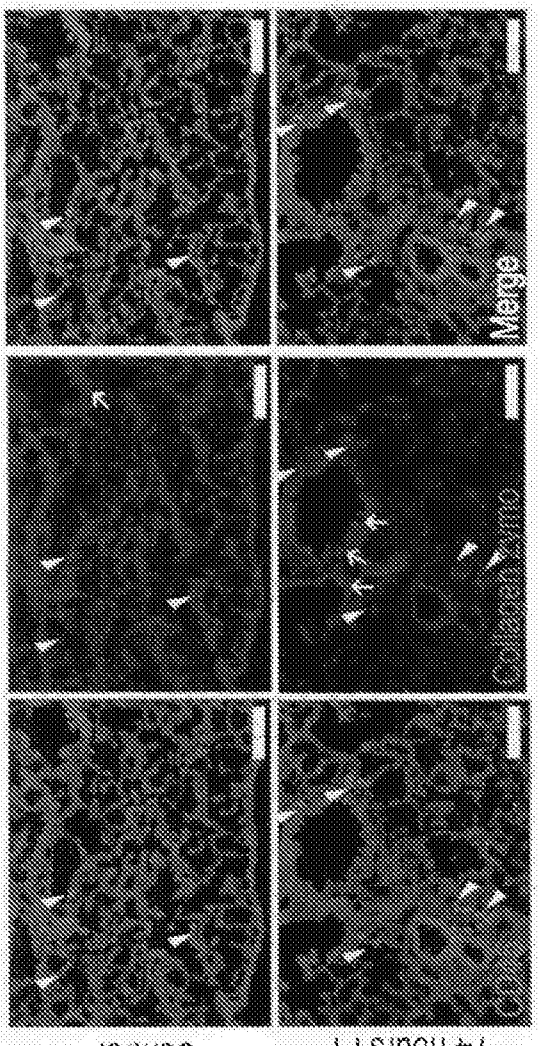


FIG. 7D

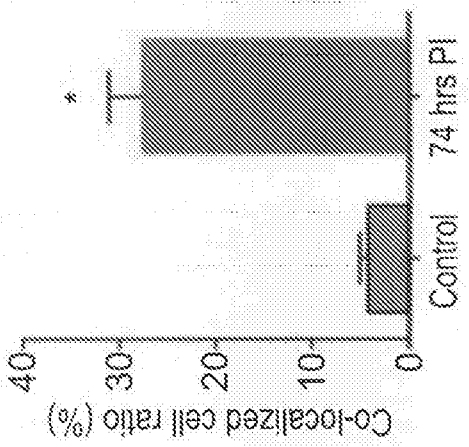


FIG. 8

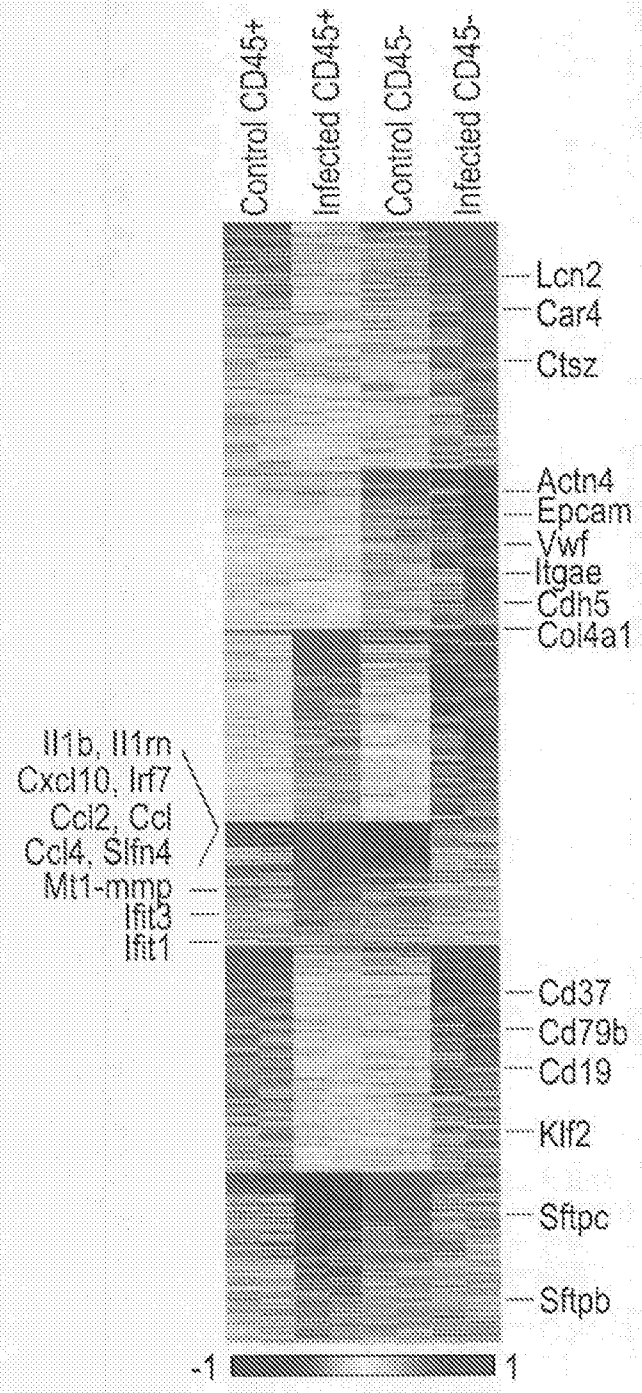

Control CD45+
Infected CD45+
Control CD45-
Infected CD45-
Lcn2
Car4
Ctsz
Actn4
Epcam
Vwf
Itgae
Cdh5
Col4a1
Il1b, Il1rn
Cxcl10, Irf7
Ccl2, Ccl
Ccl4, Slfn4
Mt1-mmp
Ifit3
Ifit1
Cd37
Cd79b
Cd19
Klf2
Sftpc
Sftpb
-1
1

FIG. 9A

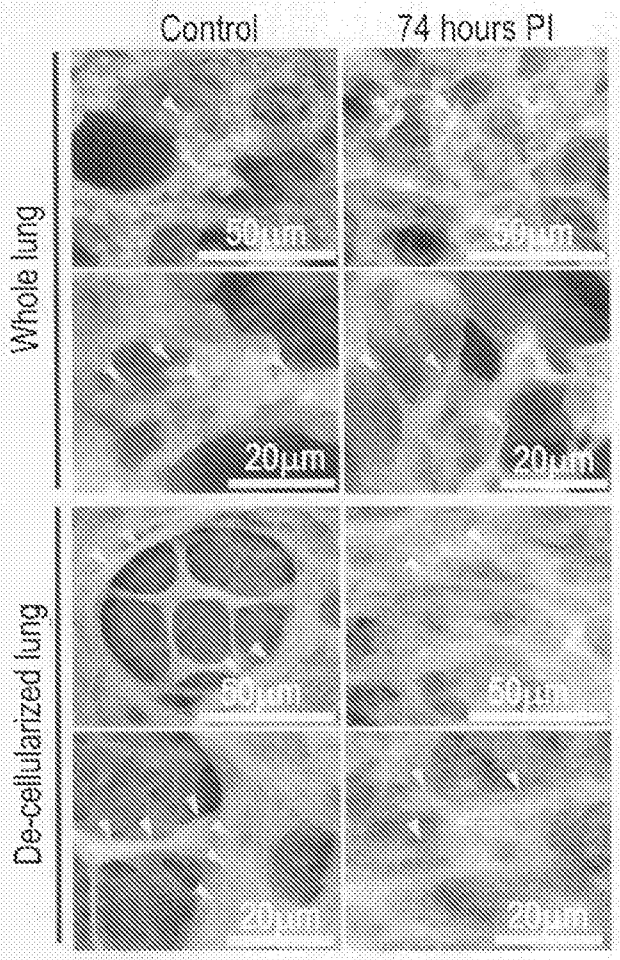
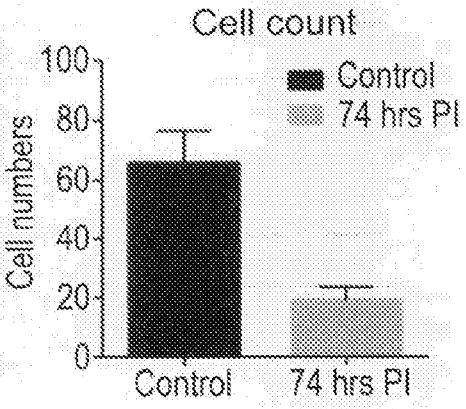


FIG. 9B

FIG. 9C



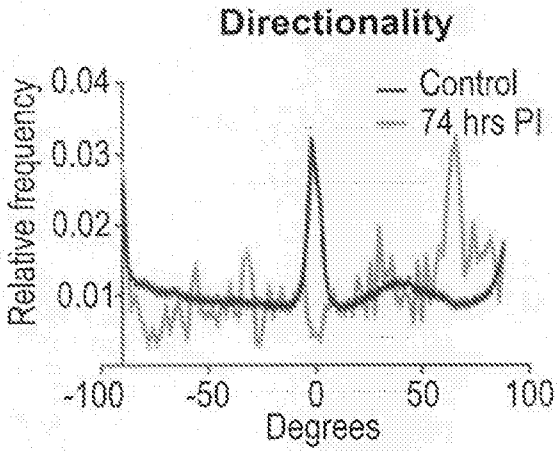


FIG. 9D

FIG. 10A

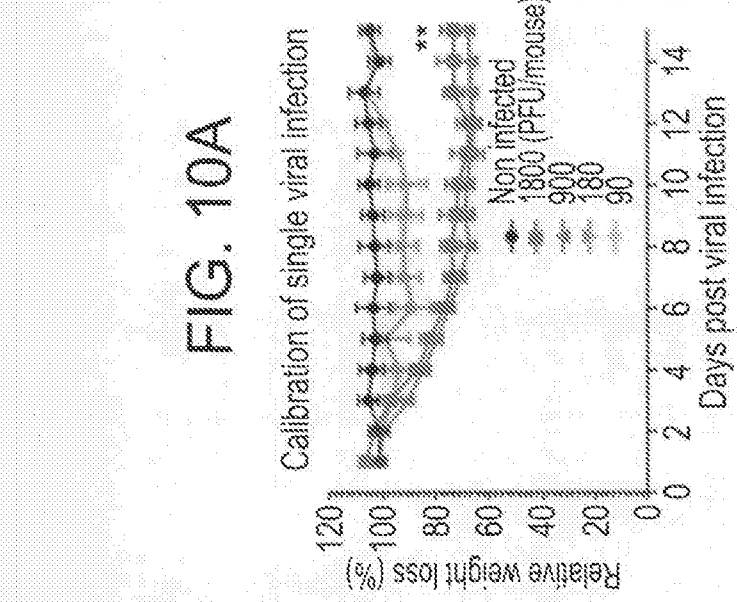


FIG. 10B

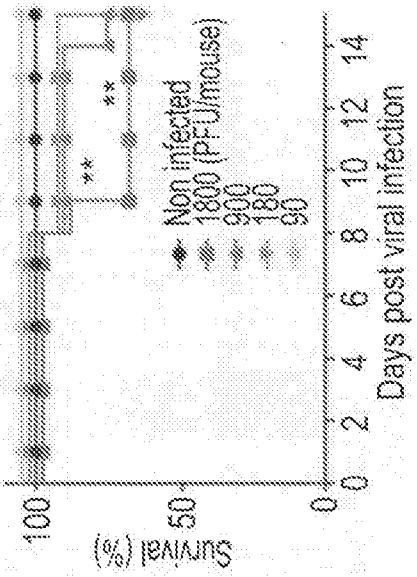


FIG. 10C

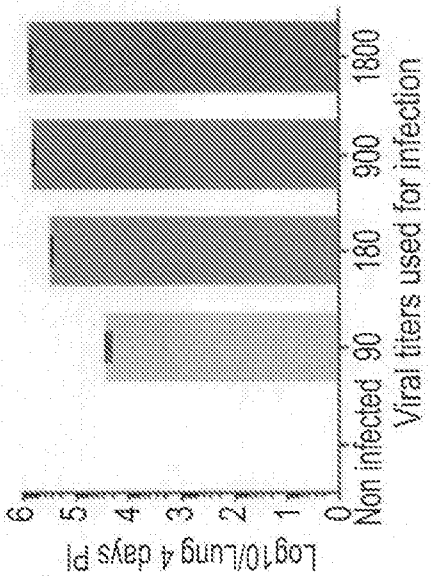
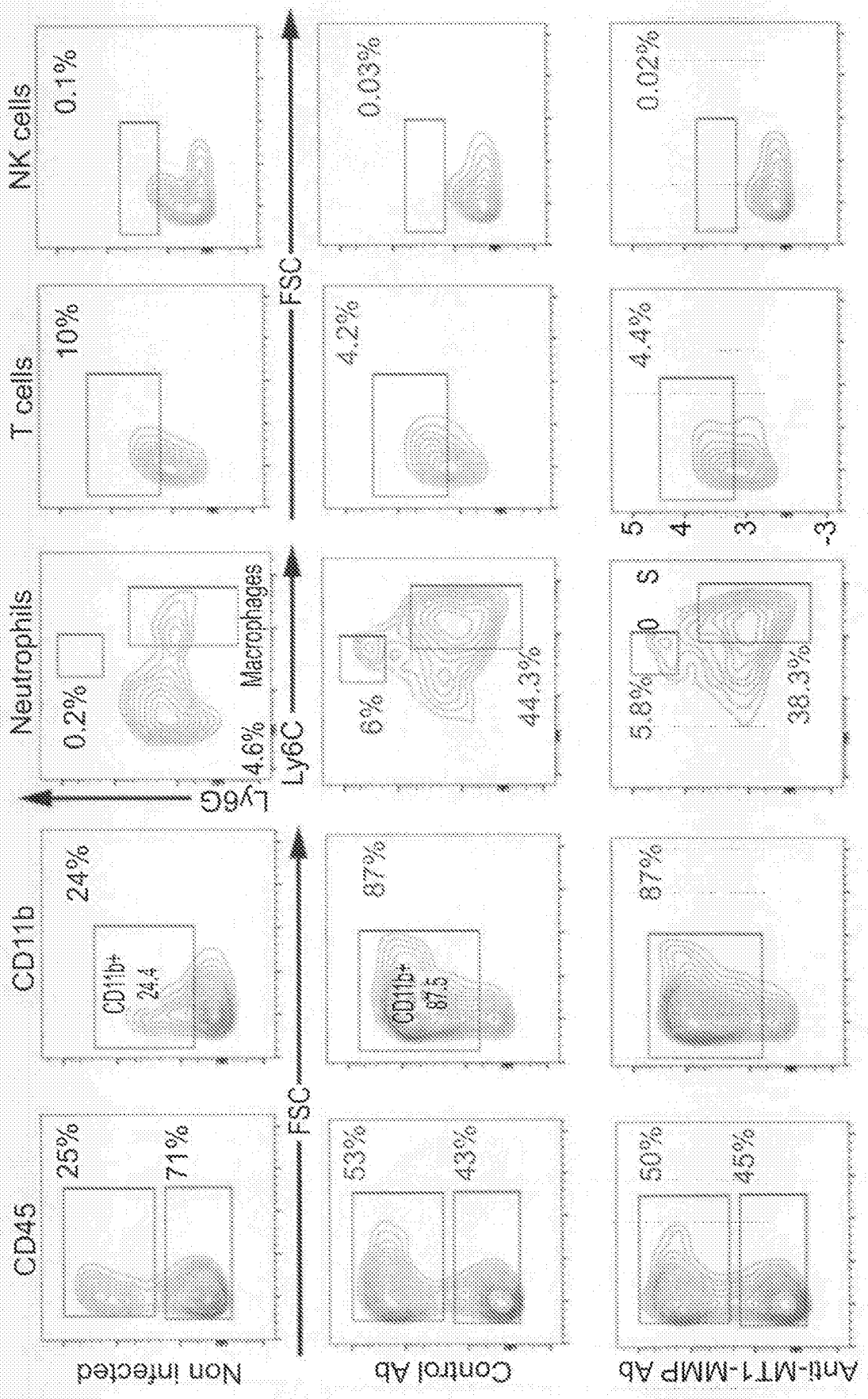


FIG. 11A


CD45
CD11b
Neutrophils
T cells
NK cells
Non infected
Control Ab
Anti-MT1-MMP Ab
FSC
Ly6G
Ly6C
25%
71%
24%
CD11b+
24.4
0.2%
4.6%
Macrophages
10%
0.1%
53%
43%
87%
CD11b+
87.5
6%
44.3%
4.2%
0.03%
50%
45%
87%
5.8%
38.3%
4.4%
0.02%
5
4
3
-3

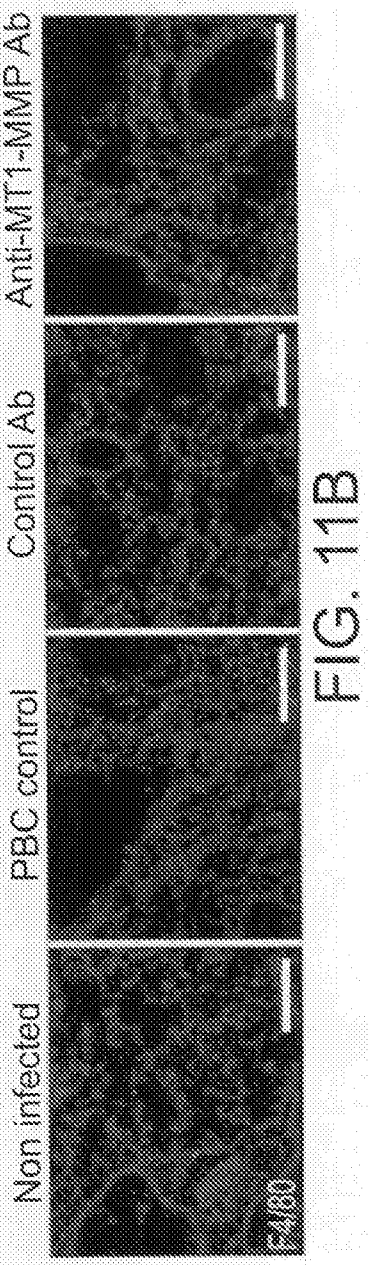


FIG. 11B

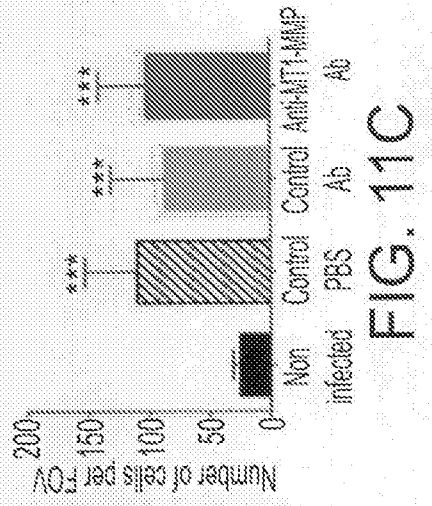


FIG. 11C

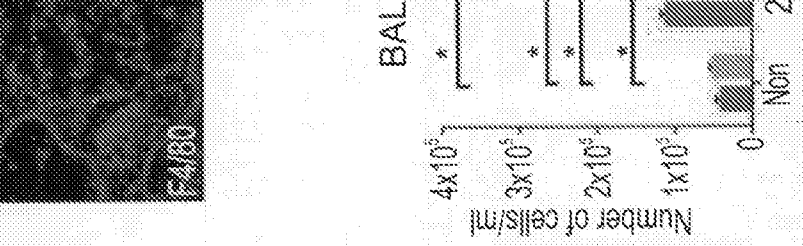


FIG. 11D

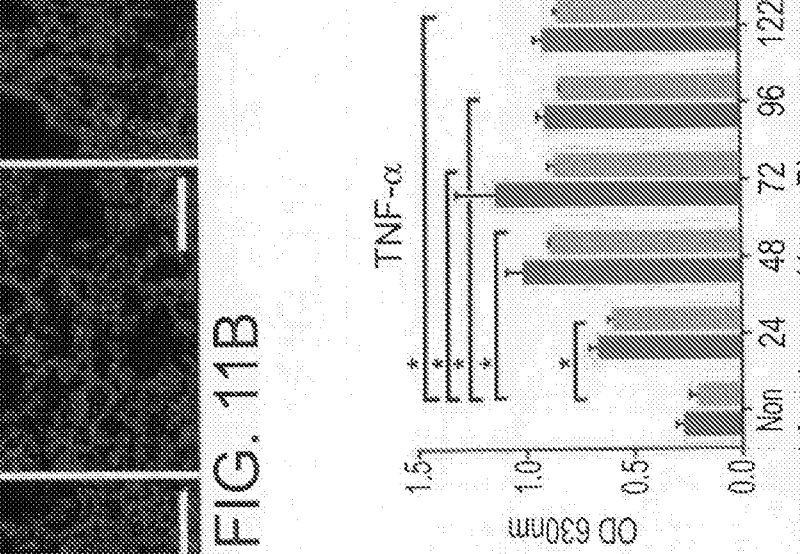


FIG. 11E

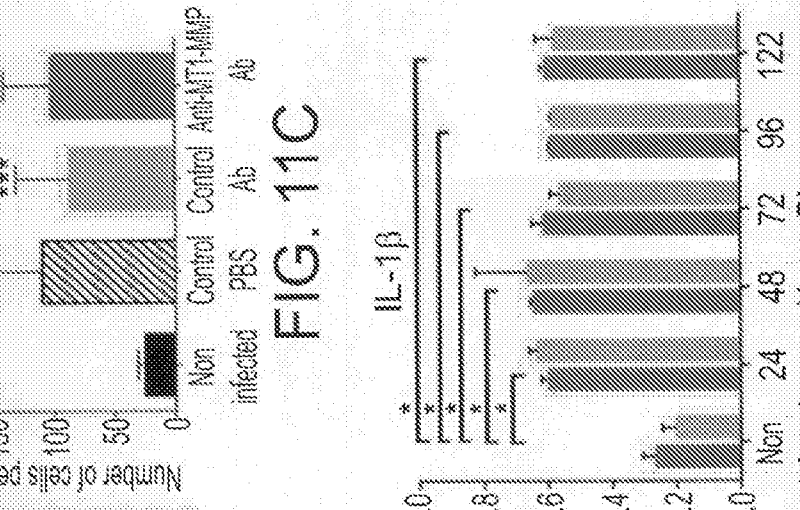


FIG. 11F

FIG. 12A

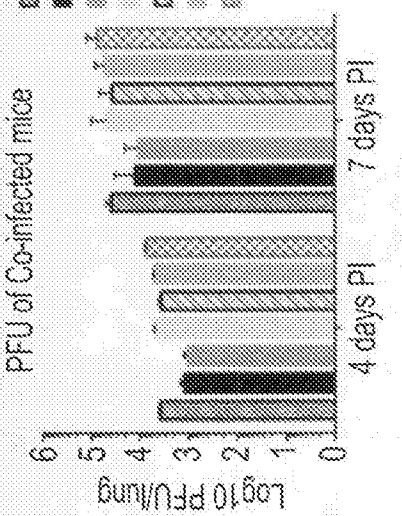


FIG. 12B

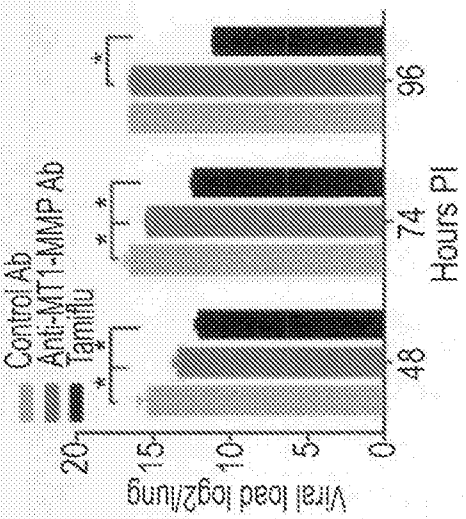


FIG. 12C

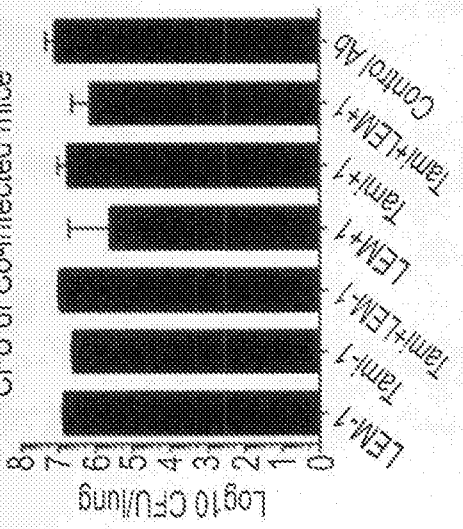


FIG. 12D

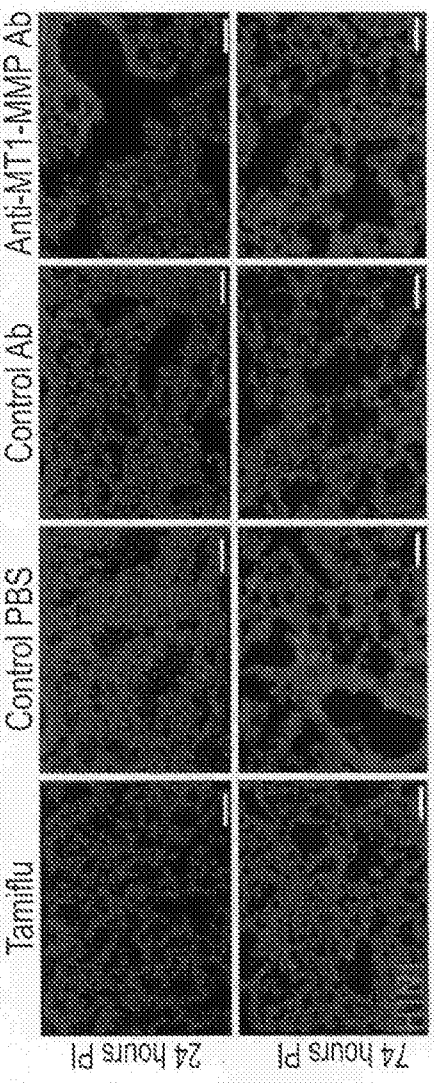
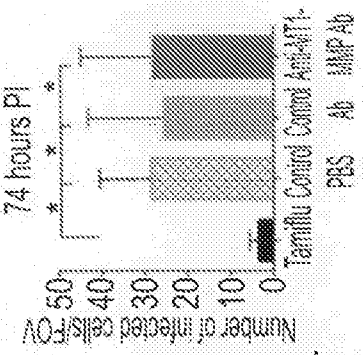


FIG. 12E

FIG. 12F



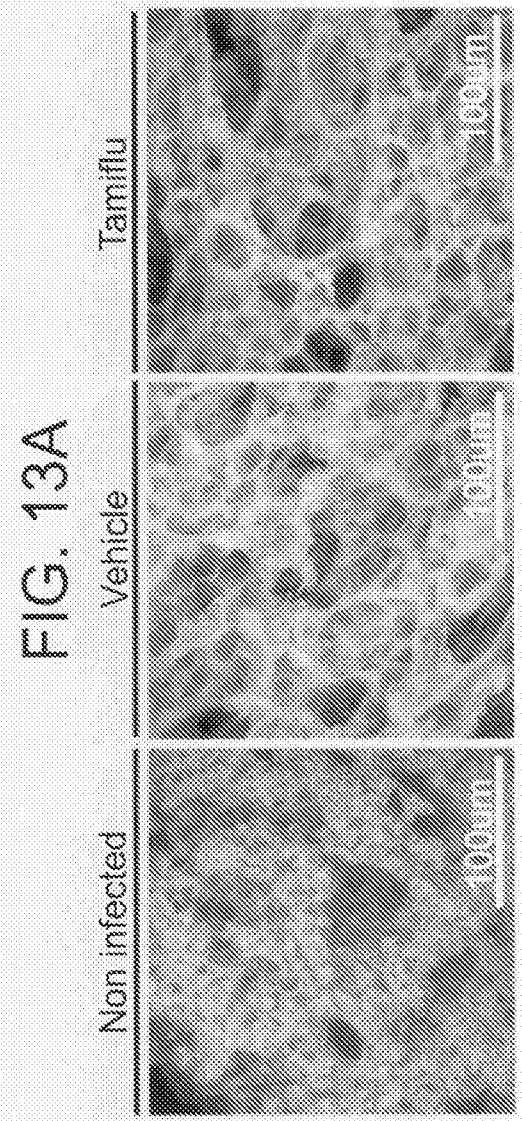


FIG. 13A

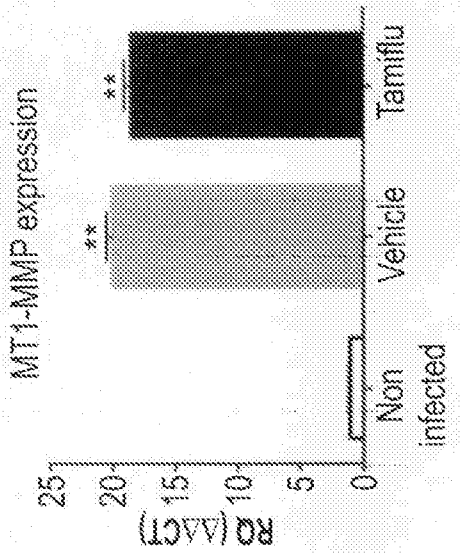


FIG. 13D

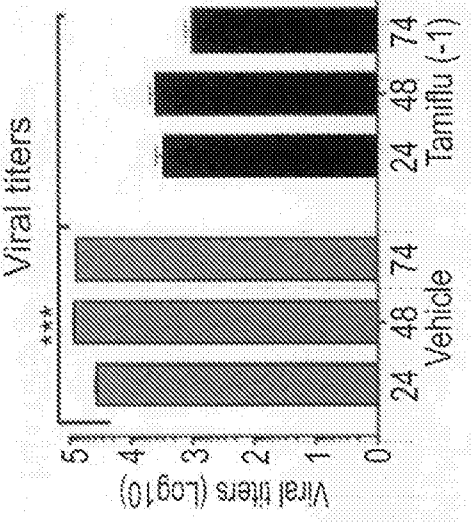


FIG. 13C

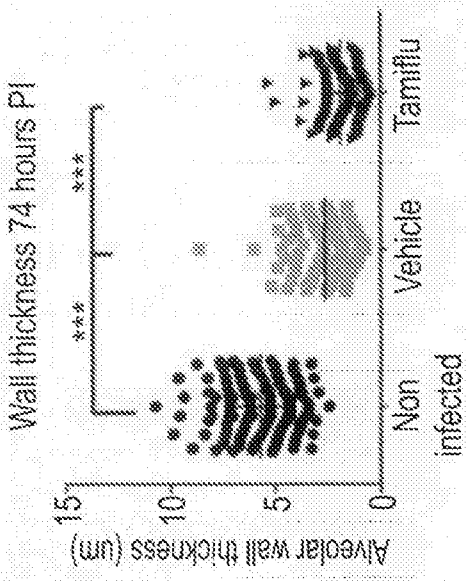


FIG. 13B

FIG. 14A

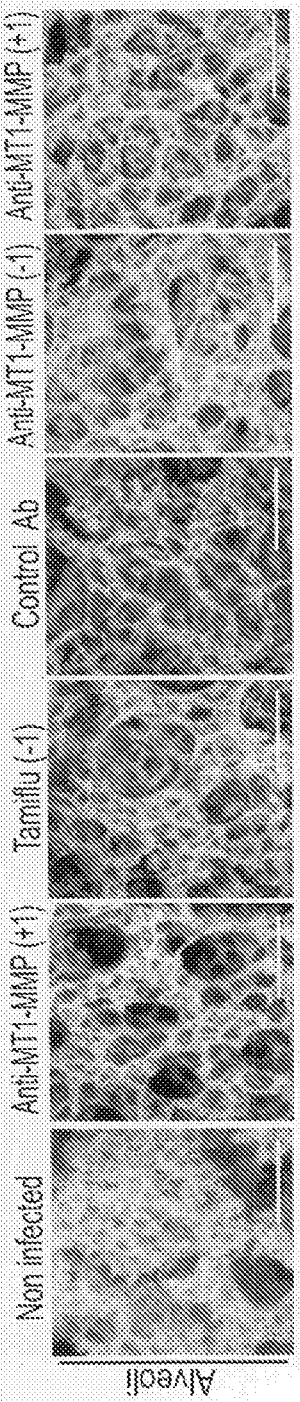


FIG. 14B

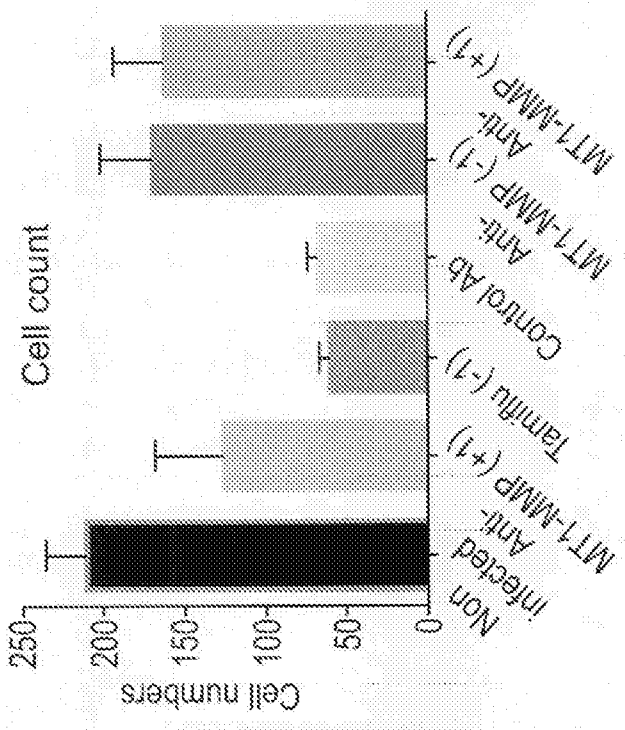
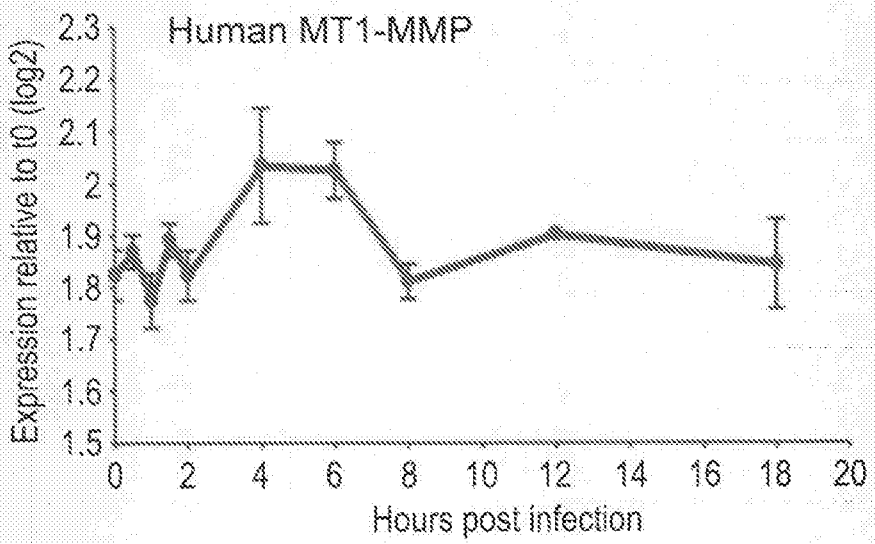
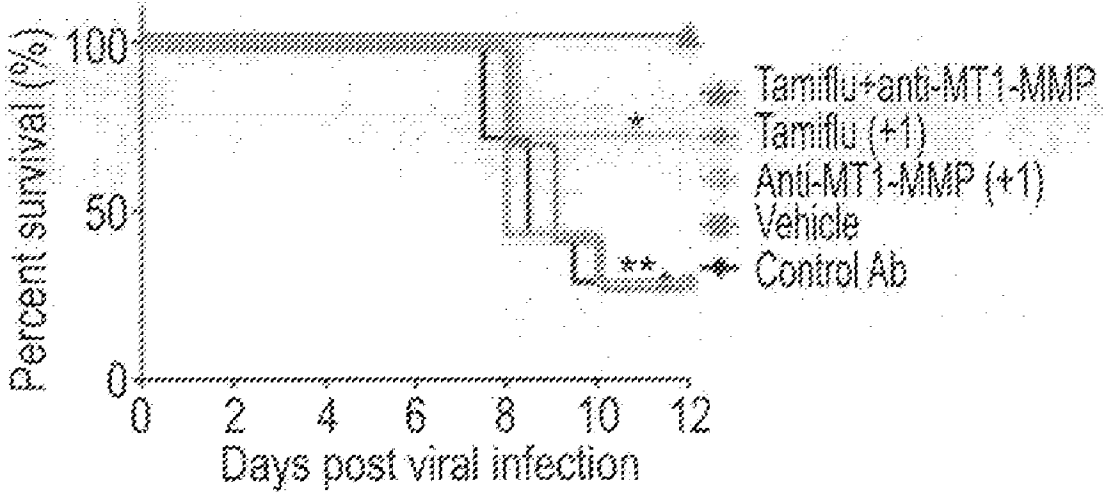


FIG. 15


Human MT1-MMP
Expression relative to t0 (log2)
2.3
2.2
2.1
2
1.9
1.8
1.7
1.6
1.5
0
2
4
6
8
10
12
14
16
18
20
Hours post infection


Percent survival (%)
100
50
0
0
2
4
6
8
10
12
Days post viral infection
*
**
Tamiflu+anti-MT1-MMP
Tamiflu (+1)
Anti-MT1-MMP (+1)
Vehicle
Control Ab