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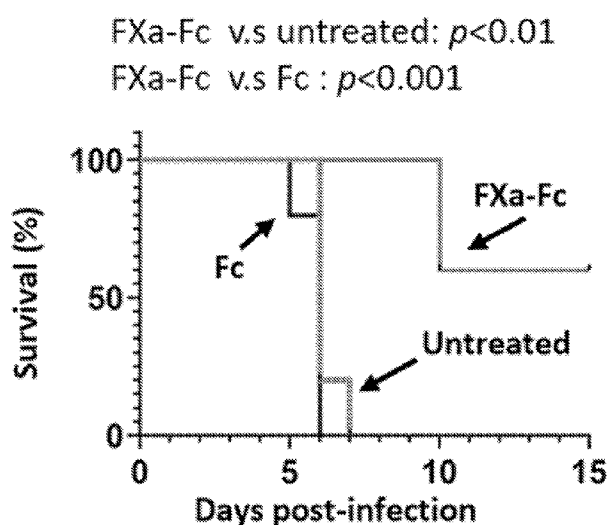
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(54) Title: FXA PROTEIN FOR TREATING AND/OR PREVENTING COVID-19

FIG. 4B



(57) Abstract: Provided herein are, inter alia, methods and compositions useful for treating or preventing COVID-19 in a subject in need thereof. The compositions include Factor Xa (FXa) protein or functional portion thereof. The compositions may further include an anticoagulant, particularly an indirect inhibitor of FXa.



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FXa PROTEIN FOR TREATING AND/OR PREVENTING COVID-19**CROSS-REFERENCES TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Application No. 63/186,042 filed May 7, 2021, the disclosure of which is incorporated by reference herein in its entirety.

BACKGROUND

[0002] Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pathogen responsible for the global coronavirus disease 2019 (COVID-19) pandemic (1). As of 2021, over 90,000,000 cases and approximately 2,000,000 deaths have been recorded with a worldwide mortality of 2% (3-6). The public health and economic consequences have been devastating (7).

[0003] Angiotensin-converting enzyme 2 (ACE2) has been identified as the receptor of SARS-CoV-2 (13, 14). SARS-CoV-2 uses its spike (S) protein to bind to ACE2 and enter host cells. Several host serine proteases (SPs) have been identified as facilitating SARS-CoV-2 entry via cleavage of its S protein into functional S1 and S2 subunits (16, 17). Furin and Transmembrane serine protease 2 (TMPRSS2) are requisite SPs that cut the S protein at the polybasic cleavage site (PRRAR) (R-R-A-R₆₈₅↓) site, cleaving S1 from S2 thereby enhancing the efficiency of SARS-CoV-2 infection (18). The SP coagulation factor Xa (FXa) binds to tissue factor (TF) to initiate conversion of prothrombin to thrombin in the clotting cascade (19). Direct FXa inhibitors rivaroxaban, apixaban, and edoxaban as well as the indirect inhibitor fondaparinux have been developed as clinical anti-coagulants (20), and at least one direct inhibitor (rivaroxaban) is currently be evaluated for use in patient at high-risk for COVID-19 (<https://clinicaltrials.gov/ct2/show/NCT04504032>).

BRIEF SUMMARY

[0004] Provided herein are, *inter alia*, compositions and methods for treating or preventing COVID-19. The methods include administering to a subject an effective amount of FXa protein or a functional portion thereof. The methods may further include administering an anticoagulant, particularly anticoagulants that are not direct FXa inhibitors. The compositions and methods are effective for treating or preventing COVID-19 in subjects who have thrombosis or who are at risk of thrombosis.

[0005] In an aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, wherein the method includes administering to the subject an effective amount of a Factor Xa (FXa) protein or functional portion thereof.

[0006] In an aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, including: i) obtaining a sample from the subject, ii) detecting a lower level of Factor Xa (FXa) in the sample relative to a standard control, and iii) administering to the subject an effective amount of an FXa protein or functional portion thereof.

[0007] In an aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, wherein the method includes: i) obtaining a sample from the subject, ii) detecting a higher level of FXa in the sample relative to a standard control, and iii) administering an effective amount of an anticoagulant.

[0008] In another aspect a pharmaceutical composition is provided including an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient.

[0009] In an aspect is provided a method a kit including a: (i) a first dosage form including an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient; and (ii) a second dosage form including an anticoagulant and a pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIGS. 1A-1J. Factor Xa (FXa) inhibits severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection by targeting viral particles. FIG. 1A and FIG. 1B: FXa protein levels in lungs (FIG. 1A) or serum (FIG. 1B) of coronavirus disease 2019 (COVID-19) patients vs. healthy donors, using an immunohistochemistry (IHC) (FIG. 1A) and enzyme-linked immunosorbent assay (ELISA) (FIG. 1B), respectively. FIG. 1C: FXa in serum of COVID-19 patients as shown post diagnosis of infection. FIG. 1D and FIG. 1E: Human embryonic kidney 293T (HEK 293T) cells co-transfected with Angiotensin-converting enzyme 2 (ACE2) and FXa or an extracellular vesicles (EV) in the absence (FIG. 1D) or presence (FIG. 1E) of Transmembrane serine protease 2 (TMPRSS2) were infected by Vesicular stomatitis virus-Severe acute respiratory syndrome coronavirus 2 (VSV-SARS-CoV-2), and quantified by flow at 16, 24, 36 and 48 hours post infection (hpi). FIG. 1F: MA104 cells transduced with FXa (MA104-FXa) or EV were infected by VSV-SARS-CoV-2 and imaged at 16, 24, 36 and 48 hpi by fluorescence microscopy. FIG. 1G and FIG. 1H:

VSV-SARS-CoV-2 was preincubated with FXa at different concentrations 1h before infection. Cells were imaged at 16, 24, 36 and 48 hpi by fluorescence microscopy (FIG. 1G) and corresponding infectivity was measured by flow (FIG. 1H). FIG. 1I: MA104-FXa and MA104-EV cells were infected with live SARS-CoV-2. At 24 hpi, infectivity was measured by plaque assay. FIG. 1J: Live SARS-CoV-2 was pretreated with or without FXa 1h before infection. At 24 hpi, infectivity was measured by plaque assay.

[0011] FIGS. 2A-2E. FXa suppresses viral entry by binding to and cleaving the SARS-CoV-2 S protein. FIG. 2A: The binding affinity of FXa with full-length S protein, subunit S1, subunit S2 and receptor binding domain (RBD) was quantified by ELISA. FIG. 2B and FIG. 2C: The interaction between FXa protein and full-length S protein was examined by pull-down assay (FIG. 2B) and the binding affinity at indicated concentrations of FXa was measured by ELISA (FIG. 2C). FIG. 2D: The cleavage of S protein by Furin, TMPRSS2, and FXa was analyzed by immunoblotting. FIG. 2E: Schema of the cleavage sites for Furin, TMPRSS2 and FXa on the full-length S protein or its RBD.

[0012] FIGS. 3A-3M. FXa cleavage blocks the binding between S protein and ACE2. FIG. 3A: The binding between ACE2 with S protein or FXa-pretreated S protein were measured by ELISA. FIG. 3B and FIG. 3C: The binding between S protein or FXa-pretreated S protein and ACE2 expressed on 293T cells was measured by flow cytometry (FIG. 3B and FIG. 3C). FIG. 3D: The binding FXa with S protein, S protein-ACE2 complex or Phosphate Buffered Saline (PBS) control, assessed by ELISA. FIG. 3E and FIG. 3F: The binding between S protein or S protein-ACE2 complex with FXa expressed on 293T cells. PBS serves as control for S protein and S protein-ACE2 complex. FIG. 3G: The effect of rivaroxaban or fondaparinux on the binding of FXa with S protein as measured by ELISA. FIG. 3H and FIG. 3I: The infectivity of FXa-pretreated vs. untreated VSV-SARS-CoV-2 in MA104 cells in the presence or absence of rivaroxaban or fondaparinux was examined with microscopy (FIG. 3H) and flow cytometry (FIG. 3I). FIG. 3J: S protein cleavage by FXa in the presence or absence of rivaroxaban or fondaparinux was examined by immunoblot. FIG. 3K and FIG. 3L: FXa pretreated with or without rivaroxaban or fondaparinux was incubated with S protein, followed by assessing binding capability of these S proteins with ACE2 coated on a plate (FIG. 3K) or expressed on 293T cells (FIG. 3L). FIG. 3M: The infectivity of FXa-pretreated vs. untreated live SARS-CoV-2 in MA104 cells in the presence or absence of rivaroxaban or fondaparinux was examined using immune-plaque assay.

[0013] FIGS. 4A-4F. The effect of FXa protein on live SARS-CoV-2 infection in a K18-hACE2 mouse model of COVID-19. FIG. 4A and FIG. 4B: Body weight (FIG. 4A) and survival (FIG. 4B) of mice infected with 3×10^5 Plaque-forming units (PFU) SARS-CoV-2 and treated with or without FXa-Fc fusion protein. Fc-protein was used as control. FIGS. 4C-4E: Viral load in the trachea (FIG. 4C), lung (FIG. 4D) and brain (FIG. 4E) of mice treated with or without FXa-Fc fusion protein or Fc control was assessed by Quantitative polymerase chain reaction (Q-PCR). FIG. 4F: Determination of the existence of live SARS-CoV-2 by IHC staining with an antibody against a viral nucleocapsid protein (NP) in the brain and lung, of mice treated with FXa-Fc or Fc-protein.

[0014] FIGS. 5A-5E. The effect of the direct FXa inhibitor rivaroxaban and the indirect inhibitor fondaparinux on FXa-mediated protection of K18-hACE2 mice from the live SARS-CoV-2 infection. FIG. 5A and FIG. 5B: Body weight (FIG. 5A) and survival (FIG. 5B) of mice infected with 3×10^5 (PFU) SARS-CoV-2 and treated with or without FXa-Fc in the presence or absence of rivaroxaban or fondaparinux. FIGS. 5C-5E: Viral load in the trachea (FIG. 5C), lung (FIG. 5D), and brain (FIG. 5E) of mice treated with or without FXa-Fc in the presence or absence of rivaroxaban or fondaparinux was assessed by Q-PCR.

[0015] FIGS. 6A-6B. Expression of serine proteases (SPs) in organs of autopsy samples of patients who died of COVID-19 vs. non-COVID-19 donors. FIG. 6A: Expression of furin, trypsin, and plasmin SP in the lung of autopsy samples of patients died of COVID-19 vs. non-COVID-19 donors. FIG. 6B: Expression of FXa SP in the liver of autopsy samples of patients died of COVID-19 vs. non-COVID-19 donors.

[0016] FIGS. 7A-7D. Infectivity and virus production of VSV-SARS-CoV-2 in MA104 cells expressing FXa or a control vector. FIG. 7A: Confirmation of forced over-expression of FXa in MA104 cells was conducted by immunoblotting. FIG. 7B: MA104 cells transduced with FXa (MA104-FXa) or an empty vector (MA104-EV) were infected by VSV-SARS-CoV-2. Infectivity of the cells were quantified by flow cytometry at 16, 24, 36 and 48 hpi. FIG. 7C and FIG. 7D: The titer of the supernatant of VSV-SARS-CoV-2-infected MA104 or -MA104-FXa cells at 24 hpi and 48 hpi was determined by re-infection of Vero cells.

[0017] FIGS. 8A-8C. FXa inhibits while TMPRSS2, trypsin, and furin promote VSV-SARS-CoV-2 infection in MA104 cells. FIG. 8A and FIG. 8B: Infectivity of MA104 cells infected with VSV-SARS-CoV-2 in the presence or absence of FXa, TMPRSS2, trypsin, or furin was determined by fluorescence microscope. FIG. 8C: Virus production MA104 cells

infected with VSV-SARS-CoV-2 in the presence or absence of FXa, TMPRSS2, trypsin, or furin was determined by re-infection of Vero cells.

[0018] FIGS. 9A-9C. Determination of the effect of FXa on virus particles and host cells. FIG. 9A: VSV-SARS-CoV-2 was preincubated with FXa at different concentration 1 hour prior to viral infection of MA104 cells. The supernatants were collected at 24 and 48 hpi for virus production assay by re-infection of Vero cells. FIG. 9B and FIG. 9C: MA104 cells were preincubated with or without FXa at different concentration 1 hour before infection. Infectivity of VSV-SARS-CoV-2 in the preincubated or untreated MA104 with FXa was determined by fluorescence microscope (FIG. 9B) and flow cytometry (FIG. 9C).

[0019] FIG. 10. Cleavage of S protein in the S protein-ACE2 complex by FXa. S protein was pre-incubated with ACE2 for 1 hour, followed by addition of FXa for another 1 hr. The cleavage of S protein in the S protein-ACE2 complex was determined by immunoblot.

[0020] FIGS. 11A-11E. Binding of FXa with and cleavage of the mutant S protein of the SARS-CoV-2 D614G variant. FIG. 11A: The binding affinity of FXa and D614G S protein was measured by ELISA. FIG. 11B: The binding of the mutant S protein of the SARS-CoV-2 D614G variant with FXa expressed on 293T cells was assessed by flow cytometry. FIG. 11C: Cleavage assay of D614G S protein by FXa was measured by immunoblotting. FIG. 11D and FIG. 11E: The binding affinity of ACE2 and D614G S protein pretreated with or without FXa was measured by flow cytometry.

[0021] FIGS. 12A-12C. The effect of rivaroxaban or fondaparinux on infectivity and virus production of VSV-SARS-CoV-2 or live SARS-CoV-2 pretreated with FXa. FIG. 12A: Virus production of FXa-pretreated vs. untreated VSV-SARS-CoV-2 in MA104 cells in the presence or absence of rivaroxaban or fondaparinux was determined by re-infection of Vero cells. FIG. 12B: FXa pretreated with or without rivaroxaban or fondaparinux was incubated with S protein, followed by assessing binding capability of these S proteins binding with ACE2 expressed on 293T cells by flow cytometry (summary data of main Fig. 3L). FIG. 12C: The infectivity of FXa-pretreated vs. untreated live SARS-CoV-2 in MA104 cells in the presence or absence of rivaroxaban or fondaparinux were examined using immune-plaque assay.

[0022] FIGS. 13A-13D. FXa inhibits wild-type SARS-CoV-2 infection by targeting viral particles. FIG. 13A: MA104 cells transduced with the plasmid encoding FXa (MA104-

FXa) or an empty vector (MA104-EV) were infected with VSV-SARS-CoV-2. Infectivity of the cells was quantified by flow cytometry at 16, 24, 36, and 48 hpi. FIG. 13B: MA104 and Vero E6 cells were infected with live wild-type SARS-CoV-2. At 24 hpi, infectivity was measured with an immune-plaque assay FIG. 13C: Summary of data from FIG. 13B. FIG. 13D: A549-ACE2 cells were either preincubated with 100 nM FXa for 1 hour and then infected with live WT SARS-CoV-2 or treated with FXa at the time of viral infection. Infectivity was measured by immune-plaque assays 24 hours post infection. Representative infection and the summary data are presented at the left and right, respectively. MFI data were log₂ transformed before running the statistical models. * $P \leq 0.05$; *** $P \leq 0.001$; **** $P \leq 0.0001$; n.s., not significant.

[0023] FIGS. 14A-14C. FXa suppresses viral entry by binding to and cleaving the SARS-CoV-2 S protein. FIG. 14A: The binding affinity of FXa to VSV-SARS-CoV-2 viral particles was quantified by ELISA. FIG. 14B: S protein was cleaved by FXa, followed by immunoblotting with an anti-RBD antibody (left) and an anti-S2 antibody (right). FIG. 14C: The cleavage of VSV-SARS-CoV-2 by FXa or furin was analyzed by immunoblotting.

[0024] FIGS. 15A-15B. FXa cleavage reduces the binding between S protein and ACE2. FIG. 15A: The binding between S protein or FXa-pretreated S protein and ACE2 expressed on A549 human lung cancer cells was measured by flow cytometry (left, representative flow cytometry histogram; right, summary data). FIG. 15B: The binding of membrane-bound (mb) FXa with S protein or the binding of mb FXa with S protein-ACE2 complex on 293T cells was measured by flow cytometry (left, representative flow cytometry histogram; right, summary data). PBS served as control for S protein and the S protein-ACE2 complex.

[0025] FIGS. 16A-16B. Effect of the direct FXa inhibitor RIVA and the indirect inhibitor FONDA on FXa-mediated protection of K18-hACE2 mice from WT SARS-CoV-2 infection. FIG. 16A: Body weight and FIG. 16B: survival of mice infected with 5×10^3 PFU of SARS-CoV-2 (WA1) and treated with or without FXa-Fc in the presence or absence of RIVA or FONDA.

[0026] FIGS. 17A-17F. FXa is less effective in blocking infection of the SARS-CoV-2 B.1.1.7 variant *in vitro* and *in vivo*. FIG. 17A: A549-ACE2 cells were preincubated or not preincubated with 100 nM FXa for 1 h, and then infected with either live SARS-CoV-2 WA1 or the SARS-CoV-2 B.1.1.7 variant. Infectivity was measured with an immuno-plaque assay

24 hours post infection and the infection inhibition ratio induced by FXa was summarized (right panel). FIG. 17B: Vero E6 cells were pre-treated with FXa and then infected with live SARS-CoV-2 WA1 or the SARS-CoV-2 B.1.1.7 variant at various MOIs. At 24 hpi, infectivity was measured with an immuno-plaque assay (left panel), and the infection inhibition ratio induced by FXa at different MOIs was summarized (right panel). FIG. 17C: Body weight and FIG. 17D: survival of mice infected with 5×10^3 PFU wild-type SARS-CoV-2 or B.1.1.7 variant and treated with or without FXa-Fc fusion protein. Viral load in the FIG. 17E: lung and FIG. 17F: brain of mice treated with or without FXa-Fc fusion protein was assessed by qPCR. All the mice were sacrificed at day 5 post infection. For all panels, data were presented as mean values $\pm$ SD and statistical analyses were performed by two-way ANOVA models and one-way ANOVA models and log-rank test. Copy number was $\log_2$ transformed before running statistical models. $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$; $****P \leq 0.0001$; n.s., not significant.

[0027] FIG. 18. Expression of serine proteases in autopsied organs of COVID-19 patients. FXa levels ($\mu\text{g/ml}$) in plasma as measured by ELISA and spike expression in patients' nasopharyngeal swabs as measured by qPCR with normalization at different time points post diagnosis of COVID-19.

[0028] FIGS. 19A-19C. FXa inhibits VSV-SARS-CoV-2 infection in MA104 cells while TMPRSS2, trypsin, and furin promote infection. FIG. 19A: VSV-SARS-CoV-2 was preincubated with furin, TMPRSS2, trypsin, or FXa at the indicated concentrations for 1 hour before it was used to infect M104 cells. Infectivity was quantified by flow cytometry at 48 hpi. FIG. 19B: Virus titration by MA104 cells infected with VSV-SARS-CoV-2 in the presence or absence of FXa, TMPRSS2, trypsin, or furin was determined by subsequently infecting Vero cells. FIG. 19C: VSV-SARS-CoV-2 was preincubated with MA104 cells for 1 hour, washed twice, and then exposed to treatment with FXa. Infectivity of the cells was quantified by flow cytometry at 24 hpi. All data are representative of three independent experiments.

[0029] FIGS. 20A-20D. FXa recombinant protein, its conversion from plasma, and TF-FVIIa-FXa complex inhibit VSV-SARS-CoV-2 infection in MA104 cells. FIG. 20A: VSV-SARS-CoV-2 was preincubated with different concentrations of FXa for 1 hour before infecting M104 cells. Cells were imaged at 16, 24, 36, and 48 hpi by fluorescence microscopy (top panel), and the corresponding infectivity was measured by flow cytometry (bottom panel). FIG. 20B: MA104 cells were infected with VSV-SARS-CoV-2 that had been preincubated with different concentrations of FXa for 1 hour. Supernatants collected at 24 and 48 hpi were used to infect

Vero cells for a virus titration assay. FIG. 20C: VSV-SARS-CoV-2 was preincubated with plasma in which FX was converted or unconverted to FXa by incubating with FIXa and Factor V Activating Enzyme from Russell's viper venom. Infectivity was measured at 24 hpi by fluorescence microscopy. FIG. 20D: VSV-SARS-CoV-2 was preincubated with PBS, TF-FVIIa-FXa complex, FXa, FVIIa, or TF, followed by infecting M104 cells. Corresponding infectivity was measured by flow cytometry.

[0030] FIGS. 21A-21F. FXa does not act on host cells and the shedding S protein by FXa.. FIG. 21A: M104 cells were incubated with or without FXa for 1 hr, washed, and then infected with VSV-SARS-CoV-2 for 24 hr. Viral infectivity was measured by flow cytometry, measuring the expression level of GFP from virally infected cells. The binding affinity of active FXa-Fc, inactive FXa-Fc, and Fc to full-length S protein FIG. 21B: or VSV-SARS-CoV-2 chimeric viral particles FIG. 21C: was quantified by ELISA. FIG. 21D: Shedding S protein by FXa. GFP and S protein surface expression on A549 cells, referred to as A549-S cells, treated with or without FXa were detected by flow cytometry. S protein from the supernatants of FXa-treated A549-S cells was measured by FIG. 21E: ELISA and FIG. 21F: immunoblotting assay. Data were presented as mean values $\pm$ SD and statistical analyses were performed by one-way ANOVA. MFI data were log₂ transformed before running statistical models. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; n.s., not significant.

[0031] FIGS. 22A-22D. The effect of low-dose and endogenous of FXa on *in vivo* live SARAS-CoV-2 infection. FIG. 22A: The survival of K18-hACE2 mice infected intranasally with 5×10^3 PFU SARS-CoV-2 WA1 strain treated intranasally with or without 2 μ g FXa-Fc fusion protein (n=6 mice/group). Expression of FXa in lungs of FXa^{+/+}-K18-hACE2 mice and K18-hACE2 control mice were measured by FIG. 22B: q-PCR and FIG. 22C: immunoblotting assay. FIG. 22D: Viral copy numbers in lungs of FXa^{+/+}-K18-hACE2 mice and K18-hACE2 mice were assessed by q-PCR. Data were presented as mean values $\pm$ SD and statistical analyses were performed by Student's *t* test. * $P \leq 0.05$.

[0032] FIG. 23. IHC staining with an antibody against viral nuclear protein (NP) to detect SARS-CoV-2 in lungs of mice treated with FXa, FXa+RIVA, or FXa+FONDA. PBS served as the control. Pathological analysis of the lung of these mice as performed by H&E staining. All the mice were sacrificed at day 5 post infection to collect lung tissues for H&E staining and IHC with an anti-NP antibody.

[0033] FIGS. 24A-24B. Effect of various doses of RIVA or FONDA on infectivity of live WA1 SARS-CoV-2 or the B.1.1.7 variant pretreated with FXa in Vero E6 cells. FIG. 24A:

Vero E6 cells were infected with live WA1 SARS-CoV-2 or the B.1.1.7 variant that had been pretreated for 1 hr with different doses of RIVA or FONDA in the presence of FXa. At 24 hpi, viral infectivity was measured by immuno-plaque assay. A representative assay is shown on the left; summary data are on the right. FIG. 24B: Vero E6 cells were infected with live WA1 SARS-CoV-2 or the B.1.1.7 variant pretreated with different doses of FXa in the presence of RIVA or FONDA. At 24 hpi, viral infectivity was measured by immuno-plaque assay (upper); summary data (lower). Data were presented as mean values $\pm$ SD and statistical analyses were performed with two-way ANOVA. *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; n.s., not significant.

[0034] FIGS. 25A-25B. Effect of various doses of RIVA or FONDA on infectivity of live WA1 SARS-CoV-2 or the B.1.1.7 variant pretreated with FXa in MA104 cells. FIG. 25A: MA104 cells were infected with live WA1 SARS-CoV-2 or the B.1.1.7 variant that had been pretreated for 1 hr with different doses of RIVA or FONDA in the presence of FXa. At 24 hpi, viral infectivity was measured with an immuno-plaque assay. Left: a representative assay; right: summary data. FIG. 25B: MA104 cells were infected with live WA1 SARS-CoV-2 or the B.1.1.7 variant. Both strains had been pretreated with different doses of FXa for 1 hr in the presence of RIVA or FONDA. At 24 hpi, viral infectivity was measured by immuno-plaque assay (upper). Summary data are presented in the lower panel. Data were presented as mean values $\pm$ SD and statistical analyses were performed with two-way ANOVA. *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; n.s., not significant.

[0035] FIGS. 26A-26H. Binding and cleavage of the mutant S protein by FXa. FIG. 26A: Binding of FXa with WT WA1 S protein or B.1.1.7 variant S protein was assessed by ELISA. FIG. 26B: Binding of WT WA1 S protein or the B.1.1.7 S protein with FXa expressed on 293T cells, as assessed by flow cytometry. PBS was the control (representative flow cytometry histograms on the left; summary data on the right). FIG. 26C: Binding affinity of FXa and the D614G S protein was measured by ELISA. FIG. 26D: Binding of the mutant S protein of the SARS-CoV-2 D614G variant with FXa expressed on 293T cells was assessed by flow cytometry. FIG. 26E: Cleavage of the D614G S protein by FXa was assayed by immunoblotting. Binding affinity of FIG. 26F: ACE2 and the FIG. 26G: D614G S protein pretreated or not treated with FXa was measured by flow cytometry. FIG. 26H: Cleavage of WT WA1 S protein or Omicron S protein by FXa after 1-hour incubation was analyzed by immunoblotting. All data are representative of at least three independent experiments. Data were presented as mean values $\pm$ SD and statistical analyses were performed Student's *t* test and one-way ANOVA. **** $P \leq 0.0001$; n.s., not significant.

[0036] **FIG. 27.** Characteristics of individual participants in the studies described herein.

DETAILED DESCRIPTION

[0037] There is a need for treatments for the global pandemic caused by novel coronavirus SARS-CoV-2 resulting in COVID-19. Provided herein, *inter alia*, are compositions including FXa or a functional portion thereof or a nucleic acid encoding the same. The compositions may further include anticoagulants, particularly anticoagulants that do not directly inhibit serine protease activity. The compositions provided herein are useful for treating COVID-19 in a subject in need thereof. The compositions are further contemplated to be useful for downregulating blood coagulation and/or inflammation in the subject.

[0038] While various embodiments and aspects of the present invention are shown and described herein, it will be obvious to those skilled in the art that such embodiments and aspects are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

[0039] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, without limitation, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0040] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

[0041] The use of a singular indefinite or definite article (e.g., “a,” “an,” “the,” etc.) in this disclosure and in the following claims follows the traditional approach in patents of meaning “at least one” unless in a particular instance it is clear from context that the term is intended

in that particular instance to mean specifically one and only one. Likewise, the term “comprising” is open ended, not excluding additional items, features, components, etc. References identified herein are expressly incorporated herein by reference in their entireties unless otherwise indicated.

[0042] The terms “comprise,” “include,” and “have,” and the derivatives thereof, are used herein interchangeably as comprehensive, open-ended terms. For example, use of “comprising,” “including,” or “having” means that whatever element is comprised, had, or included, is not the only element encompassed by the subject of the clause that contains the verb.

[0043] “Nucleic acid” refers to nucleotides (e.g., deoxyribonucleotides or ribonucleotides) and polymers thereof in either single-, double- or multiple-stranded form, or complements thereof, or nucleosides (e.g., deoxyribonucleosides or ribonucleosides). In embodiments, “nucleic acid” does not include nucleosides. The terms “polynucleotide,” “oligonucleotide,” “oligo” or the like refer, in the usual and customary sense, to a linear sequence of nucleotides. The term “nucleoside” refers, in the usual and customary sense, to a glycosylamine including a nucleobase and a five-carbon sugar (ribose or deoxyribose). Non-limiting examples, of nucleosides include, cytidine, uridine, adenosine, guanosine, thymidine and inosine. The term “nucleotide” refers, in the usual and customary sense, to a single unit of a polynucleotide, i.e., a monomer. Nucleotides can be ribonucleotides, deoxyribonucleotides, or modified versions thereof. Examples of polynucleotides contemplated herein include single and double stranded DNA, single and double stranded RNA, and hybrid molecules having mixtures of single and double stranded DNA and RNA. Examples of nucleic acid, e.g. polynucleotides contemplated herein include any types of RNA, e.g. mRNA, siRNA, miRNA, and guide RNA and any types of DNA, genomic DNA, plasmid DNA, and minicircle DNA, and any fragments thereof. The term “duplex” in the context of polynucleotides refers, in the usual and customary sense, to double strandedness. Nucleic acids can be linear or branched. For example, nucleic acids can be a linear chain of nucleotides or the nucleic acids can be branched, e.g., such that the nucleic acids comprise one or more arms or branches of nucleotides. Optionally, the branched nucleic acids are repetitively branched to form higher ordered structures such as dendrimers and the like.

[0044] As may be used herein, the terms “nucleic acid,” “nucleic acid molecule,” “nucleic acid oligomer,” “oligonucleotide,” “nucleic acid sequence,” “nucleic acid fragment” and

“polynucleotide” are used interchangeably and are intended to include, but are not limited to, a polymeric form of nucleotides covalently linked together that may have various lengths, either deoxyribonucleotides or ribonucleotides, or analogs, derivatives or modifications thereof. Different polynucleotides may have different three-dimensional structures, and may perform various functions, known or unknown. Non-limiting examples of polynucleotides include a gene, a gene fragment, an exon, an intron, intergenic DNA (including, without limitation, heterochromatic DNA), messenger RNA (mRNA), transfer RNA, ribosomal RNA, a ribozyme, cDNA, a recombinant polynucleotide, a branched polynucleotide, a plasmid, a vector, isolated DNA of a sequence, isolated RNA of a sequence, a nucleic acid probe, and a primer. For example, the nucleic acid provided herein may be part of a vector. For example, the nucleic acid provided herein may be part of an adenoviral vector, which may be transduced into a cell. Polynucleotides useful in the methods of the disclosure may comprise natural nucleic acid sequences and variants thereof, artificial nucleic acid sequences, or a combination of such sequences.

[0045] Nucleic acids can include nonspecific sequences. As used herein, the term "nonspecific sequence" refers to a nucleic acid sequence that contains a series of residues that are not designed to be complementary to or are only partially complementary to any other nucleic acid sequence. By way of example, a nonspecific nucleic acid sequence is a sequence of nucleic acid residues that does not function as an inhibitory nucleic acid when contacted with a cell or organism.

[0046] A polynucleotide is typically composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); and thymine (T) (uracil (U) for thymine (T) when the polynucleotide is RNA). Thus, the term “polynucleotide sequence” is the alphabetical representation of a polynucleotide molecule; alternatively, the term may be applied to the polynucleotide molecule itself. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching. Polynucleotides may optionally include one or more non-standard nucleotide(s), nucleotide analog(s) and/or modified nucleotides.

[0047] The term “amino acid” refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the

genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. The terms “non-naturally occurring amino acid” and “unnatural amino acid” refer to amino acid analogs, synthetic amino acids, and amino acid mimetics which are not found in nature.

[0048] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0049] The terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues, wherein the polymer may In embodiments be conjugated to a moiety that does not consist of amino acids. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. A “fusion protein” refers to a chimeric protein encoding two or more separate protein sequences that are recombinantly expressed as a single moiety.

[0050] An amino acid or nucleotide base “position” is denoted by a number that sequentially identifies each amino acid (or nucleotide base) in the reference sequence based on its position relative to the N-terminus (or 5'-end). Due to deletions, insertions, truncations, fusions, and the like that must be taken into account when determining an optimal alignment, in general the amino acid residue number in a test sequence determined by simply counting from the N-terminus will not necessarily be the same as the number of its corresponding position in the reference sequence. For example, in a case where a variant has a deletion relative to an aligned reference sequence, there will be no amino acid in the variant that corresponds to a position in the reference sequence at the site of deletion. Where there is an

insertion in an aligned reference sequence, that insertion will not correspond to a numbered amino acid position in the reference sequence. In the case of truncations or fusions there can be stretches of amino acids in either the reference or aligned sequence that do not correspond to any amino acid in the corresponding sequence.

[0051] The terms "numbered with reference to" or "corresponding to," when used in the context of the numbering of a given amino acid or polynucleotide sequence, refers to the numbering of the residues of a specified reference sequence when the given amino acid or polynucleotide sequence is compared to the reference sequence. An amino acid residue in a protein "corresponds" to a given residue when it occupies the same essential structural position within the protein as the given residue. One skilled in the art will immediately recognize the identity and location of residues corresponding to a specific position in a protein (*e.g.*, spike protein) in other proteins with different numbering systems. For example, by performing a simple sequence alignment with a protein (*e.g.*, spike protein) the identity and location of residues corresponding to specific positions of the protein are identified in other protein sequences aligning to the protein. For example, a selected residue in a selected protein corresponds to glutamic acid at position 138 when the selected residue occupies the same essential spatial or other structural relationship as a glutamic acid at position 138. In some embodiments, where a selected protein is aligned for maximum homology with a protein, the position in the aligned selected protein aligning with glutamic acid 138 is the to correspond to glutamic acid 138. Instead of a primary sequence alignment, a three-dimensional structural alignment can also be used, *e.g.*, where the structure of the selected protein is aligned for maximum correspondence with the glutamic acid at position 138, and the overall structures compared. In this case, an amino acid that occupies the same essential position as glutamic acid 138 in the structural model is the to correspond to the glutamic acid 138 residue.

[0052] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids that encode identical or essentially identical amino acid sequences. Because of the degeneracy of the genetic code, a number of nucleic acid sequences will encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which

are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0053] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the disclosure.

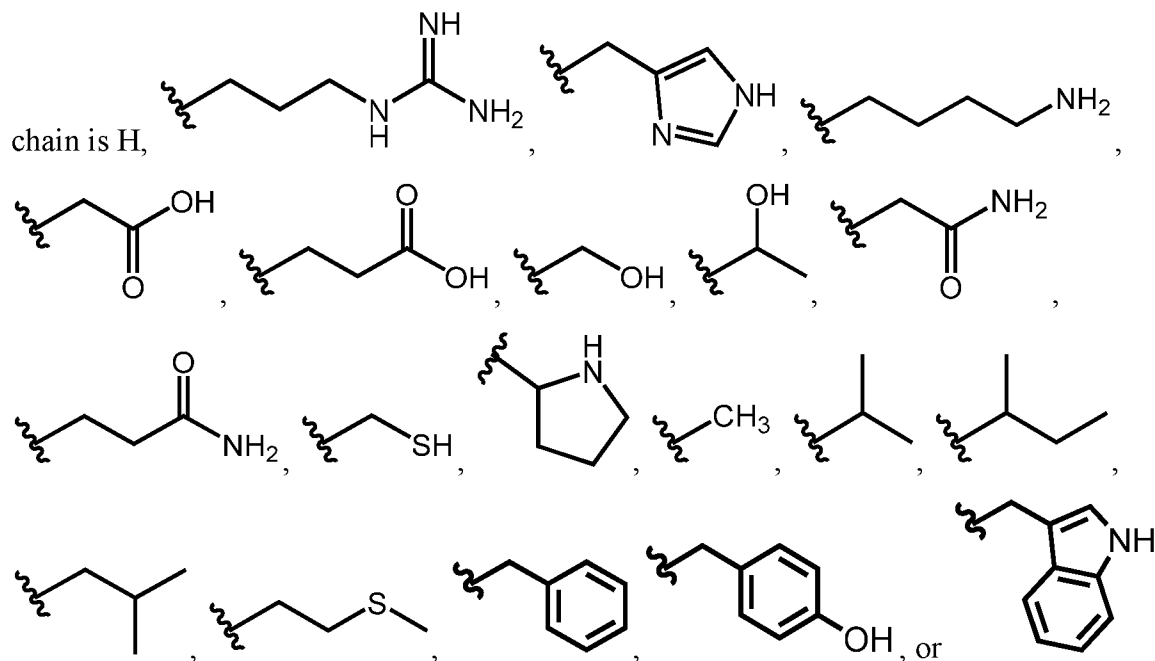
[0054] The following eight groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Glycine (G);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
- 7) Serine (S), Threonine (T); and
- 8) Cysteine (C), Methionine (M)

(see, e.g., Creighton, *Proteins* (1984)).

[0055] The term “amino acid side chain” refers to the functional substituent contained on amino acids. For example, an amino acid side chain may be the side chain of a naturally occurring amino acid. Naturally occurring amino acids are those encoded by the genetic code

(e.g., alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine), as well as those amino acids that are later modified, e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. In embodiments, the amino acid side chain may be a non-natural amino acid side chain. In embodiments, the amino acid side



[0056] The term “non-natural amino acid side chain” refers to the functional substituent of compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium, allylalanine, 2-aminoisobutyric acid. Non-natural amino acids are non-proteinogenic amino acids that either occur naturally or are chemically synthesized. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Non-limiting examples include *exo-cis*-3-Aminobicyclo[2.2.1]hept-5-ene-2-carboxylic acid hydrochloride, *cis*-2-Aminocycloheptanecarboxylic acid hydrochloride, *cis*-6-Amino-3-cyclohexene-1-carboxylic acid hydrochloride, *cis*-2-Amino-2-methylcyclohexanecarboxylic acid hydrochloride, *cis*-2-Amino-2-methylcyclopentanecarboxylic acid hydrochloride, 2-(Boc-aminomethyl)benzoic acid, 2-(Boc-amino)octanedioic acid, Boc-4,5-dehydro-Leu-OH (dicyclohexylammonium), Boc-4-(Fmoc-amino)-L-phenylalanine, Boc- β -Homopyr-OH, Boc-(2-indanyl)-Gly-OH, 4-Boc-3-morpholineacetic acid, 4-Boc-3-morpholineacetic acid, Boc-pentafluoro-D-phenylalanine, Boc-pentafluoro-L-phenylalanine, Boc-Phe(2-Br)-OH, Boc-Phe(4-Br)-OH,

Boc-D-Phe(4-Br)-OH, Boc-D-Phe(3-Cl)-OH, Boc-Phe(4-NH₂)-OH, Boc-Phe(3-NO₂)-OH, Boc-Phe(3,5-F₂)-OH, 2-(4-Boc-piperazino)-2-(3,4-dimethoxyphenyl)acetic acid purum, 2-(4-Boc-piperazino)-2-(2-fluorophenyl)acetic acid purum, 2-(4-Boc-piperazino)-2-(3-fluorophenyl)acetic acid purum, 2-(4-Boc-piperazino)-2-(4-fluorophenyl)acetic acid purum, 2-(4-Boc-piperazino)-2-(4-methoxyphenyl)acetic acid purum, 2-(4-Boc-piperazino)-2-phenylacetic acid purum, 2-(4-Boc-piperazino)-2-(3-pyridyl)acetic acid purum, 2-(4-Boc-piperazino)-2-[4-(trifluoromethyl)phenyl]acetic acid purum, Boc-β-(2-quinolyl)-Ala-OH, N-Boc-1,2,3,6-tetrahydro-2-pyridinecarboxylic acid, Boc-β-(4-thiazolyl)-Ala-OH, Boc-β-(2-thienyl)-D-Ala-OH, Fmoc-N-(4-Boc-aminobutyl)-Gly-OH, Fmoc-N-(2-Boc-aminoethyl)-Gly-OH, Fmoc-N-(2,4-dimethoxybenzyl)-Gly-OH, Fmoc-(2-indanyl)-Gly-OH, Fmoc-pentafluoro-L-phenylalanine, Fmoc-Pen(Trt)-OH, Fmoc-Phe(2-Br)-OH, Fmoc-Phe(4-Br)-OH, Fmoc-Phe(3,5-F₂)-OH, Fmoc-β-(4-thiazolyl)-Ala-OH, Fmoc-β-(2-thienyl)-Ala-OH, 4-(Hydroxymethyl)-D-phenylalanine.

[0057] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region, when compared and aligned for maximum correspondence over a comparison window or designated region) as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*see, e.g.*, NCBI web site <http://www.ncbi.nlm.nih.gov/BLAST/> or the like). Such sequences are then said to be “substantially identical.” This definition also refers to, or may be applied to, the complement of a test sequence. The definition also includes sequences that have deletions and/or additions, as well as those that have substitutions. As described below, the preferred algorithms can account for gaps and the like. Preferably, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0058] “Percentage of sequence identity” is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the

number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[0059] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of, e.g., a full length sequence or from 20 to 600, about 50 to about 200, or about 100 to about 150 amino acids or nucleotides in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman (1970) *Adv. Appl. Math.* 2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Ausubel *et al.*, *Current Protocols in Molecular Biology* (1995 supplement)).

[0060] An example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) *Nuc. Acids Res.* 25:3389-3402, and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the

cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0061] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0062] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0063] The term “ACE2”, “ACE-2”, “ACE2 receptor”, or “ACE-2 receptor” refer to angiotensin-converting enzyme 2, which is an enzyme attached to the membranes of cells in the lungs, arteries, heart, kidney, and intestines. ACE2 serves as the entry point into cells for some coronaviruses, including HCoV-NL63, SARS-CoV, and SARS-CoV-2. The human

version of the enzyme is often referred to as hACE2. In embodiments, ACE2 is encoded by the ACE2 gene. In embodiments, ACE2 includes any of the recombinant or naturally-occurring forms of ACE2 protein, or variants or homologs thereof that maintain ACE2 protein activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to ACE2 protein). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring ACE2 protein. In embodiments, the ACE2 protein is substantially identical to the protein identified by the Entrez Accession No. 59272 or a variant or homolog having substantial identity thereto. In embodiments, the ACE2 protein is substantially identical to the protein identified by the UniProt reference number Q9BYF1 or a variant or homolog having substantial identity thereto. In embodiments, the ACE2 protein is substantially identical to the protein identified by the Accession No. NP_068576 or a variant or homolog having substantial identity thereto. In embodiments, the ACE2 protein is substantially identical to the protein identified by the Accession No. NP_001358344 or a variant or homolog having substantial identity thereto. In embodiments, ACE2 has the sequence of SEQ ID NO:1. In embodiments, ACE2 has the sequence of SEQ ID NO:2.

[0064] The term “SARS-CoV-2 spike protein”, “S protein” or “spike protein” refers to the protein that is responsible for allowing the virus to attach to and fuse with the membrane of a host cell. Specifically, the SARS-CoV-2 spike (S) protein binds angiotensin converting enzyme 2 (ACE2) as an entry receptor. The S1 subunit of the spike protein catalyzes attachment, and the S2 subunit of the spike protein catalyzes fusion. The term includes any of the recombinant or naturally-occurring forms of spike protein, or variants or homologs thereof that maintain spike protein activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to spike protein). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring spike protein. In embodiments, the spike protein is substantially identical to the protein identified by the UniProt reference number P0DTC2 or a variant or homolog having substantial identity thereto. In embodiments, the spike protein has the amino acid sequence of the B.1.1.7 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.2 variant,

BA.3 variant, BA.4 variant, or BA.5 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.2 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.2.12 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.2.12.1 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.3 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.4 variant or BA.5 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.4 variant. In embodiments, the spike protein has the amino acid sequence of the Omicron BA.5 variant.

[0065] The term “factor Xa,” “FXa,” or “FXa protein” refers to a serine protease in the blood coagulation pathway. Factor X (fX) is cleaved to produce the active form of the protein, factor Xa. FXa includes two peptide chains linked by a disulfide bridge. In embodiments, the wild type FXa includes a heavy chain and a light chain. Factor Xa typically cleaves a substrate after the arginine residue upon recognition of the preferred cleavage sequence Ile-(Glu or Asp)-Gly-Arg. Factor Xa may cleave the peptide bond at other basic residues. In embodiments, Factor Xa cleaves the peptide bond at the C-terminal end of the arginine residue of Ile-(Glu or Asp)-Gly-Arg-X, where X is any amino acid other than proline or arginine.

[0066] FXa converts prothrombin to thrombin in the coagulation pathway. Factor Xa may be activated by factor IXa and its cofactor (factor VIIIa) in a complex known as intrinsic Xase, or by factor VIIa with its cofactor (tissue factor) in a complex known as extrinsic Xase. Factor FXa typically forms a membrane-bound prothrombinase complex with factor Va, and is the active component in the prothrombinase complex that catalyzes the conversion of prothrombin to thrombin. Factor Xa is a two chain molecule linked by one disulfide bond between the two chains. The heavy chain contains the serine protease, trypsin-like active site and the N-terminal activation peptide, which is typically glycosylated. Thrombin is the enzyme that catalyzes the conversion of fibrinogen to fibrin, which ultimately leads to blood clot formation. Thus, the biological activity of FXa may be referred to as “procoagulant activity.” The nucleic acid sequence coding human factor X (“fX”) can be found in GenBank, “NM_000504” (<https://www.ncbi.nlm.nih.gov/nuccore/89142731>).

[0067] FXa as used herein includes any of the recombinant or naturally-occurring forms of coagulation factor Xa protein (FXa), or variants or homologs thereof that maintain FXa activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity

compared to FXa). In aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring FXa protein.

[0068] In embodiments, the FXa protein includes a sequence having at least 80% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 85% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 90% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 95% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes the sequence of SEQ ID NO:6. In embodiments, the FXa protein is the sequence of SEQ ID NO:6.

[0069] In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 80% sequence identity to SEQ ID NO:4 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide having at least 85% sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 85% sequence identity to SEQ ID NO:4 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide having at least 90% sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 90% sequence identity to SEQ ID NO:4 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide having at least 95% sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 95% sequence identity to SEQ ID NO:4 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide including the sequence of SEQ ID NO:3 (e.g. light chain) and a second peptide including the sequence of SEQ ID NO:4 (e.g. heavy chain). In embodiments, the first peptide and the second peptide are covalently attached. In embodiments, the first peptide and the second peptide are attached by disulfide bonds.

[0070] In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 80% sequence identity to SEQ ID NO:5 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide having at least 85% sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 85% sequence identity to SEQ ID NO:5 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide having at least 90%

sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 90% sequence identity to SEQ ID NO:5 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide having at least 95% sequence identity to SEQ ID NO:3 (e.g. light chain) and a second peptide having at least 95% sequence identity to SEQ ID NO:5 (e.g. heavy chain). In embodiments, the FXa protein includes a first peptide including the sequence of SEQ ID NO:3 (e.g. light chain) and a second peptide including the sequence of SEQ ID NO:5 (e.g. heavy chain). In embodiments, the first peptide and the second peptide are covalently attached. In embodiments, the first peptide and the second peptide are attached by disulfide bonds.

[0071] The term "Factor X protein" or "Factor X" as used herein includes any of the recombinant or naturally-occurring forms of Coagulation factor X protein, also known as Stuart factor, or variants or homologs thereof that maintain Factor X activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to Factor X). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring Factor X protein. In embodiments, the Factor X protein is substantially identical to the protein identified by the UniProt reference number P00742 or a variant or homolog having substantial identity thereto. In embodiments, Factor X protein is cleaved into its active form Factor Xa (FXa).

[0072] The term "serine protease" refers to an enzyme that has peptide bond cleaving activity, wherein a serine residue is a nucleophilic amino acid at the (enzyme's) active site. Accordingly, "serine protease activity" is the peptide cleaving activity associated with the enzyme. In embodiments, procoagulant activity may be detected by measuring serine protease activity of one or more serine proteases (e.g. FXa protein) in the coagulation pathway. For example, serine protease activity may be measured by detecting the cleavage product of a serine protease substrate (e.g. prothrombin).

[0073] The term "prothrombin protein" or "prothrombin" as used herein includes any of the recombinant or naturally-occurring forms of prothrombin protein, also known as Coagulation factor II, or variants or homologs thereof that maintain prothrombin activity (e.g. within at least 50%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100% activity compared to prothrombin). In some aspects, the variants or homologs have at least 90%, 95%, 96%, 97%,

98%, 99% or 100% amino acid sequence identity across the whole sequence or a portion of the sequence (e.g. a 50, 100, 150 or 200 continuous amino acid portion) compared to a naturally occurring prothrombin protein. In embodiments, the prothrombin protein is substantially identical to the protein identified by the UniProt reference number P00734 or a variant or homolog having substantial identity thereto. In embodiments, prothrombin is cleaved to its active form thrombin. In embodiments, thrombin includes a first peptide sequence corresponding to residues 328-363 of the sequence identified by the UniProt reference number P00734 and a second peptide sequence corresponding to residues 364-622 of the sequence identified by the UniProt reference number P00734.

[0074] The term "gene" means the segment of DNA involved in producing a protein; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). The leader, the trailer as well as the introns include regulatory elements that are necessary during the transcription and the translation of a gene. Further, a "protein gene product" is a protein expressed from a particular gene.

[0075] The terms "plasmid", "vector" or "expression vector" refer to a nucleic acid molecule that encodes for genes and/or regulatory elements necessary for the expression of genes. Expression of a gene from a plasmid can occur in cis or in trans. If a gene is expressed in cis, the gene and the regulatory elements are encoded by the same plasmid. Expression in trans refers to the instance where the gene and the regulatory elements are encoded by separate plasmids.

[0076] As used herein, the term "expression cassette" refers to a distinct component of vector DNA including a gene and regulatory sequence to be expressed by a transfected cell. In each successful transformation, the expression cassette directs the cell's machinery to make RNA and protein(s). Some expression cassettes are designed for modular cloning of protein-encoding sequences so that the same cassette can easily be altered to make different proteins. An expression cassette is composed of one or more genes and the sequences controlling their expression. An expression cassette comprises three components: a promoter sequence, an open reading frame, and a 3' untranslated region that, in eukaryotes, usually contains a polyadenylation site. Different expression cassettes can be transfected into different organisms including bacteria, yeast, plants, and mammalian cells as long as the correct regulatory sequences are used.

[0077] The word "expression" or "expressed" as used herein in reference to a gene means the transcriptional and/or translational product of that gene. The level of expression of a DNA molecule in a cell may be determined on the basis of either the amount of corresponding mRNA that is present within the cell or the amount of protein encoded by that DNA produced by the cell. The level of expression of non-coding nucleic acid molecules (e.g., siRNA) may be detected by standard PCR or Northern blot methods well known in the art. See, Sambrook *et al.*, 1989 *Molecular Cloning: A Laboratory Manual*, 18.1-18.88.

[0078] The term "recombinant" when used with reference, e.g., to a virus, cell, nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. In instances, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all. Transgenic cells and plants are those that express a heterologous gene or coding sequence, typically as a result of recombinant methods. In embodiments, the FXa protein or functional portion thereof as provided herein is a recombinant protein.

[0079] Antibodies are large, complex molecules (molecular weight of ~150,000 Da or about 1320 amino acids) with intricate internal structure. A natural antibody molecule contains two identical pairs of polypeptide chains, each pair having one light chain and one heavy chain. Each light chain and heavy chain in turn consists of two regions: a variable ("V") region involved in binding the target antigen, and a constant ("C") region that interacts with other components of the immune system. The light and heavy chain variable regions come together in 3-dimensional space to form a variable region that binds the antigen (for example, a receptor on the surface of a cell). Within each light or heavy chain variable region, there are three short segments (averaging 10 amino acids in length) called the complementarity determining regions ("CDRs"). The six CDRs in an antibody variable domain (three from the light chain and three from the heavy chain) fold up together in 3-dimensional space to form the actual antibody binding site (paratope), which docks onto the target antigen (epitope). The position and length of the CDRs have been precisely defined by Kabat, E. *et al.*, Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services, 1983, 1987. The part of a variable region not contained in the CDRs is called the framework ("FR"), which forms the environment for the CDRs.

[0080] The term "antibody" refers to a polypeptide encoded by an immunoglobulin gene or functional fragments thereof that specifically binds and recognizes an antigen. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0081] An "antibody variant" as provided herein refers to a polypeptide capable of binding to an antigen and including one or more structural domains (*e.g.*, light chain variable domain, heavy chain variable domain) of an antibody or fragment thereof. Non-limiting examples of antibody variants include single-domain antibodies or nanobodies, monospecific Fab₂, bispecific Fab₂, trispecific Fab₃, monovalent IgGs, scFv, bispecific diabodies, trispecific triabodies, scFv-Fc, minibodies, IgNAR, V-NAR, hcIgG, VhH, or peptibodies. A "peptibody" as provided herein refers to a peptide moiety attached (through a covalent or non-covalent linker) to the Fc domain of an antibody. Further non-limiting examples of antibody variants known in the art include antibodies produced by cartilaginous fish or camelids. A general description of antibodies from camelids and the variable regions thereof and methods for their production, isolation, and use may be found in references WO97/49805 and WO 97/49805 which are incorporated by reference herein in their entirety and for all purposes. Likewise, antibodies from cartilaginous fish and the variable regions thereof and methods for their production, isolation, and use may be found in WO2005/118629, which is incorporated by reference herein in its entirety and for all purposes.

[0082] The term "antibody" is used according to its commonly known meaning in the art. Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)₂, a dimer of Fab which itself is a light chain joined to V_H-C_{H1} by a disulfide bond. The F(ab)₂ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)₂ dimer into an Fab' monomer. The Fab' monomer is essentially a Fab with part of the hinge region (see Fundamental Immunology (Paul ed., 3rd ed. 1993). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes

antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (*e.g.*, single chain Fv) or those identified using phage display libraries (*see, e.g.*, McCafferty *et al.*, *Nature* 348:552-554 (1990)).

[0083] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kD) and one “heavy” chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) or light chain variable region and variable heavy chain (VH) or heavy chain variable region refer to these light and heavy chain regions, respectively. The terms variable light chain (VL) and light chain variable region as referred to herein may be used interchangeably. The terms variable heavy chain (VH) and heavy chain variable region as referred to herein may be used interchangeably. The Fc (*i.e.*, fragment crystallizable region) is the “base” or “tail” of an immunoglobulin and is typically composed of two heavy chains that contribute two or three constant domains depending on the class of the antibody. By binding to specific proteins, the Fc region ensures that each antibody generates an appropriate immune response for a given antigen. The Fc region also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins.

[0084] A single-chain variable fragment (scFv) is typically a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of 10 to about 25 amino acids. The linker may usually be rich in glycine for flexibility, as well as serine or threonine for solubility. The linker can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa.

[0085] The term “antibody” is used according to its commonly known meaning in the art. Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)₂, a dimer of Fab which itself is a light chain joined to V_H-C_{H1} by a disulfide bond. The F(ab)₂ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)₂ dimer into an Fab' monomer. The Fab' monomer is essentially Fab with part of the hinge region (*see* Fundamental Immunology (Paul ed., 3d ed. 1993). While

various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized *de novo* using recombinant DNA methodologies (e.g., single chain Fv) or those identified using phage display libraries (see, e.g., McCafferty *et al.*, *Nature* 348:552-554 (1990)). The term “antibody” as referred to herein further includes antibody variants such as single domain antibodies. Thus, in embodiments an antibody includes a single monomeric variable antibody domain. Thus, in embodiments, the antibody, includes a variable light chain (VL) domain or a variable heavy chain (VH) domain. In embodiments, the antibody is a variable light chain (VL) domain or a variable heavy chain (VH) domain.

[0086] “Biological sample” or “sample” refer to materials obtained from or derived from a subject or patient. A biological sample includes sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histological purposes. Such samples include bodily fluids such as blood and blood fractions or products (e.g., serum, plasma, platelets, red blood cells, and the like), sputum, tissue, cultured cells (e.g., primary cultures, explants, and transformed cells) stool, urine, synovial fluid, joint tissue, synovial tissue, synoviocytes, fibroblast-like synoviocytes, macrophage-like synoviocytes, immune cells, hematopoietic cells, fibroblasts, macrophages, T cells, etc. A biological sample is typically obtained from a eukaryotic organism, such as a mammal such as a primate e.g., chimpanzee or human; cow; dog; cat; a rodent, e.g., guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish. In some embodiments, the sample is obtained from a human. In embodiments, the sample is blood. In embodiments, the sample is serum.

[0087] A “cell” as used herein, refers to a cell carrying out metabolic or other functions sufficient to preserve or replicate its genomic DNA. A cell can be identified by well-known methods in the art including, for example, presence of an intact membrane, staining by a particular dye, ability to produce progeny or, in the case of a gamete, ability to combine with a second gamete to produce a viable offspring. Cells may include prokaryotic and eukaryotic cells. Prokaryotic cells include but are not limited to bacteria. Eukaryotic cells include but are not limited to yeast cells and cells derived from plants and animals, for example mammalian, insect (e.g., spodoptera) and human cells. In embodiments, the cell is a human cell. Cells may be useful when they are naturally nonadherent or have been treated not to adhere to surfaces, for example by trypsinization.

[0088] The terms “virus” or “virus particle” are used according to its plain ordinary meaning within Virology and refers to a virion including the viral genome (e.g. DNA, RNA, single strand, double strand), viral capsid and associated proteins, and in the case of enveloped viruses (e.g. SARS-CoV-2, herpesvirus, poxvirus), an envelope including lipids and optionally components of host cell membranes, and/or viral proteins.

[0089] The term “replicate” is used in accordance with its plain ordinary meaning and refers to the ability of a cell or virus to produce progeny. A person of ordinary skill in the art will immediately understand that the term replicate when used in connection with DNA, refers to the biological process of producing two identical replicas of DNA from one original DNA molecule. Thus, the term “replicate” includes passaging and re-infecting progeny cells. In the context of a virus, the term “replicate” includes the ability of a virus to replicate (duplicate the viral genome and packaging said genome into viral particles) in a host cell and subsequently release progeny viruses from the host cell, which results in the lysis of the host cell. A “replication-competent” virus as provided herein refers to a virus that is capable of replicating in a cell.

[0090] The term “plaque forming units” is used according to its plain ordinary meaning in Virology and refers to the amount of plaques in a cell monolayer that can be formed per volume of viral particles. In some embodiments the units are based on the number of plaques that could form when infecting a monolayer of susceptible cells. For example, in embodiments 1,000 PFU/μl indicates that 1 μl of a solution including viral particles contains enough virus particles to produce 1000 infectious plaques in a cell monolayer. In embodiments, plaque forming units are abbreviated “PFU”.

[0091] The terms “multiplicity of infection” or “MOI” are used according to its plain ordinary meaning in Virology and refers to the ratio of infectious agent (e.g., SARS-CoV-2, vesicular stomatitis virus, etc.) to the target (e.g. cell) in a given area or volume. In embodiments, the area or volume is assumed to be homogenous.

[0092] The term “coronavirus” is used in accordance with its plain ordinary meaning and refers to an RNA virus that in humans causes respiratory tract infections. Coronaviruses constitute the subfamily *Orthocoronavirinae*, in the family *Coronaviridae*, order *Nidovirales*, and realm *Riboviria*. In embodiments, the coronavirus is an enveloped viruses with a positive-sense single-stranded RNA genome. Non-limiting examples of coronaviruses include human coronavirus OC43 (HCoV-OC43), human coronavirus HKU1 (HCoV-

HKU1), human coronavirus 229E (HCoV-229E), human coronavirus NL63 (HCoV-NL63), Middle East respiratory syndrome-related coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

[0093] SARS-CoV-2 belongs to the family of betacoronaviruses, whose members include other zoonotic viruses including severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV-2 shows nearly 80 percent genetic similarity to SARS-CoV, which triggered the severe acute respiratory syndrome (SARS) epidemic in 2002-2003. SARS-CoV-2 is more distantly related to MERS-CoV, which is responsible for the Middle East respiratory syndrome (MERS) epidemic that began in 2012 and still persists. See, e.g., Yuki et al., 2020, Clin. Immun. 215, 108427; Chen et al. 2020, J. Med. Virol. 92, 418-423.

[0094] The term “severe acute respiratory syndrome coronavirus” or “SARS-CoV-1” refers to the strain of coronavirus that causes severe acute respiratory syndrome (SARS). In embodiments, SARS-CoV-1 is an enveloped, positive-sense, single-stranded RNA virus that infects the epithelial cells within the lungs. In embodiments, SARS-CoV-1 enters the host cell by binding to the angiotensin-converting enzyme 2 (ACE2) receptor.

[0095] The term “severe acute respiratory syndrome coronavirus 2” or “SARS-CoV-2” refers to the strain of coronavirus that causes coronavirus disease 2019 (COVID-19). In embodiments, SARS-CoV-2 is a positive-sense single-stranded RNA virus. Like other coronaviruses, SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins. The N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope. In embodiments, SARS-CoV-2 enters the host cell by binding to the angiotensin-converting enzyme 2 (ACE2) receptor.

[0096] “MERS-CoV” refers to Middle Eastern respiratory syndrome-associated coronavirus. See, e.g., Chung et al, Genetic Characterization of Middle East Respiratory Syndrome Coronavirus, South Korea, 2018. Emerging Infectious Diseases, 25(5):958-962 (2019).

[0097] The terms “disease” or “condition” refer to a state of being or health status of a patient or subject capable of being treated with the compositions or methods provided herein.

The disease may be an infectious disease.

[0098] The term “infection” or “infectious disease” refers to a disease or condition that can be caused by organisms such as a bacterium, virus, fungi or any other pathogenic microbial agents. In embodiments, the infectious disease is caused by a virus. In embodiments, the virus is a coronavirus. In embodiments, the virus is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In embodiments, the virus is severe acute respiratory syndrome coronavirus (SARS-CoV or SARS-CoV-1). In embodiments, the virus is MERS-coronavirus (MERS-CoV). In embodiments, the infectious disease is COVID-19.

[0099] The term “viral infection” or “viral disease” refers to a disease or condition that is caused by a virus. Non-limiting examples of viral infections include hepatic viral diseases (e.g., hepatitis A, B, C, D, E), herpes virus infection (e.g., HSV-1, HSV-2, herpes zoster), flavivirus infection, Zika virus infection, cytomegalovirus infection, a respiratory viral infection (e.g., adenovirus infection, influenza, severe acute respiratory syndrome, coronavirus infection (e.g., SARS-CoV-1, SARS-CoV-2, MERS-CoV, COVID-19, MERS)), a gastrointestinal viral infection (e.g., norovirus infection, rotavirus infection, astrovirus infection), an exanthematous viral infection (e.g., measles, shingles, smallpox, rubella), viral hemorrhagic disease (e.g., Ebola, Lassa fever, dengue fever, yellow fever), a neurologic viral infection (e.g., West Nile viral infection, polio, viral meningitis, viral encephalitis, Japanese encephalitis, rabies), and human papilloma viral infection.

[0100] The terms “COVID-19”, “2019-nCoV”, “2019 novel coronavirus”, “HCoV-19”, “hCoV-19”, or “human coronavirus 2019” refer to coronavirus disease 2019, which is the respiratory illness responsible for the COVID-19 pandemic.

[0101] “Middle Eastern respiratory syndrome” or “MERS” refers to the disease caused by MERS- coronavirus.

[0102] “Contacting” is used in accordance with its plain ordinary meaning and refers to the process of allowing at least two distinct species (e.g. chemical compounds including biomolecules or cells) to become sufficiently proximal to react, interact or physically touch. It should be appreciated; however, the resulting reaction product can be produced directly from a reaction between the added reagents or from an intermediate from one or more of the added reagents that can be produced in the reaction mixture.

[0103] The term “contacting” may include allowing two species to react, interact, or

physically touch, wherein the two species may be a compound as described herein and a protein (e.g. FXa or a portion thereof) or enzyme. In some embodiments contacting includes allowing a compound described herein to interact with a protein or enzyme that is involved in a signaling pathway.

[0104] The term “isolated”, when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It can be, for example, in a homogeneous state and may be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified.

[0105] “Selective” or “selectivity” or the like of a compound refers to the compound’s ability to discriminate between molecular targets.

[0106] “Specific”, “specifically”, “specificity”, or the like of a compound refers to the compound’s ability to cause a particular action, such as inhibition, to a particular molecular target with minimal or no action to other proteins in the cell.

[0107] As defined herein, the term “activation”, “activate”, “activating”, “activator” and the like in reference to a protein-inhibitor interaction means positively affecting (e.g. increasing) the activity or function of the protein relative to the activity or function of the protein in the absence of the activator. In embodiments activation means positively affecting (e.g. increasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the activator. The terms may reference activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein decreased in a disease. Thus, activation may include, at least in part, partially or totally increasing stimulation, increasing or enabling activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein associated with a disease (e.g., a protein which is decreased in a disease relative to a non-diseased control). Activation may include, at least in part, partially or totally increasing stimulation, increasing or enabling activation, or activating, sensitizing, or up-regulating signal transduction or enzymatic activity or the amount of a protein

[0108] The terms “agonist,” “activator,” “upregulator,” etc. refer to a substance capable of

detectably increasing the expression or activity of a given gene or protein. The agonist can increase expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the agonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or higher than the expression or activity in the absence of the agonist.

[0109] As defined herein, the term “inhibition”, “inhibit”, “inhibiting” and the like in reference to a protein-inhibitor interaction means negatively affecting (e.g. decreasing) the activity or function of the protein relative to the activity or function of the protein in the absence of the inhibitor. In embodiments inhibition means negatively affecting (e.g. decreasing) the concentration or levels of the protein relative to the concentration or level of the protein in the absence of the inhibitor. In embodiments inhibition refers to reduction of a disease or symptoms of disease. In embodiments, inhibition refers to a reduction in the activity of a particular protein target. Thus, inhibition includes, at least in part, partially or totally blocking stimulation, decreasing, preventing, or delaying activation, or inactivating, desensitizing, or down-regulating signal transduction or enzymatic activity or the amount of a protein. In embodiments, inhibition refers to a reduction of activity of a target protein resulting from a direct interaction (e.g. an inhibitor binds to the target protein). In embodiments, inhibition refers to a reduction of activity of a target protein from an indirect interaction (e.g. an inhibitor binds to a protein that activates the target protein, thereby preventing target protein activation).

[0110] The terms “inhibitor,” “repressor” or “antagonist” or “downregulator” interchangeably refer to a substance capable of detectably decreasing the expression or activity of a given gene or protein. The antagonist can decrease expression or activity 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a control in the absence of the antagonist. In certain instances, expression or activity is 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold or lower than the expression or activity in the absence of the antagonist.

[0111] The term “modulate” is used in accordance with its plain ordinary meaning and refers to the act of changing or varying one or more properties. “Modulation” refers to the process of changing or varying one or more properties. For example, as applied to the effects of a modulator on a target protein, to modulate means to change by increasing or decreasing a property or function of the target molecule or the amount of the target molecule.

[0112] The term “modulator” refers to a composition that increases or decreases the level of a target molecule or the function of a target molecule or the physical state of the target of the molecule relative to the absence of the modulator.

[0113] “Control” or “control experiment” is used in accordance with its plain ordinary meaning and refers to an experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment except for omission of a procedure, reagent, or variable of the experiment. In some instances, the control is used as a standard of comparison in evaluating experimental effects. In some embodiments, a control is the measurement of the activity of a protein in the absence of a compound as described herein (including embodiments and examples).

[0114] A “control” or “standard control” refers to a sample, measurement, or value that serves as a reference, usually a known reference, for comparison to a test sample, measurement, or value. For example, a test sample can be taken from a patient suspected of having a given disease and compared to a known normal (non-diseased) individual (e.g. a standard control subject). A standard control can also represent an average measurement or value gathered from a population of similar individuals (e.g. standard control subjects) that do not have a given disease (i.e. standard control population), e.g., healthy individuals with a similar medical background, same age, weight, etc. A standard control value can also be obtained from the same individual, e.g. from an earlier-obtained sample from the patient prior to disease onset. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (e.g., half-life) or therapeutic measures (e.g., comparison of side effects). Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered as significant. One of skill will recognize that standard controls can be designed for assessment of any number of parameters (e.g. RNA levels, protein levels, specific cell types, specific bodily fluids, specific tissues, etc).

[0115] The term “signaling pathway” as used herein refers to a series of interactions between cellular and optionally extra-cellular components (e.g. proteins, nucleic acids, small molecules, ions, lipids) that conveys a change in one component to one or more other components, which in turn may convey a change to additional components, which is optionally propagated to other signaling pathway components.

[0116] The term “associated” or “associated with” in the context of a substance or

substance activity or function associated with a disease (e.g. a protein associated disease, a cancer (e.g., cancer, inflammatory disease, autoimmune disease, or infectious disease)) means that the disease (e.g. cancer, inflammatory disease, autoimmune disease, or infectious disease) is caused by (in whole or in part), or a symptom of the disease is caused by (in whole or in part) the substance or substance activity or function. As used herein, what is described as being associated with a disease, if a causative agent, could be a target for treatment of the disease.

[0117] The term “aberrant” as used herein refers to different from normal. When used to describe enzymatic activity or protein function, aberrant refers to activity or function that is greater or less than a normal control or the average of normal non-diseased control samples. Aberrant activity may refer to an amount of activity that results in a disease, wherein returning the aberrant activity to a normal or non-disease-associated amount (e.g. by administering a compound or using a method as described herein), results in reduction of the disease or one or more disease symptoms.

[0118] As used herein, the term "administering" is used in accordance with its plain and ordinary meaning and includes oral administration, administration as a suppository, topical contact, intravenous, parenteral, intraperitoneal, intramuscular, intralesional, intrathecal, intranasal or subcutaneous administration, or the implantation of a slow-release device, e.g., a mini-osmotic pump, to a subject. Administration is by any route, including parenteral and transmucosal (e.g., buccal, sublingual, palatal, gingival, nasal, vaginal, rectal, or transdermal). Parenteral administration includes, e.g., intravenous, intramuscular, intra-arteriole, intradermal, subcutaneous, intraperitoneal, intraventricular, and intracranial. Other modes of delivery include, but are not limited to, the use of liposomal formulations, intravenous infusion, transdermal patches, etc. In embodiments, the administering does not include administration of any active agent other than the recited active agent.

[0119] “Administration,” “administering” and the like, when used in connection with a composition refer both to direct administration, which may be administration to cells *in vitro*, administration to cells *in vivo*, administration to a subject by a medical professional or by self-administration by the subject and/or to indirect administration, which may be the act of prescribing a composition of the disclosure. Typically, an effective amount is administered, which amount can be determined by one of skill in the art. Compositions (e.g., FXa protein or a functional portion thereof) may be administered to cells by, for example, addition of the

composition to the cell culture media or injection *in vivo*.

[0120] “Co-administer” means that a composition described herein is administered at the same time, just prior to, or just after the administration of one or more additional therapies. For example, the FXa protein or functional fragment thereof may be co-administered with an anticoagulant. The compounds provided herein can be administered alone or can be coadministered to the patient. Coadministration is meant to include simultaneous or sequential administration of the compounds individually or in combination (more than one compound). Thus, the preparations can also be combined, when desired, with other active substances (e.g. to reduce metabolic degradation). The compositions of the present disclosure can be delivered transdermally, by a topical route, or formulated as applicator sticks, solutions, suspensions, emulsions, gels, creams, ointments, pastes, jellies, paints, powders, and aerosols.

[0121] The terms “disease” or “condition” refer to a state of being or health status of a patient or subject capable of being treated with the compounds or methods provided herein. The disease may be an inflammatory disease. The disease may be an infectious disease. The disease may be a viral disease.

[0122] “Treating” or “treatment” as used herein (and as well-understood in the art) also broadly includes any approach for obtaining beneficial or desired results in a subject’s condition, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of the extent of a disease, stabilizing (*i.e.*, not worsening) the state of disease, prevention of a disease’s transmission or spread, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission, whether partial or total and whether detectable or undetectable. In other words, “treatment” as used herein includes any cure, amelioration, or prevention of a disease. Treatment may prevent the disease from occurring; inhibit the disease’s spread; relieve the disease’s symptoms, fully or partially remove the disease’s underlying cause, shorten a disease’s duration, or do a combination of these things.

[0123] “Treating” and “treatment” as used herein include prophylactic treatment. Treatment methods include administering to a subject a therapeutically effective amount of an active agent. The administering step may consist of a single administration or may include a series of administrations. The length of the treatment period depends on a variety of

factors, such as the severity of the condition, the age of the patient, the concentration of active agent, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment or prophylaxis may increase or decrease over the course of a particular treatment or prophylaxis regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient. In embodiments, the treating or treatment is no prophylactic treatment.

[0124] The term “prevent” refers to a decrease in the occurrence of disease symptoms in a patient. As indicated above, the prevention may be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur absent treatment.

[0125] “Patient” or “subject in need thereof” refers to a living organism suffering from or prone to a disease (e.g. COVID-19) or condition that can be treated by administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goat, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0126] An “effective amount” is an amount sufficient for a compound to accomplish a stated purpose relative to the absence of the compound (e.g. achieve the effect for which it is administered, treat a disease(e.g. COVID-19), reduce enzyme activity, increase enzyme activity, reduce a signaling pathway, or reduce one or more symptoms of a disease or condition). An example of an “effective amount” is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease (e.g. COVID-19), which could also be referred to as a “therapeutically effective amount.” A “reduction” of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). A “prophylactically effective amount” of a drug is an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of an injury, disease, pathology or condition, or reducing the likelihood of the onset (or reoccurrence) of an injury, disease, pathology, or condition, or their symptoms. The full prophylactic effect does not necessarily occur by administration of one dose, and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An “activity decreasing

amount,” as used herein, refers to an amount of antagonist required to decrease the activity of an enzyme relative to the absence of the antagonist. A “function disrupting amount,” as used herein, refers to the amount of antagonist required to disrupt the function of an enzyme or protein relative to the absence of the antagonist. The exact amounts will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); Pickar, *Dosage Calculations* (1999); and *Remington: The Science and Practice of Pharmacy*, 20th Edition, 2003, Gennaro, Ed., Lippincott, Williams & Wilkins).

[0127] The term “EC50” or “half maximal effective concentration” as used herein refers to the concentration of a molecule (e.g., antibody, chimeric antigen receptor or bispecific antibody) capable of inducing a response which is halfway between the baseline response and the maximum response after a specified exposure time. In embodiments, the EC50 is the concentration of a molecule (e.g., antibody, chimeric antigen receptor or bispecific antibody) that produces 50% of the maximal possible effect of that molecule.

[0128] The term “pharmaceutical composition” refers to a composition comprising a compound (e.g. FXa or a portion thereof) of the invention in combination with at least one additional pharmaceutically acceptable carrier. A “pharmaceutically acceptable carrier” refers to media generally accepted in the art for the delivery of biologically active agents to animals, in particular, mammals, including, *i.e.*, adjuvant, excipient or vehicle, such as diluents, preserving agents, fillers, flow regulating agents, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms. Pharmaceutically acceptable carriers are formulated according to a number of factors well within the purview of those of ordinary skill in the art. These include, without limitation: the type and nature of the active agent being formulated; the subject to which the agent-containing composition is to be administered; the intended route of administration of the composition; and the therapeutic indication being targeted. Pharmaceutically acceptable carriers include both aqueous and non-aqueous liquid media, as well as a variety of solid and semi-solid dosage forms. Such carriers can include a number of different ingredients and additives in addition to the active agent, such additional ingredients being included in the formulation for a variety of reasons, *e.g.*, stabilization of the active agent, binders, etc., well

known to those of ordinary skill in the art. Descriptions of suitable pharmaceutically acceptable carriers, and factors involved in their selection, are found in a variety of readily available sources such as, for example, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990).

[0129] As used herein, “dosage forms” or “unit dosage forms” may be administered orally, ophthalmically, via inhalation, as a parenteral, topically, as a suppository. In embodiments, an oral dosage form may include, but is not limited to, pill (tablet or capsule), syrups, specialty tablets (e.g., buccal, sub-lingual, orally-disintegrating), thin film, liquid solution or suspension (syrup or drink), powder, and pastes. They may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form, all using dosage forms well known in the pharmaceutical arts. In embodiments, oral dosage forms can be administered alone, but generally will be administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice. In embodiments, ophthalmic dosage forms may be a liquid solution. In embodiments, inhaled dosage forms may include an aerosol, inhaler, nebulizer, and vaporizer.

[0130] The pharmaceutical compositions provided herein may be formulated into a unit dosage form. Such formulations are well known to one of ordinary skill in the art. In embodiments, the dosage form may be a liquid, solid, or semisolid dosage form. In embodiments, solid dosage forms may include, but are not limited to, pills, tablets, capsules, granules, powders, sachets, reconstitutable powders, dry powder inhalers and chewables. In embodiments, liquid dosage forms may include, but are not limited to syrups, suspensions, emulsions, and elixers. As used herein, semisolid dosage forms may include, but are not limited to creams, gels, ointments, suppositories, pastes, chewables, gummies, and soft-chews.

[0131] In embodiments, parenteral dosage forms may include, but are not limited to, intradermal, subcutaneous, intramuscular, intraosseous, intraperitoneal, and intravenous. In embodiments, topical dosage forms may include, but are not limited to, cream, gel, liniment, balm, lotion, ointment, dermal patch, ear drops, eye drops, and powder. When administered intravenously or intra-arterially, the parenteral can be given continuously or intermittently. Furthermore, the formulation can be developed for intramuscularly and subcutaneous delivery to ensure a gradual release of the active pharmaceutical ingredient.

[0132] In embodiments, suppository dosage forms may include, but are not limited to nasal suppositories.

[0133] As used herein, “liquid dosage forms” refer to pourable pharmaceutical formulations which contain a mixture of active drug components and excipients dissolved or suspended in a suitable solvent or mixtures of solvents. Liquid dosage forms are broadly classified as monophasic dosage forms and biphasic dosage forms. In embodiments, the monophasic dosage form may be a parenteral.

[0134] In embodiments, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration preferably contain a water soluble salt of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl-or propyl-paraben, and chlorobutanol.

[0135] For the pharmaceutical compositions provided herein, the dosage and frequency (single or multiple doses) administered to a subject can vary depending upon a variety of factors, for example, whether the subject suffers from another disease, its route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated, kind of concurrent treatment, complications from the disease being treated or other health-related problems. Other therapeutic regimens or agents can be used in conjunction with the methods and compositions described herein including embodiments thereof. Adjustment and manipulation of established dosages (e.g., frequency and duration) are well within the ability of those skilled in the art.

[0136] By way of general guidance, the daily oral dosage of each active ingredient, when used for the indicated effects, will range between about 0.001 to about 1000 mg/kg of body weight, preferably between about 0.01 to about 100 mg/kg of body weight per day, and most preferably between about 0.1 to about 20 mg/kg/day. Intravenously, the most preferred doses will range from about 0.001 to about 10 mg/kg/minute during a constant rate infusion. Compounds of this invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three, or four times daily.

[0137] In embodiments, dosage forms suitable for administration may contain from about 1 milligram to about 1000 milligrams of active ingredient per dosage unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.1-95% by weight based on the total weight of the composition.

FXA PROTEIN COMPOSITIONS

[0138] Applicant has discovered that FXa protein and functional portions thereof have potent antiviral activity. For example, Applicant has demonstrated that FXa inhibits entry of SARS-CoV-2 into host cell cells. Without wishing to be bound by scientific theory, FXa protein cleaves S protein into fragments which are unable to effectively bind host cell receptors necessary for gaining entry into the host cell. In an aspect is provided compositions including FXa protein or a functional portion thereof. In embodiments, the FXa protein or function portion thereof is a recombinant protein.

[0139] In embodiments, the FXa protein includes a sequence having at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity across the whole sequence or a portion of the sequence (e.g. a 40, 50, 60, 70, 80, 90 or 100 continuous amino acid portion) of SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 80% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 85% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 90% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 92% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 94% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 96% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 98% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes a sequence having at least 99% sequence identity to SEQ ID NO:6. In embodiments, the FXa protein includes the sequence of SEQ ID NO:6. In embodiments, the FXa protein is a sequence having the sequence of SEQ ID NO:6.

[0140] In embodiments, the FXa protein includes a sequence having at least 80% sequence identity to SEQ ID NO:6, and the sequence having at least 80% sequence identity is contiguous. In embodiments, the FXa protein includes a sequence having at least 85% sequence identity to SEQ ID NO:6, and the sequence having at least 85% sequence identity is contiguous. In embodiments, the FXa protein includes a sequence having at least 90%

sequence identity to SEQ ID NO:6, and the sequence having at least 90% sequence identity is contiguous. In embodiments, the FXa protein includes a sequence having at least 92% sequence identity to SEQ ID NO:6, and the sequence having at least 92% sequence identity is contiguous. In embodiments, the FXa protein includes a sequence having at least 94% sequence identity to SEQ ID NO:6, and the sequence having at least 94% sequence identity is contiguous. In embodiments, the FXa protein includes a sequence having at least 96% sequence identity to SEQ ID NO:6, and the sequence having at least 96% sequence identity is contiguous. In embodiments, the FXa protein includes a sequence having at least 98% sequence identity to SEQ ID NO:6, and the sequence having at least 98% sequence identity is contiguous. In embodiments, the FXa protein includes a sequence having at least 99% sequence identity to SEQ ID NO:6, and the sequence having at least 99% sequence identity is contiguous.

[0141] In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a second peptide having at least 80% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4. In embodiments, the first peptide and the second peptide are covalently attached. In embodiments, the first peptide and the second peptide are covalently attached via one or more disulfide bonds.

[0142] In embodiments, the FXa protein includes a first peptide having at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity across the whole sequence or a portion of the sequence (e.g. a 40, 50, 60, 70, 80, 90 or 100 continuous amino acid portion) of SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 85% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 90% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 92% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 94% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 96% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 98% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 99% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide including

the sequence of SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having the sequence of SEQ ID NO:3.

[0143] In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3, and the sequence having at least 80% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 85% sequence identity to SEQ ID NO:3, and the sequence having at least 85% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 90% sequence identity to SEQ ID NO:3, and the sequence having at least 90% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 92% sequence identity to SEQ ID NO:3, and the sequence having at least 92% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 94% sequence identity to SEQ ID NO:3, and the sequence having at least 94% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 96% sequence identity to SEQ ID NO:3, and the sequence having at least 96% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 98% sequence identity to SEQ ID NO:3, and the sequence having at least 98% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 99% sequence identity to SEQ ID NO:3, and the sequence having at least 99% sequence identity is contiguous.

[0144] In embodiments, the FXa protein includes a second peptide having at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity across the whole sequence or a portion of the sequence (e.g. a 40, 50, 60, 70, 80, 90, 100, 150, or 200 continuous amino acid portion) of SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 80% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 85% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 90% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 92% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 94% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 96% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 98% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having at least 99% sequence identity to SEQ ID NO:4. In embodiments, the

FXa protein includes a second peptide including the sequence of SEQ ID NO:4. In embodiments, the FXa protein includes a second peptide having the sequence of SEQ ID NO:4.

[0145] In embodiments, the FXa protein includes a second peptide having at least 80% sequence identity to SEQ ID NO:4, and the sequence having at least 80% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 85% sequence identity to SEQ ID NO:4, and the sequence having at least 85% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 90% sequence identity to SEQ ID NO:4, and the sequence having at least 90% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 92% sequence identity to SEQ ID NO:4, and the sequence having at least 92% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 94% sequence identity to SEQ ID NO:4, and the sequence having at least 94% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 96% sequence identity to SEQ ID NO:4, and the sequence having at least 96% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 98% sequence identity to SEQ ID NO:4, and the sequence having at least 98% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 99% sequence identity to SEQ ID NO:4, and the sequence having at least 99% sequence identity is contiguous.

[0146] In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3. In embodiments, the FXa protein includes a second peptide having at least 80% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5. In embodiments, the first peptide and the second peptide are covalently attached. In embodiments, the first peptide and the second peptide are covalently attached via one or more disulfide bonds.

[0147] In embodiments, the FXa protein includes a first peptide having at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity across the whole sequence or a portion of the sequence (e.g. a 40, 50, 60, 70, 80, 90 or 100 continuous amino acid portion) of SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a

first peptide having at least 85% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 90% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 92% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 94% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 96% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 98% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having at least 99% sequence identity to SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide including the sequence of SEQ ID NO:3. In embodiments, the FXa protein includes a first peptide having the sequence of SEQ ID NO:3.

[0148] In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3, and the sequence having at least 80% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 85% sequence identity to SEQ ID NO:3, and the sequence having at least 85% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 90% sequence identity to SEQ ID NO:3, and the sequence having at least 90% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 92% sequence identity to SEQ ID NO:3, and the sequence having at least 92% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 94% sequence identity to SEQ ID NO:3, and the sequence having at least 94% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 96% sequence identity to SEQ ID NO:3, and the sequence having at least 96% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 98% sequence identity to SEQ ID NO:3, and the sequence having at least 98% sequence identity is contiguous. In embodiments, the FXa protein includes a first peptide having at least 99% sequence identity to SEQ ID NO:3, and the sequence having at least 99% sequence identity is contiguous.

[0149] In embodiments, the FXa protein includes a second peptide having at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity across the whole sequence or a portion of the sequence (e.g. a 40, 50, 60, 70, 80, 90, 100, 150, or 200 continuous amino acid portion) of SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having at least 80% sequence identity to SEQ ID NO:5. In embodiments, the

FXa protein includes a second peptide having at least 85% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having at least 90% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having at least 92% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having at least 94% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having at least 96% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having at least 98% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having at least 99% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide including the sequence of SEQ ID NO:5. In embodiments, the FXa protein includes a second peptide having the sequence of SEQ ID NO:5.

[0150] In embodiments, the FXa protein includes a second peptide having at least 80% sequence identity to SEQ ID NO:5, and the sequence having at least 80% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 85% sequence identity to SEQ ID NO:5, and the sequence having at least 85% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 90% sequence identity to SEQ ID NO:5, and the sequence having at least 90% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 92% sequence identity to SEQ ID NO:5, and the sequence having at least 92% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 94% sequence identity to SEQ ID NO:5, and the sequence having at least 94% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 96% sequence identity to SEQ ID NO:5, and the sequence having at least 96% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 98% sequence identity to SEQ ID NO:5, and the sequence having at least 98% sequence identity is contiguous. In embodiments, the FXa protein includes a second peptide having at least 99% sequence identity to SEQ ID NO:5, and the sequence having at least 99% sequence identity is contiguous.

[0151] A "functional portion thereof" or "portion thereof" refers to a fragment of a polypeptide or a fragment of a nucleic acid encoding the polypeptide, wherein the fragment retains at least a fraction of the biological activity of the wild type polypeptide or wild type nucleic acid. For example, with regard to FXa protein, a functional portion thereof includes a

fragment of an FXa polypeptide that retains at least a fraction of the procoagulant activity of wild type FXa. In embodiments, a functional portion thereof retains at least 50% of the procoagulant activity of wild type FXa protein. In embodiments, a functional portion thereof retains at least 60% of the procoagulant activity of wild type FXa protein. In embodiments, a functional portion thereof retains at least 70% of the procoagulant activity of wild type FXa protein. In embodiments, a functional portion thereof retains at least 80% of the procoagulant activity of wild type FXa protein. In embodiments, a functional portion thereof retains at least 90% of the procoagulant activity of wild type FXa protein. In embodiments, a functional portion thereof retains at least 95% of the procoagulant activity of wild type FXa protein. In embodiments, a functional portion thereof retains at least 98% of the procoagulant activity of wild type FXa protein. In embodiments, a functional portion thereof has the procoagulant activity of wild type FXa protein.

[0152] "Procoagulant activity" is referred to as the ability of any one of the proteins (e.g. FXa, thrombin, etc.) in the coagulation pathway to cause blood coagulation or clot formation. For example, with regards to FXa protein, procoagulant activity may be measured by the serine protease activity of FXa. Procoagulant activity may be measured by any method known in the art, including direct or indirect methods. Methods for measuring procoagulant activity include but are not limited to detecting conversion of prothrombin to thrombin, detecting cleavage of an FXa substrate, and detecting blood clotting, for example by way of clot based assays (e.g. detecting the length of time for blood to clot (e.g. prothrombin time (PT) test), etc.). In embodiments, procoagulant activity of FXa may be measured by detecting conversion of prothrombin to thrombin. In embodiments, procoagulant activity of FXa may be measured by a clot based assay.

[0153] Reduced procoagulant activity means that procoagulant activity has been reduced by at least about 50%, at least about 90%, or at least about 95% as compared to wild-type FXa during the same time period. In embodiments, reduced procoagulant activity is reduced conversion of prothrombin to thrombin, or reduced blood clotting ability (e.g. increased prothrombin time). For example, recombinant fX-S395A essentially has no procoagulant activity as measured by in vitro assays, such as in FXa activity assays (e.g. chromogenic assays to detect cleavage of an FXa substrate). In embodiments, procoagulant activity is reduced by at least 50% as compared to wild-type FXa during the same time period. In embodiments, procoagulant activity is reduced by at least 60% as compared to wild-type FXa during the same time period. In embodiments, procoagulant activity is reduced by at least

70% as compared to wild-type FXa during the same time period. In embodiments, procoagulant activity is reduced by at least 80% as compared to wild-type FXa during the same time period. In embodiments, procoagulant activity is reduced by at least 90% as compared to wild-type FXa during the same time period. In embodiments, procoagulant activity is reduced by at least 95% as compared to wild-type FXa during the same time period. In embodiments, procoagulant activity is reduced by at least 98% as compared to wild-type FXa during the same time period. In embodiments, procoagulant activity is reduced by 100% as compared to wild-type FXa during the same time period (e.g. coagulation, blood clotting, or prothrombin to thrombin conversion is not detectable when measured by methods known in the art).

[0154] “Native FXa” or “wild-type FXa” refers to the FXa protein naturally present in plasma, or to the FXa protein isolated from a biological sample or being. In embodiments, wild-type FXa has the biological activity of converting prothrombin to thrombin, therefore promoting formation of blood clot. For example, FXa activity can be measured by the conversion of prothrombin to thrombin or by blood clot formation (e.g. via the prothrombin time assay, etc.).

[0155] In embodiments, the FXa protein or functional portion thereof further includes an Fc domain. In embodiments, the FXa protein is a recombinant protein including an Fc domain. For example, the FXa protein may be a fusion protein including the FXa protein or portion thereof and the Fc domain. The term “Fc domain” or “Fc region” refers to the fragment crystallizable region (Fc region) which is the “base” or “tail” of an immunoglobulin. An Fc domain does not include any antibody variable domains (e.g. VH). The Fc domain is typically composed of two heavy chains that each contribute two or three constant domains (CH domain) depending on the class of the antibody. By binding to specific proteins, the Fc domain ensures that each antibody generates an appropriate immune response for a given antigen. The Fc domain also binds to various cell receptors, such as Fc receptors, and other immune molecules, such as complement proteins. In embodiments, IgG, IgA and IgD Fc domains include two heavy chain constant domains (e.g. CH2 and CH3 domain). In embodiments, IgM and IgE Fc domains include three heavy chain constant domains (e.g. CH2, CH3, CH4 domains). In embodiments, the Fc domain includes a constant heavy chain domain 3 (CH3 domain) and a constant heavy chain domain 2 (CH2 domain) in each heavy chain. In embodiments, the Fc domain includes a constant heavy chain domain 2 (CH2 domain), a constant heavy chain domain 3 (CH3 domain), and a constant heavy chain domain

4 (CH4 domain) in each heavy chain. The Fc domains of IgGs typically include a highly conserved N-glycosylation site necessary for Fc receptor-mediated activity. The N-glycans attached to this site may be core-fucosylated diantennary structures of the complex type. In embodiments, small amounts of these N-glycans also bear bisecting GlcNAc and α -2,6 linked sialic acid residues. In embodiments, the Fc domain has been engineered to contain an antigen-binding site.

[0156] In embodiments, the Fc domain has at least 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity across the whole sequence or a portion of the sequence (e.g. a 40, 50, 60, 70, 80, 90 or 100 continuous amino acid portion) of SEQ ID NO:7. In embodiments, the Fc domain has at least 80% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain has at least 85% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain has at least 90% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain has at least 92% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain has at least 94% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain has at least 96% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain has at least 98% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain has at least 99% sequence identity to SEQ ID NO:7. In embodiments, the FXa protein includes the sequence of SEQ ID NO:7. In embodiments, the Fc domain is the sequence of SEQ ID NO:7.

[0157] In embodiments, the Fc domain has at least 80% sequence identity to SEQ ID NO:7, and the sequence having at least 80% sequence identity is contiguous. In embodiments, the Fc domain has at least 85% sequence identity to SEQ ID NO:7, and the sequence having at least 85% sequence identity is contiguous. In embodiments, the Fc domain has at least 90% sequence identity to SEQ ID NO:7, and the sequence having at least 90% sequence identity is contiguous. In embodiments, the Fc domain has at least 92% sequence identity to SEQ ID NO:7, and the sequence having at least 92% sequence identity is contiguous. In embodiments, the Fc domain has at least 94% sequence identity to SEQ ID NO:7, and the sequence having at least 94% sequence identity is contiguous. In embodiments, the Fc domain has at least 96% sequence identity to SEQ ID NO:7, and the sequence having at least 96% sequence identity is contiguous. In embodiments, the Fc domain has at least 98% sequence identity to SEQ ID NO:7, and the sequence having at least 98% sequence identity is contiguous. In embodiments, the Fc domain has at least 99% sequence identity to SEQ ID NO:7, and the sequence having at least 99% sequence identity is contiguous.

[0158] In embodiments, the FXa protein is an FXa protein derivative. The term "FXa derivative" or "modified FXa" or "derivative of a factor Xa protein" refers to an FXa protein that has been modified such that it binds, either directly or indirectly, to a factor Xa inhibitor. For example, the FXa protein may include one or more amino acid substitutions to increase binding affinity of the protein to the factor Xa inhibitor. In embodiments, the factor Xa inhibitor is an indirect factor Xa inhibitor. In embodiments, the FXa derivative is not capable of binding to factor Va or has reduced binding to factor Va compared to wild type FXa. Structurally, an FXa derivative has no procoagulant activity or has reduced procoagulant activity. In embodiments, the FXa derivative has about 50% procoagulant activity as compared to wild-type FXa during the same time period. In embodiments, the FXa derivative has about 40% procoagulant activity as compared to wild-type FXa during the same time period. In embodiments, the FXa derivative has about 30% procoagulant activity as compared to wild-type FXa during the same time period. In embodiments, the FXa derivative has about 20% procoagulant activity as compared to wild-type FXa during the same time period. In embodiments, the FXa derivative has about 10% procoagulant activity as compared to wild-type FXa during the same time period. In embodiments, the FXa derivative has about 5% procoagulant activity as compared to wild-type FXa during the same time period. In embodiments, the FXa derivative has about 2% procoagulant activity as compared to wild-type FXa during the same time period. In embodiments, the FXa derivative does not have procoagulant activity (e.g. procoagulant activity is undetectable when measured by methods known in the art (e.g. prothrombin to thrombin conversion, blood clot formation).

[0159] The term "FXa inhibitor binding activity" refers to the ability of a molecule (e.g. FXa and portions thereof) to bind an inhibitor of FXa. Provided herein are compounds (e.g. FXa and portions thereof) having FXa inhibitor binding activity. In embodiments, a compound upstream or downstream of FXa has FXa inhibitor binding activity.

PHARMACEUTICAL COMPOSITIONS

[0160] In an aspect is provided a pharmaceutical composition including an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient. In embodiments, the pharmaceutical composition further includes an anticoagulant. In embodiments, the FXa protein or functional portion thereof the anticoagulant are in a single dosage form.

[0161] The term "anticoagulant" refers to a substance that directly or indirectly inhibits or downregulates the biological activity (e.g. procoagulant activity) of any one of the proteins in

the coagulation pathway. An anticoagulant may therefore inhibit blood clot formation or prolong blood clotting time. Thus, an anticoagulant may inhibit or downregulate the enzymatic activity (e.g. protease activity) of a protein or enzyme (e.g. FXa protein) in the coagulation pathway. An anticoagulant may directly inhibit the activity of a protein, for example, by blocking the active site of the protein. Alternatively, an anticoagulant may indirectly inhibit the activity of the protein, for example, by modulating the activity or expression of a molecule upstream of the protein that affects the protein's activity. An anticoagulant may bind to a second protein, in which binding of the second protein causes the protein to become inactive or decreases the protein's activity. For example, the anticoagulant fondaparinux binds to antithrombin, and potentiates the neutralization effect of FXa by binding antithrombin. Binding of antithrombin and fondaparinux results in conformational changes to antithrombin. The conformational changes to antithrombin enables it to bind FXa, causing inhibition of FXa activity.

[0162] Thus, an anticoagulant may decrease or downregulate procoagulant activity. An anticoagulant may prevent or reduce blood clotting. An anticoagulant may therefore prolong blood clotting time. In instances, an anticoagulant may break down an existing blood clot. In embodiments, an anticoagulant is a directly acting oral anticoagulant, novel oral anticoagulant, or non-vitamin K antagonist oral anticoagulant. In embodiments, an anticoagulant is a direct thrombin inhibitor (e.g., dabigatran) or factor Xa inhibitor (rivaroxaban, apixaban, betrixaban and edoxaban). In embodiments, the anticoagulant is coumarin (e.g. vitamin K antagonist) or a derivative thereof, heparin or a derivative thereof, or a low molecular weight heparin. In embodiments, the anticoagulant is a synthetic pentasaccharide inhibitor of factor Xa (e.g., fondaparinux, idraparinux, idrabiotaparinux).

[0163] For the pharmaceutical composition provided herein, in embodiments, the anticoagulant is an FXa inhibitor. In embodiments, the anticoagulant is not a direct inhibitor of FXa (e.g. is an indirect FXa inhibitor). In embodiments, the anticoagulant is fondaparinux, heparin, or low molecular weight heparin. In embodiments, the anticoagulant is heparin. In embodiments, the anticoagulant is or low molecular weight heparin. In embodiments, the FXa inhibitor is fondaparinux.

[0164] The term "factor Xa inhibitor", "FXa inhibitor", "inhibitor of factor Xa", or "inhibitor of FXa" refers to a compound that downregulates or inhibits, either directly or indirectly, the ability of Factor Xa to convert prothrombin to thrombin. In embodiments, the

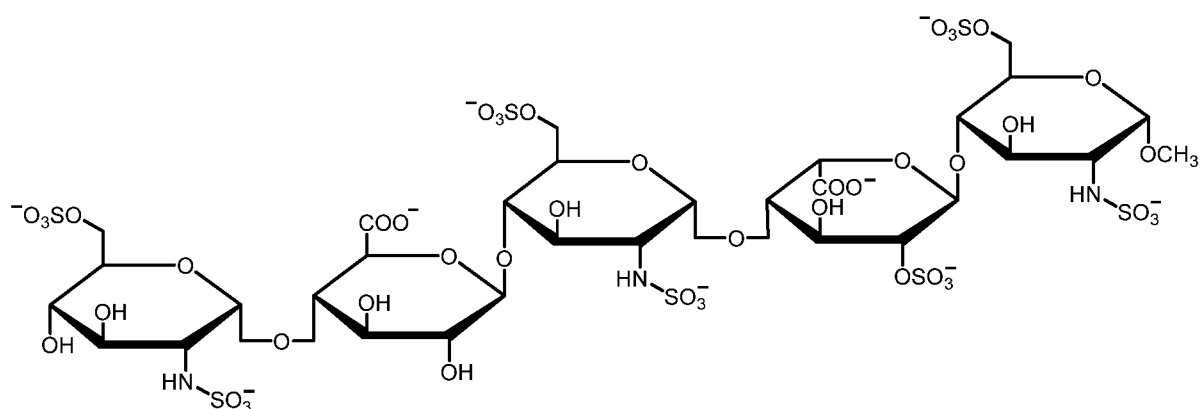
FXa inhibitor decreases conversion of prothrombin to thrombin at least 50% compared to conversion in the absence of the FXa inhibitor. In embodiments, the FXa inhibitor decreases conversion of prothrombin to thrombin at least 60% compared to conversion in the absence of the FXa inhibitor. In embodiments, the FXa inhibitor decreases conversion of prothrombin to thrombin at least 70% compared to conversion in the absence of the FXa inhibitor. In embodiments, the FXa inhibitor decreases conversion of prothrombin to thrombin at least 80% compared to conversion in the absence of the FXa inhibitor. In embodiments, the FXa inhibitor decreases conversion of prothrombin to thrombin at least 90% compared to conversion in the absence of the FXa inhibitor. In embodiments, the FXa inhibitor decreases conversion of prothrombin to thrombin at least 95% compared to conversion in the absence of the FXa inhibitor. In embodiments, the FXa inhibitor decreases conversion of prothrombin to thrombin at least 98% compared to conversion in the absence of the FXa inhibitor. In embodiments, the FXa inhibitor decreases conversion of prothrombin to thrombin 100% compared to conversion in the absence of the FXa inhibitor (e.g. conversion of prothrombin to thrombin is undetectable using methods known in the art). Methods of detecting conversion of prothrombin to thrombin are known in the art and include SDS-PAGE gel, antibody-based assays, chromogenic assays, or blood clotting assays. In embodiments, the FXa inhibitor does not inhibit the activity of FXa to cleave non-thrombin substrates (e.g. S protein).

[0165] In embodiments, examples of known FXa inhibitors include, but are not limited to, edoxaban, fondaparinux, idraparinux, biotinylated idraparinux, enoxaparin, fragmin, NAP-5, rNAPc2, tissue factor pathway inhibitor, otamixaban, razaxaban (DPC906), and betrixaban. In embodiments, the FXa inhibitor is edoxaban, fondaparinux, idraparinux, biotinylated idraparinux, enoxaparin, fragmin, NAP-5, rNAPc2, tissue factor pathway inhibitor, otamixaban, razaxaban, or betrixaban. In embodiments, the FXa inhibitor is edoxaban. In embodiments, the FXa inhibitor is fondaparinux. In embodiments, the FXa inhibitor is idraparinux. In embodiments, the FXa inhibitor is biotinylated idraparinux. In embodiments, the FXa inhibitor is enoxaparin. In embodiments, the FXa inhibitor is fragmin. In embodiments, the FXa inhibitor is NAP-5. In embodiments, the FXa inhibitor is rNAPc2. In embodiments, the FXa inhibitor is tissue factor pathway inhibitor. In embodiments, the FXa inhibitor is otamixaban. In embodiments, the FXa inhibitor is oraxaban. In embodiments, the FXa inhibitor is razaxaban (DPC906). In embodiments, the FXa inhibitor is betrixaban. In embodiments, the factor Xa inhibitor is low molecular weight heparin ("LMWH"). In

embodiments, the FXa inhibitor is not a direct FXa inhibitor. In embodiments, the FXa inhibitor is an indirect FXa inhibitor.

[0166] A “direct FXa inhibitor” or “direct inhibitor of FXa” binds to FXa. For example, a direct inhibitor may bind to the active site of FXa. A direct FXa inhibitor may directly downregulate or decrease serine protease activity. An “indirect FXa inhibitor” or “indirect inhibitor of FXa” does not bind FXa. An indirect FXa inhibitor therefore does not directly inhibit serine protease activity. For example, an indirect FXa inhibitor downregulates or decreases FXa activity by modulating the activity of a molecule that interacts with FXa. In instances, an indirect FXa inhibitor binds a molecule that inhibits or decreases FXa activity or expression. In embodiments, an indirect FXa inhibitor may bind to antithrombin. In embodiments, an indirect inhibitor may bind to prothrombin, thereby inhibiting binding of FXa to prothrombin. In embodiments, the direct FXa inhibitor is rivaroxaban. In embodiments, the indirect FXa inhibitor is fondaparinux.

[0167] The term “fondaparinux” refers to a synthetic pentasaccharide factor Xa inhibitor which binds to antithrombin assists in its inhibition of factor Xa. Because fondaparinux influences the interaction of a molecule that interacts with FXa, it is an indirect inhibitor of FXa. The sequence of monosaccharides is D-GlcNS6S- α -(1,4)-D-GlcA- β -(1,4)-D-GlcNS3,6S- α -(1,4)-L-IdoA2S- α -(1,4)-D-GlcNS6S-OMe. In an embodiment, the structure of fondaparinux is represented as:



[0168] For the pharmaceutical compositions provided herein, in embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity to SEQ ID NO:3 and

a second peptide having at least 80% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a sequence at least 80% sequence identity SEQ ID NO:6.

[0169] For the pharmaceutical compositions provided herein, in embodiments, the FXa protein further includes an Fc domain. In embodiments, the Fc domain has at least 80% sequence identity to SEQ ID NO:7. For example, the FXa protein or functional portion thereof may be a fusion protein including FXa and the Fc domain. For example, the FXa protein may be a fusion protein including FXa and the Fc domain, wherein the C-terminus of FXa protein is attached to the N-terminus of a heavy chain of the Fc domain. In embodiments, the FXa protein is a fusion protein including FXa and the Fc domain, wherein the C-terminus of a first FXa protein is attached to the N-terminus of a first heavy chain of the Fc domain, and the C-terminus of a second FXa protein is attached to the N-terminus of the second heavy chain of the Fc domain. Attachment may be direct or indirect attachment. For example, the FXa protein may be attached to the Fc domain via a peptide linker (e.g. an amino acid sequence that links the Fc domain to the FXa protein). For example, a first FXa protein may be attached to a first heavy chain of the Fc domain through a first peptide linker and a second FXa protein may be attached to a second heavy chain of the Fc domain through a second peptide linker. In embodiments, the peptide linker includes from about 2 to about 100 amino acid residues in length. In embodiments, the peptide linker is about 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acid residues in length.

[0170] Peptide linkers may independently be from 2 amino acids to 500 amino acids in length. In embodiments, the peptide linkers are independently from 5 to 100 amino acids in length. In embodiments, the peptide linkers are independently from 5 to 50 amino acids in length. In embodiments, the peptide linkers are independently less than 500 amino acids in length. In embodiments, the peptide linkers are independently less than 250 amino acids in length. In embodiments, the peptide linkers are independently less than 100 amino acids in length. In embodiments, the peptide linkers are independently less than 90 amino acids in length. In embodiments, the peptide linkers are independently less than 80 amino acids in length. In embodiments, the peptide linkers are independently less than 70 amino acids in length. In embodiments, the peptide linkers are independently less than 60 amino acids in length. In embodiments, the peptide linkers are independently less than 50 amino acids in length. In embodiments, the peptide linkers are independently less than 40 amino acids in length. In embodiments, the peptide linkers are independently less than 30 amino acids in length. In embodiments, the peptide linkers are independently less than 25 amino acids in

length. In embodiments, the peptide linkers are independently less than 20 amino acids in length. In embodiments, the peptide linkers are independently less than 15 amino acids in length. In embodiments, the peptide linkers are independently less than 10 amino acids in length.

[0171] In embodiments, the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain. In embodiments, the Fc domain is an IgG Fc domain. In embodiments, the Fc domain is an IgA Fc domain. In embodiments, the Fc domain is an IgD Fc domain. In embodiments, the Fc domain is an IgM Fc domain. In embodiments, the Fc domain is an IgG, IgE Fc domain.

[0172] The compositions provided herein may include nucleic acids encoding the FXa protein or functional portion thereof as provided herein including embodiments thereof. Thus, provided herein are pharmaceutical compositions including a nucleic acid encoding the FXa protein or functional portion thereof. In embodiments, the nucleic acid forms part of a vector. In embodiments, the vector is an expression vector.

[0173] In embodiments, the composition provided herein including embodiments thereof are provided as a pulmonary pharmaceutical composition comprising a pulmonary pharmaceutical excipient. The terms “pulmonary pharmaceutical composition” and the like refer to pharmaceutical compositions intended for pulmonary administration (e.g. intranasal route, oro-nasal route). The terms “pulmonary administration” and the like refer, in the usual and customary sense, to administration to achieve inhalation therapy (e.g. intranasal route, oro-nasal route). The term “inhalation therapy” and the like refer to direct delivery of medications to the lungs by inhalation. In embodiments, the complexes provided herein including embodiments thereof are effective when delivered directly to the lung by an inhaled drug delivery system. The term “pulmonary pharmaceutical liquid” refers to a pulmonary pharmaceutical composition which is a liquid. The terms “pulmonary pharmaceutical solid,” “pulmonary pharmaceutical solid” and the like refer to a pulmonary pharmaceutical composition which is a solid (e.g., a powder).

[0174] In embodiments, the composition provided herein is provided in an inhaled drug delivery systems. In embodiments, the inhaled drug delivery system is a (i) nebulizer; (ii) a pressurized metered-dose inhaler (pMDI); or (iii) a dry powder inhaler (DPI). Nebulizers are distinctly different from both pMDIs and DPIs, in that the active agent is dissolved or suspended in a polar liquid, e.g., water. In contrast, pMDIs and DPIs are bolus drug delivery devices that contain active agent (e.g., nanoparticle complex), suspended or dissolved in a

nonpolar volatile propellant or in a dry powder mix that is fluidized when the patient inhales. pMDIs and DPIs have considerably reduced treatment time compared with nebulizers. The term “pulmonary pharmaceutical delivery device” and the like refer to an inhaled drug delivery system suitable for delivery (e.g., intranasal, oro-nasal delivery, etc.) of a pharmaceutical composition.

[0175] In embodiments, the composition is administered to the respiratory tract. In embodiments, the composition is administered to the lungs.

METHODS OF USE

[0176] The compositions (e.g. FXa protein, nucleic acid, etc.) provided herein including embodiments thereof are useful for treating and preventing coronavirus (e.g. SARS CoV-2) infection in a subject need thereof. For example, the compositions have been shown to be effective for preventing entry of the virus into a host cell. Without wishing to be bound by scientific theory, FXa has been found to cleave coronavirus S protein into fragments that are unable to bind to the host cell ACE2 receptor, thereby preventing entry of the virus into the cell. Thus, in an aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, wherein the method includes administering to the subject an effective amount of a Factor Xa (FXa) protein or functional portion thereof. Applicant has further discovered that the combination of FXa protein and anticoagulant have antiviral activity FXa. Thus, in embodiments, the method further includes administering an anticoagulant. In embodiments, the anticoagulant is not a direct inhibitor of FXa. For example, the anticoagulant does not directly inhibit FXa serine protease activity. For example, the anticoagulant may inhibit prothrombin to thrombin conversion by FXa, but may not inhibit the ability of FXa to cleave non-thrombin substrates (e.g. protein S). In embodiments, the anticoagulant is fondaparinux, heparin, or low molecular weight heparin. In embodiments, the anticoagulant is heparin. In embodiments, the anticoagulant is low molecular weight heparin. In embodiments, the anticoagulant is fondaparinux.

[0177] As described throughout the specification, classes of anticoagulants do not inhibit FXa antiviral activity. The compositions provided herein are therefore effective for treating or preventing coronavirus infection in a subject with a blood clotting disorder (e.g. thrombosis), is at risk of developing a blood clot (e.g. thrombophilia), or has a condition that may indirectly cause a blood clot (e.g. has a stent). Thus, in embodiments, the subject has or previously had an atrial fibrillation, coronary artery disease, deep vein thrombosis, ischemic

stroke, or a hypercoagulable state. In embodiments, the subject has or previously had atrial fibrillation. In embodiments, the subject has or previously had coronary artery disease. In embodiments, the subject has or previously had deep vein thrombosis. In embodiments, the subject has or previously had ischemic stroke. In embodiments, the subject has or previously had a hypercoagulable state. In embodiments, the hypercoagulable state is antiphospholipid syndrome, factor V Leiden (FVL) mutation, prothrombin gene G20210A mutations, elevated factor VIII, or hyperhomocysteinemia. In embodiments, the hypercoagulable state is antithrombin deficiency, protein C deficiency, or protein S deficiency. In embodiments, the subject has a mechanical heart valve. In embodiments, the subject has or previously had a myocardial infarction. In embodiments, the subject has or previously had a myocardial infarction pulmonary embolism. In embodiments, the subject has or previously had a myocardial infarction restenosis from stents. In embodiments, the subject has or previously had a myocardial infarction. In embodiments, the subject has or previously had heart failure.

[0178] In embodiments, treating COVID-19 includes treating one or more symptoms of COVID-19. In embodiments, the symptom is cough, shortness of breath or difficulty breathing, fever, chills, repeated shaking with chills, muscle pain, headache, sore throat, or new loss of taste or smell. In embodiments, the method includes treating respiratory symptoms. In embodiments, the method includes treating shortness of breath or difficulty breathing. In embodiments, the method includes treating fever. In embodiments, the method includes treating cough. In embodiments, the method includes treating fatigue. In embodiments, the method includes treating body aches. In embodiments, the method includes treating headache.

[0179] In embodiments, the subject is not hospitalized. In embodiments, the subject is hospitalized. In embodiments, the subject is in an intensive care unit.

[0180] In an aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, wherein the method includes administering to the subject an effective amount of a nucleic acid encoding an FXa protein or functional portion thereof as provided herein including embodiments thereof. In embodiments, the method further includes administering an anticoagulant. In embodiments, the anticoagulant is not a direct inhibitor of FXa. In embodiments, the anticoagulant is fondaparinux, heparin, or low molecular weight heparin. In embodiments, the anticoagulant is heparin. In embodiments, the the anticoagulant is low molecular weight heparin. In embodiments, the anticoagulant is fondaparinux.

[0181] The compositions provided therefore may be used to treat or prevent COVID-19 in subjects with low levels of FXa relative to a standard control. In an aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, including i) detecting a lower level of FXa in a sample obtained from the subject relative to a standard control, and ii) administering an effective amount of an FXa protein or functional portion thereof to the subject. In another aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, including: i) obtaining a sample from the subject, ii) detecting a lower level of Factor Xa (FXa) in the sample relative to a standard control, and iii) administering to the subject an effective amount of an FXa protein or functional portion thereof. In embodiments, a standard control is the level of FXa in a sample from a subject who has COVID-19. In embodiments, the standard control is the level of FXa in a sample from a subject who previously had COVID-19 and no longer has COVID-19. In embodiments, the standard control is from a subject who does not have COVID-19 (e.g. a healthy subject). In embodiments, a standard control is the level of FXa in a subject who does not have a blood clotting disorder or is not at risk of developing a blot clot.

[0182] The level of FXa in the sample may be at least about 5% lower, 10% lower, 20% lower, 30% lower, 40% lower, 50% lower, 60% lower, 70% lower, 80% lower, 90% lower, or 95% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 5% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 10% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 15% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 20% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 25% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 30% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 35% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 40% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 45% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 50% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 55% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 60% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 65% lower than the

level of FXa in the standard control. The level of FXa in the sample may be at least about 70% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 75% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 80% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 85% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 90% lower than the level of FXa in the standard control. The level of FXa in the sample may be at least about 95% lower than the level of FXa in the standard control. The level of FXa in a sample may be detected by any method known in the art, including antibody-based methods (e.g. immunohistochemistry) and flow cytometry etc.

[0183] In embodiments, the sample is blood. In embodiments, the sample is plasma.

[0184] As described throughout the specification, including the examples and figures, Applicant has discovered that classes of anticoagulants do not inhibit FXa antiviral activity. Thus, in embodiments, the method provided herein further includes administering an anticoagulant. In embodiments, the anticoagulant is not a direct inhibitor of FXa. For example, the anticoagulant does not directly inhibit FXa serine protease activity. For example, the anticoagulant may not directly bind to the active site of a serine protease enzyme (e.g. FXa). In embodiments, the anticoagulant is fondaparinux, heparin, or low molecular weight heparin. In embodiments, the anticoagulant is heparin. In embodiments, the anticoagulant is low molecular weight heparin. In embodiments, the anticoagulant is fondaparinux.

[0185] In embodiments, the anticoagulant and FXa protein are administered sequentially. For example, the anticoagulant may be administered prior to administration of the FXa protein. The anticoagulant may be administered after administration of the FXa protein. In embodiments, the anticoagulant and FXa protein are administered simultaneously (e.g. in a single unit dosage form).

[0186] For the methods provided herein, in embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a sequence at least 80% sequence identity SEQ ID NO:6.

[0187] For the methods provided herein, in embodiments, the FXa protein or functional portion thereof further includes an Fc domain. In embodiments, the Fc domain has at least 80% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain. In embodiments, the Fc domain is an IgG Fc domain. In embodiments, the Fc domain is an IgA Fc domain. In embodiments, the Fc domain is an IgD Fc domain. In embodiments, the Fc domain is an IgM Fc domain. In embodiments, the Fc domain is an IgG, IgE Fc domain.

[0188] Because FXa protein is known to induce blood coagulation, and may potentially cause inflammation, compositions provided herein may include an anticoagulant. The compositions are therefore contemplated to be useful for downregulating blood coagulation and/or inflammation while treating or preventing COVID-19 in subjects in need thereof. In an aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, wherein the method includes: i) detecting a higher level of FXa in a sample obtained from the subject relative to a standard control, and ii) administering an effective amount of an anticoagulant to the subject. In another aspect is provided a method of treating or preventing COVID-19 in a subject in need thereof, wherein the method includes: i) obtaining a sample from the subject, ii) detecting a higher level of FXa in the sample relative to a standard control, and iii) administering an effective amount of an anticoagulant. In embodiments, the sample is blood or plasma. In embodiments, the sample is blood. In embodiments, the sample is plasma.

[0189] In embodiments, a standard control is the level of FXa in a sample from a subject who has COVID-19. In embodiments, the standard control is the level of FXa in a sample from a subject who previously had COVID-19 and no longer has COVID-19. In embodiments, the standard control is from a subject who does not have COVID-19 (e.g. a healthy subject). In embodiments, a standard control is the level of FXa in a subject who does not have a blood clotting disorder or is not at risk of developing a blot clot. The level of FXa in the sample may be at least about 5% higher, 10% higher, 20% higher, 30% higher, 40% higher, 50% higher, 60% higher, 70% higher, 80% higher, 90% higher, or 95% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 5% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 10% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 15% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 20% higher than the level of FXa in the

standard control. The level of FXa in the sample may be at least about 25% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 30% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 35% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 40% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 45% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 50% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 55% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 60% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 65% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 70% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 75% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 80% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 85% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 90% higher than the level of FXa in the standard control. The level of FXa in the sample may be at least about 95% higher than the level of FXa in the standard control. The level of FXa in a sample may be detected by any method known in the art, including antibody-based methods (e.g. immunohistochemistry) and flow cytometry etc.

[0190] In embodiments, the anticoagulant is not a direct inhibitor of FXa. For example, the anticoagulant may not directly inhibit FXa serine protease activity. In embodiments, the anticoagulant is fondaparinux, heparin, or low molecular weight heparin. In embodiments, the anticoagulant is heparin. In embodiments, the anticoagulant is low molecular weight heparin. In embodiments, the anticoagulant is fondaparinux.

[0191] In embodiments, the method further includes administering to the subject FXa or a functional portion thereof. In embodiments, the anticoagulant and FXa or functional portion thereof are administered sequentially. In embodiments, the anticoagulant and FXa or functional portion thereof are administered simultaneously. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3 and a

second peptide having at least 80% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a sequence at least 80% sequence identity SEQ ID NO:6. In embodiments, the FXa protein further includes an Fc domain. In embodiments, the Fc domain has at least 80% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain. In embodiments, the Fc domain is an IgG Fc domain.

KITS

[0192] Provided herein are kits, including pharmaceutical kits, comprising the compositions provided herein including embodiments thereof. In an aspect is provided a kit including a: (i) a first dosage form including an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient; and (ii) a second dosage form including an anticoagulant and a pharmaceutically acceptable excipient. A “first dosage form” as provided herein refers to a discrete composition of an FXa protein or functional portion thereof and is separate from other dosage forms (e.g., the second dosage form of the anticoagulant). In embodiments, the first dosage form does not include any other active agents. In embodiments, the first dosage form does not include any other therapeutic agent. Likewise, a “second dosage form” as provided herein refers to a discrete composition of the anticoagulant and is separate from other dosage forms (e.g., the first dosage form of the FXa protein or functional portion thereof). In embodiments, the second dosage form does not include any other active agents. In embodiments, the second dosage form does not include any other therapeutic agent.

[0193] In embodiments, the anticoagulant is not a direct FXa inhibitor. In embodiments, the anticoagulant is fondaparinux, heparin, or low molecular weight heparin. In embodiments, the anticoagulant is fondaparinux. In embodiments, the anticoagulant is heparin. In embodiments, the anticoagulant is low molecular weight heparin.

[0194] In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5. In embodiments, the FXa protein includes a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4. In embodiments, the FXa protein includes a sequence at least 80% sequence identity SEQ ID NO:6. In embodiments, the FXa protein or functional portion thereof further includes an Fc domain. In embodiments, the Fc domain has at least 80% sequence identity to SEQ ID NO:7. In embodiments, the Fc domain is an IgG, IgA, IgD, IgM

or IgE Fc domain. In embodiments, the Fc domain is an IgG Fc domain.

[0195] In embodiments, the first or second dosage form includes more than one active agent. In embodiments, the active agent is a different functional portion of FXa protein. In embodiments, the active agent is a different anticoagulant. Thus, the first dosage form may include multiple functional portions of FXa protein. Thus, the second dosage form may include multiple anticoagulants.

[0196] In embodiments, the first dosage form and the second dosage are in separate containers. In embodiments, the kit further includes instructions for treatment of COVID-19 or thrombosis. In embodiments, the kit further includes instructions for treatment of COVID-19. In embodiments, the kit further includes instructions for treatment of thrombosis.

[0197] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

P EMBODIMENTS

[0198] P Embodiment 1. A method of preventing or treating COVID-19 in a subject in need thereof, wherein the method comprises administering to the subject an effective amount of an FXa protein or functional portion thereof.

[0199] P Embodiment 2. The method of P Embodiment 1, wherein the method further comprises administering an anticoagulant, wherein said anticoagulant does not directly inhibit FXa serine protease activity.

[0200] P Embodiment 3. The method of P Embodiment 2, wherein the anticoagulant is fondaparinux.

[0201] P Embodiment 4. The method of any of P Embodiments 1-3, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0202] P Embodiment 5. A method of preventing or treating COVID-19 in a subject in need thereof, wherein the method comprises: i) obtaining a sample from the subject, ii) detecting a level of FXa in said sample lower than a standard control, and iii) administering

an effective amount of an FXa protein or functional portion thereof to the subject.

[0203] P Embodiment 6. The method of P Embodiment 5, wherein the sample is a blood or plasma sample.

[0204] P Embodiment 7. The method of P Embodiment 5 or 6, wherein the method further comprises administering an anticoagulant, wherein said anticoagulant does not directly inhibit FXa serine protease activity.

[0205] P Embodiment 8. The method of P Embodiment 7, wherein the anticoagulant is fondaparinux.

[0206] P Embodiment 9. The method protein of any of P Embodiments 5-8, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0207] P Embodiment 10. A method of preventing or treating COVID-19 in a subject in need thereof, wherein the method comprises: i) obtaining a sample from the subject, ii) detecting a level of FXa in said sample higher than a standard control, and iii) administering an effective amount of an anticoagulant, wherein said anticoagulant does not directly inhibit FXa serine protease activity.

[0208] P Embodiment 11. The method of P Embodiment 10, wherein the sample is a blood or plasma sample.

[0209] P Embodiment 12. The method of P Embodiment 10 or 11, wherein the anticoagulant is fondaparinux.

[0210] P Embodiment 13. A pharmaceutical composition comprising an effective amount of an FXa protein or functional portion thereof and an effective amount of an anticoagulant in a single dosage form, wherein said anticoagulant does not directly inhibit FXa serine protease activity.

[0211] P Embodiment 14. The pharmaceutical composition of P Embodiment 13, wherein the anticoagulant is fondaparinux.

[0212] P Embodiment 15. The pharmaceutical composition of P Embodiment 13 or 14, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0213] P Embodiment 16. A kit comprising an effective amount of an FXa protein or functional portion thereof in a first dosage form and an effective amount of an anticoagulant

in a second dosage form, wherein said anticoagulant does not directly inhibit FXa serine protease activity.

[0214] P Embodiment 17. The kit of P Embodiment 16, wherein the anticoagulant is fondaparinux.

[0215] P Embodiment 18. P Embodiment 16 or 17, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

EMBODIMENTS

[0216] Embodiment. 1. A method of treating or preventing COVID-19 in a subject in need thereof, wherein the method comprises administering to the subject an effective amount of a Factor Xa (FXa) protein or functional portion thereof.

[0217] Embodiment. 2. The method of Embodiment 1, wherein the method further comprises administering an anticoagulant

[0218] Embodiment. 3. The method of Embodiment 2, wherein the anticoagulant is not a direct inhibitor of FXa.

[0219] Embodiment. 4. The method of Embodiment 2 or 3, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin

[0220] Embodiment. 5. The method of Embodiment 4, wherein the anticoagulant is fondaparinux.

[0221] Embodiment. 6. The method of any one of Embodiments 1-5, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.

[0222] Embodiment. 7. The method of any one of Embodiments 1-5, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

[0223] Embodiment. 8. The method of any one of Embodiments 1-5, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.

[0224] Embodiment. 9. The method of any of Embodiments 1-8, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0225] Embodiment. 10. The method of Embodiment 9, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0226] Embodiment. 11. The method of Embodiment 9, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

[0227] Embodiment. 12. The method of Embodiment 11, wherein the Fc domain is an IgG Fc domain.

[0228] Embodiment 13. A method of treating or preventing COVID-19 in a subject in need thereof, comprising: i) obtaining a sample from the subject, ii) detecting a lower level of Factor Xa (FXa) in the sample relative to a standard control, and iii) administering to the subject an effective amount of an FXa protein or functional portion thereof.

[0229] Embodiment. 14. The method of Embodiment 13, wherein the sample is blood or plasma.

[0230] Embodiment. 15. The method of Embodiment 13 or 14, wherein the method further comprises administering an anticoagulant.

[0231] Embodiment. 16. The method of Embodiment 15, wherein the anticoagulant is not a direct inhibitor of FXa

[0232] Embodiment. 17. The method of Embodiment 15 or 16, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin

[0233] Embodiment. 18. The method of Embodiment 17, wherein the anticoagulant is fondaparinux.

[0234] Embodiment. 19. The method of any of Embodiments 15-18, wherein the anticoagulant and FXa or functional portion thereof are administered sequentially.

[0235] Embodiment. 20. The method of any of Embodiments 15-18, wherein the anticoagulant and FXa or functional portion thereof are administered simultaneously.

[0236] Embodiment 21. The method of any one of Embodiments 13-20, wherein the FXa protein comprises a sequence having at least 80% sequence identity to SEQ ID NO:6.

[0237] Embodiment 22. The method of any one of Embodiments 13-20, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

[0238] Embodiment 23. The method of any one of Embodiments 13-20, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.

[0239] Embodiment. 24. The method of any of Embodiments 15-23, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0240] Embodiment. 25. The method of Embodiment 24, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.

[0241] Embodiment. 26. The method of Embodiment 24, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

[0242] Embodiment. 27. The method of Embodiment 26, wherein the Fc domain is an IgG Fc domain.

[0243] Embodiment. 28. A method of treating or preventing COVID-19 in a subject in need thereof, wherein the method comprises: i) obtaining a sample from the subject, ii) detecting a higher level of FXa in the sample relative to a standard control, and iii) administering an effective amount of an anticoagulant.

[0244] Embodiment. 29. The method of Embodiment 28, wherein the sample is blood or plasma.

[0245] Embodiment. 30. The method of Embodiment 28 or 29, wherein the anticoagulant is not a direct inhibitor of FXa.

[0246] Embodiment. 31. The method of any of Embodiments 28-30, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin.

[0247] Embodiment. 32. The method of any of Embodiments 28-31, wherein the anticoagulant is fondaparinux.

[0248] Embodiment. 33. The method of any of Embodiments 28-32, further comprising administering to the subject FXa or a functional portion thereof.

[0249] Embodiment. 34. The method of Embodiment 33, wherein the anticoagulant and FXa or functional portion thereof are administered sequentially.

[0250] Embodiment. 35. The method of Embodiment 33, wherein the anticoagulant and FXa or functional portion thereof are administered simultaneously.

[0251] Embodiment. 36. The method of any one of Embodiments 33-35, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.

[0252] Embodiment. 37. The method of any one of Embodiments 33-35, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

[0253] Embodiment. 38. The method of any one of Embodiments 33-35, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5

[0254] Embodiment. 39. The method of any of Embodiments 33-38, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0255] Embodiment. 40. The method of Embodiment 39, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.

[0256] Embodiment. 41. The method of Embodiment 39, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

[0257] Embodiment. 42. The method of Embodiment 41, wherein the Fc domain is an IgG Fc domain.

[0258] Embodiment. 43. A pharmaceutical composition comprising an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient.

[0259] Embodiment. 44. The pharmaceutical composition of Embodiment 43, further comprising an anticoagulant

[0260] Embodiment. 45. The pharmaceutical composition of Embodiment 44, wherein the anticoagulant is not a direct inhibitor of FXa.

[0261] Embodiment. 46. The pharmaceutical composition of Embodiment 44 or 45, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin

[0262] Embodiment. 47. The pharmaceutical composition of any of Embodiments 44-46, wherein the anticoagulant is fondaparinux.

[0263] Embodiment. 48. The pharmaceutical composition of any of Embodiments 43-47, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.

[0264] Embodiment. 49. The pharmaceutical composition of any of Embodiments 43-47, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

[0265] Embodiment. 50. The pharmaceutical composition of any of Embodiments 43-47, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.

[0266] Embodiment. 51. The pharmaceutical composition of any of Embodiments 43-50, wherein the FXa protein or functional portion thereof further comprises an Fc domain

[0267] Embodiment. 52. The pharmaceutical composition of Embodiment 51, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.

[0268] Embodiment. 53. The pharmaceutical composition of Embodiment 51, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

[0269] Embodiment. 54. The pharmaceutical composition Embodiment 53, wherein the Fc domain is an IgG Fc domain.

[0270] Embodiment. 55. A kit comprising a: (i) a first dosage form comprising an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient; and (ii) a second dosage form comprising an anticoagulant and a pharmaceutically acceptable excipient

[0271] Embodiment. 56. The kit of Embodiment 55, wherein the anticoagulant is not a direct FXa inhibitor.

[0272] Embodiment. 57. The kit of Embodiment 55 or 56, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin.

[0273] Embodiment. 58. The kit of any of Embodiments 55-57, wherein the anticoagulant is fondaparinux.

[0274] Embodiment. 59. The kit of any of any of Embodiments 55-58, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.

[0275] Embodiment. 60. The kit of any of any of Embodiments 55-58, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

[0276] Embodiment. 61. The kit of any of any of Embodiments 55-58, wherein the FXa

protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5

[0277] Embodiment. 62. The kit of any of Embodiments 55-61, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

[0278] Embodiment. 63. The kit of any of Embodiment 62, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.

[0279] Embodiment. 64. The kit of Embodiment 62, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

[0280] Embodiment. 65. The kit of Embodiment 64, wherein the Fc domain is an IgG Fc domain.

EXAMPLES

Example 1: Introduction to Exemplary Experiments

[0281] SARS-CoV-2 is the pathogen responsible for the global COVID-19 pandemic(1). To date, nearly 425,000,000 cases and 6,000,000 deaths have been recorded, with a worldwide mortality rate of 2%(2). Not surprisingly, the public health and economic consequences have been devastating. Although some strategies such as FDA-approved vaccines and oral drugs have been effective in the clinic thus far, the pandemic rages on, resulting in persistent concerns regarding the emergence of new variants(3-5).

[0282] Angiotensin-converting enzyme 2 (ACE2) is the host receptor for SARS-CoV-2(6, 7), which uses its spike (S) protein to bind to ACE2 and enter host cells. Cleavage of S protein to S1 and S2 subunits and then to S2' is essential to initiate the membrane-fusion process(8). For this purpose, the virus solicits the help of several host serine proteases (SPs)(9, 10). Furin cuts S protein at the PRRAR (R-R-A-R685↓) site into the S1 and S2 subunits at virus budding, while TMPRSS2 cleaves S protein at the S2' site (P-S-K-R815↓) at viral entry; therefore, both cleavages are essential for SARS-CoV-2 infection (8, 9, 11, 12).

[0283] Another SP family member, activated coagulation factor X (FXa), binds to tissue factor to initiate the conversion of prothrombin to thrombin in the clotting cascade(13). Direct FXa inhibitors (rivaroxaban, apixaban, edoxaban, and betrixaban) as well as an indirect inhibitor (fondaparinux) have been developed as clinical anticoagulants(14), and several

direct inhibitors are currently being evaluated for use in patients at high-risk for COVID-19(15).

[0284] Applicant demonstrates herein that FXa inhibits the entry of SARS-CoV-2 into cells. Mechanistically, FXa binds to and cleaves S protein, but with a different cleavage pattern than that produced by furin and then TMPRSS2, and blocks S protein binding to ACE2. This inhibition of infectivity by FXa was found both *in vitro* and *in vivo* and was most pronounced with the ancestral wild-type SARS-CoV-2 virus but was diminished in variants such as B.1.1.7 and Delta that harbor the D614G mutation. Loss of endogenous FXa resulted in increased viral copy numbers following infection *in vivo*. Exogenous administration of FXa protected mice from lethal infection in a humanized hACE2 mouse model of COVID-19 when the wild-type SARS-CoV-2 was used, but not the B.1.1.7 variant. The antiviral effect of FXa was attenuated by the direct FXa inhibitor rivaroxaban (RIVA) but not the indirect inhibitor fondaparinux (FONDA) both *in vivo* and *in vitro*.

Example 2: FXa blocks viral infection

[0285] To identify changes in serine proteases (SPs) during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, coronavirus disease 2019 (COVID-19) patients' expression was examined using an immunohistochemistry assay (IHC). Due to the lack of specific antibodies directly against FXa, FX expression was instead quantified, because ~100% of FX can be activated to FXa at injury sites when platelets are exposed to both collagen and thrombin. IHC analysis indicated that thrombin was highly expressed in lungs from COVID-19 patients compared to that from patients without COVID-19, as also previously reported. Further, Factor Xa (FXa) was significantly increased in the lungs of COVID-19 patients compared to healthy donors, while consistent upregulation of other tested SPs was not observed (FIG. 6A and FIG. 1A). FXa expression in the liver and serum was tested and found that FXa expression had also increased in the liver (FIG. 6B) and serum (FIG. 1B and FIG. 1C) of COVID-19 patients compared to healthy donors. The correlation between the expression of FXa and that of spike (S) protein in COVID-19 patients was analyzed. Expression of FXa and S protein show a similar trend.

[0286] To investigate the increase in FXa during SARS-CoV-2 infection, FXa was cloned into the pCDH-mCherry vector and its function was assessed using using a chimera of SARS-CoV-2 and the vesicular stomatitis virus, (VSV)-SARS-CoV-2 (23). 293T cells were co-transfected with Angiotensin-converting enzyme 2 (ACE2) and FXa or control empty vector

(EV). Cells were infected by vesicular stomatitis virus- severe acute respiratory syndrome coronavirus 2 (VSV-SARS-CoV-2) 24 hours later. The percentage of infected cells (Green fluorescent protein (GFP positive cells)) was examined at the indicated time points using flow cytometry. Surprisingly, the group transfected with the FXa expression plasmid had a significantly lower percentage of infected cells than the group transfected with EV. Thus, the presence of FXa efficiently blocked viral infection (FIG. 1D). SARS-CoV-2 infection depends not only on ACE2 but also Transmembrane serine protease 2 (TMPRSS2). When 293T cells were co-transfected with FXa, ACE2 and TMPRSS2, FXa again blocked viral infection, with stronger effect during the early timepoints in the presence of TMPRSS2 (FIG. 1E). The MA104 epithelial lung cell line stably expressing FXa (MA104-FXa) was generated (FIG. 7A). The cells were infected by VSV-SARS-CoV-2 at different MOIs and the infectivity was determined at 16, 24, 36 and 48 hours post infection (hpi). MA104-FXa cells showed markedly decreased infection at each time point and at various MOIs compared to MA104-EV control cells (FIG. 1F and FIG. 7B). The titer of the supernatant from the viral infected MA104-FXa cells at 24 and 48 hpi was significantly decreased compared to that from MA104-EV cells, suggesting over-expressing FXa also impaired viral production (FIG. 7C and FIG. 7D). The role of FXa and other SPs in virus infection was also compared. Unlike furin, TMPRSS2 and trypsin which increased VSV-SARS-CoV-2 infection, FXa pre-treatment inhibited viral infection (FIG. 8A and FIG. 8B). The titer of the supernatant from the viral infected MA104-FXa cells was again significantly decreased compared to those treated with furin, TMPRSS2 or trypsin or any of the other SPs (FIG. 8C). Consistent with this, FXa significantly decreased the viral titer in supernatant from MA104 cells infected with VSV-SARS-CoV-2, while furin, TMPRSS2, or trypsin increased the viral titer compared to vehicle control (PBS). The role of FXa on viral infection was also explored when the SARS-CoV-2 was bound to cell receptors. For this purpose, VSV-SARS-CoV-2 virus was added to MA104 cells first. One hour later, the media with free VSV-SARS-CoV-2 virus was removed, and cells with bound virus were washed twice. Then the cells with bound virus were treated with FXa or PBS (control). Data showed that compared to PBS, FXa treatment still inhibited viral infection when SARS-CoV-2 was already bound to cell receptors.

[0287] Furthermore, to determine if FXa blocks viral infection by targeting the SARS-CoV-2 S protein, the FXa-Fc fusion protein expression plasmid was constructed and the FXa-Fc fusion protein purified from Chinese hamster ovary (CHO) cells. Further, the VSV-SARS-CoV-2 was co-incubated with or without FXa-Fc fusion protein *in vitro* for 1 hour

before adding the mixture into MA104 cells. The rate of infection was examined at the indicated timepoints. Pre-incubation of VSV-SARS-CoV-2 with FXa-Fc fusion protein significantly inhibited viral infection in a dose-dependent manner, suggesting that FXa could block viral infection by targeting viral proteins (FIG. 1G and FIG. 1H). This suggests that FXa blocked viral infection by targeting SARS-CoV-2. Of note, inactivated FXa had no effect on viral infection. To confirm the effects of FXa on viral infectivity, MA104 cells were infected with VSV-SARS-CoV-2 at a very low MOI (0.001) in the presence or absence of purified FXa protein at the indicated concentrations. The viral infectivity was found to have decreased in a dose-dependent manner as the concentration of FXa increased, suggesting that FXa plays an essential role in inhibiting viral infectivity.

[0288] To assess whether endogenous or natural FXa in human peripheral blood exhibits antiviral activity against the SARS-CoV-2 coronavirus, FX was first converted into its active form, FXa, in plasma from healthy donors. Next, MA104 cells were infected with VSV-SARS-CoV-2 chimeric virus that had been pre-treated with human plasma unconverted or converted from FX to FXa. The converted plasma significantly decreased infection with the chimeric VSV-SARS-CoV-2 virus. The infection experiment was also performed with the tissue factor (TF)-FVIIa-FXa complex and used the VSV-SARS-CoV-2 chimera. The TF-FVIIa-FXa complex inhibited infection by the chimeric virus compared to PBS, FVIIa, and TF controls, indicating that FXa in a natural complex can also reduce viral infection.

[0289] To determine the effects of FXa on viral production, the MA104 cells were infected with VSV-SARS-CoV-2 at a very low Multiplicity of infection (MOI) (0.001) and with FXa protein at the indicated concentrations. The viral titer showed a dose-dependent decrease with the increase of FXa protein, suggesting that FXa plays an essential role in inhibiting viral production (FIG. 9A). To examine whether FXa also inhibits viral infection through interaction with host proteins (such as ACE2 or TMPRSS2), FXa-Fc fusion protein with MA104 cells for 1 hour was preincubated and then washed out the medium before infection. FXa-Fc fusion protein pre-treatment with the MA104 cells did not significantly affect viral infection (FIG. 9B and FIG. 9C). SARS-CoV-2 was used next to infect MA104-EV and MA104-FXa cells followed by quantitative assessment of viral load by an immune-plaque assay. The infected MA104-FXa cells showed significantly fewer plaques compared to MA104-EV cells (FIG. 1I). Moreover, pre-incubation FXa with live SARS-CoV-2 prior to infection also significantly reduced viral infection in MA104 cells, consistent with the above VSV-SARS-CoV-2 pseudovirus data (FIG. 1J). Results showed that FXa, a serine protease

that is upregulated following SARS-CoV-2 infection in host cells, inhibited viral infection and thus possessed an anti-viral activity, in distinct contrast to other serine proteases such as furin and TMPRSS2.

[0290] Next live SARS-CoV-2 virus was used to infect Vero E6 and MA104 cells followed by quantitative assessment of viral load using an immuno-plaque assay. Pre-incubation of FXa with live SARS-CoV-2 prior to infection was found to significantly reduce viral infection in both Vero E6 and MA104 cells compared to the buffer control, consistent with the above data from the chimeric VSV-SARS-CoV-2 virus. Given that lung cells are more physiologically relevant to COVID-19 than Vero E6 and MA104 cells, the A549 lung cell line expressing ACE2 (A549-ACE2) was infected with live SARS-CoV-2 while in the presence or absence of FXa. Concomitant treatment of FXa and live SARS-CoV-2 in A549-ACE2 cells gave results that were similar to those obtained when live SARS-CoV-2 was pre-treated with FXa before attempting to infect A549-ACE2 cells. Collectively, results show that FXa, a SP that is upregulated following SARS-CoV-2 infection in host cells, inhibits SARS-CoV-2 infection and thus possesses an anti-viral activity, in distinct contrast to other SPs such as furin, trypsin, and TMPRSS2.

[0291] To study the mechanism(s) of the anti-viral activity of FXa, the binding between FXa and different subunits of SARS-CoV-2 S protein was compared. FXa has the strongest binding affinity toward the full-length S protein and to a lesser extent to subunit S1, subunit S2 and receptor binding domain (RBD) compared to the control Fc protein (FIG. 2A). Pull-down assays showed that FXa but not the Fc control co-precipitated with S protein (FIG. 2B). The binding affinity of FXa and the full-length S protein was measured. The results showed that the binding affinity of FXa to S protein is in the nanogram range and was dose-dependent (FIG. 2C). The binding affinity between active or heat-inactivated FXa with S protein or with VSV-SARS-CoV-2 was measured. Data show there is no difference in binding when comparing active FXa to heat-inactivated FXa. These results suggest that FXa binds to the S protein, which might inhibit viral entry efficiency.

[0292] Virus entry is followed by important conformational changes of viral proteins via cleavage of the S protein by host SPs. To determine whether FXa could cleave S protein, full-length S protein was incubated with FXa, followed by immunoblotting. Furin and TMPRSS2 serve as positive controls, as they are known to induce functional conformational changes of S protein. Full-length S was cut into three fragments by FXa with the size of approximately

60 Kilodalton (KD), then 50 KD and 29 KD (FIG. 2D), consistent with *in silico* prediction of two FXa cleavage sites on S protein, Ile-(Asp/Glu)-Gly-Arg (R1000) and Gly-Arg (R567) (FIG. 2E). This is in contrast to Furin and TMPRSS2, which cut the full-length S protein into the ~80 KD subunit S1. Cleavage by FXa did not produce the ~80 KD subunit S1.

[0293] A cleavage assay of the native S protein on virus particles by FXa was performed. For this purpose, VSV-SARS-CoV-2 chimeric viral particles were incubated with FXa. Furin was included as control, which assumably cleaved the VSV-SARS-CoV-2 virus into S1 and S2 fragments. The immunoblotting assay data showed that VSV-SARS-CoV-2 virus was cleaved into three fragments by FXa with the sizes of approximately 75 kD, 50 kD, and 29 kD. These fragments resembled those detected in the cleavage assay using full-length S protein. That cleavage pattern is consistent with the *in silico* prediction of two FXa cleavage sites on S protein: Gly-Arg (R567) and Ile-(Asp/Glu)-Gly-Arg (R1000). Of note, the appearance of a 75kD fragment, instead of a 60 kD one, in the cleavage assay of VSV-SARS-CoV-2 virus could have resulted from glycosylation of S at its N-terminal, as S proteins on native viral particles should be glycosylated trimers(20). For the furin control, as expected, the cleavage of viral particles by it generated an S1 band.

[0294] The shedding experiment was performed with S protein to clarify the mechanism underlying FXa-mediated inhibition of SARS-CoV-2 entry. For this purpose, A549 cells were transduced with a pCDH lentiviral vector expressing S protein, which are referred to as A549-S cells. GFP was co-expressed with S protein for FACS-sorting to purify transduced cells. 25 nM or 1 μ M FXa was used to treat the cells in PBS for 12 hours. After FXa treatment, the supernatants from each group were collected to measure S protein by ELISA with anti-RBD antibody and immunoblotting assay with anti-S antibody. The expression of S protein on the surface of A549-S cells was detected by FACS. GFP was detected as a control, as it cannot be cleaved by FXa. As expected, data showed that, compared to the untreated control group, FXa treatment decreased S protein on the A549-S cell surface while increasing S protein concentration in supernatants, both in a dose-dependent manner. The immunoblotting assay result of the supernatant showed that the 60 kD and 50 kD fragments existed but without 29 kD fragment. The reason may be that R1000 cleavage site is near the transmembrane domain of S protein (21), which may result in the remaining of the 29 kD fragment on the cell surface.

[0295] Since FXa could cut S protein into different sizes, the cleavage of FXa was tested for any effect on the binding ability of S protein to ACE2. Enzyme-linked immunosorbent assay (ELISA) data indicated that S protein pre-treated with FXa resulted in decreased binding affinity to ACE2 (FIG. 3A). Flow cytometry further confirmed this result, as FXa-pre-treated S protein could not efficiently bind to ACE2 expressing HEK293T cells (FIG. 3B and FIG. 3C). FXa still bound to S protein when S protein was already bound to ACE2 (FIGS. 3D-3F), yet FXa still cleaved the S protein bound to ACE2 (FIG. 10). Overall, results indicated that FXa could be an efficient inhibitor of viral entry.

[0296] An emergent SARS-CoV-2 strain was found to substitute aspartic acid-614 for glycine (D614G) in the S protein (24). Further, FXa having similar functional interactions with the D614G S protein was tested. FXa could still bind with and cleave the D614G S protein (FIGS. 11A-11C). Furthermore, the binding affinity between ACE2 and D614G S protein decreased if S protein was pretreated with FXa (FIG. 11D and FIG. 11E).

[0297] COVID-19 patients with increased risk of thrombosis were treated with direct FXa inhibitors (rivaroxaban) or indirect inhibitors (fondaparinux) (25, 26). Applicant therefore asked if rivaroxaban (RIVA) or fondaparinux (FONDA) affect the anti-viral activity of FXa. Neither rivaroxaban nor fondaparinux blocked the binding of FXa to S protein (FIG. 3G), and neither drug alone had any effect on VSV-SARS-CoV-2 infectivity, however, the direct FXa inhibitor rivaroxaban blocked FXa-induced antiviral activity, while the indirect FXa inhibitor fondaparinux did not (FIG. 3H and FIG. 3I). Measurement of viral production in the supernatants collected from this inhibitor experiment validated that rivaroxaban rather than fondaparinux affected FXa-induced antiviral activity (FIG. 12A). Furthermore, cleavage assay showed that the direct FXa inhibitor rivaroxaban, but not the indirect inhibitor fondaparinux, inhibited cleavage of S protein by FXa (FIG. 3J). Consistent with this, pretreatment of the mixture of S protein and FXa with rivaroxaban or fondaparinux, followed by incubation with ACE2, showed that rivaroxaban, but not fondaparinux, diminished the effect of FXa on inhibiting the binding of S protein to ACE2, presumably by inhibiting cleavage of S protein by rivaroxaban, but not by fondaparinux (FIG. 3K). ELISA results in were validated by flow cytometry analysis (FIG. 3L and FIG. 12B). Data indicate that the direct, rather than indirect, inhibitor of FXa could diminish FXa-mediated blockade of viral entry by inhibiting the cleavage of S protein by FXa so that intact S protein can efficiently bind to ACE2. Repetition of these experiment with live SARS-CoV-2 and MA104 cells demonstrated identical results (FIG. 3M and FIG. 12C).

[0298] To evaluate the potential effect of FXa in vivo, a humanized K18-hACE2 SARS-CoV-2 mice was inoculated with 3×10^5 plaque-forming units (pfu) SARS-CoV-2, followed by intranasal administration of the FXa-Fc protein or two controls, saline and the Fc protein. Body weight of the mice were monitored. The majority of mice in the untreated and Fc-treated groups dramatically decreased their body weight at day 5 and were euthanized at that time or shortly thereafter, while three of the five mice treated with FXa-Fc began recovery of their body weight at day 7 (FIG. 4A). The FXa-Fc treated group lived significantly longer than the two control groups, with no difference between the control groups (FIG. 4B).

[0299] To measure copy numbers in trachea, lung, and brain tissue, RNA was isolated and used quantitative real-time PCR. The viral load of trachea, lung, and brain in the FXa-Fc treated group was approximately 1,000-fold lower than that of the two control groups, indicating that FXa-Fc could significantly restrict SARS-CoV-2 infection in vivo (FIGS. 4C-E). Viral nucleocapsid protein (NP) also showed a marked decrease in the FXa-Fc treated group compared to the untreated and Fc-treated group (FIG. 4F). The histological study showed that FXa-Fc-treated mice had more intact lung structure and less pathological damage compared to the two control groups (FIG. 4G). Mice were also treated with 100-fold less FXa per mouse, i.e., 2 μ g per mouse, using vehicle as a negative control. 2 μ g FXa treatment still showed a protection ratio 33.3% at day 15 post treatment while all mice died by day 7 in the vehicle control group. A protective role was observed at a 0.2 μ g dose per mouse (data not shown). Of note, these lower doses are close to the physiological dose level in humans (16).

[0300] To evaluate the effects of the two types of FXa inhibitors, the direct inhibitor rivaroxaban was orally administered into FXa-treated (intranasally) SARS-CoV-2-infected mice, while the indirect inhibitor fondaparinux was administered via intraperitoneal injection (i.p), each following FDA-approved clinical-use guidelines (27). Consistent with the in vitro data, direct FXa inhibitor rivaroxaban significantly blocked the antiviral and survival advantage afforded by intranasal administration of FXa, while the indirect FXa inhibitor fondaparinux had no significant effect on the antiviral and survival advantage afforded by intranasal administration of FXa alone (FIGS. 5A-E). These in vivo results with live SARS-CoV-2 provided preclinical support for the use of an indirect FXa inhibitor such as fondaparinux as an anti-coagulant when preventing or treating thrombotic complications of COVID-19, while avoided the use of a direct FXa inhibitor such as the anti-coagulant rivaroxaban under similar clinical circumstances.

[0301] The effect of low-dose FXa in protecting mice from SARS-CoV-2 infection, as described above, prompted Applicant to investigate the potential endogenous role of FXa on SARS-CoV-2 infection, using a knock-out approach. For this purpose, FXa knockout mice were generated by CRISPR/Cas9 gene-editing technology. Mice were crossed with K18-hACE2 mice and obtained the FXa (heterozygote)-K18-hACE2 strain. FXa was knockdown successfully in the heterozygotes, confirmed by qPCR and immunoblotting assay, while FXa homozygote knockouts are embryonic lethal. To clarify the endogenous FXa function, FXa (heterozygote)-K18-hACE2 mice and K18-hACE2 mice (control) were inoculated with SARS-CoV-2, followed by measuring viral copy numbers of lung tissues. The results showed that the viral copy numbers in lungs of FXa (heterozygote)-K18-hACE2 were significantly increased compared to the K18-hACE2 control group, indicating that the endogenous FXa also showed anti-SARS-CoV-2 infection. These data, together with the infection inhibition of converted FXa from human plasma, substantiate the endogenous anti-SARS-CoV-2 role of FXa.

[0302] To evaluate the direct FXa inhibitor RIVA and the indirect FXa inhibitor FONDA *in vivo*, RIVA and FONDA were administered intranasally into SARS-CoV-2-infected mice treated with FXa. Consistent with the *in vitro* data, the direct FXa inhibitor RIVA significantly blocked the anti-viral and survival advantage afforded by intranasal administration of FXa-Fc. In contrast, the indirect FXa inhibitor FONDA had no significant effect on the anti-viral and survival advantage afforded by intranasal administration of FXa-Fc alone. The NP IHC data and histological study also showed that RIVA abolished FXa's anti-viral infection function while FONDA had no such an effect. In the absence of a prospective, double blind randomized clinical trial comparing the direct and indirect inhibitors of FXa in COVID-19 patients, these *in vivo* results with live SARS-CoV-2 provide preclinical support for using an indirect FXa inhibitor such as FONDA as an anti-coagulant when attempting to prevent or treat thrombotic complications of COVID-19. They also suggest that a direct FXa inhibitor, such as the anti-coagulant RIVA, should be avoided under such clinical circumstances as it would likely negate any beneficial effects of FXa.

[0303] The B.1.1.7 SARS-CoV-2 variant, Alpha, which emerged in the United Kingdom in September 2020, has many mutations associated with increased transmissibility and higher risk of death(25, 26). To evaluate FXa's effectiveness against that variant, the A549-ACE2 cells were infected with the original emergent SARS-CoV-2 (WA1; wild-type, WT) or the B.1.1.7 variant that had been pre-treated or concomitantly treated of FXa. The immuno-

plaque results showed that FXa was less efficient in inhibiting infection by the B.1.17 variant compared to WT virus. Furthermore, the results were confirmed by viral infection at various MOIs, which showed that FXa could still block WT infection even at a very high MOI (MOI=8) but had little effect on B.1.1.7 infection blockade at the same MOI.

[0304] The effects of the direct (RIVA) and indirect (FONDA) FXa inhibitors were tested on the B.1.1.7 SARS-CoV-2 variant using the WT strain as control. RIVA efficiently blocked the antiviral effect of FXa in a dose-dependent manner, starting as low as 0.05 µg/ml dose, while FONDA did not even at the high dose of 50 µg/ml, against the B.1.1.7 variant infection in both Vero E6 and MA104 cells. These data were validated in similar experiments with dose concentration gradients of FXa in both Vero E6 and MA104 host cells.

[0305] The anti-viral effect of FXa against the WT and B.1.1.7 SARS-CoV-2 variant *in vivo* was compared. Consistent with the *in vitro* data, the anti-viral and survival advantage afforded by FXa-Fc was abolished or significantly decreased in the B.1.1.7 variant-infected group compared to the WT-infected group. Thus, our data showed that the B.1.1.7 variant, with its mutated spike protein, was less efficient in FXa-mediated infectivity inhibition *in vitro* and *in vivo* compared to the WT strain.

[0306] To explore the mechanism of this difference of FXa targeting different variants, the binding affinity between FXa and WT S protein was compared with that between FXa and B.1.1.7 S protein. Both ELISA and flow cytometry results showed that FXa had a significantly lower binding affinity with B.1.1.7 S protein compared with WT S protein. As B.1.1.7 has several mutations in its S protein, it was determined which one was most important for resisting binding and cleavage by FXa. Applicant focused on the aspartic acid–614 to glycine (D614G) substitution in S protein, as it had been linked to enhanced SARS-CoV-2 infection(27, 28). Whether FXa had a similar functional interaction with the D614G S protein as with the WT S protein was determined. FXa could still bind to and cleave the D614G S protein, and the binding affinity between ACE2 and D614G S protein decreased if S protein was pre-treated with FXa. However, FXa cleaved the D614G S protein less efficiently than it cleaved the WA1 S protein after one-hour incubation. This implied that the D614G variant might be resistant to FXa-mediated anti-viral activity. When cleavage of the WA1 and B.1.1.7 S proteins by FXa was compared, there was less efficient cleavage of B.1.1.7 S protein by FXa compared to WA1 S protein after one-hour incubation.

[0307] As both D614G and A570D in the B.1.1.7 variant are close to FXa's predicted cleavage site 567, whether either or both mutations are responsible for the reduction in FXa-mediated anti-viral activity was tested. For this purpose, a custom variant carrying both D614G and A570D was used, referred to as "D614G+A570D"(29, 30) or D614G alone in the WA1 background. Vero E6 cells were infected with the original emergent WA1 SARS-CoV-2 (wild-type; WT), the D614G variant, or the custom-made variant D614G+A570D. The immuno-plaque results revealed that FXa showed significantly less antiviral activity against the D614G variant compared to the WT strain. However, there was no difference between the D614G variant and the D614G+A570D custom-made variant after FXa treatment, indicating that the D614G mutation was the important mutation for significantly reducing FXa's anti-viral activity. Cleavage assays were also performed with S protein of the Delta and Omicron variants, both of which contain the D614G mutation relative to WA1. Similarly, the cleavage of S protein of Delta and Omicron variants by FXa was less efficient than that of the WA1 S protein after one-hour incubation. The viral infection of the WA1 and the Delta SARS-CoV-2 variants in the presence or absence of FXa was compared. The Delta variant, which contains the D614G mutation, showed much less efficiency for FXa-dependent inhibition of SARS-CoV-2 infection when compared to WA1 strain.

[0308] Collectively, the experimental data demonstrate that variant strains carrying the D614G mutation in their S protein (all dominant pandemic variants to date) are relatively resistant to the FXa anti-viral effect compared to the WT strain. This observation may in part explain the emergence, higher transmission(27, 31), and higher mortality(32) rates of variants containing the D614G mutation.

[0309] Here, Applicant identifies a novel mechanism of human host anti-viral defense involving the human SP FXa which, at the time of SARS-CoV-2 infection, binds to and cleaves the SARS-CoV-2 S protein, blocking viral entry into host cells. In contrast to other SPs, the precursor to FX was found to be increased in COVID-19 patient tissues and serum compared to normal donors. FXa administration reduced viral load and protected a humanized angiotensin-converting enzyme 2 (hACE2) mouse model of COVID-19 from lethal infection, an effect that was attenuated by a direct but not an indirect FXa inhibitor and anti-coagulant, which may have implications for clinical therapeutic responses.

[0310] SARS-CoV-2 is newly emergent human pathogen that utilizes the ACE2 receptor to enter host cells. SARS-CoV-2 is a newly emergent human pathogen that belongs to the beta-

coronavirus containing a single-stranded RNA associated with a nucleoprotein within a capsid. Unlike bats, which display immune tolerance, humans infected with SARS-CoV-2 sometimes over activate inflammatory components of the immune system, triggering cytokine release syndrome(34), which can be fatal in some people though nonexistent in others with the same exposure(35). Proteolytic processing S protein by SPs such as TMPRSS2, furin, and trypsin enhanced the binding affinity between ACE2 and the processed S protein (29). In the course of infection, SARS-CoV-2 at times excessively activated the inflammatory component immune system leading to the cytokine release syndrome, which can be fatal in some, yet non-existent in others with the same exposure (30). Therefore, identification of the body's natural defense mechanisms against SARS-CoV-2 was important for developing effective prevention as well as therapeutic strategies. A natural defense mechanism, as disclosed herein, involving the binding to and cleavage of the SARS-CoV-2 S protein by FXa that blocked its entry into host cells may lend to the development of such strategies. Like TMPRSS2 and furin, FXa belongs to the family of SPs that each cleave S protein. However, unlike FXa, the cleavage sites of the others are different in that they enhance viral entry into host cells. TMPRSS2 and furin cleave S protein at the S1/S2 site of at RRAR (685R), followed by the S2' site KPSKR (R856), resulted in priming the SARS-CoV-2 fusion step. The FXa cleavage of the S protein blocking entry of SARS-CoV-2 into host cells is currently unknown. In one embodiment, in silico modeling predicted that the cleavage sites of FXa on S protein were at Ile-(Asp/Glu)-Gly-Arg (R1000) or Gly-Arg (R567), distinct from the S1/S2 or the S2' site, resulting in a unique conformational change of S protein when forming a syncytium (31). The most conserved region of the RBD was from AA 306 to 527, which was close to the FXa cleavage site (R567) (32). As such, the cleavage by FXa near the RBD might adversely impact the conserved conformation of the RBD. The S protein is a type 1 viral fusion protein with two conserved heptad repeat regions, HR-N (916-950) and HR-C (1150-1185) that may form a 6-helix bundle allowed for a better fusion between the viral and host cell membranes (33). The two likely cleavage sites of FXa were located within the HR-N and the HR-C repeat. However, whether the cleavage at this site will affect the formation of the 6-helix bundle remains to be determined. In addition, the amino acid sequences of SARS-CoV-2 and SARS-CoV-1 are different, and this may result in opposite effects of FXa on the two viruses(41-43).

[0311] Since the B.1.1.7 variant emerged in the United Kingdom in September 2020, it has demonstrated higher transmissibility(44, 45) and mortality(32). Some vaccines and

neutralizing antibodies are less effective against the B.1.1.7 variant than against the WT strain(46-49). However, the reasons for the higher transmissibility and mortality of the B.1.1.7 variant remain elusive(50). Applicant's description of the previously unknown FXa response to SARS-CoV-2 infection and its differential effect on the B.1.1.7 variant is a possible, or at least partial, explanation. Data showed that the B.1.1.7 variant with its mutated S protein, was more resistant to inhibition by FXa *in vitro* and *in vivo* when compared to the WT strain. This should not be attributed to the recently identified loss of furin PRRAR cleavage site found in the B.1.1.7 variant, as the loss of the site resulted in an attenuated variant(51) and as other variants lacking this deletion, e.g., D614G and Delta, showed similar less efficiency in the FXa-mediated infection inhibition. Therefore, a host antiviral defense system depending on FXa might not be strong enough to protect humans from infection by the SARS-CoV-2 B.1.1.7 variant. The basis for the difference between variants could be the D614G mutation, which might also be responsible for the success of other variants such as Delta and Omicron. Of note, all pandemic variants identified to date carry the D614G mutation(52). In agreement with Applicant's speculation, the data show that all the variants tested, including B.1.1.7, Delta, and Omicron as well as the engineered variant D614G+A570D contain the D614G mutation and are relatively resistant to cleavage of S protein by FXa and therefore more infectious than the WT strain at least in the presence of FXa. Moreover, others have reported that the D614G mutation in S protein increases SARS-CoV-2 infection of multiple human cell types and increases transmission rates(28, 29). One speculation is that this mutation may change the conformation of S protein, thereby affecting the S protein's interaction with FXa.

[0312] FXa is required for the conversion of prothrombin to thrombin in the clotting cascade (19), and might have a role in inflammation (38). Both of these processes are dysregulated in some patients with COVID-19. Many coagulation factors were important predictors of the clinical outcome in COVID-19 patients(55, 56). However, prior to this study, the role of FXa in viral infection has been unclear. The bulk of the literature on this topic was based on theoretical assumptions rather than experimental data(15, 36, 57-60). In fact, limited data based only on SARS-CoV-1 and a peptide of ~10 amino acids used to represent the entire S protein of SARS-CoV-2 suggested that FXa might facilitate S1/S2 cleavage and thereby promote, rather than inhibit, SARS-CoV-2 infection(41, 61). However, some reports favor the notion that FXa itself may have anti-SARS-CoV-2 effect(57, 62). Up until now, no *in vivo* studies with live SARS-CoV-2 have been performed to support or refute

this assertion(41, 61).

[0313] For the first time, with extensive *in vitro* and *in vivo* studies—now including live SARS-CoV-2—a gap in the field is filled by demonstrating that FXa cleaves S protein into non-S1 and S2 fragments, thus inhibiting infection by SARS-CoV-2 or VSV-SARS-CoV-2. By comparing other SPs in parallel and using dose gradients, Applicant showed that furin, TMPRSS2, and trypsin facilitate—rather than impede—infection. Applicant also demonstrated that direct inhibition of FXa abrogates the protein's anti-SARS-CoV-2 activity while indirect FXa inhibition does not. Moreover, administration of exogenous FXa during experimental SARS-CoV-2 infection produced antiviral activity in a dose-dependent manner. It has been reported that both liver and extrahepatic immune cells can produce FXa(65). It will be interesting to explore whether different sources of FXa play different functions and whether FXa produced by pulmonary macrophages has anti-SARS-CoV-2 effect, based on the mechanism characterized in the current study.

[0314] Thus, while it is likely that the viral defense mechanism described herein was active and important in controlling SARS-CoV-2 infection in asymptomatic and mildly symptomatic individuals, the endogenous overexpression of FXa during serious SARS-CoV-2 infection might also contribute to the pathogenesis and complications of COVID-19, especially the thrombotic events (28, 35). There are at least two pathways forward in considering FXa as a therapeutic agent for severe SARS-CoV infection. The first would be to co-administer an indirect FXa inhibitor as an anti-coagulant in combination with the FXa-Fc fusion protein, as described herein, or with a recombinant FXa, since the indirect FXa inhibitor fondaparinux does not interfere with FXa's cleavage of S protein or its therapeutic effect against live virus *in vivo*. The second approach would be to modify chemical structure of FXa such that its enzymatic activity for cleavage of the S protein was retained while that of prothrombin conversion was lost. This latter approach can also be a preventative approach for individuals who are not vaccinated and highly susceptible to severe COVID-19, or those individuals who were vaccinated yet failed to develop effective immunity to SARS-CoV.

[0315] There are four clinically approved direct FXa inhibitors including rivaroxaban, apixaban, edoxaban as well as betrixaban) and one indirect FXa inhibitor fondaparinux for use as anti-thrombotic agents in those with hypercoagulable states (37). In the presence of a direct inhibitor of FXa, rivaroxaban, the anti-SARS-CoV-2 activity of FXa was affected not by the binding of the S protein, but by the cleavage of the S protein. Further, rivaroxaban

completely abrogated the decrease in viral load and the protective effective of FXa administration against lethal SARS-CoV-2 infection in the current *in vivo* model. Moreover, the protective effect of endogenous FXa during RS-CoV-2 infection in human being is yet to be proven, the use of direct FXa inhibitors such as rivaroxaban, in patients highly susceptible for severe COVID-19, as is currently being evaluated in a number of studies (25), should likely proceed with caution as it is at least conceivable from this work that such studies could result in an increase in viral load. Importantly, the indirect inhibitor of FXa, fondaparinux, which was found not to block the protective effects of FXa against SARS-CoV-2, was found to be safe and efficacious for venous thrombosis prophylaxis in hospitalized COVID-19 patients (26).

[0316] In summary, Applicant shows that FX, the precursor of FXa, is upregulated in COVID-19 patients. Applicant identified a new mechanism of anti-viral defense involving FXa in humans and demonstrate its protection against SARS-CoV-2 infection *in vitro* and *in vivo* with the K18-hACE2 animal model that mimics the human disease. Accordingly, FXa-Fc can be developed as a therapeutic agent to treat COVID-19. Applicant's demonstrates that when necessary, indirect FXa inhibitors should be considered over direct inhibitors when anticoagulation is indicated in COVID-19 patients.

Example 2: Materials and Methods

[0317] Patient sample collection

[0318] Patient samples were collected and tested positive for SARS-CoV-2 at City of Hope. The autopsy samples were provided by Dr. Ross Zumwalt at the University of New Mexico School of Medicine. The concentration of FXa in serum of patient samples was measured by ELISA (LS-F10420-1, LSBIO). The protocols for human specimen collection were approved by the institutional review boards of City of Hope.

[0319] Cells

[0320] Monkey kidney epithelial-derived MA104 cells were maintained with medium 199 supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml). To overexpress FXa in MA104 cells, the cells were infected with lentivirus encoding FXa to generate MA104-FXa cells. Monkey kidney epithelium-derived Vero cells, human embryonic kidney derived HEK293T cells, and CHO cells were cultured with DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml). All cell lines were routinely

tested for absence of mycoplasma using the MycoAlert Plus Mycoplasma Detection Kit from Lonza (Walkersville, MD).

[0321] VSV-SARS-CoV-2 infection

[0322] The VSV-SARS-CoV-2 chimeric virus expressing GFP was kindly provided by Sean Whelan at Washington University School of Medicine. The virus was decorated with SARS-CoV-2 S protein in place of the native glycoprotein (G) (28). For VSV-SARS-CoV-2 infection, MA104 cells were seeded 24 hours before the infection at confluency of 70% in a 96-well plate. VSV-SARS-CoV virus and indicated amount (12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml) of the FXa-Fc fusion protein were co-incubated at 37 °C for 1 hour and then were added to the cells. For assessment of the effect of FXa inhibitors, FXa protein was preincubated with or without 50 µg/ml fondaparinux or rivaroxaban separately for 1 hour at room temperature. The infectivity was detected by detecting GFP fluorescence using a Zeiss fluorescence microscope (AXIO observer 7) and/or determined by the percentage of GFP(+) cells analyzed by Fortessa X20 flow cytometer (BD Biosciences) at 16, 24, 36, and 48 hpi. To determine viral production, Vero cells were pre-seeded for 24 hours and infected with the supernatants collected from the MA104 cells infected by VSV-SARS-CoV at 24 or 48 hpi. The supernatants were diluted by 5-fold before the virus production assay.

[0323] Generation and purification of FXa-Fc

[0324] CHO cells were transduced with the pCDH lentiviral vector expressing FXa to produce FXa-Fc fusion protein for functionality assays. For this purpose, FXa fused with human IgG4 was reconstructed using the method as previously reported (29). mCherry was co-expressed with FXa for FACS-sorting to purify transduced cells by using a FACS Aria II cell sorter (BD Biosciences, San Jose, CA, USA). The conditional supernatants from the lentivirus-infected CHO cells sorted by FACS were used to purify the FXa-Fc fusion protein using a protein G column (89927, Thermo Fisher). For the *in vivo* test, the purified FXa-Fc fusion protein by the protein G column was desalted by fast protein liquid chromatography (FPLC).

[0325] SARS-CoV-2 neutralization, cell infection, plaque assay, and immune-plaque assay

[0326] The following reagents were obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, NR-52281 (wild-type, WT) and SARS-Related Coronavirus 2, Isolate USA/CA_CDC_5574/2020, NR-54011 (B.1.1.7). SARS-

Related Coronavirus 2 isolate TG898390, B.1.617.2 (Delta) was kindly provided by Dr. Pei Yong Shi (University of Texas Medical Branch) and the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). These recombinant isolates were previously described(29, 30). Virus isolates were passaged in Vero E6 cells (ATCC CRL-1586) or Calu3 cells as previously described(69). Virus concentrations were determined using immuno-plaque assays (also called focus forming assays)(70). For the immune-plaque assay, 100 PFU of live SARS-CoV-2 variants were incubated with diluted serum for 1 hour; then the virus-antibody mixture was added to Vero E6 cells for 1 hour at 37°C. The medium containing virus was then removed, overlaid with medium containing methylcellulose and 2% FBS DMEM, and incubated at 37°C. At 24-36 hours after infection, infected cells were fixed with 4% paraformaldehyde for 20 minutes at room temperature and then permeabilized in 0.5% Triton X-100/ PBS solution for 210 minutes at room temperature. SARS-CoV-2 viral nucleocapsid protein (NP) was detected using the anti-NP protein antibody (PA5-81794, Thermo Fisher) diluted 1:10000 in 0.1% tween-20/1% BSA/PBS solution as a primary antibody. Detection with an anti-rabbit secondary antibody (ab6721, Abcam) at a 1:20,000 dilution followed. Plates were washed three times between antibody solutions, using 0.5% tween-20 in PBS. The plates were developed using TrueBlue Peroxidase Substrate (5510-0030, Sera Care) and then scanned using Immunospot S6 Sentry (C.T.L Analyzers). Neutralization titers for the immune-plaque assay are defined as a 50% reduction in plaque-forming units relative to the untreated wells.

[0327] Binding assay between S protein and FXa by ELISA

[0328] Full-length coronavirus S protein with His tag (500 ng) (40589-V08B1, Sino Biological), Soronavirus S protein S1 subunit with His tag (500 ng) (40591-V08B1, Sino Biological), Soronavirus S protein S2 subunit with His tag (500 ng) (40070-V08B, Sino Biological). and S protein RBD-His recombinant protein (500 ng) (40592-V08B-B, Sino Biological) were used as coating reagents. The plate (3361, Corning) was incubated with FXa protein at a concentration of 1 µg/ml for 2 hours at room temperature. FXa-HRP conjugated anti-human Fc antibody (05-4220, Invitrogen) was used as a detecting antibody. Absorbance was measured at OD450 nm by Multiskan™ FC Microplate Photometer (Fisher Scientific).

[0329] Pull-down assay

[0330] HEK293T cells were transduced with the pCDH lentiviral vector expressing the full-length spike (S) protein for 48 hours. The cells were lysed and incubated with FXa-Fc or

Fc at a concentration of 10 µg/ml for 3 hours. 20 µl protein A agarose resin beads (P-400-25, Invitrogen) were added and incubated overnight. After incubation, the beads were washed and collected. The S protein binding assay between FXa-Fc and S protein was detected by immunoblotting using the anti-S protein antibody (ab272504, Abcam).

[0331] Cleavage assay

[0332] 1 µg full-length S protein was treated with 1 µg FXa (P8010L, NEB), furin (P8077S, NEB) or TMPRSS2 (TMPRSS2-1856H, Creative BioMart) protein for 3 hours following the manufacturer's instruction. Cleavage assays were detected using by immunoblotting with an anti-S protein antibody. For the inhibitors assay, the FXa protein was preincubated with or without 50 µg/ml fondaparinux or rivaroxaban separately for 1 hour advance at room temperature, followed by the same procedures mentioned above. For the cleavage assay towards S protein-ACE2 complex, 1 µg S protein and 1 µg ACE2 were pre-incubated 1 hour prior to incubation with FXa, followed by the same procedures mentioned above. Of note, different buffer conditions for all binding assays and cleavage assays were used.

[0333] Binding assay between S protein and FXa by flow cytometry

[0334] HEK293T cells were transduced with lentiviral vector expressing FXa for 48 hours. The cells were incubated with 10 µg/ml S protein for 20 minutes at room temperature. The cells were then washed and incubated with an anti-S protein antibody for 20 minutes at room temperature followed by staining with an FITC-labeled secondary antibody (111-605-045, Jackson ImmunoResearch). The percentage of FITC positive cells was determined by Fortessa X20 flow cytometer (BD Biosciences).

[0335] Detection of FXa binding to the complex of S protein and ACE2 by ELISA

[0336] ACE2 protein was used as a coating reagent. The plate was incubated with 1 µg/ml S protein with His tag pretreated with or without FXa (P8010L, NEB) for 2 hours at room temperature. The HRP-conjugated anti-His tag antibody (ab1187, Abcam) was used as a detecting antibody. Absorbance was measured under OD450 nm by Multiskan™ FC Microplate Photometer (Fisher Scientific).

[0337] Detection of FXa binding to the complex of S protein and ACE2 by flow cytometry

[0338] HEK293T cells stably expressing ACE2 protein were incubated with the full-length S protein or the FXa-pretreated full-length S protein for 20 minutes at room temperature. The cells were then washed and incubated with an anti-S protein antibody for 20 minutes at room temperature, followed by staining with an APC-labeled secondary antibody (111-005-003, Jackson ImmunoResearch). The percentage of APC-positive cells was determined by Fortessa X20 flow cytometer (BD Biosciences).

[0339] *In vivo* infection model

[0340] 6-8-week-old K18-hACE2 mice were anesthetized with ketamine (80 mg/kg)/xylazine (8 mg/kg) and intranasally infected with 5×10^3 PFU wild type SARS-CoV-2 or B.1.1.7 variant in 25 μ l DMEM, followed by intranasal treatment with PBS, FXa-Fc (200 μ g), FXa-Fc (2 μ g), or Fc (200 μ g) in 25 μ l DMEM. Infected mice were maintained in bio-containment unit isolator cages (Allentown, NJ, USA) in the NAU ABL3S3. Mice were then treated with PBS or rivaroxaban (30 mg/kg) via gavage or fondaparinux (30 mg/kg) via intraperitoneal injection for 4 times at a frequency of every other day. Body weights of mice were monitored daily. Mice were euthanized using ketamine (100 mg/kg)/xylazine (10 mg/kg) when body weights dropped below 20% of their original body weights. RNA was isolated from trachea, lung, and brain tissues to assess viral load using quantitative real-time PCR as described below. Expression of SARS-CoV-2 viral protein NP was examined using immunohistochemistry (IHC) in the trachea, lung, and brain sections from infected mice as described below.

[0341] FXa knock-out mice were generated by CRISPR/Cas9 gene-editing technology. The mice were crossed with K18-hACE2 mice and obtained the FXa(heterozygotes)-K18-hACE2 strain. FXa(heterozygotes)-K18-hACE2 mice and K18-hACE2 mice were inoculated with 5×10^3 PFU SARS-CoV-2 at day 0. On day 5, mice were euthanized, and lung tissues were collected to measure viral load as described below.

[0342] Quantitative real time PCR

[0343] Mouse tissues were homogenized in DMEM and RNA was isolated using the PureLink RNA isolation kit (K156002, Invitrogen). Viral copy numbers were detected using the One-Step QPCR kit (1725150, BioRad).

[0344] H&E and immunohistochemistry assay

[0345] 4- μ m-thick sections were cut from of the lung and liver from COVID-19 patient and

non-COVID-19 donor paraffin blocks of tissues. Immunohistochemical staining with an anti-FXa protein antibody (PIPA529118, Invitrogen), an anti-furin antibody (ab183495, Abcam), an anti-trypsin antibody (ab200997, Abcam) or an anti-plasmin antibody (LS-C150813-1, LSBio) as a primary antibody was performed by the Pathology Core of Shared Resources at City of Hope Beckman Research Institute and National Medical Center. Stained slides were mounted and scanned for observation.

[0346] Mouse tissues isolated from experimental mice were placed in 10% neutral buffered formalin for a minimum of 72 hours. After paraffin embedding, 4- μ m-thick sections were cut from the blocks. H&E staining and immunohistochemical staining with the anti-NP protein antibody (NB100-56576, Novus) as the primary antibody were performed by the Pathology Core of Shared Resources at City of Hope Beckman Research Institute and National Medical Center. Stained slides were mounted and scanned for observation.

[0347] Statistical analysis

[0348] Prism software v.8 (GraphPad, CA, USA) and SAS v.9.4 (SAS Institute, NC, USA) were used to perform statistical analyses. For continuous endpoints that are normally distributed or normally distributed after logarithm data transformation such as MFI or copy number, Student's *t* test or paired *t* test was used to compare two independent or matched groups, respectively. One-way ANOVA models or generalized linear models were used to compare three or more independent groups. For data with repeated measures from the same subject, linear mixed models were used to account for the variance and covariance structure due to repeated measures. Survival functions were estimated by the Kaplan–Meier method and compared by the two-sided log rank test. All tests were two-sided. P values were adjusted for multiple comparisons by Holm's procedure. A P value of 0.05 or less was considered statistically significant. The p-values are represented as: * <0.05, ** <0.01, *** <0.001, and **** <0.0001.

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INFORMAL SEQUENCE LISTING

[0387] ACE 2 (SEQ ID NO:1)

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEENVQNM
 NNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKSKRLNTILNTM
 STIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRSEVGKQLRPLYEEYV
 VLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIEDVEHTFEEIKPLYEHLHAYV
 RAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSLTVPGQKPNIDVTDAMVDQAWDA

QRIFKEAEKFFVSVGLPNMTQGFWENSMLTDPGNVQKAVCHPTAWDLGKGDFRILMCTKVT
 MDDFLTAHHEMGHIQYDMAYAAQPFLLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSP
 DFQEDNETEINFLKQALTIVGTLPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVG
 VVEPVPHDETYCDPASLFHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTE
 AGQKLFNMLRLGKSEPWTALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTD
 WSPYADQSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYAMRQYFLKVKNQMILFGEEDV
 RVANLKPRISFNFFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPPN
 QPPVSIWLIVFGVVMGVIVVGIVILIFTGIRDRKKKNKARSGENPYASIDISKGENNPGFQNTD
 DVQTSF

[0388] ACE 2 (SEQ ID NO:2)

MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSSLASWNYNTNITEE
 NVQMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQNGSSVLSEDKS
 KRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNERLWAWESWRS
 EVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYSRGQLIE
 DVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWTNLYSL
 TVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLTDP
 GNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPF
 LLRNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLKQALTIVGT
 LPFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASL
 FHVSNDYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRL
 GKSEPWTALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYAD
 QSIKVRISLKSALGDKAYEWNNDNEMYLFRSSVAYAMRQYFLKVKNQMILFGEEDVR
 VANLKPRISFNFFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTL
 GPPNQPPVSIWLIVFGVVMGVIVVGIVILIFTGIRDRKKKNKARSGENPYASIDISKGE
 NNPGFQNTDDVQTSF.

[0389] FXa light chain (SEQ ID NO:3)

ANSFLEEMKKGHLERECMEETCSYEEAREVFEDSDKTNEFWNKYKDGDQCETSPCQ
 NQGKCKDGLGEYTCTCLEGFEGKNCELFTRKLCSLDNGDCDQFCHEEQNSVVCSCA
 RGYTLADNGKACIPTGPYPGKQTLER

[0390] FXa heavy chain with activation peptide (SEQ ID NO:4)

SVAQATSSSGEAPDSITWKPYDAADLDPTENPFDLLDFNQTPERGDNNLTRIVGGQ
 ECKDGECPWQALLINEENEGFCGGTILSEFYILTAAHCLYQAKRFKVRVGDRNTEQE

EGGEAVHEVEVVIKHNRF TKETYDFDIAVLRLKTPITFRMNVAPACLPERDWAESTL
MTQKTGIVSGFGR THEKGRQSTR LKMLEVPYVDRNSCKLSSSFII TQNMFCAGYDTK
QEDACQGDSGGPHVTRFKDTYFVTGIVSWGEGCARKGKYGIYTKVTAFLKWIDRSM
KTRGLPKAKSHAPEVITSSPLK

[0391] FXa heavy chain – activated (SEQ ID NO:5)

IVGGQECKDGECPWQALLINEENEGFCGGTILSEFYIL TAAHCLYQAKRFKVRVGDR
NTEQEEGGEAVHEVEVVIKHNRF TKETYDFDIAVLRLKTPITFRMNVAPACLPERDW
AESTLMTQKTGIVSGFGR THEKGRQSTR LKMLEVPYVDRNSCKLSSSFII TQNMFCA
GYDTKQEDACQGDSGGPHVTRFKDTYFVTGIVSWGEGCARKGKYGIYTKVTAFLK
WIDRSMKTRGLPKAKSHAPEVITSSPLK

[0392] FXa (SEQ ID NO:6)

MGRPLHLVLLSASLAGLLLLGESLFIRREQANNILARVTRANSFLEEMKKGHLEREC
MEETCSYEEAREVFEDSDKTNEFWNKYKDG DQCETSPCQNQGKCKDGLGEYTCTC
LEGFEGKNCELFTRKLCSLDNGDCDQFCHEEQNSVVCSCARGYTLADNGKACIPTGP
YPCGKQTLERRKRSVAQATSSSGEAPDSITWKPYDAADLDPTENPFDLLDFNQTQPE
RGDNNLTRIVGGQECKDGECPWQALLINEENEGFCGGTILSEFYIL TAAHCLYQAKR
FKVRVGDRNTEQEEGGEAVHEVEVVIKHNRF TKETYDFDIAVLRLKTPITFRMNVAP
ACLPERDWAESTLMTQKTGIVSGFGR THEKGRQSTR LKMLEVPYVDRNSCKLSSSFII
TQNMFCAGYDTKQEDACQGDSGGPHVTRFKDTYFVTGIVSWGEGCARKGKYGIY
KVTAFLKWIDRSMKTRGLPKAKSHAPEVITSSPLK

[0393] Fc domain (SEQ ID NO:7)

PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK

WHAT IS CLAIMED IS:

1. A method of treating or preventing COVID-19 in a subject in need thereof, wherein the method comprises administering to the subject an effective amount of a Factor Xa (FXa) protein or functional portion thereof.
2. The method of claim 1, wherein the method further comprises administering an anticoagulant.
3. The method of claim 2, wherein the anticoagulant is not a direct inhibitor of FXa.
4. The method of claim 2, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin.
5. The method of claim 4, wherein the anticoagulant is fondaparinux.
6. The method of claim 1, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.
7. The method of claim 1, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.
8. The method of claim 1, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.
9. The method of claim 1, wherein the FXa protein or functional portion thereof further comprises an Fc domain.
10. The method of claim 9, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.
11. The method of claim 9, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.
12. The method of claim 11, wherein the Fc domain is an IgG Fc domain.

13. A method of treating or preventing COVID-19 in a subject in need thereof, comprising: i) obtaining a sample from the subject, ii) detecting a lower level of Factor Xa (FXa) in the sample relative to a standard control, and iii) administering to the subject an effective amount of an FXa protein or functional portion thereof.

14. The method of claim 13, wherein the sample is blood or plasma.

15. The method of claim 13, wherein the method further comprises administering an anticoagulant.

16. The method of claim 15, wherein the anticoagulant is not a direct inhibitor of FXa.

17. The method of claim 15, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin.

18. The method of claim 17, wherein the anticoagulant is fondaparinux.

19. The method of claim 15, wherein the anticoagulant and FXa or functional portion thereof are administered sequentially.

20. The method of claim 15, wherein the anticoagulant and FXa or functional portion thereof are administered simultaneously.

21. The method of claim 13, wherein the FXa protein comprises a sequence having at least 80% sequence identity to SEQ ID NO:6.

22. The method of claim 13, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

23. The method of claim 13, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.

24. The method of claim 15, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

25. The method of claim 24, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.
26. The method of claim 24, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.
27. The method of claim 26, wherein the Fc domain is an IgG Fc domain.
28. A method of treating or preventing COVID-19 in a subject in need thereof, wherein the method comprises: i) obtaining a sample from the subject, ii) detecting a higher level of FXa in the sample relative to a standard control, and iii) administering an effective amount of an anticoagulant.
29. The method of claim 28, wherein the sample is blood or plasma.
30. The method of claim 28, wherein the anticoagulant is not a direct inhibitor of FXa.
31. The method of claim 28, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin.
32. The method of claim 28, wherein the anticoagulant is fondaparinux.
33. The method of claim 28, further comprising administering to the subject FXa or a functional portion thereof.
34. The method of claim 33, wherein the anticoagulant and FXa or functional portion thereof are administered sequentially.
35. The method of claim 33, wherein the anticoagulant and FXa or functional portion thereof are administered simultaneously.
36. The method of claim 33, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.
37. The method of claim 33, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

38. The method of claim 33, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.

39. The method of claim 33, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

40. The method of claim 39, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.

41. The method of claim 39, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

42. The method of claim 41, wherein the Fc domain is an IgG Fc domain.

43. A pharmaceutical composition comprising an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient.

44. The pharmaceutical composition of claim 43, further comprising an anticoagulant.

45. The pharmaceutical composition of claim 44, wherein the anticoagulant is not a direct inhibitor of FXa.

46. The pharmaceutical composition of claim 44, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin.

47. The pharmaceutical composition of claim 44, wherein the anticoagulant is fondaparinux.

48. The pharmaceutical composition of claim 43, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.

49. The pharmaceutical composition of claim 43, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

50. The pharmaceutical composition of claim 43, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.

51. The pharmaceutical composition of claim 43, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

52. The pharmaceutical composition of claim 51, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.

53. The pharmaceutical composition claim 51, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

54. The pharmaceutical composition claim 53, wherein the Fc domain is an IgG Fc domain.

55. A kit comprising a: (i) a first dosage form comprising an FXa protein or functional portion thereof and a pharmaceutically acceptable excipient; and (ii) a second dosage form comprising an anticoagulant and a pharmaceutically acceptable excipient.

56. The kit of claim 55, wherein the anticoagulant is not a direct FXa inhibitor.

57. The kit of claim 55, wherein the anticoagulant is fondaparinux, heparin, or low molecular weight heparin.

58. The kit of claim 55, wherein the anticoagulant is fondaparinux.

59. The kit of claim 55, wherein the FXa protein comprises a sequence having at least 80% sequence identity SEQ ID NO:6.

60. The kit of claim 55, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:4.

61. The kit of claim 55, wherein the FXa protein comprises a first peptide having at least 80% sequence identity SEQ ID NO:3 and a second peptide having at least 80% sequence identity to SEQ ID NO:5.

62. The kit of claim 55, wherein the FXa protein or functional portion thereof further comprises an Fc domain.

63. The kit of claim 62, wherein the Fc domain has at least 80% sequence identity to SEQ ID NO:7.

64. The kit of claim 62, wherein the Fc domain is an IgG, IgA, IgD, IgM or IgE Fc domain.

65. The kit of claim 64, wherein the Fc domain is an IgG Fc domain.

FIG. 1A

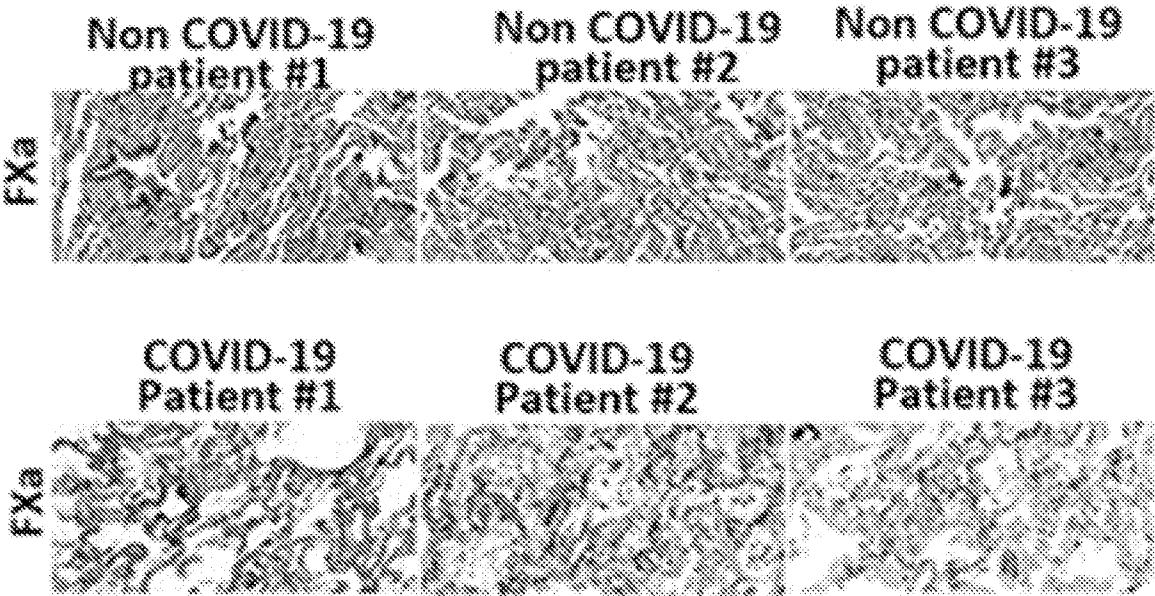


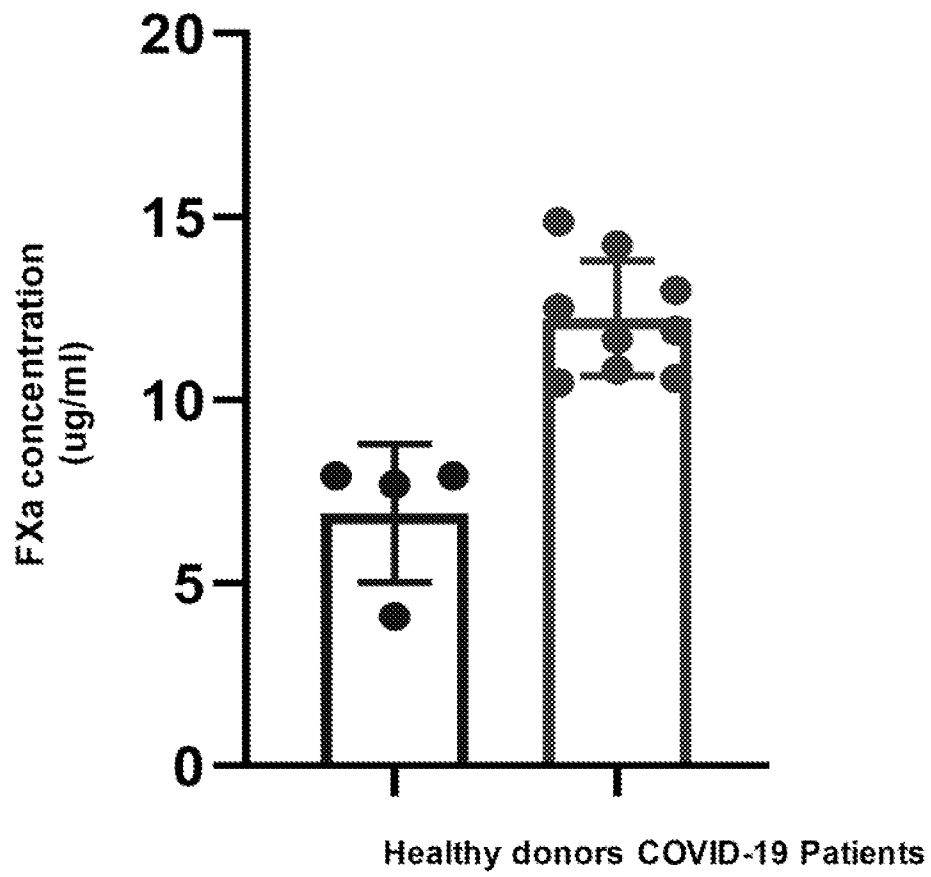
FIG. 1B

FIG. 1C

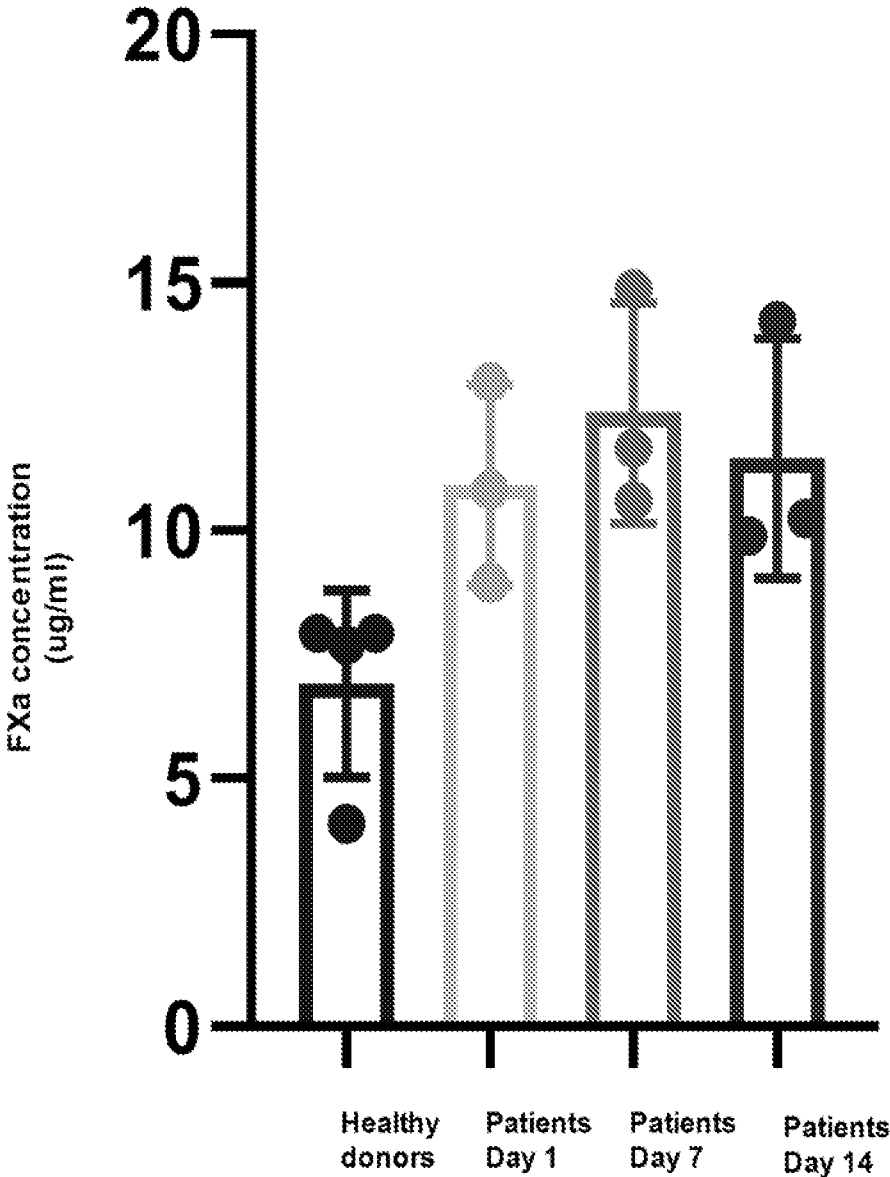


FIG. 1D

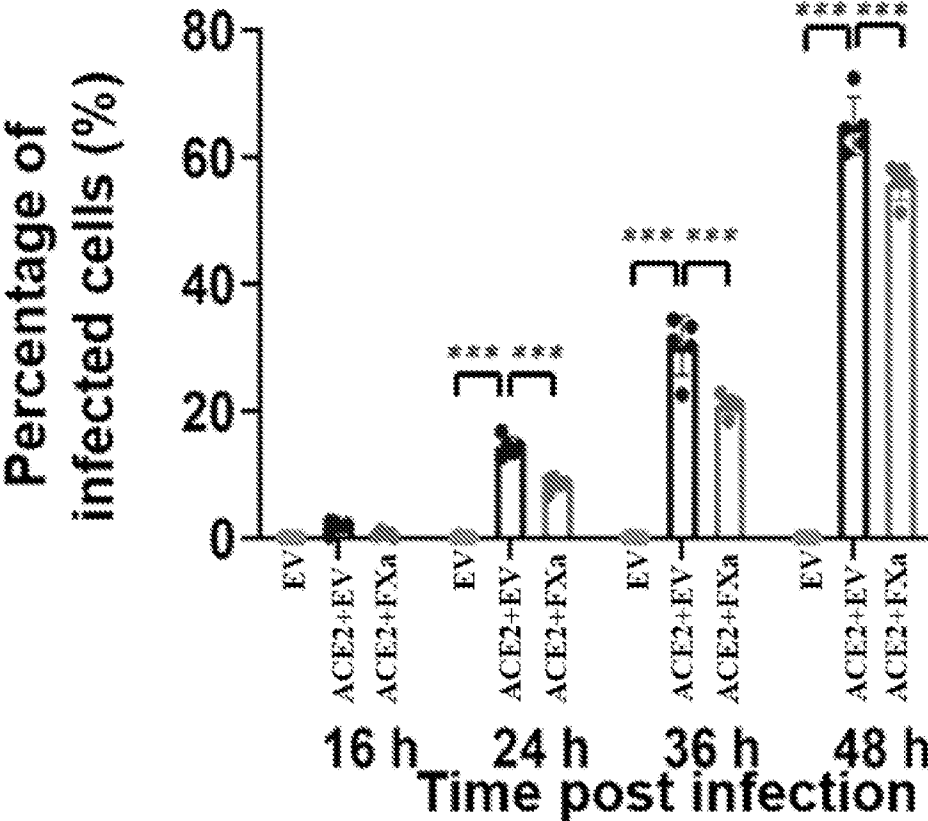


FIG. 1E

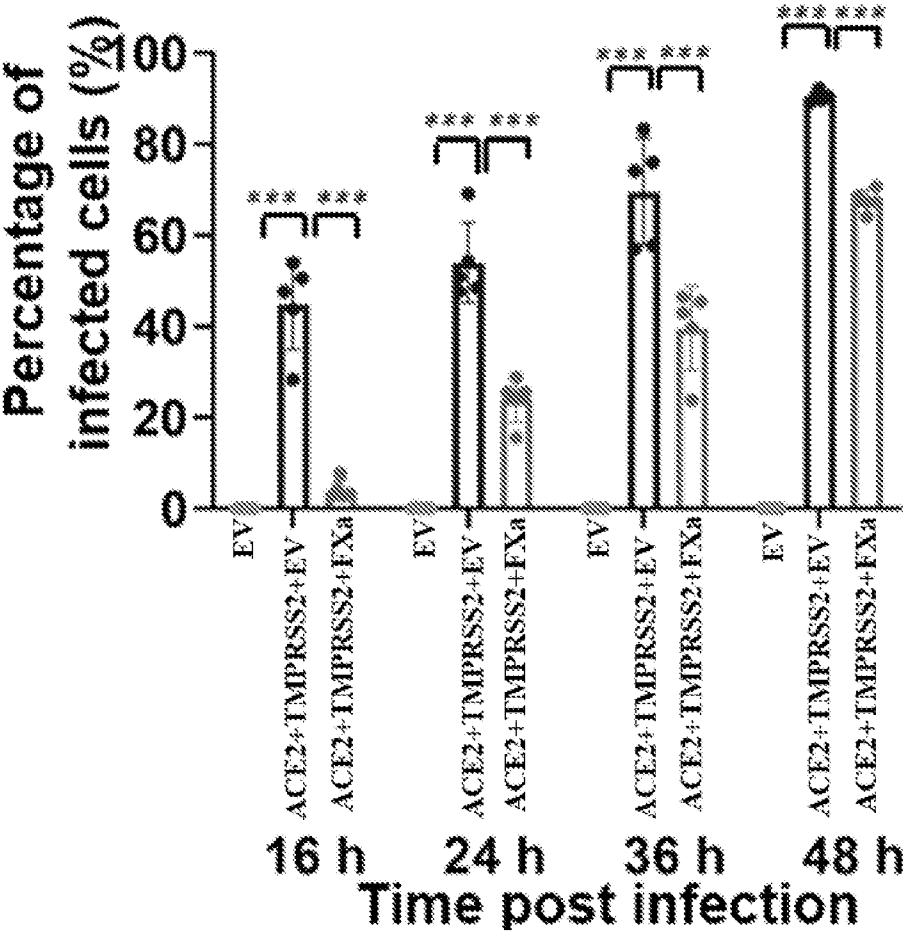


FIG. 1G

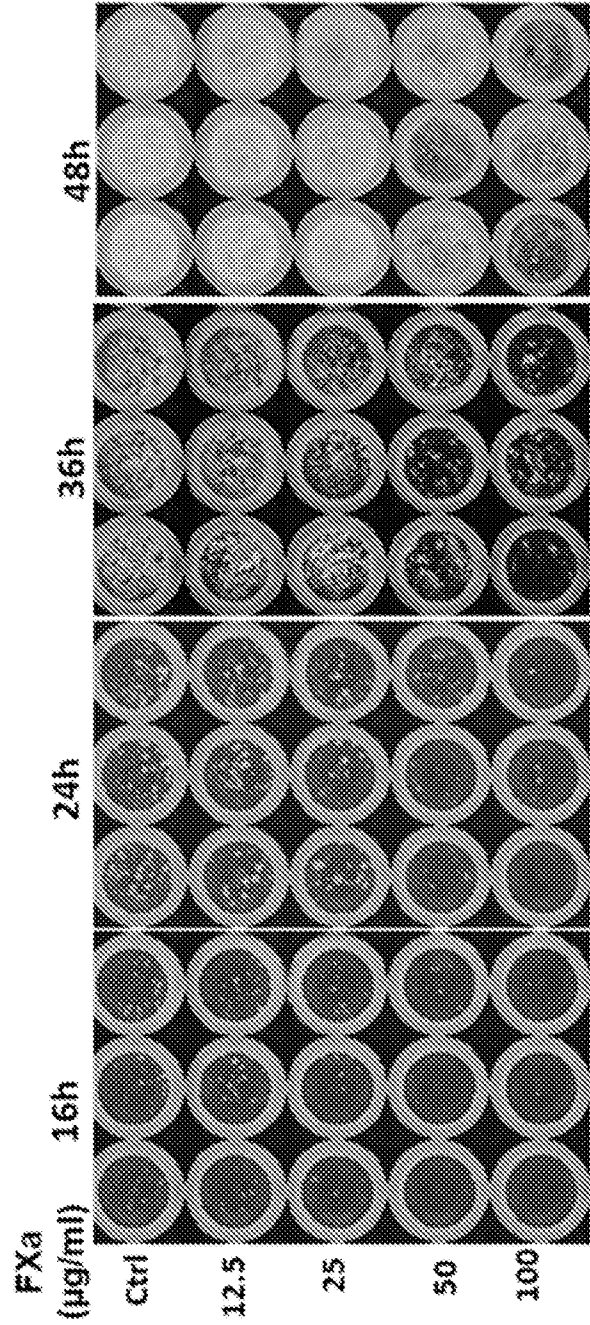


FIG. 1H

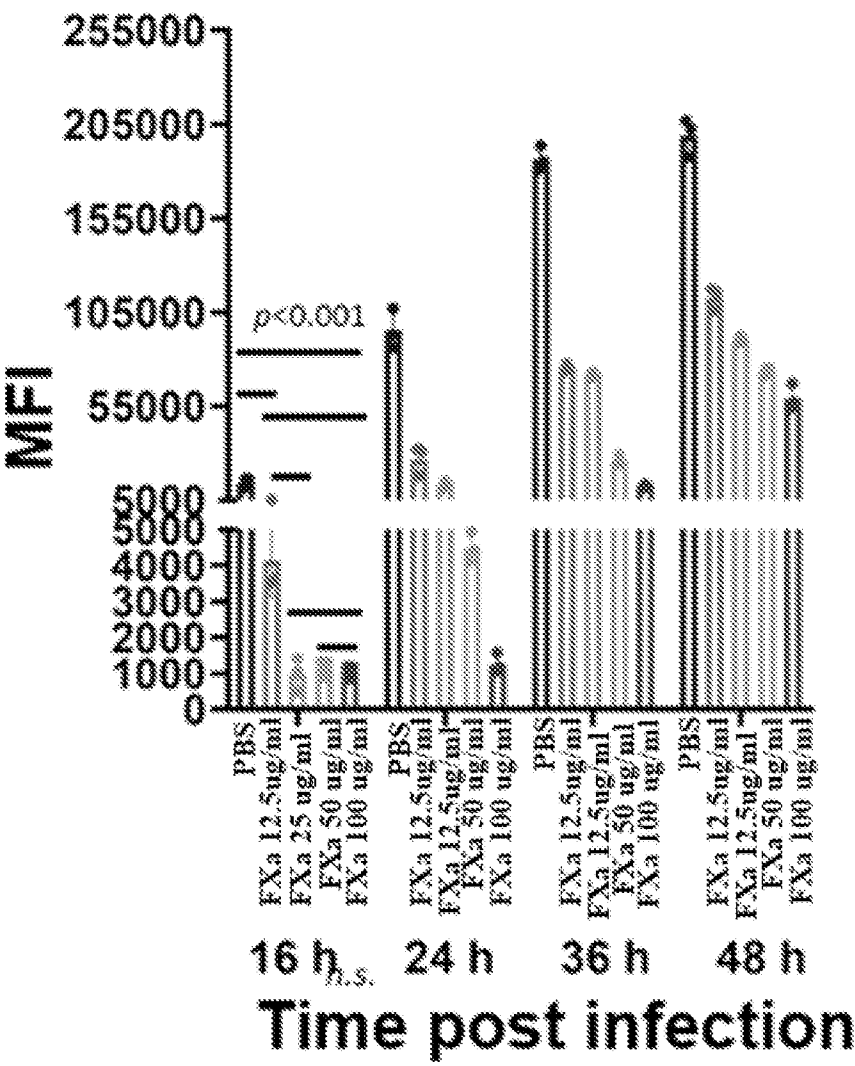


FIG. 1I
MA104-EV cells MA104-FXa cells

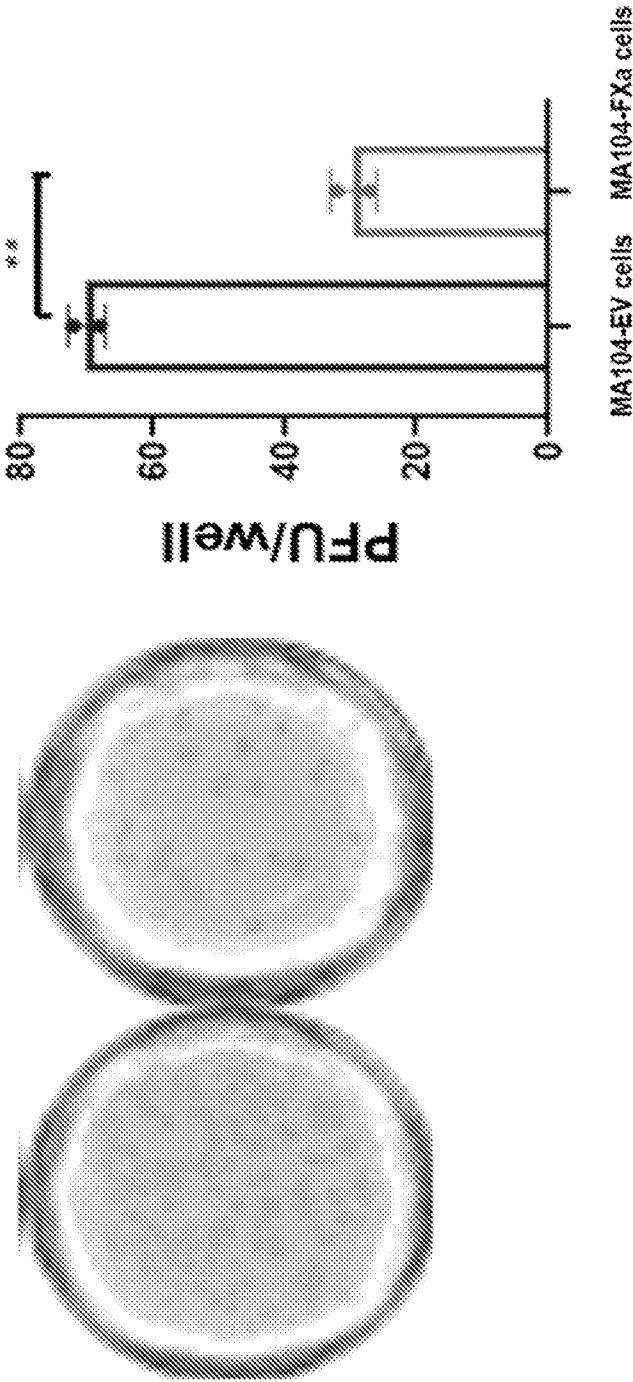


FIG. 1J

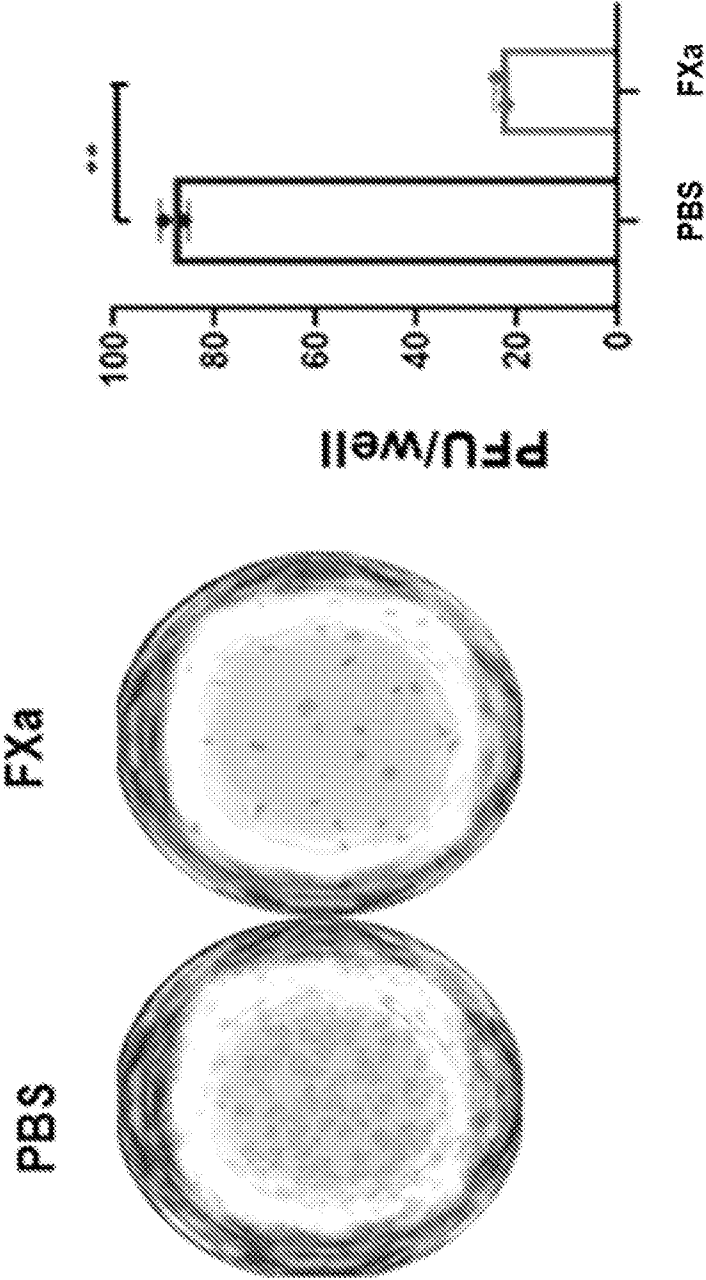


FIG. 2A

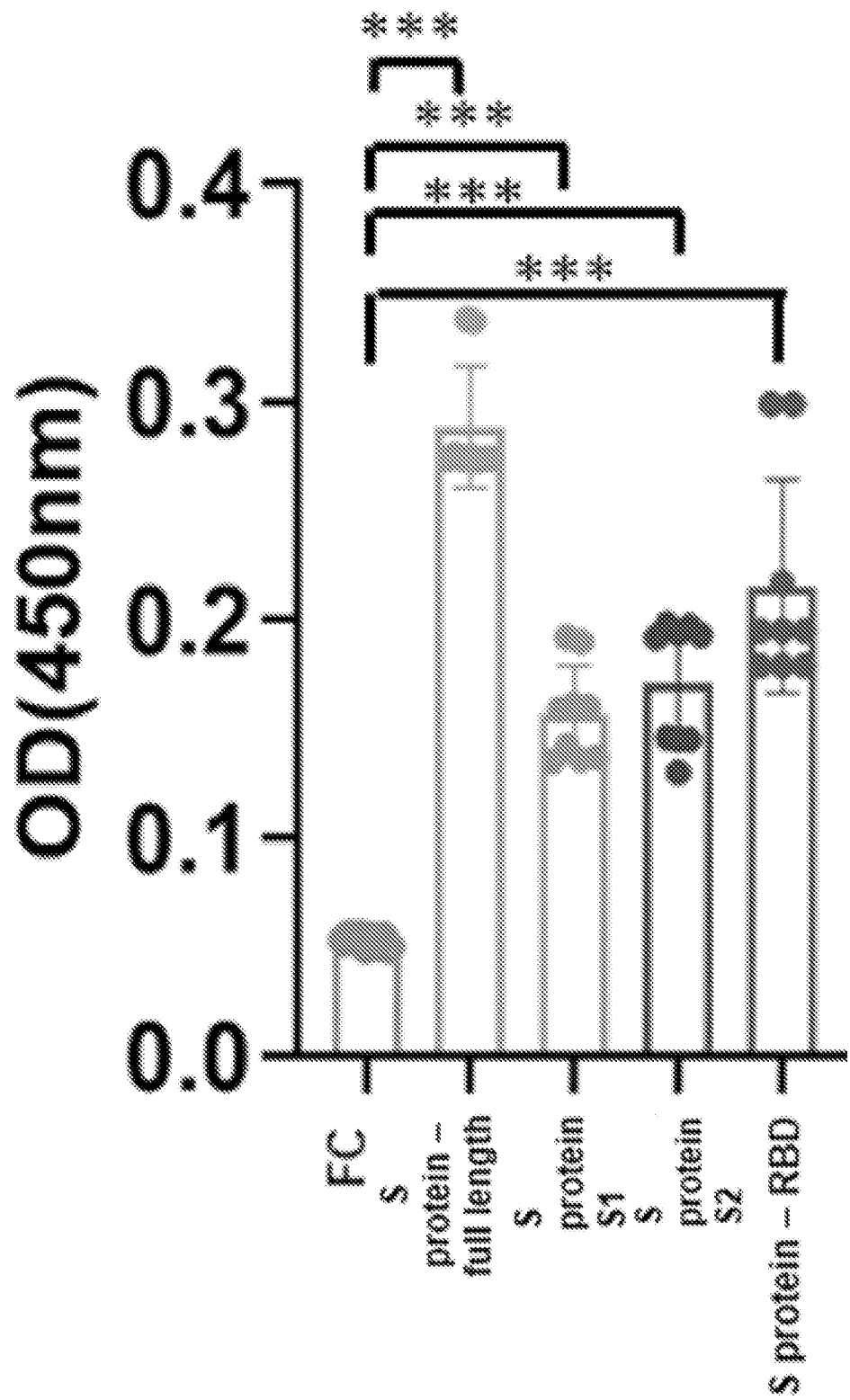


FIG. 2B

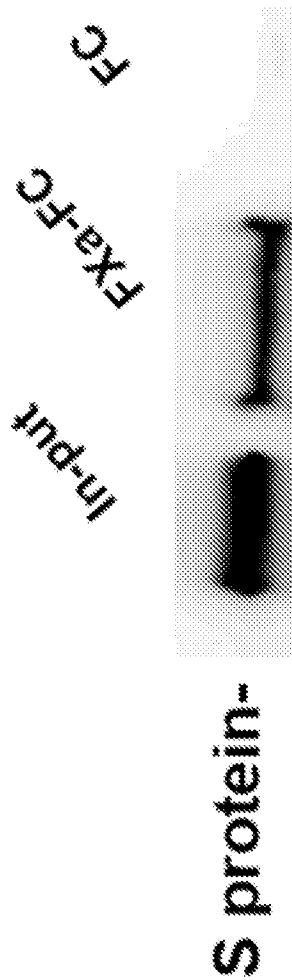


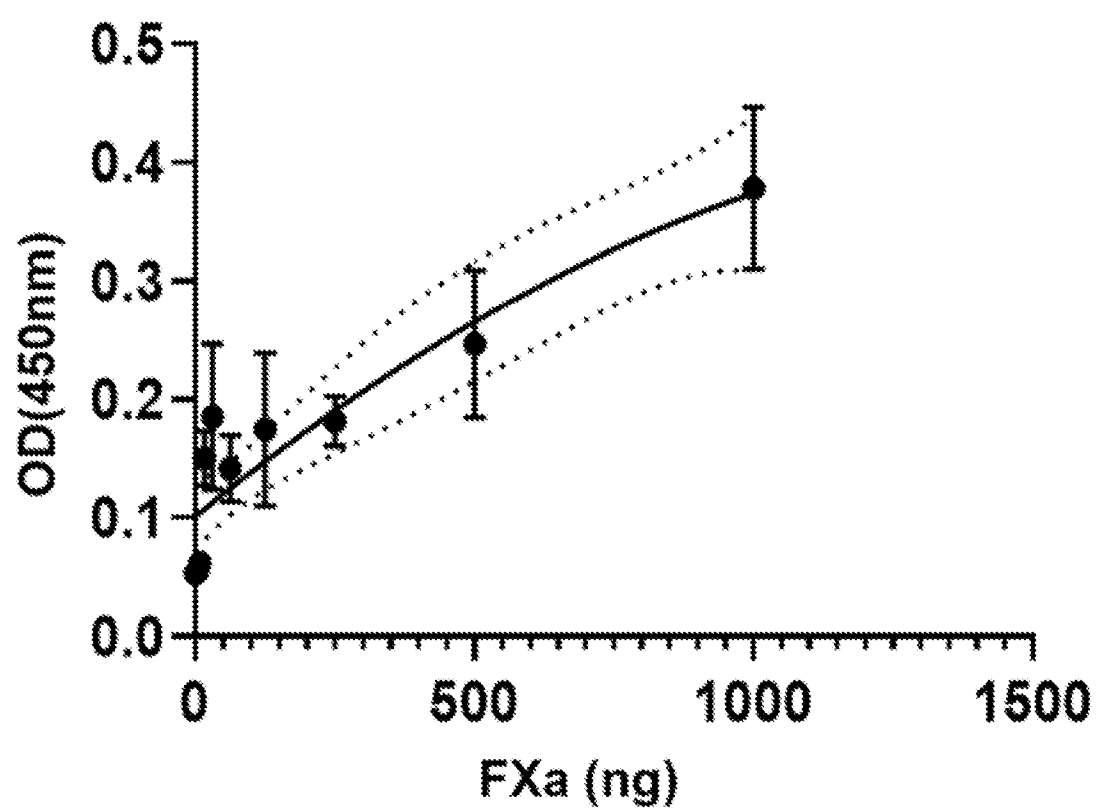
FIG. 2C

FIG. 2D

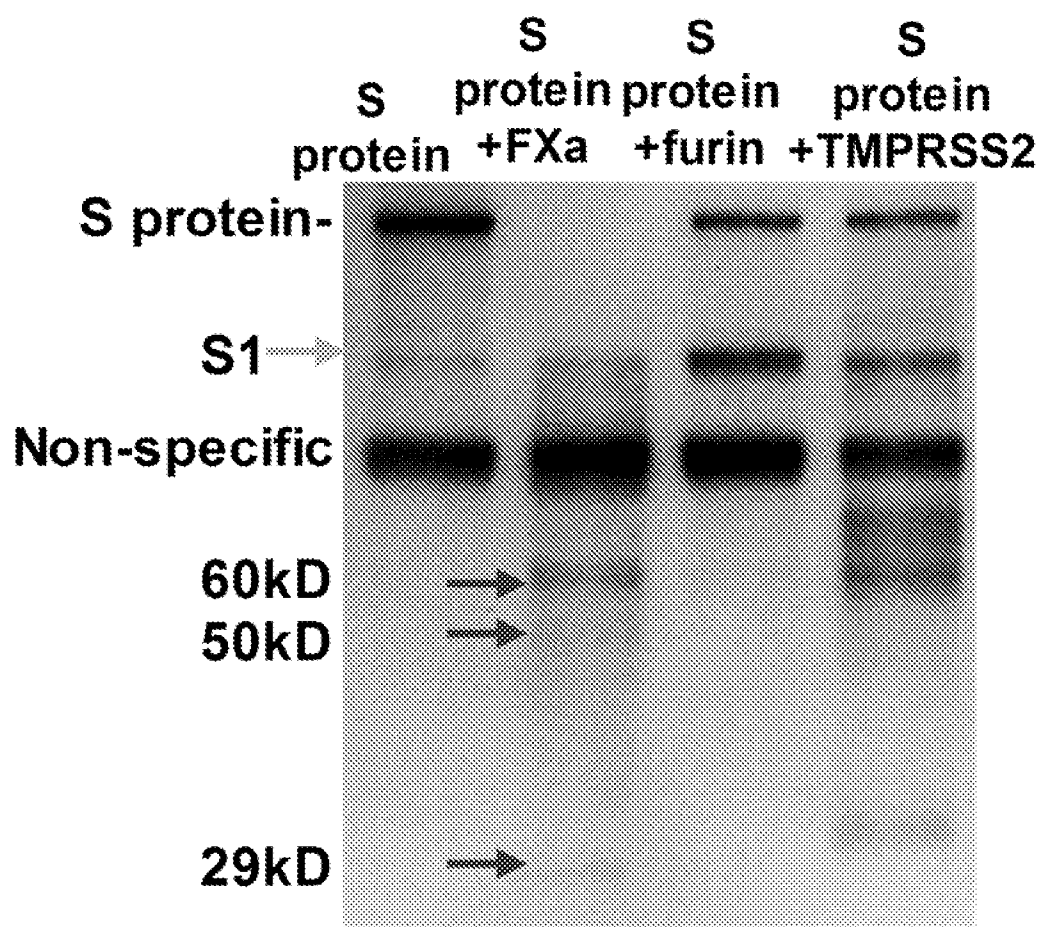


FIG. 2E

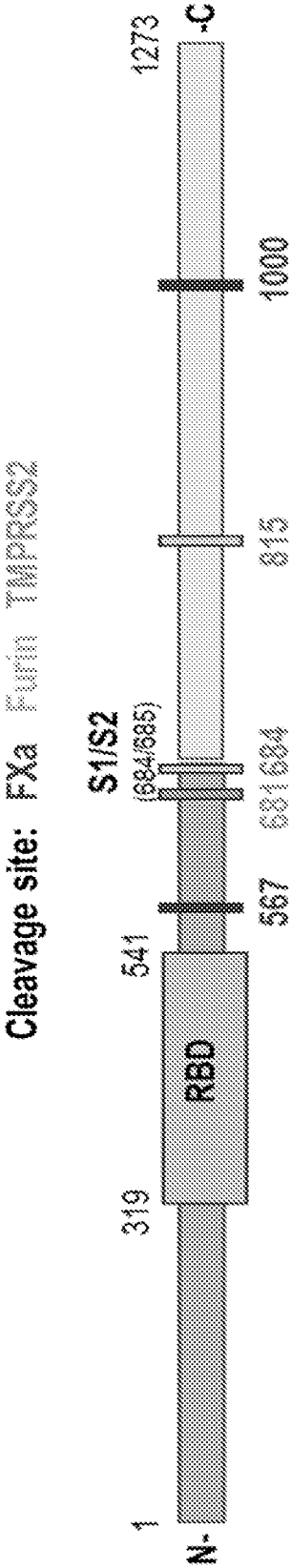


FIG. 3A

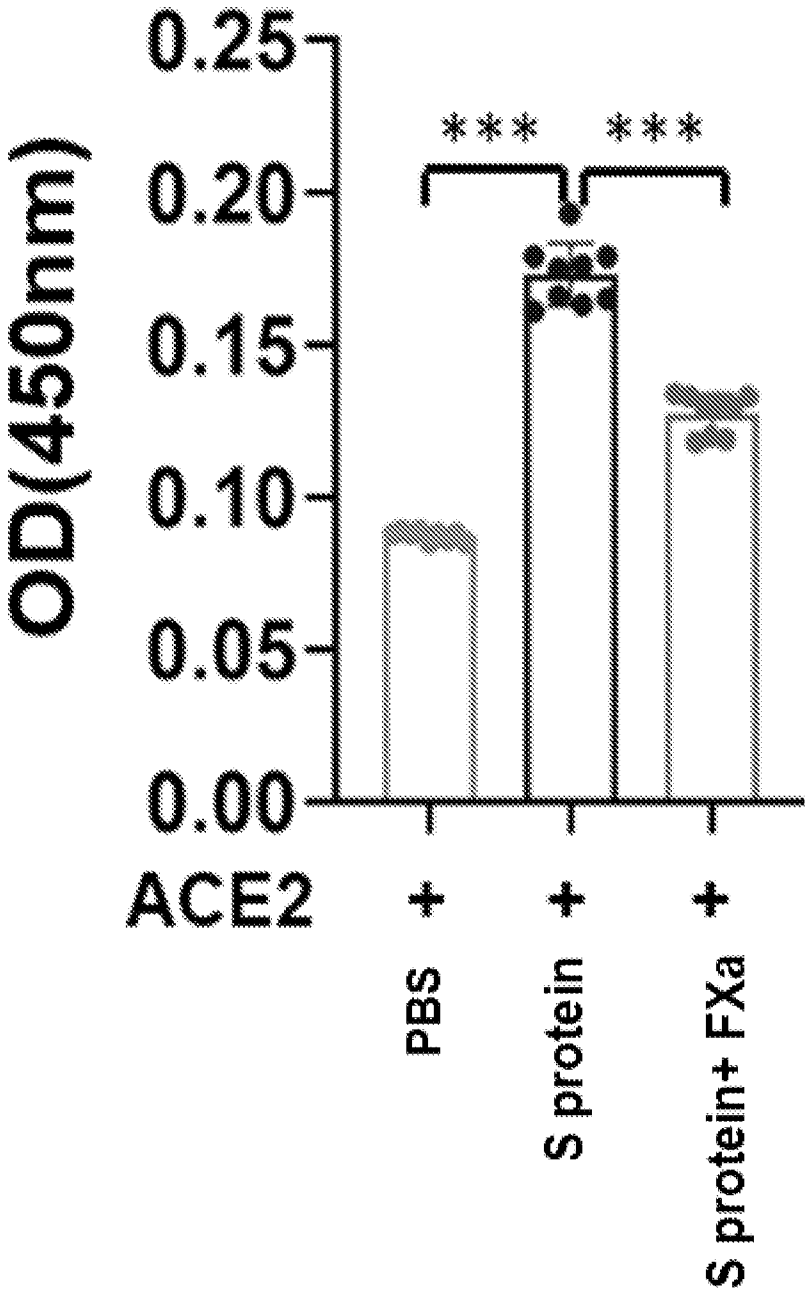


FIG. 3B

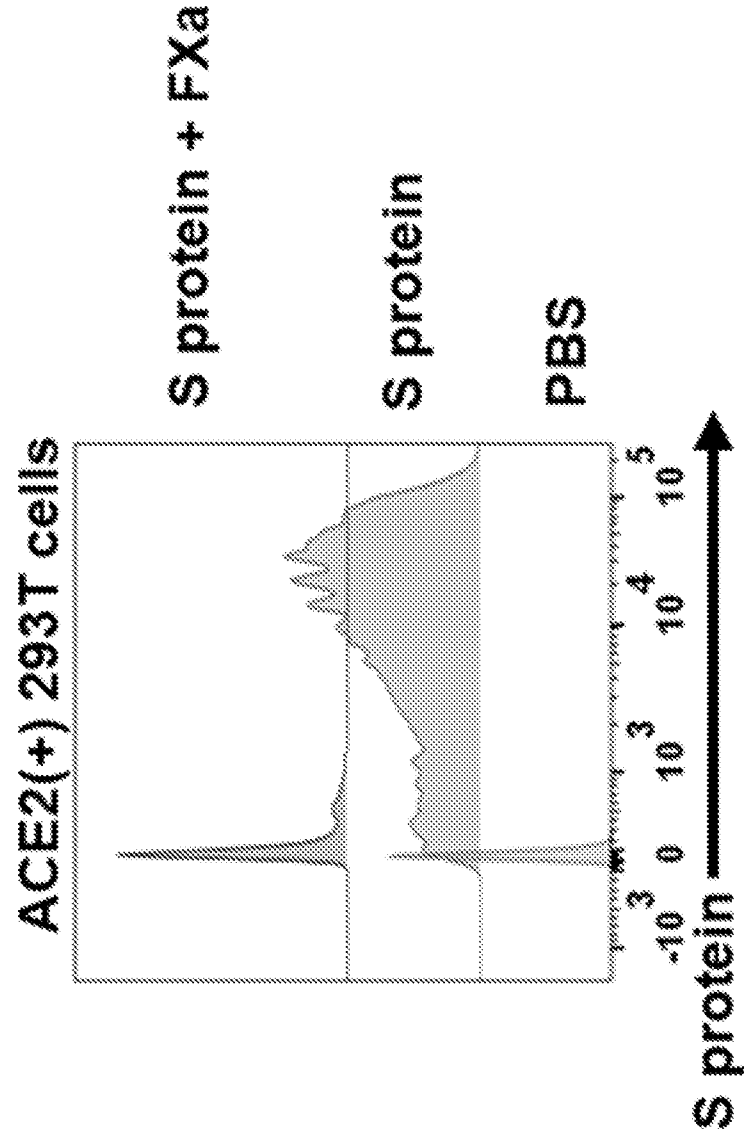


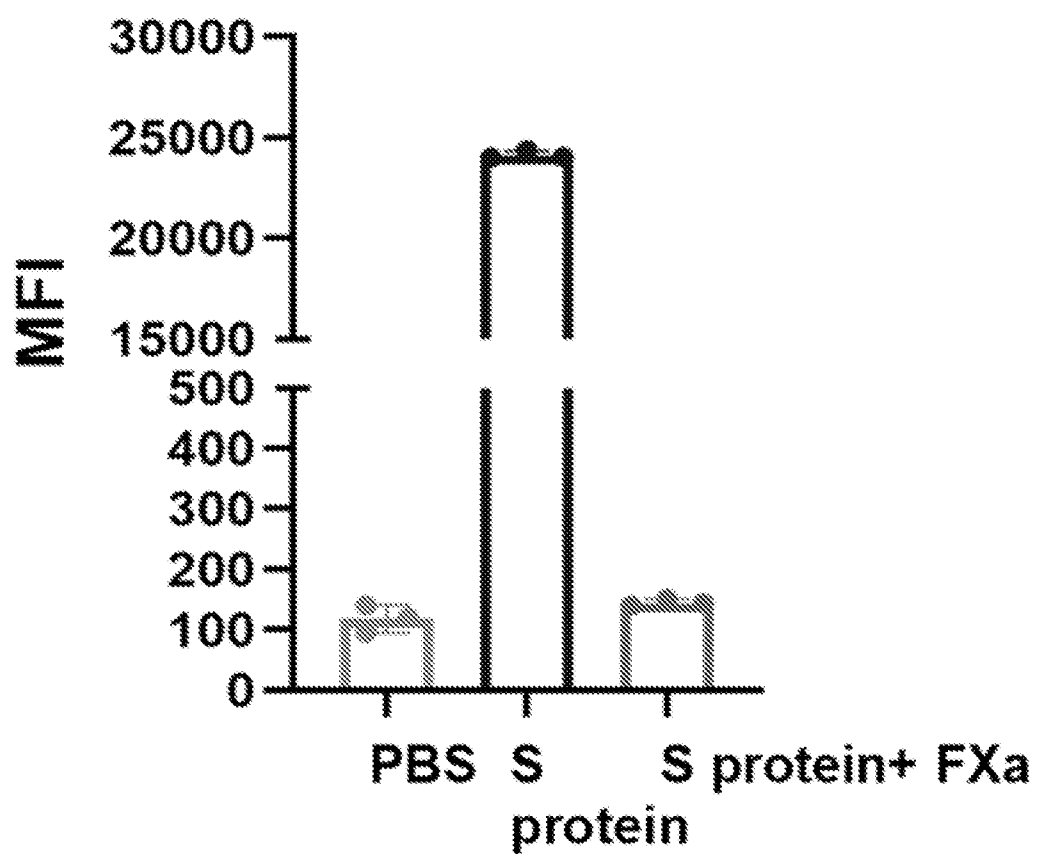
FIG. 3C

FIG. 3D

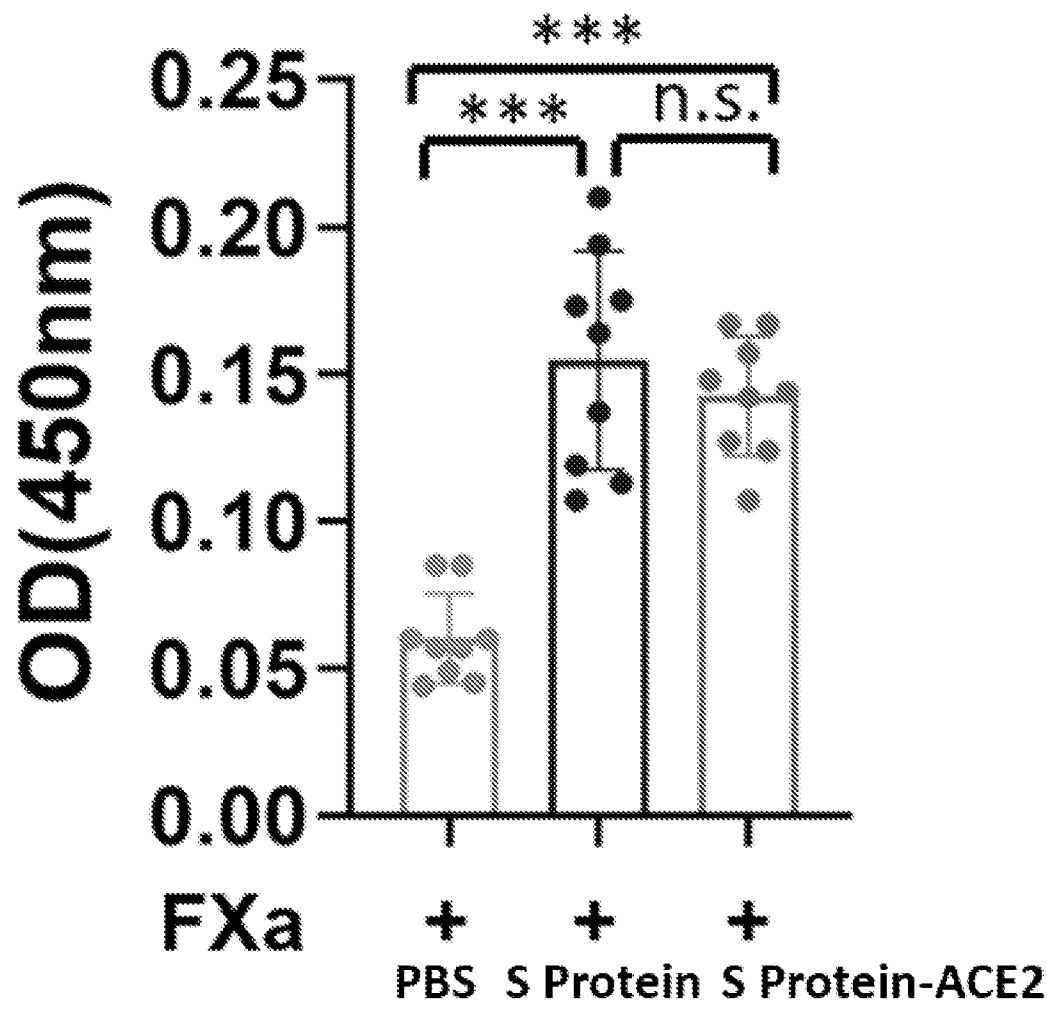


FIG. 3E

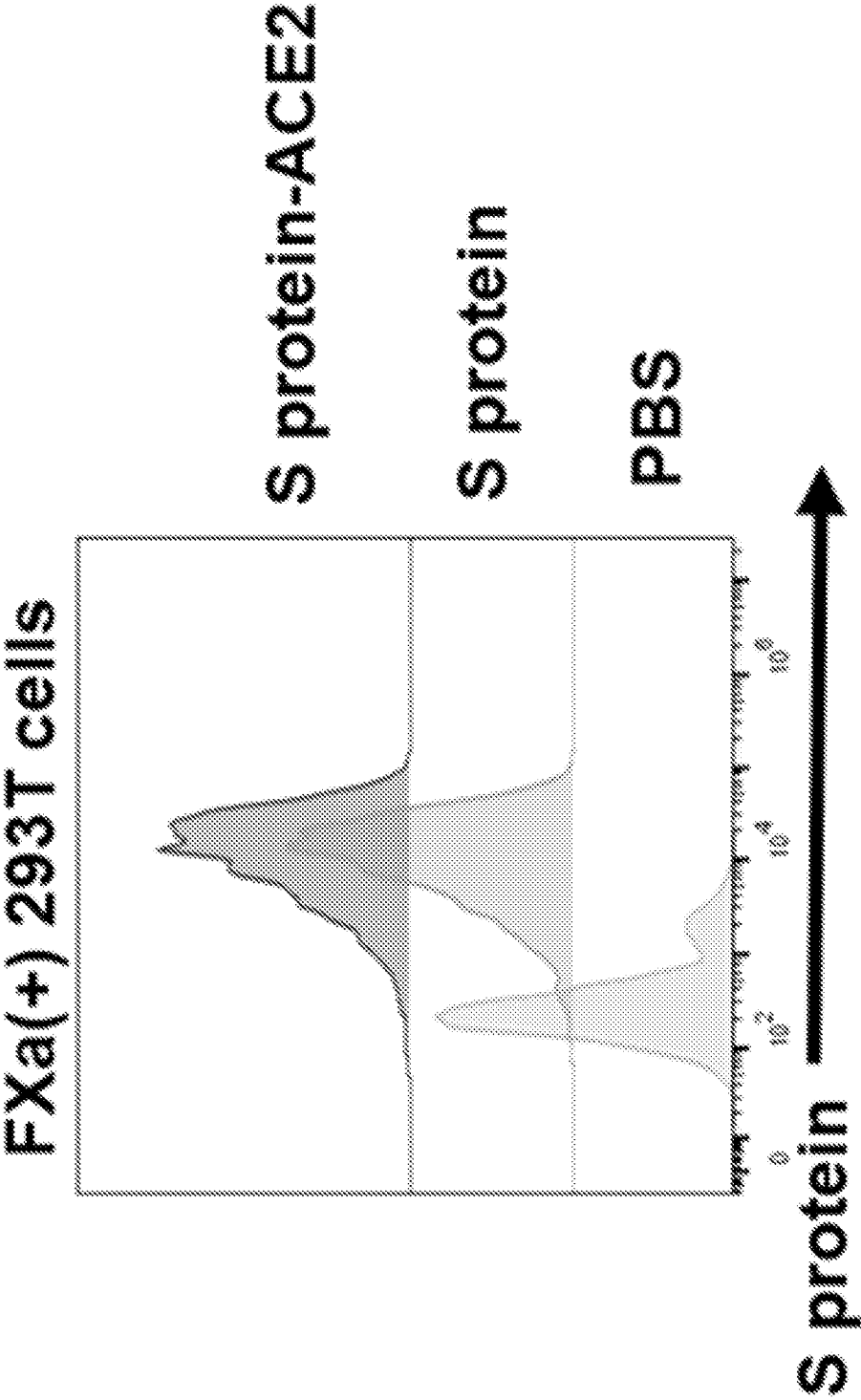


FIG. 3F

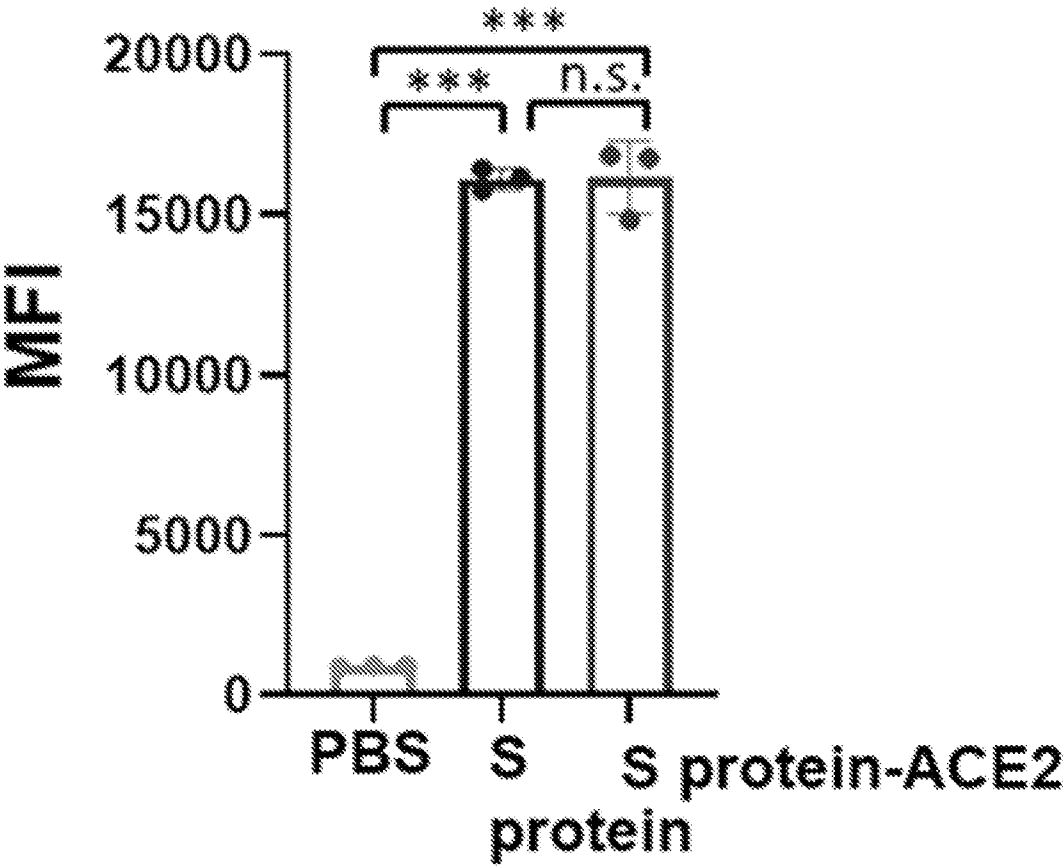


FIG. 3G

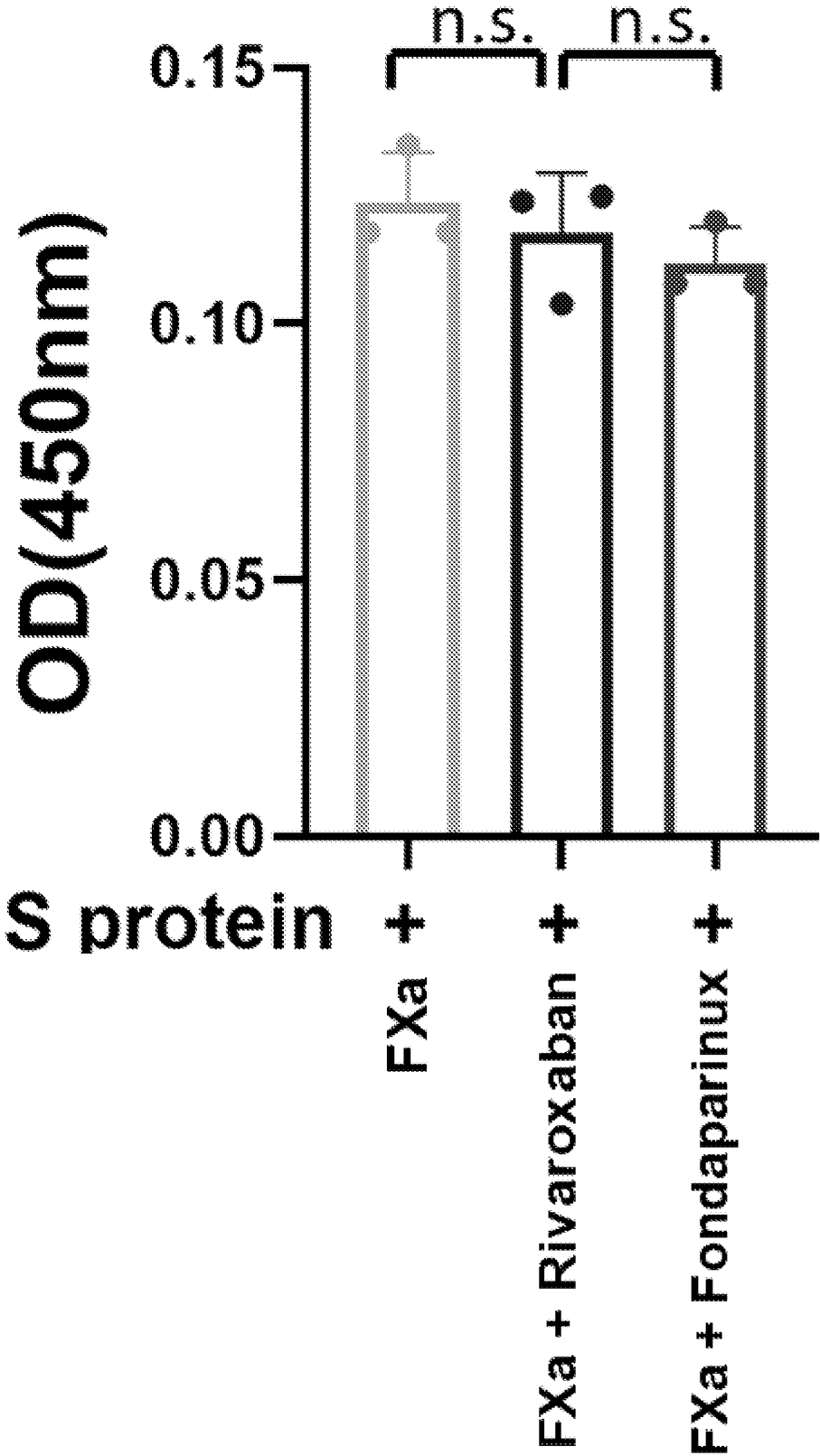


FIG. 3H

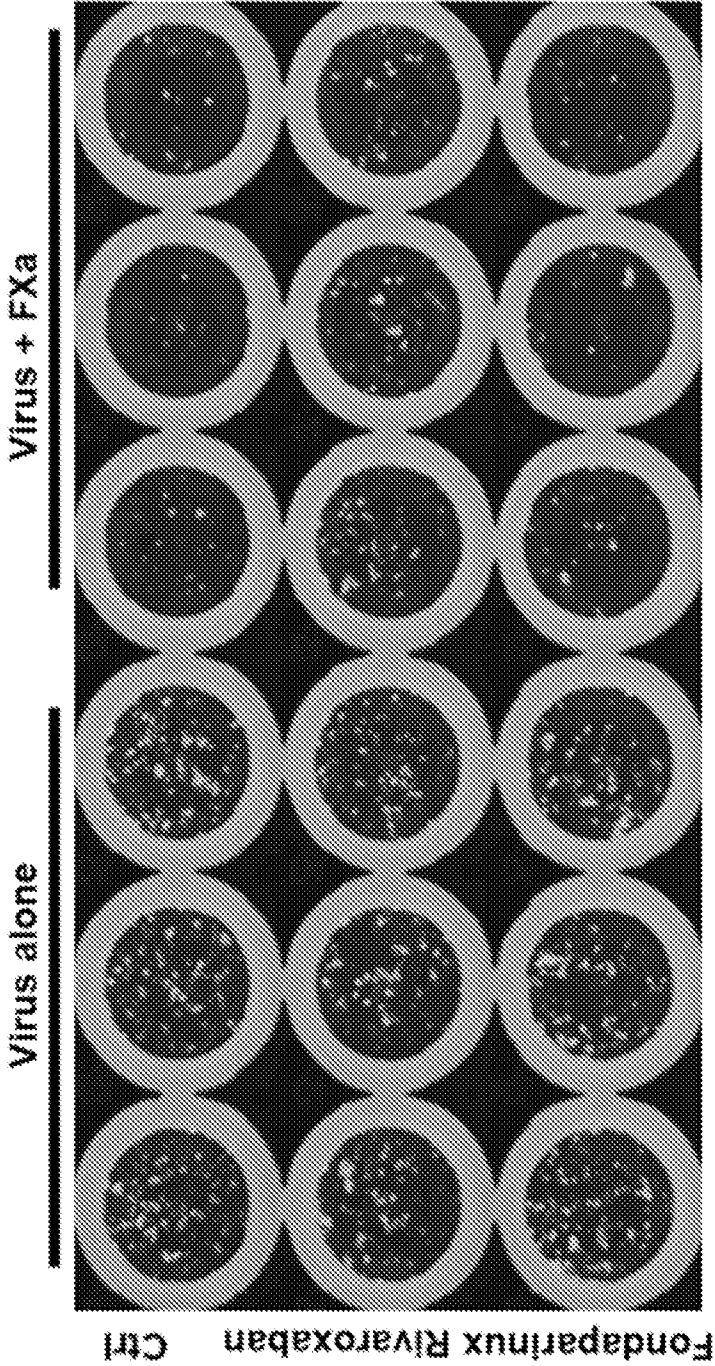


FIG. 3J

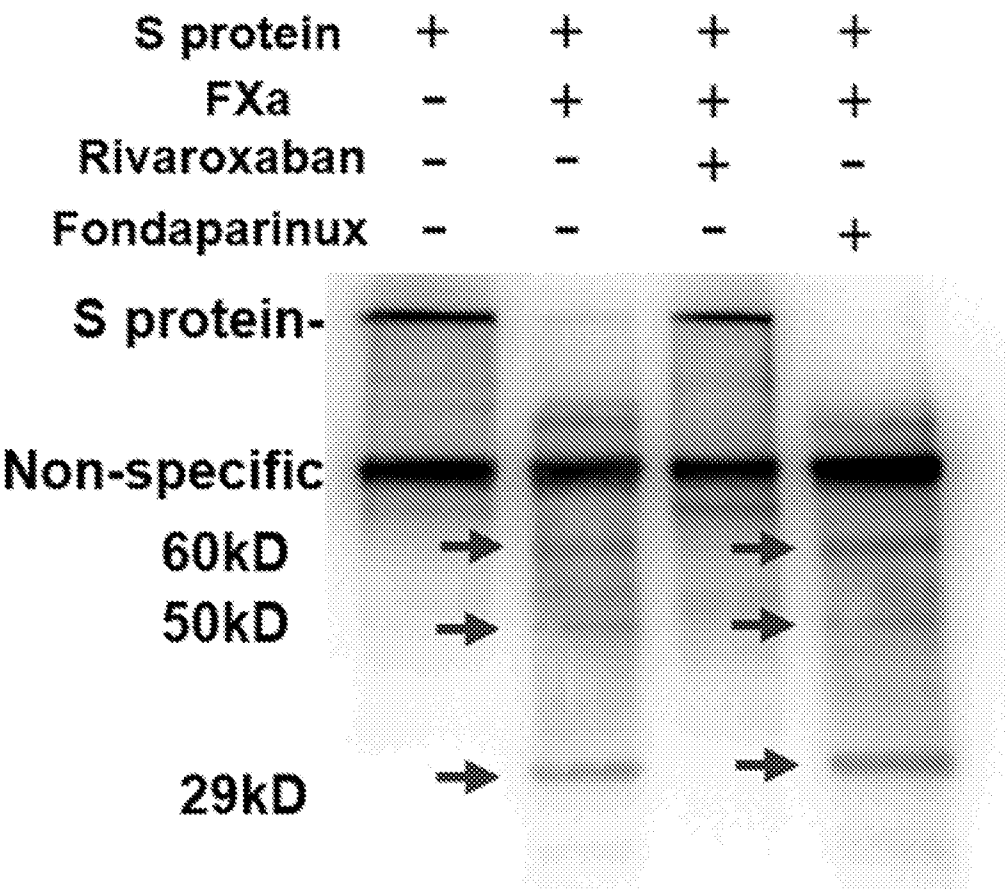


FIG. 3K

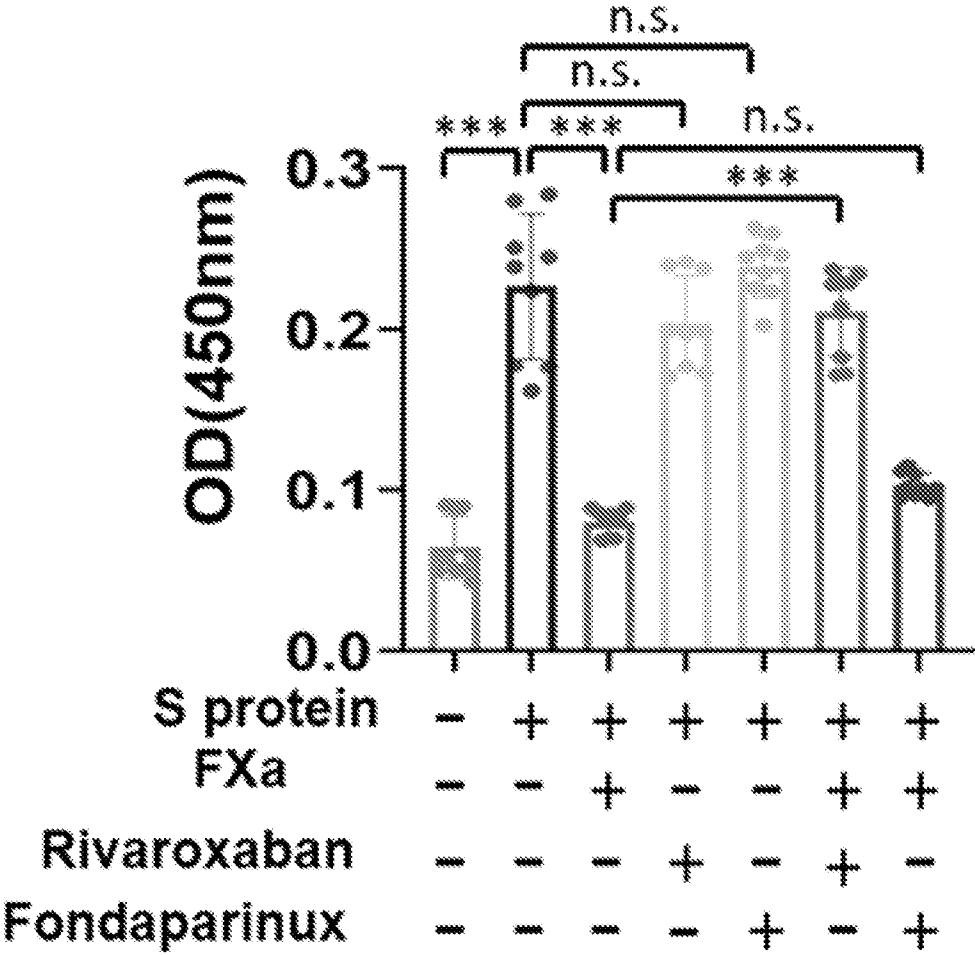


FIG. 3L

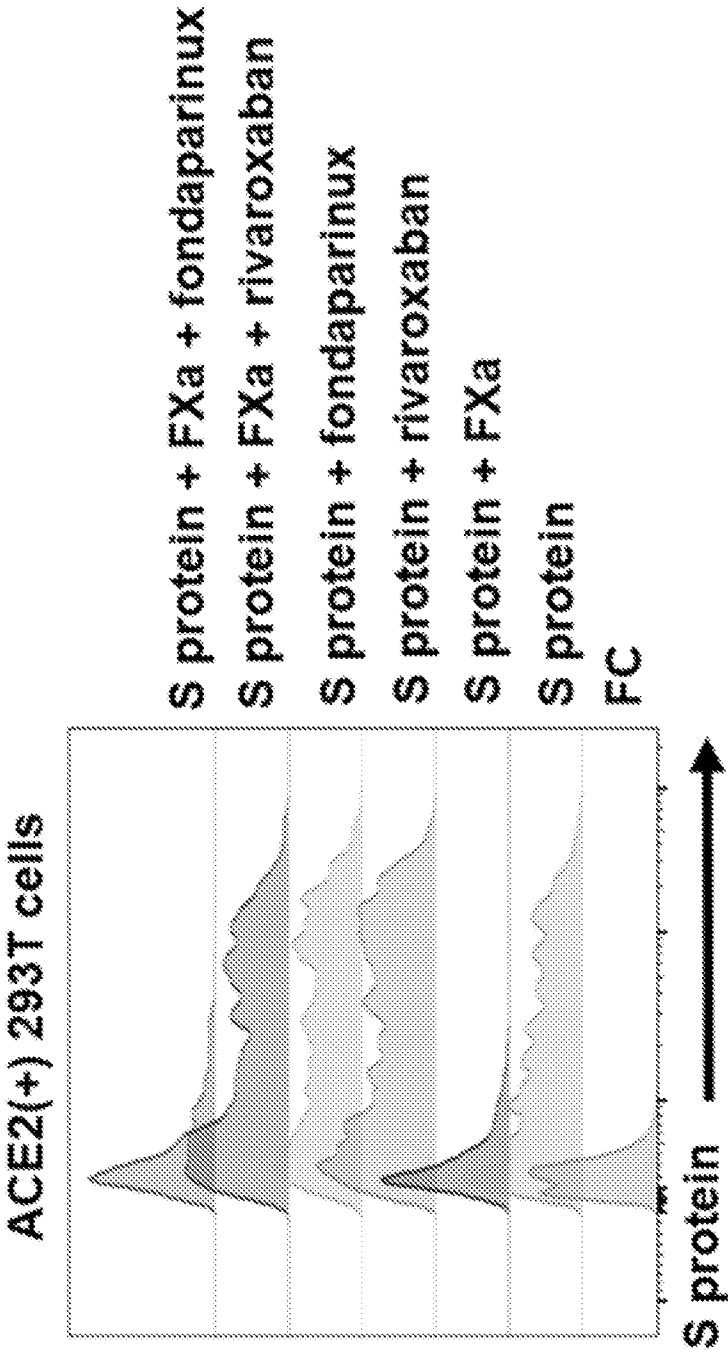


FIG. 3M

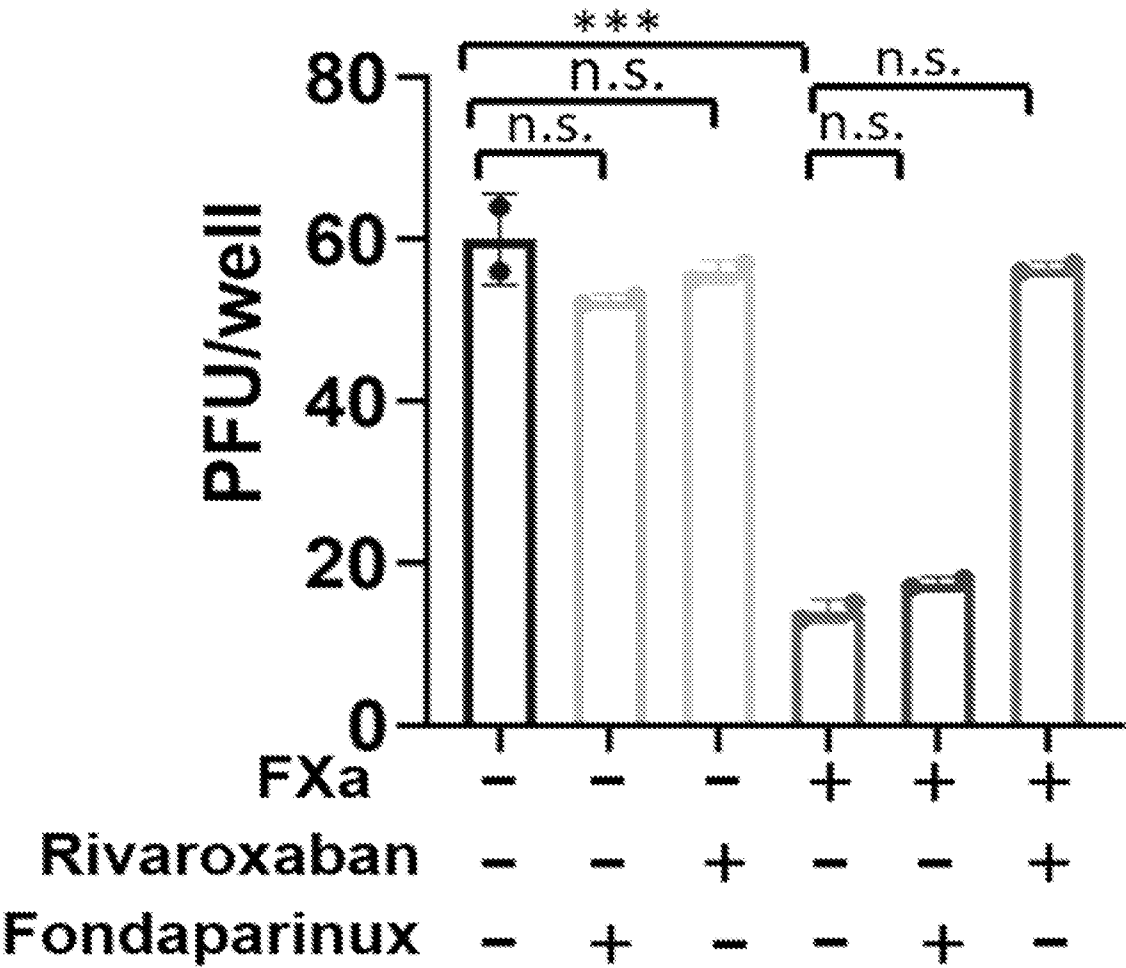


FIG. 4A

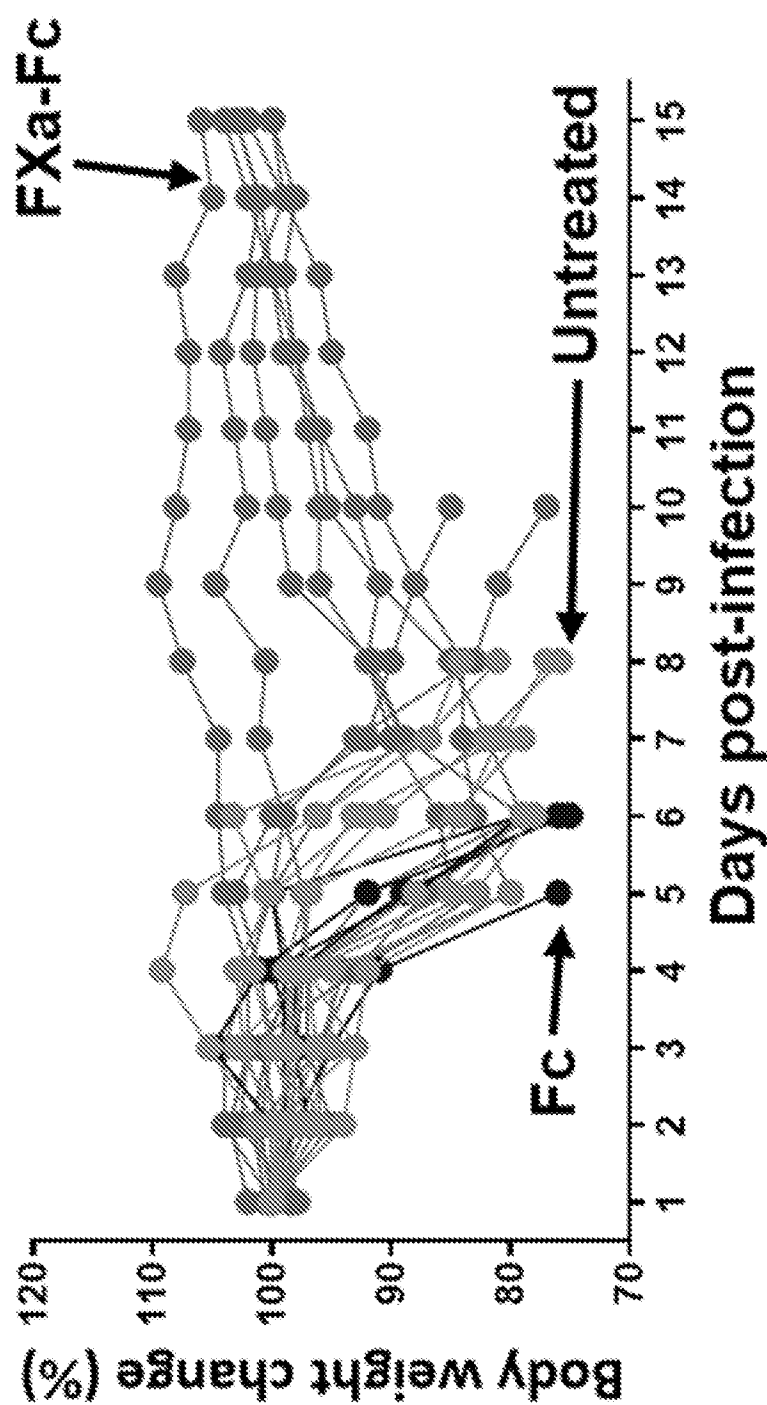


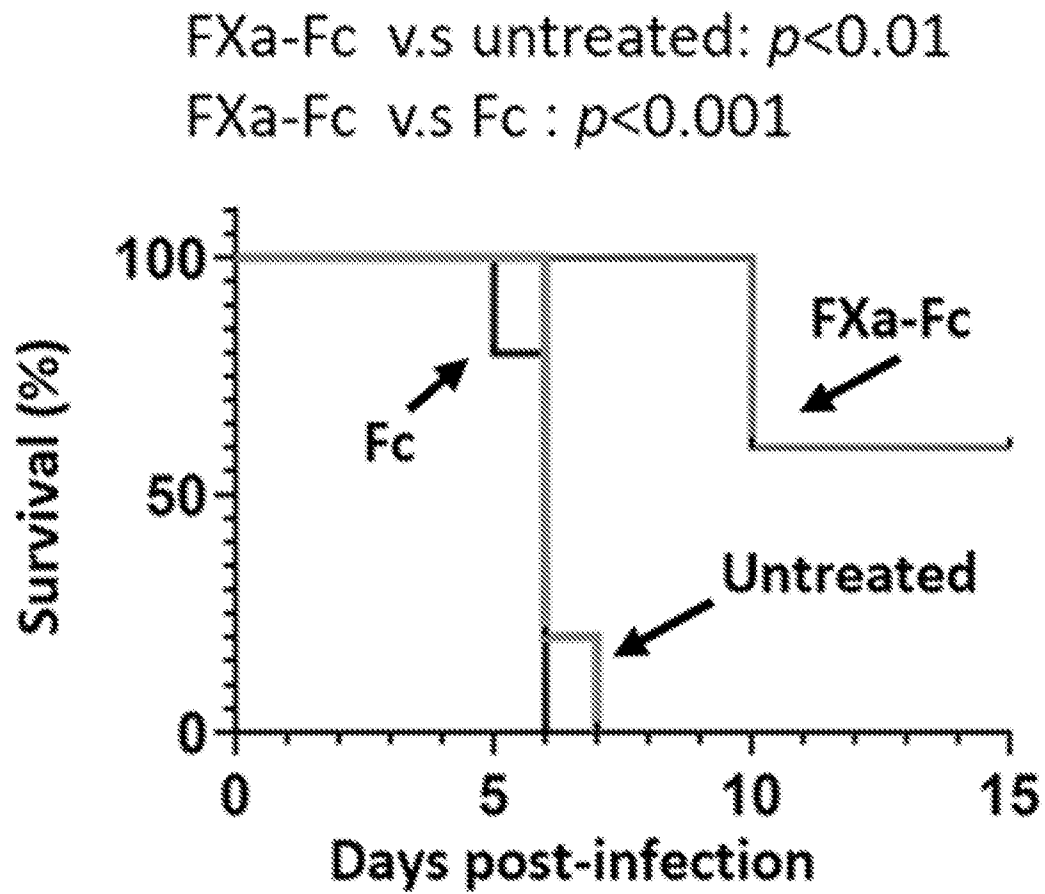
FIG. 4B

FIG. 4C

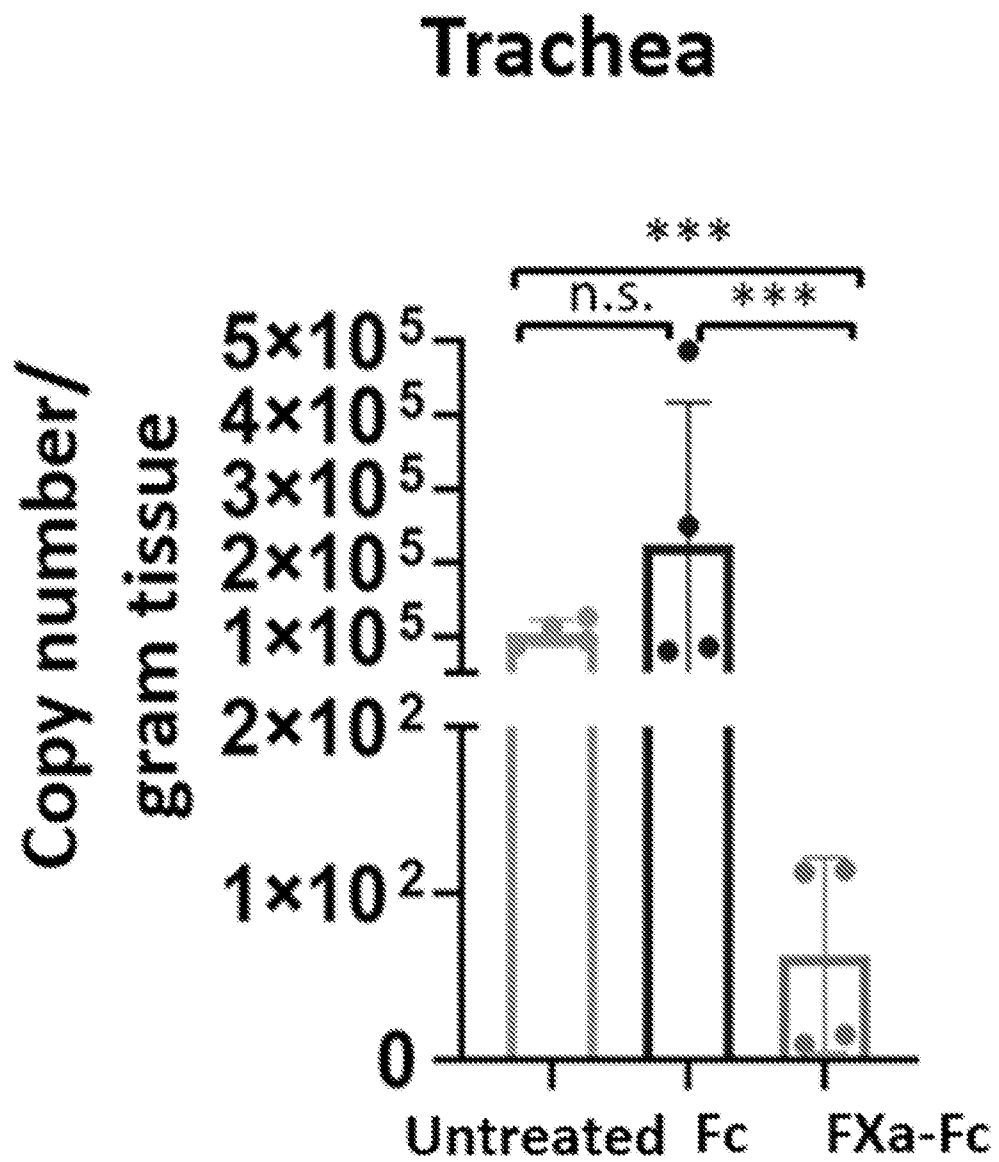


FIG. 4D

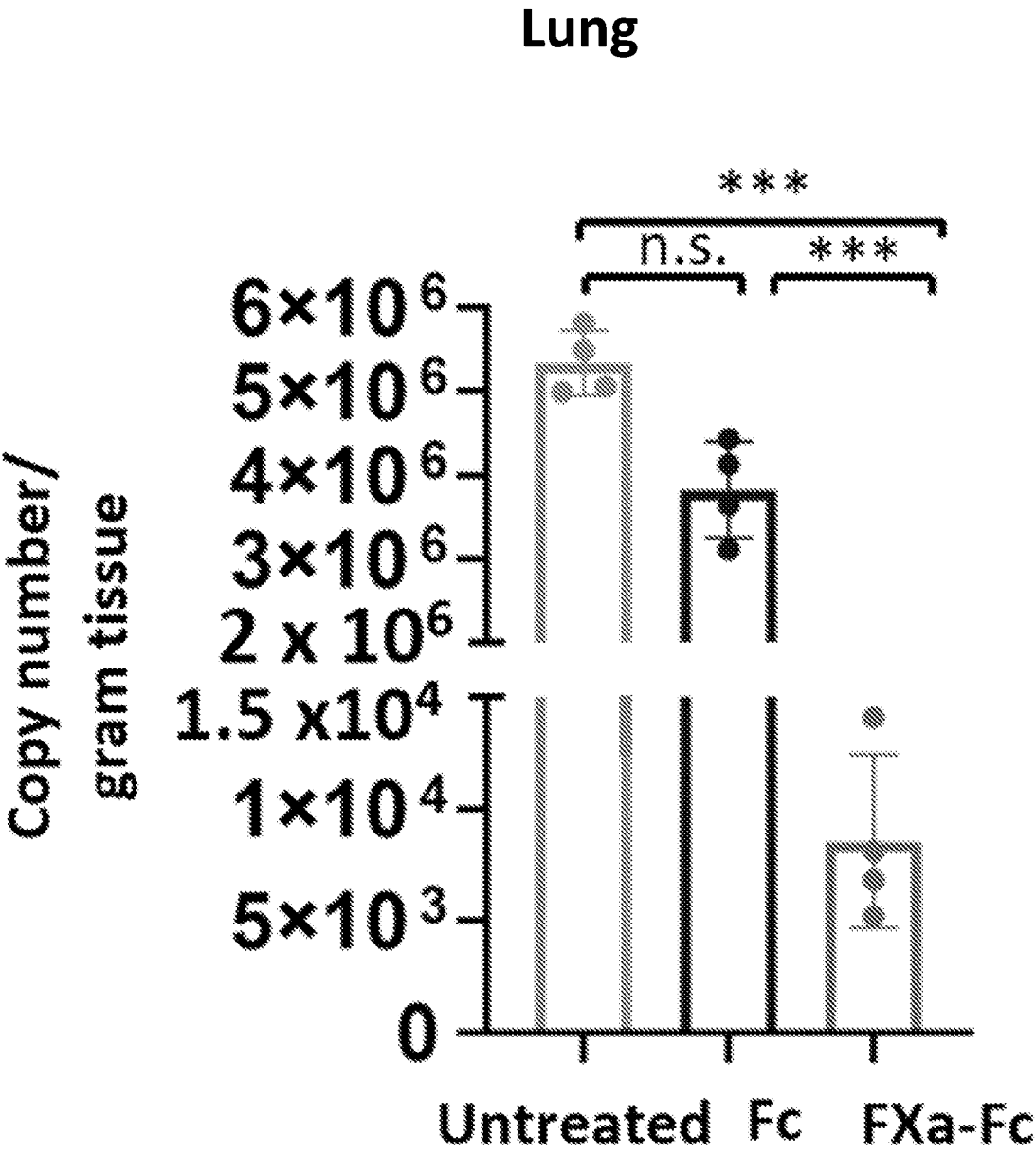


FIG. 4E

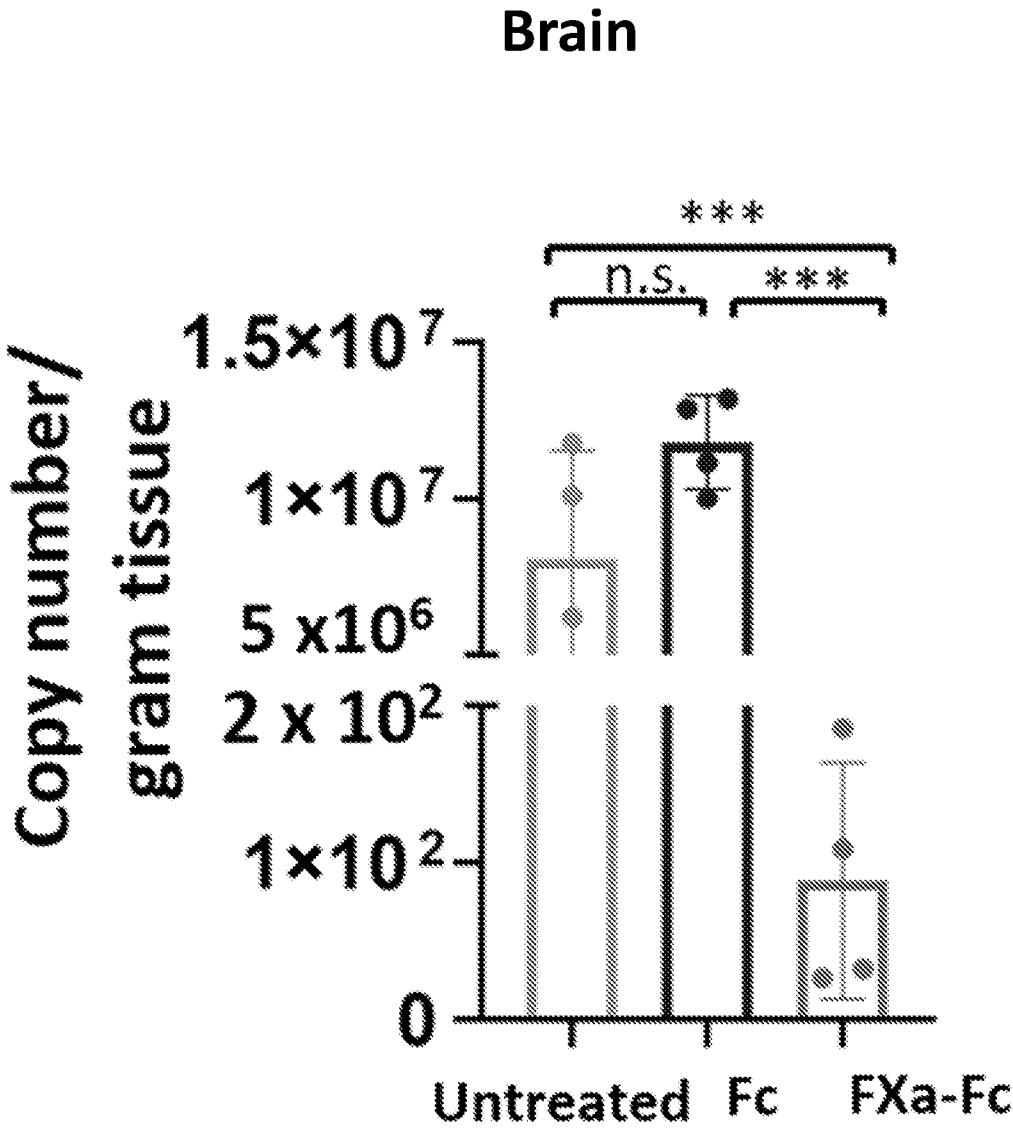


FIG. 4F

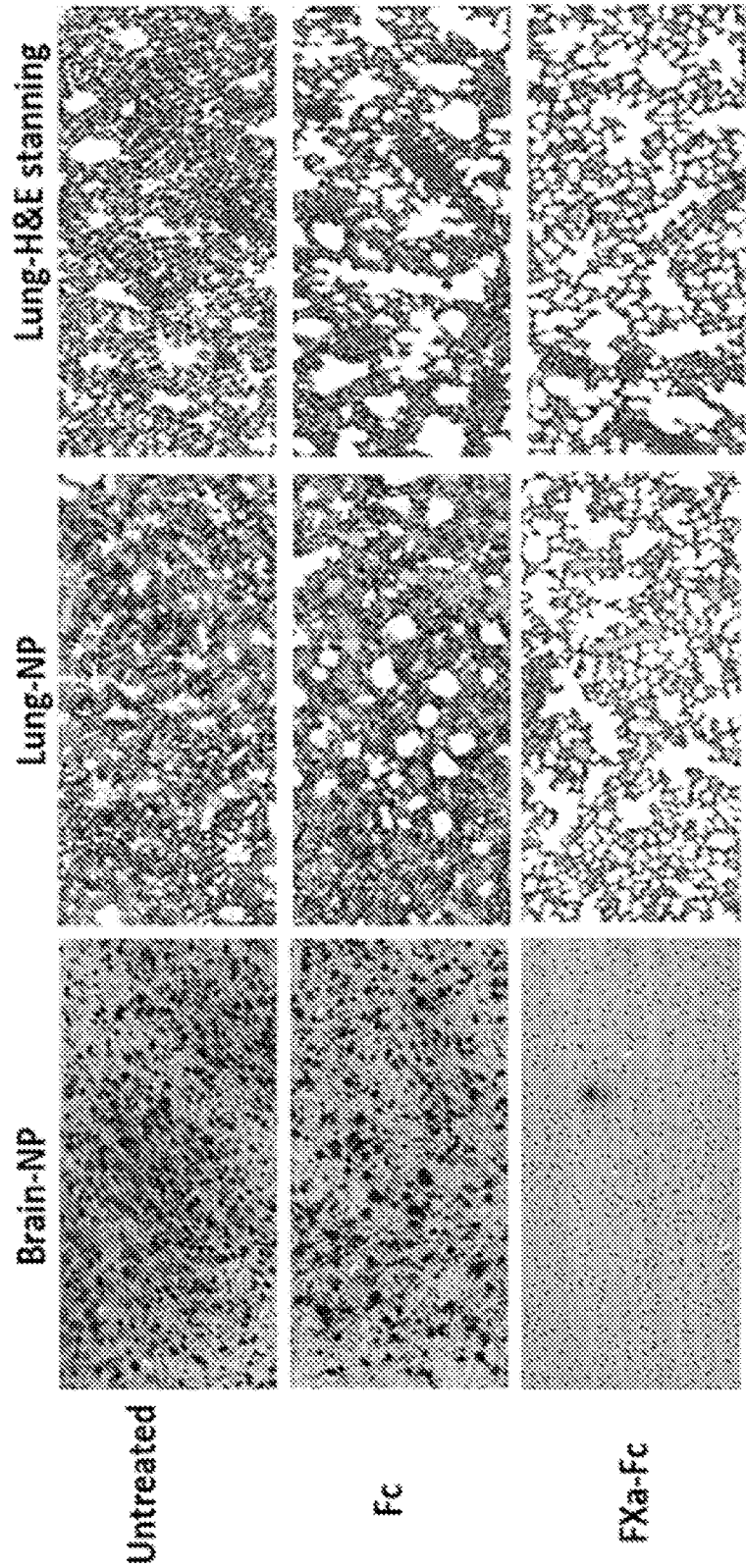


FIG. 5A

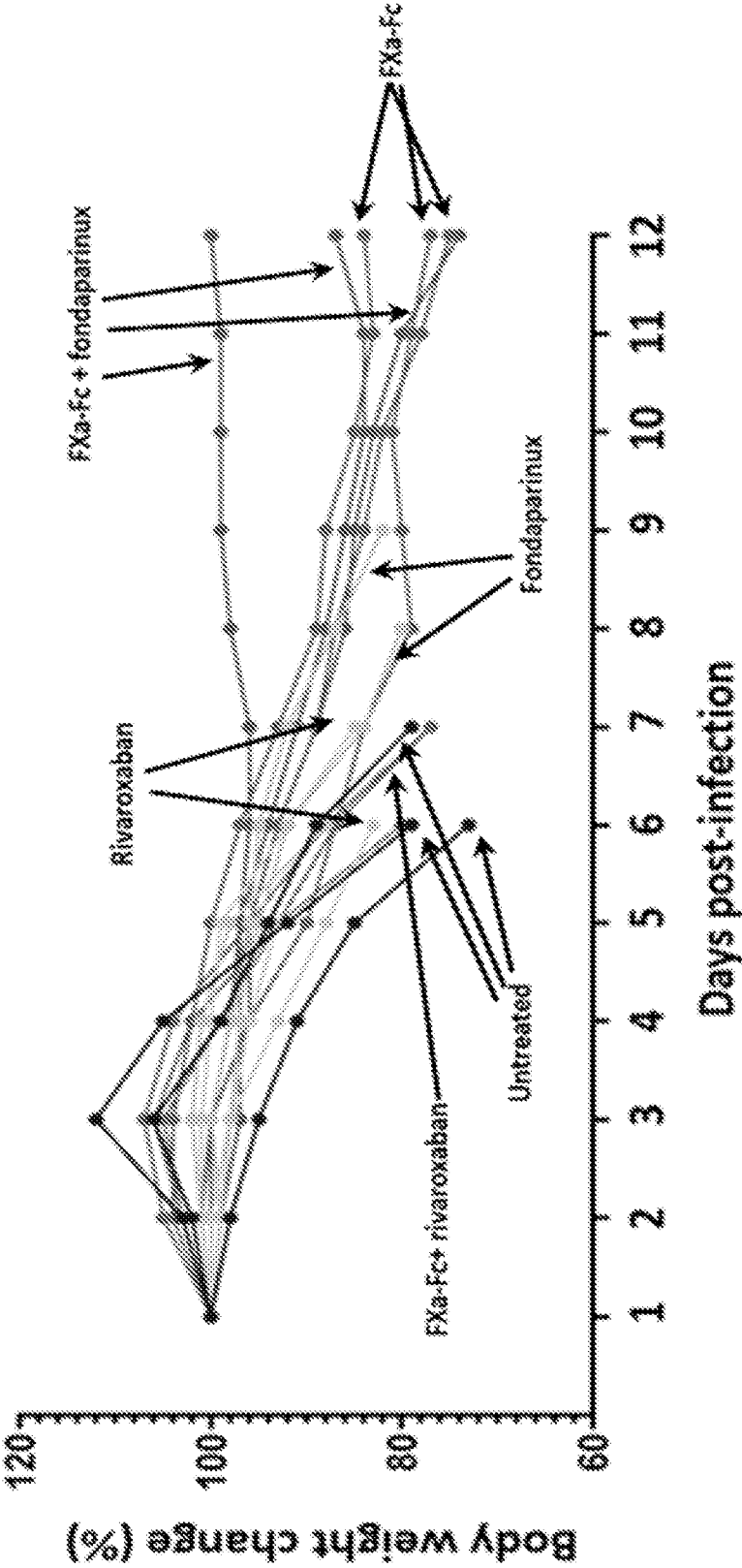


FIG. 5B

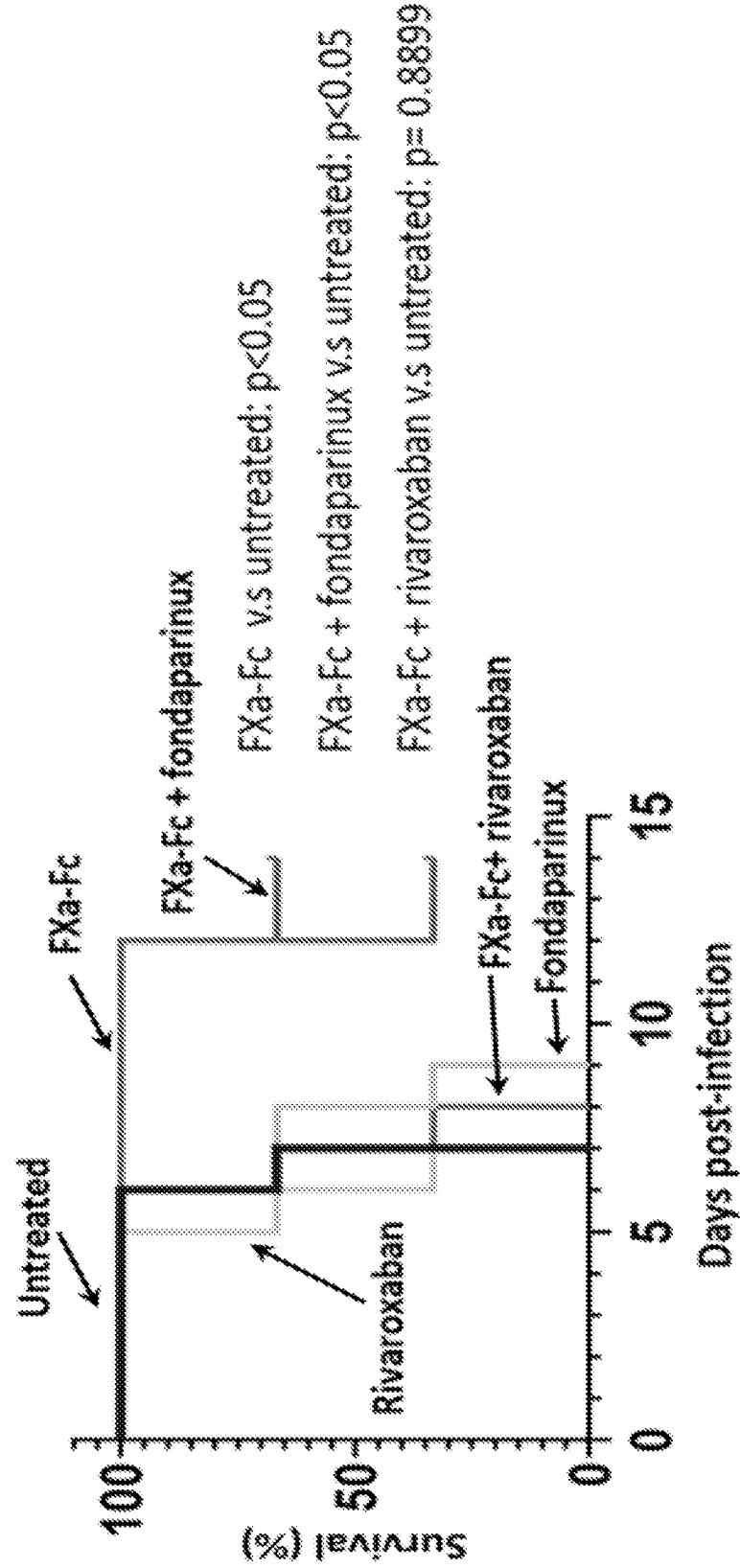


FIG. 5C

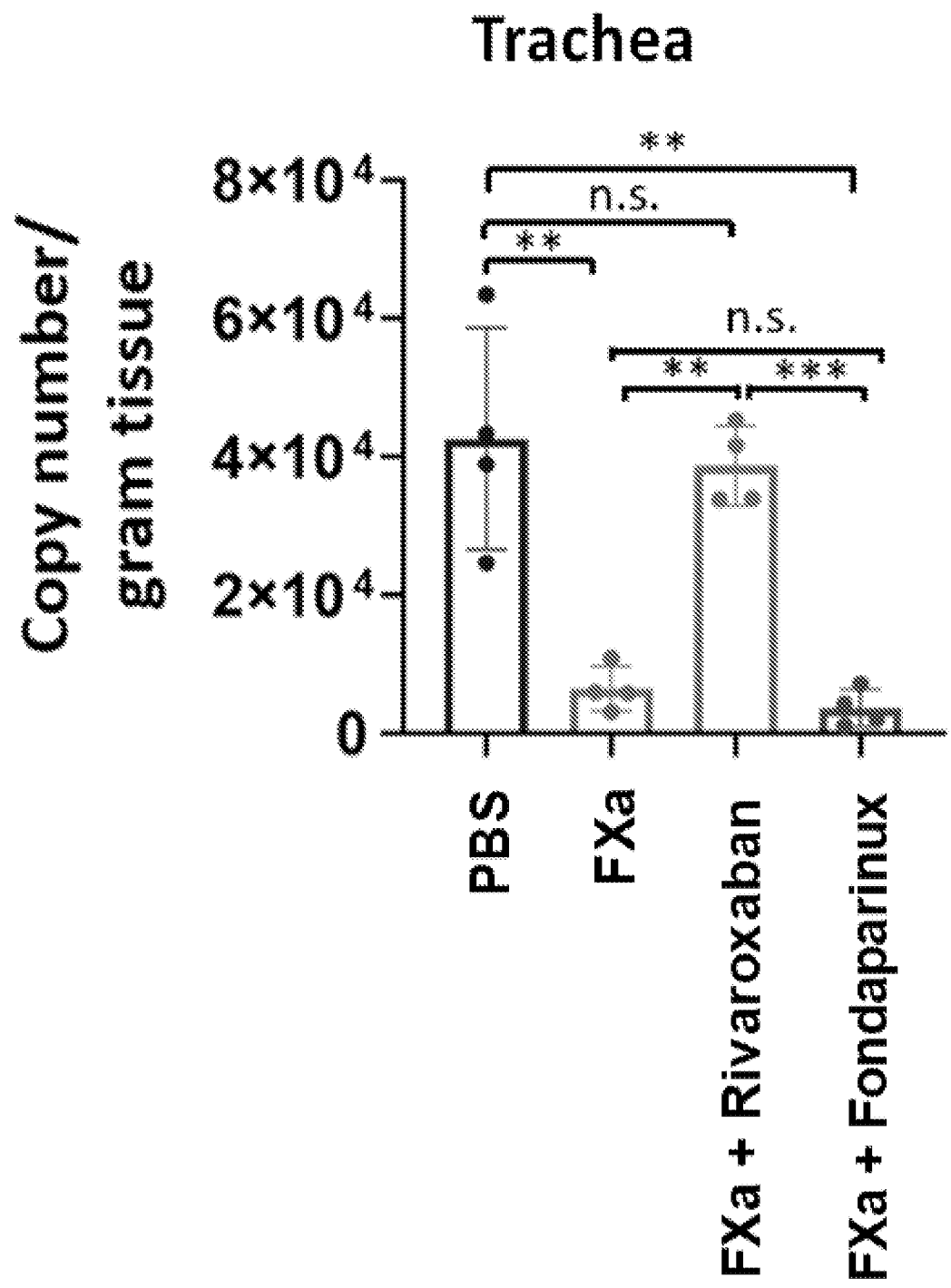


FIG. 5D

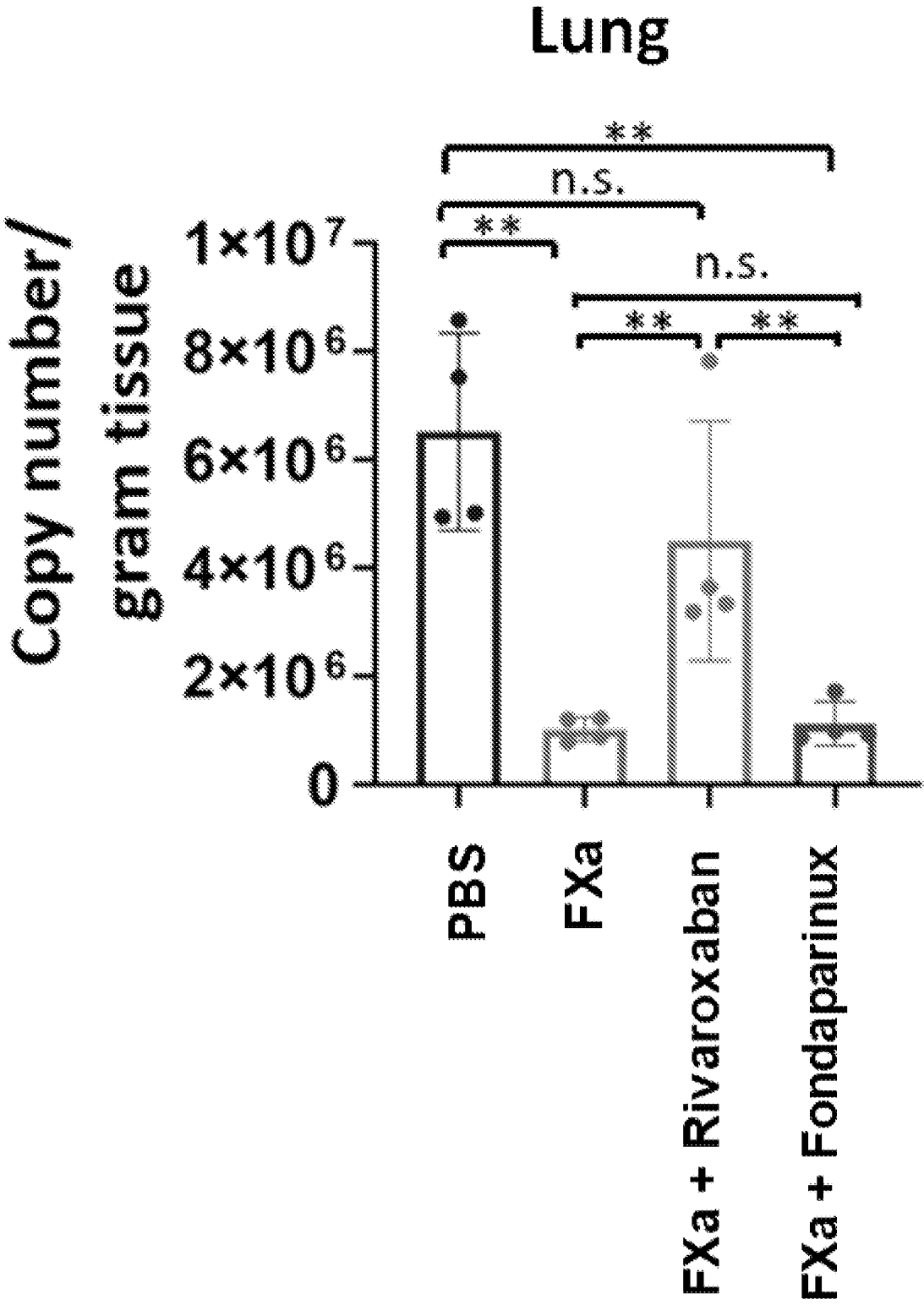


FIG. 5E

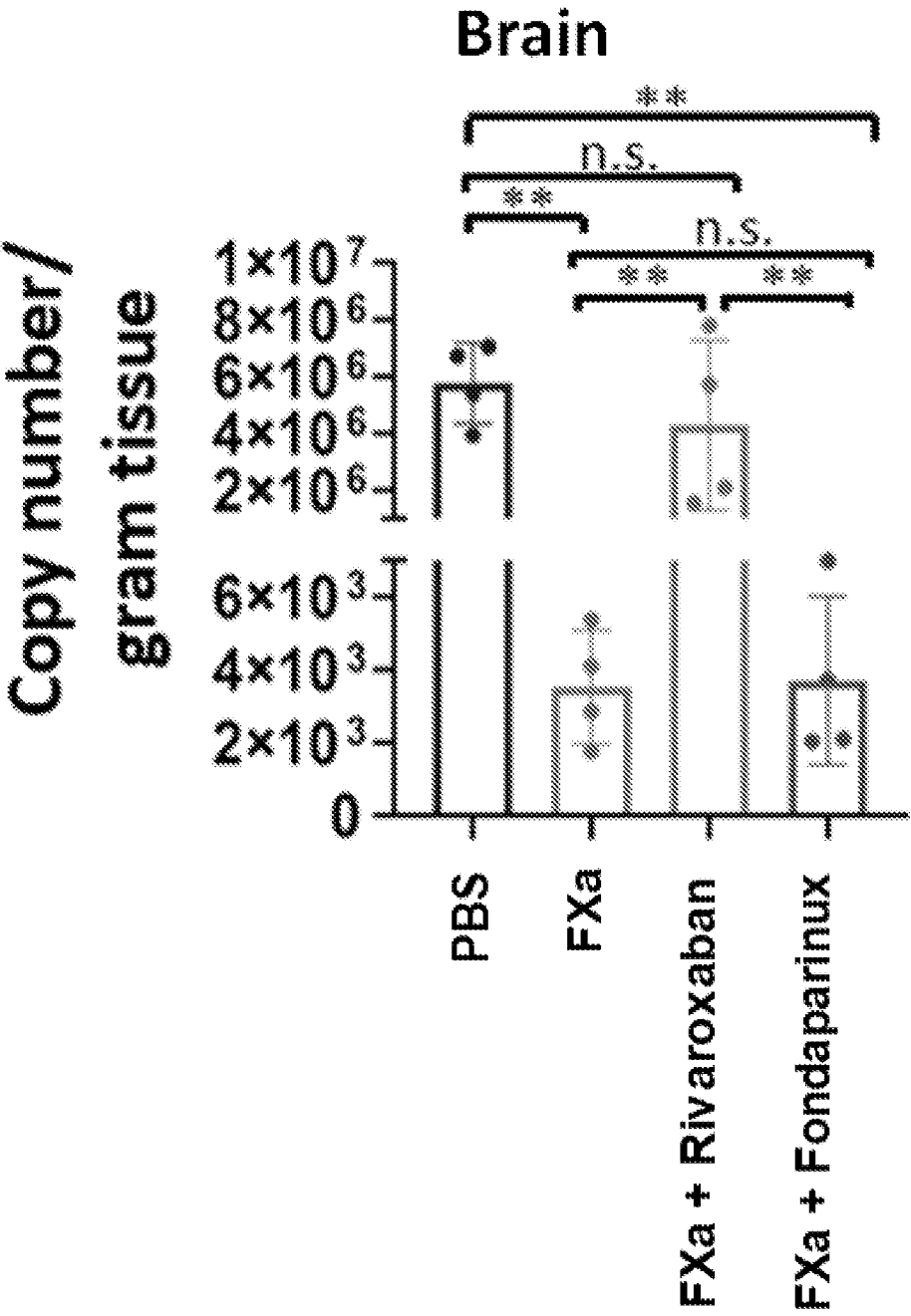


FIG. 6A

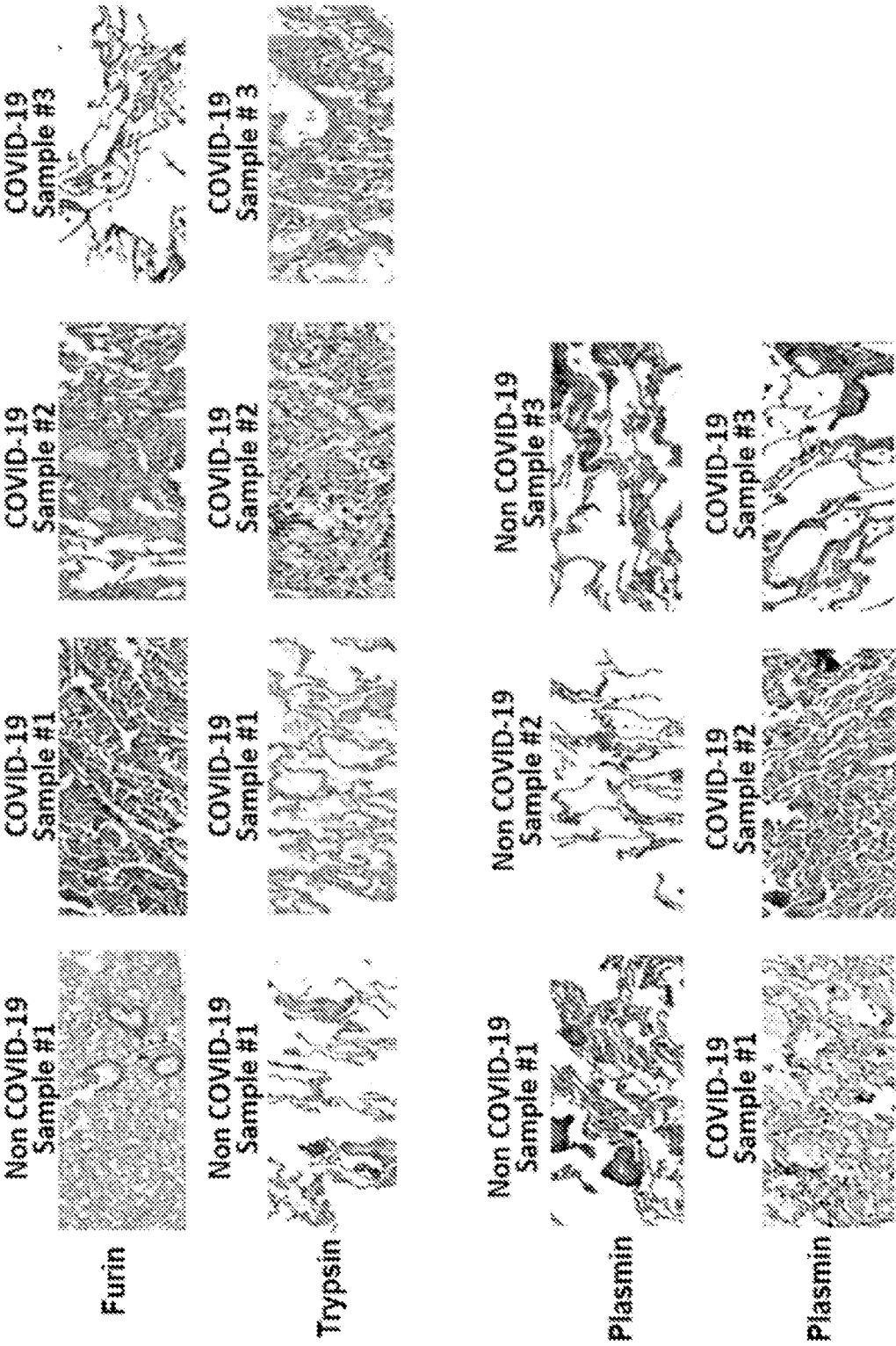
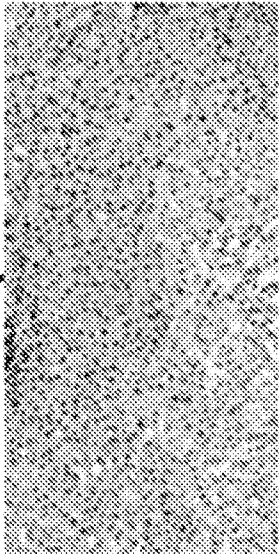


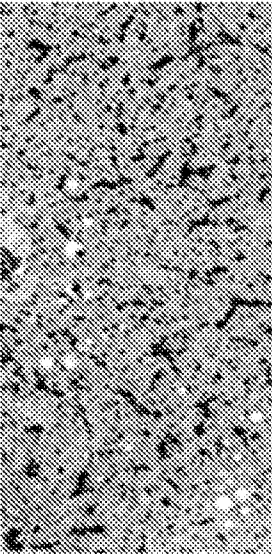
FIG. 6B

Non COVID-19
Sample #1



FXa

COVID-19
Sample #1



FXa

COVID-19
Sample #2

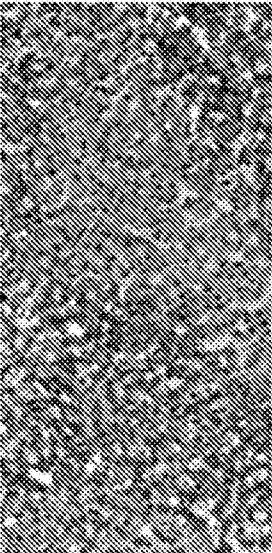


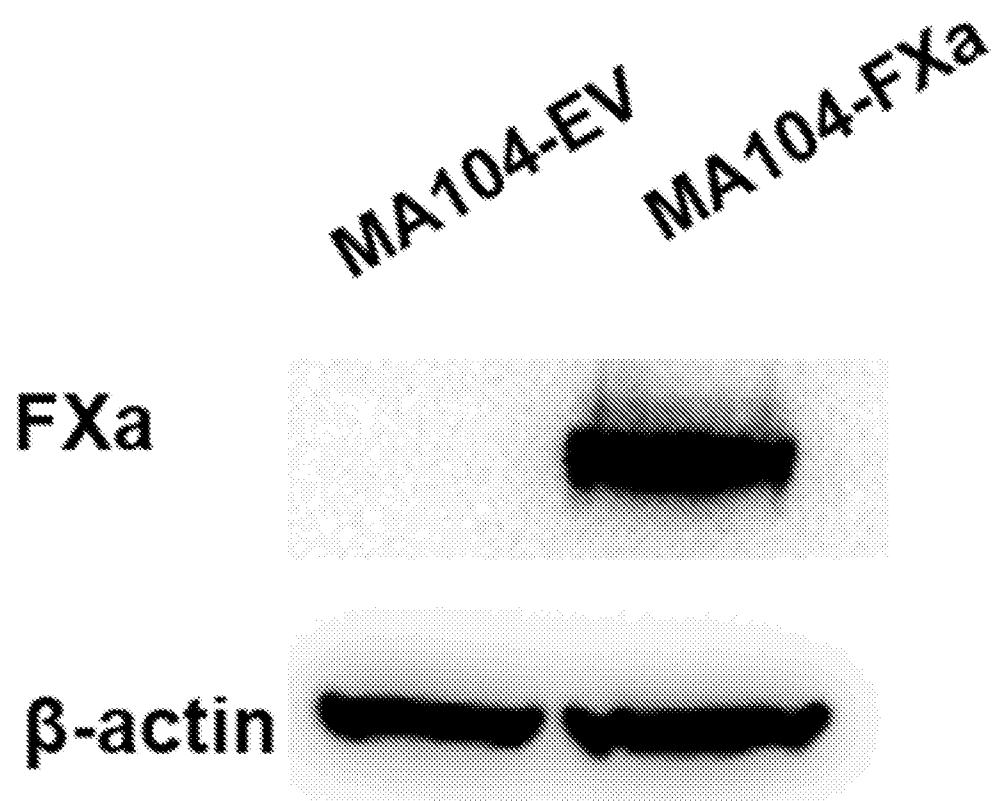
FIG. 7A

FIG. 7B

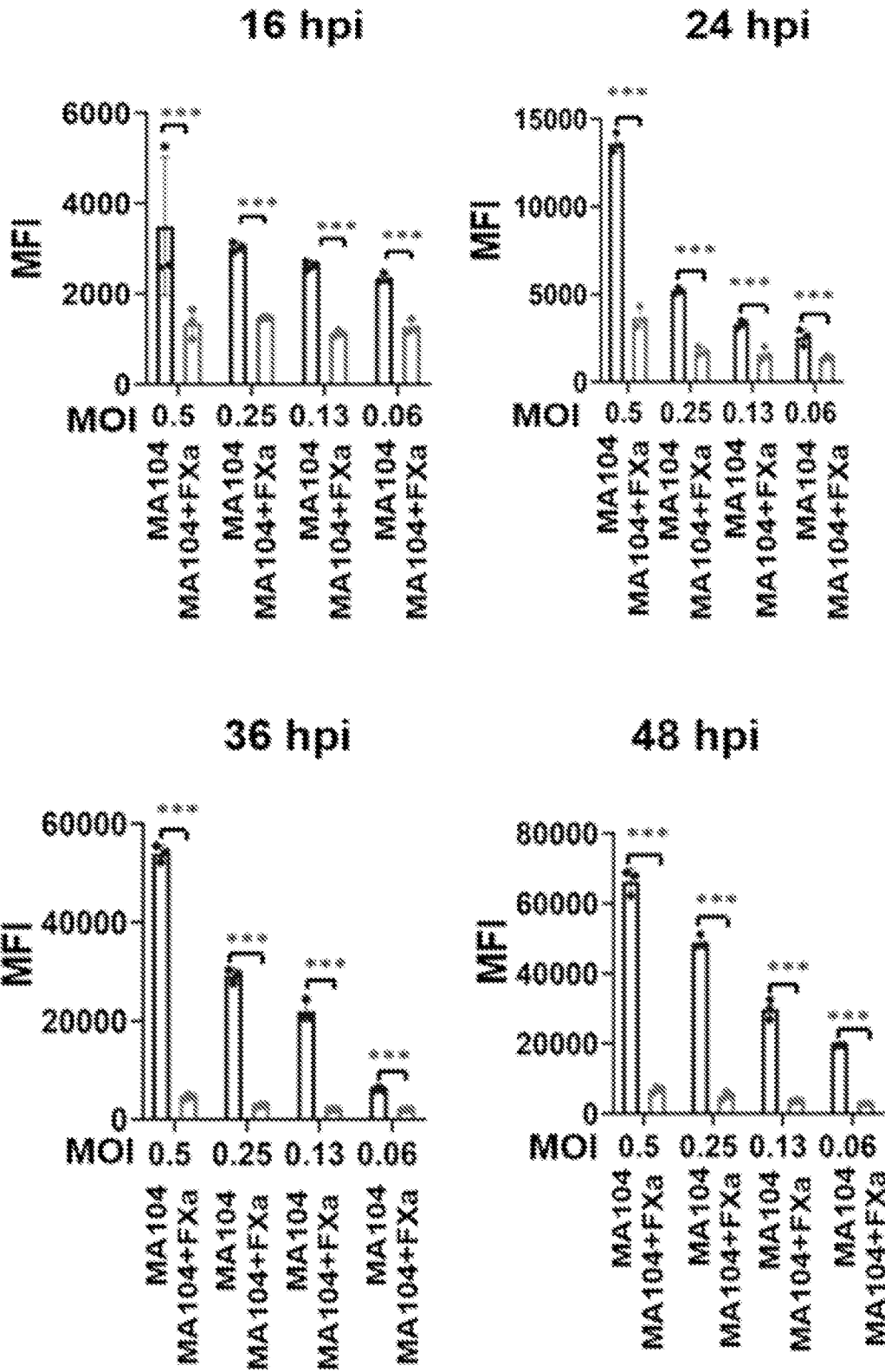


FIG. 7C

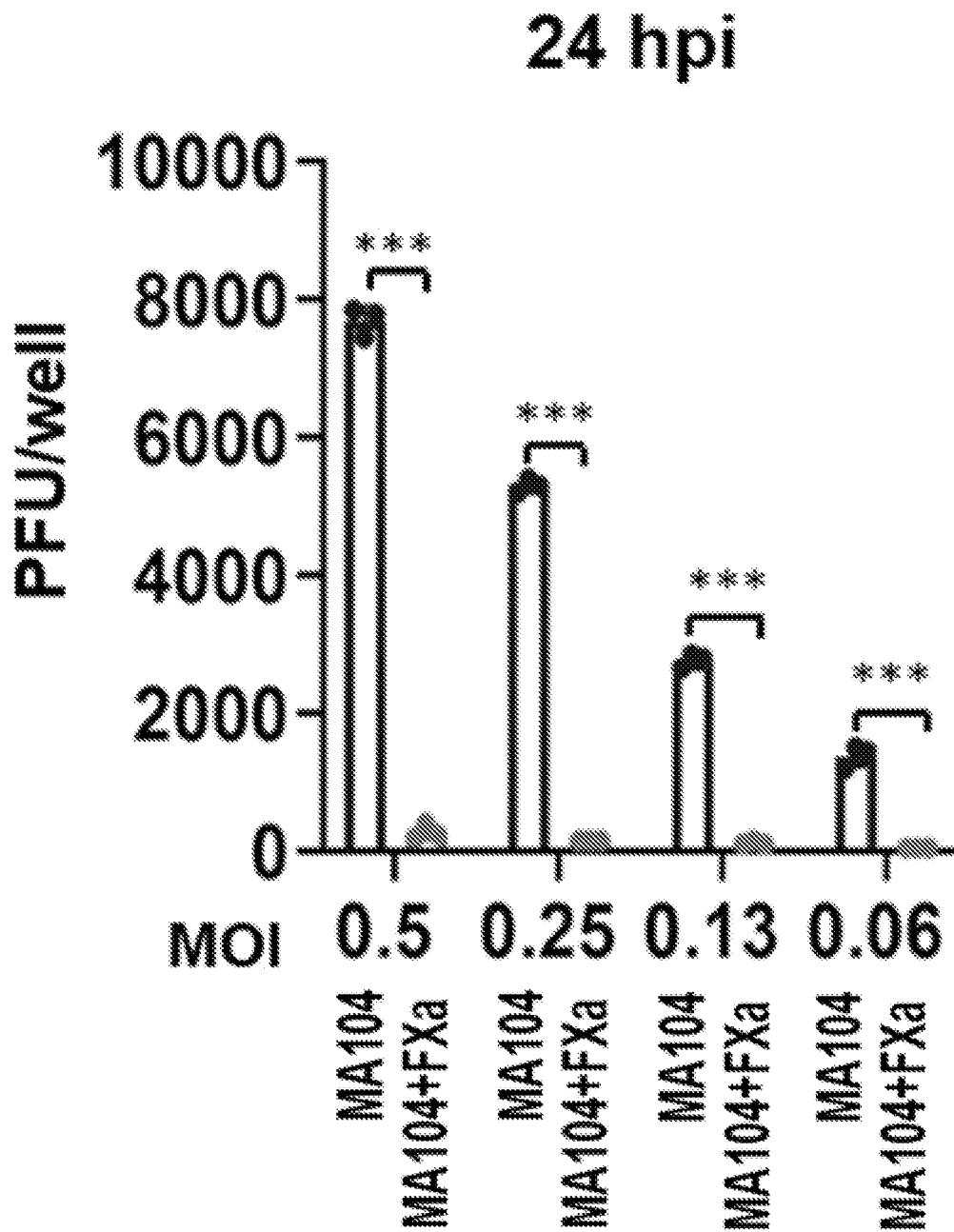


FIG. 7D

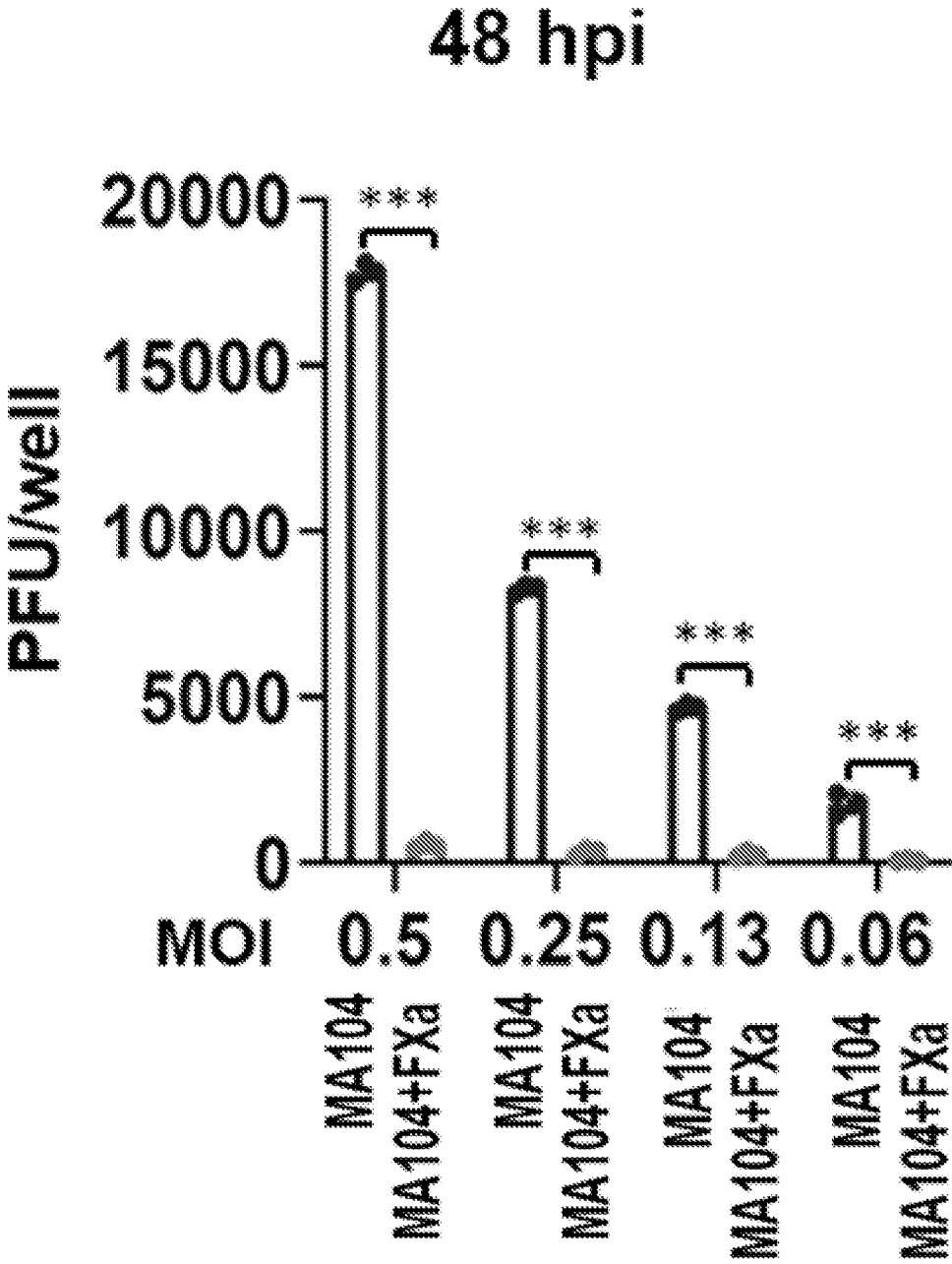


FIG. 8B

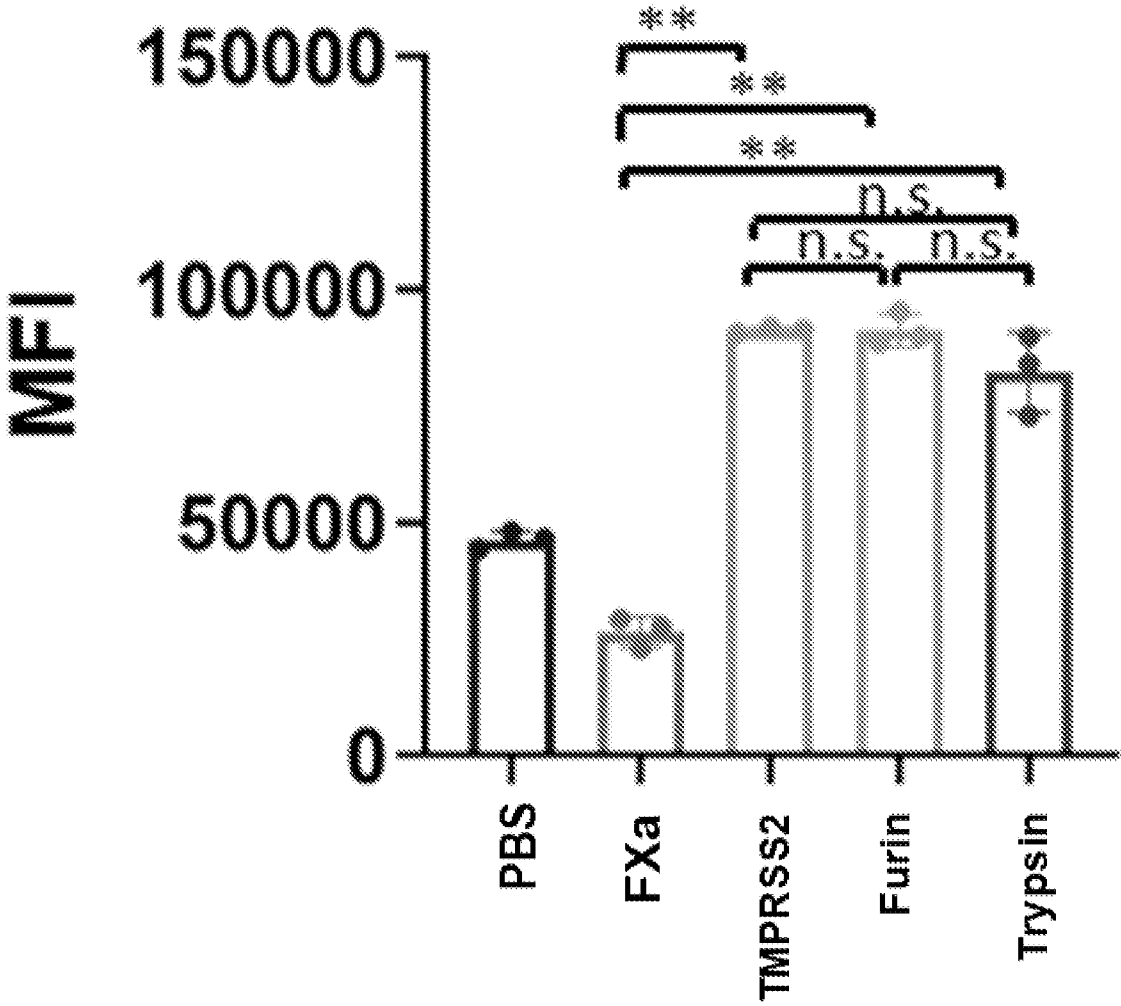


FIG. 8C

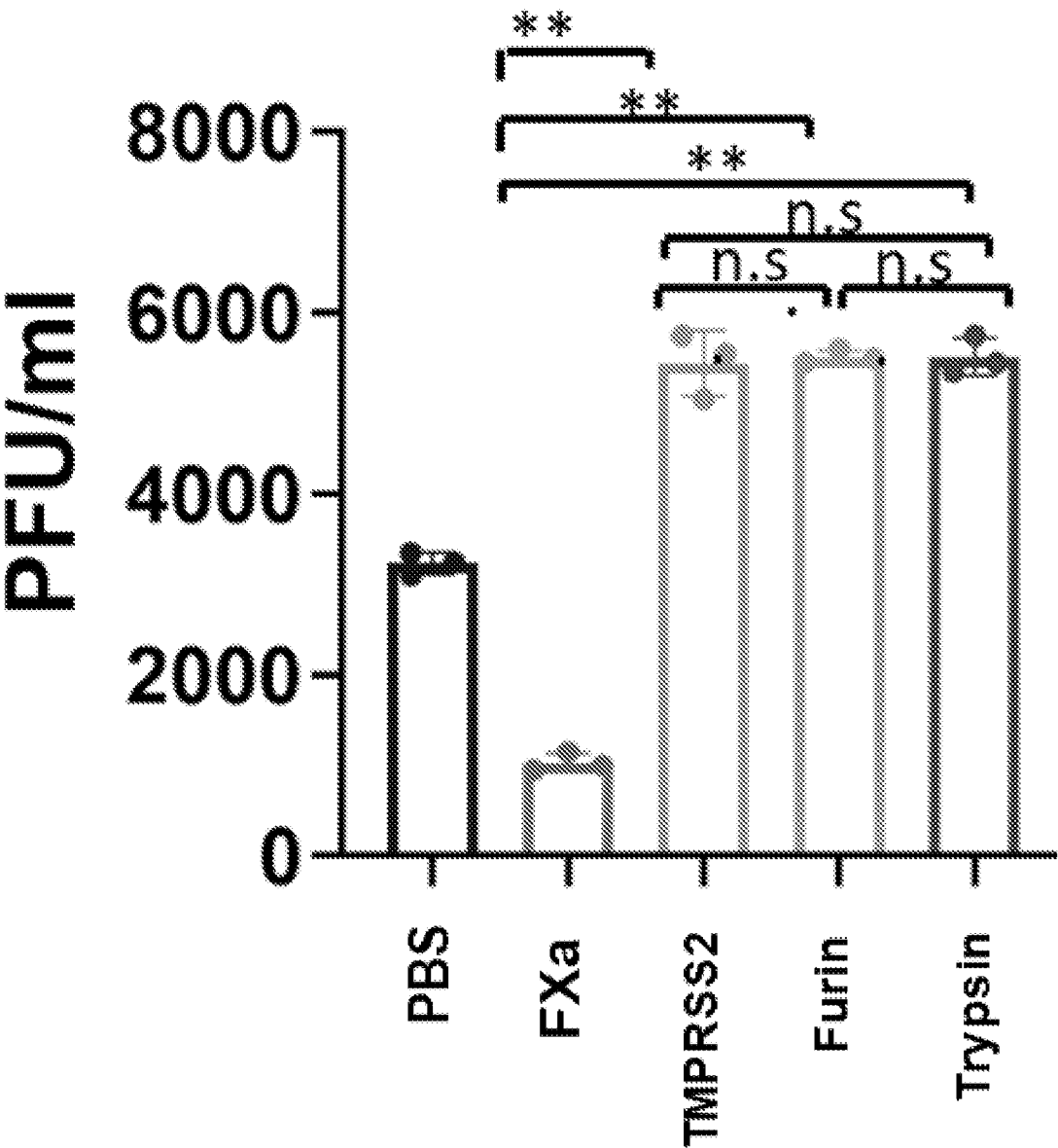


FIG. 9A

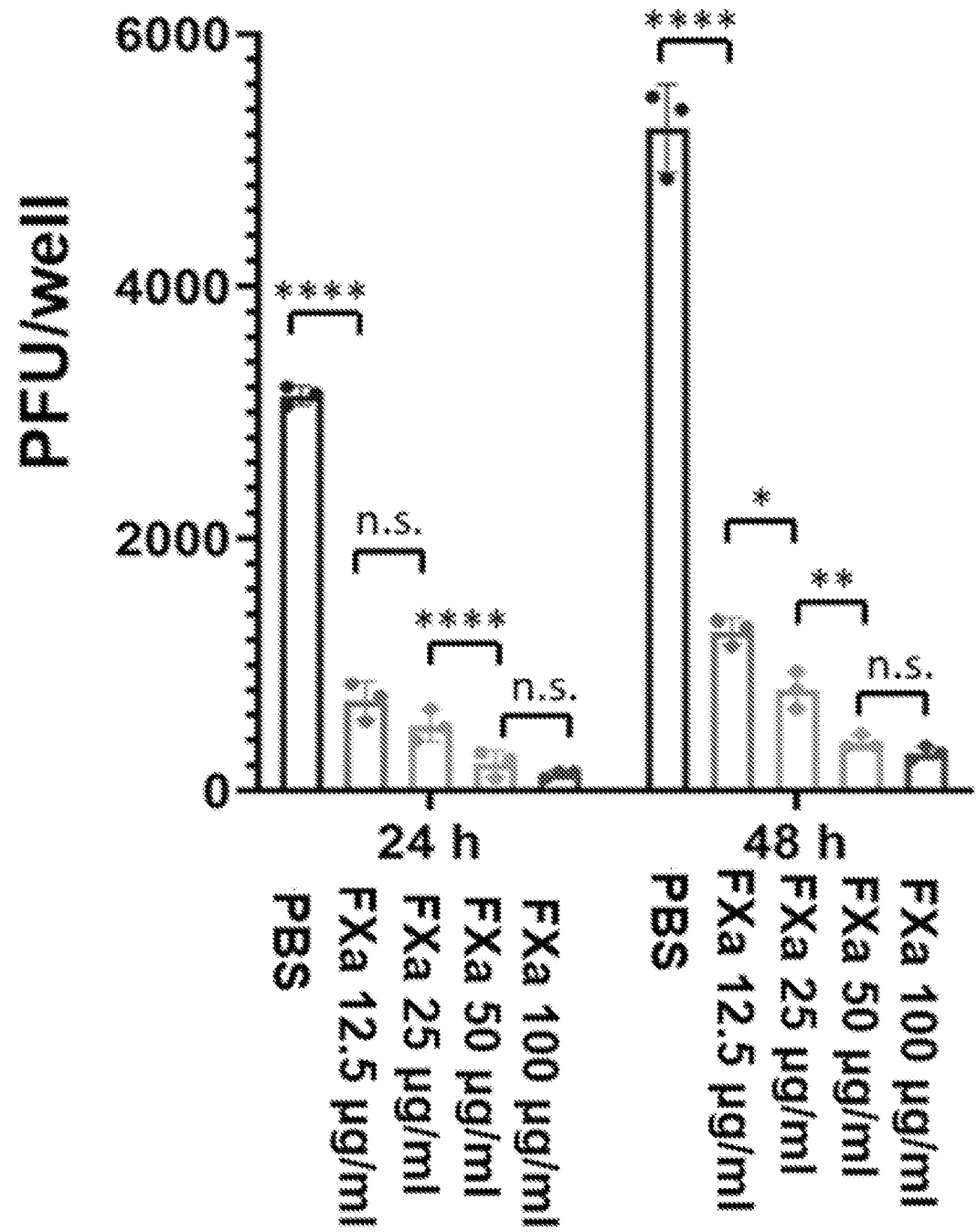


FIG. 9B

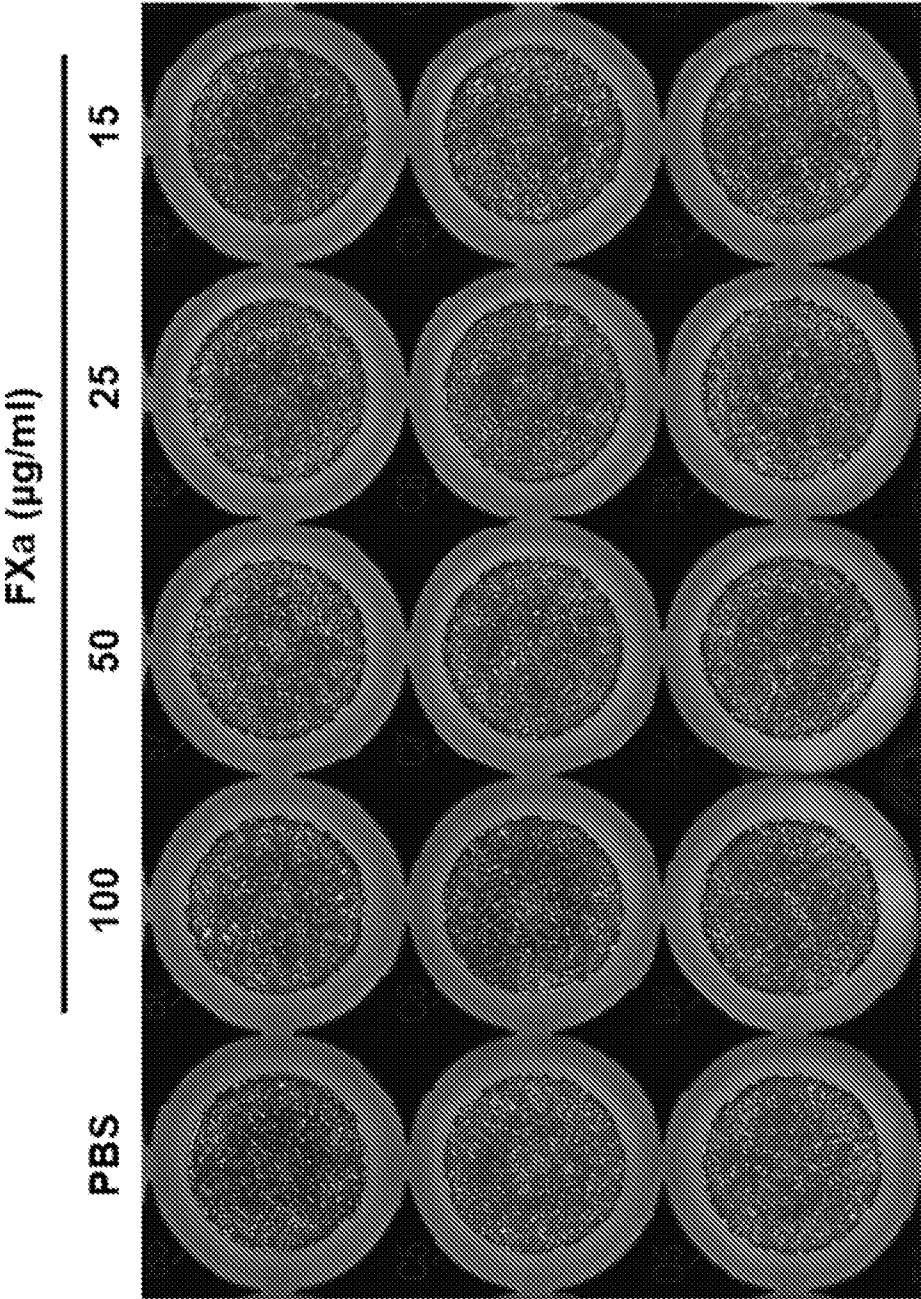


FIG. 9C

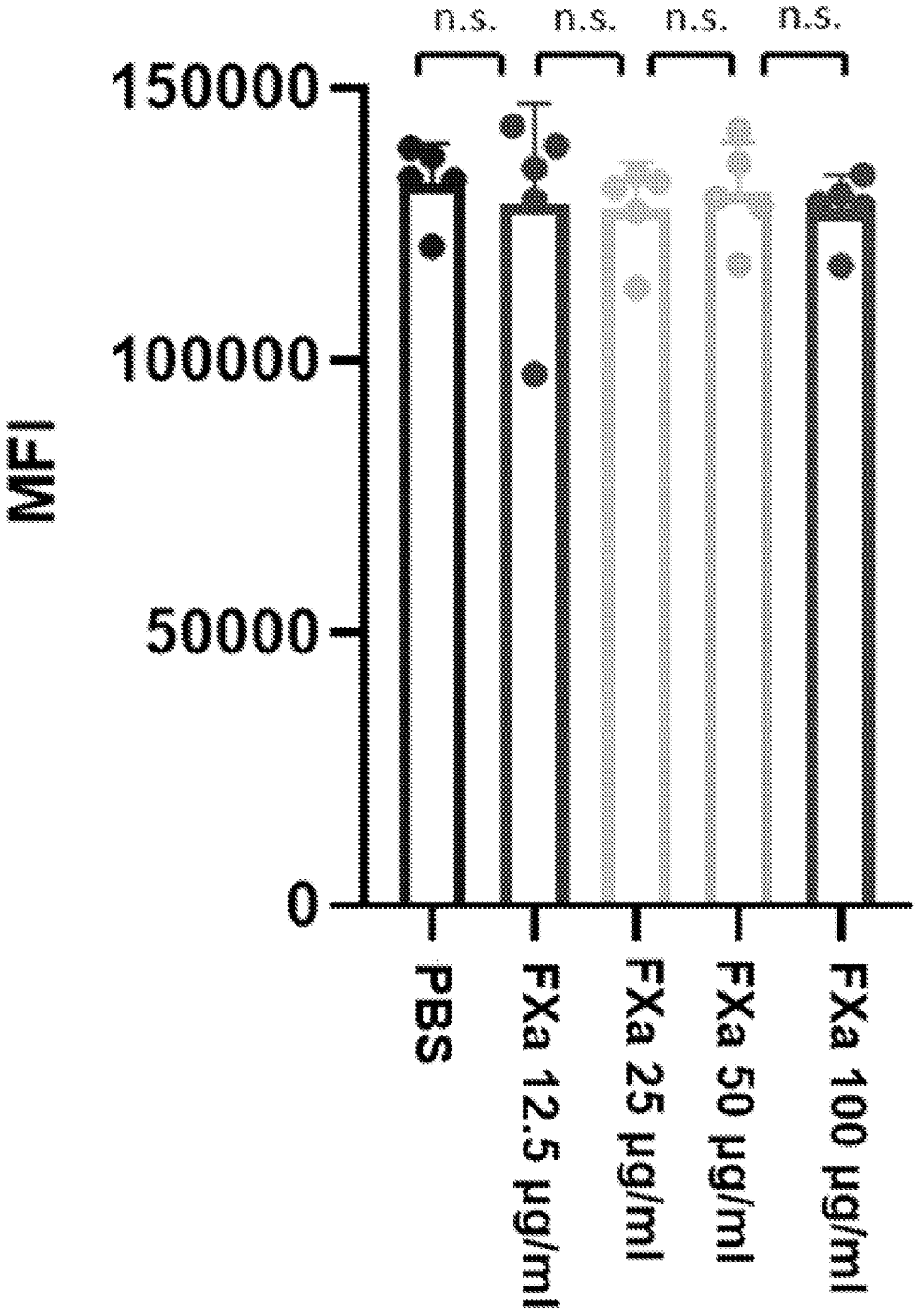


FIG. 10

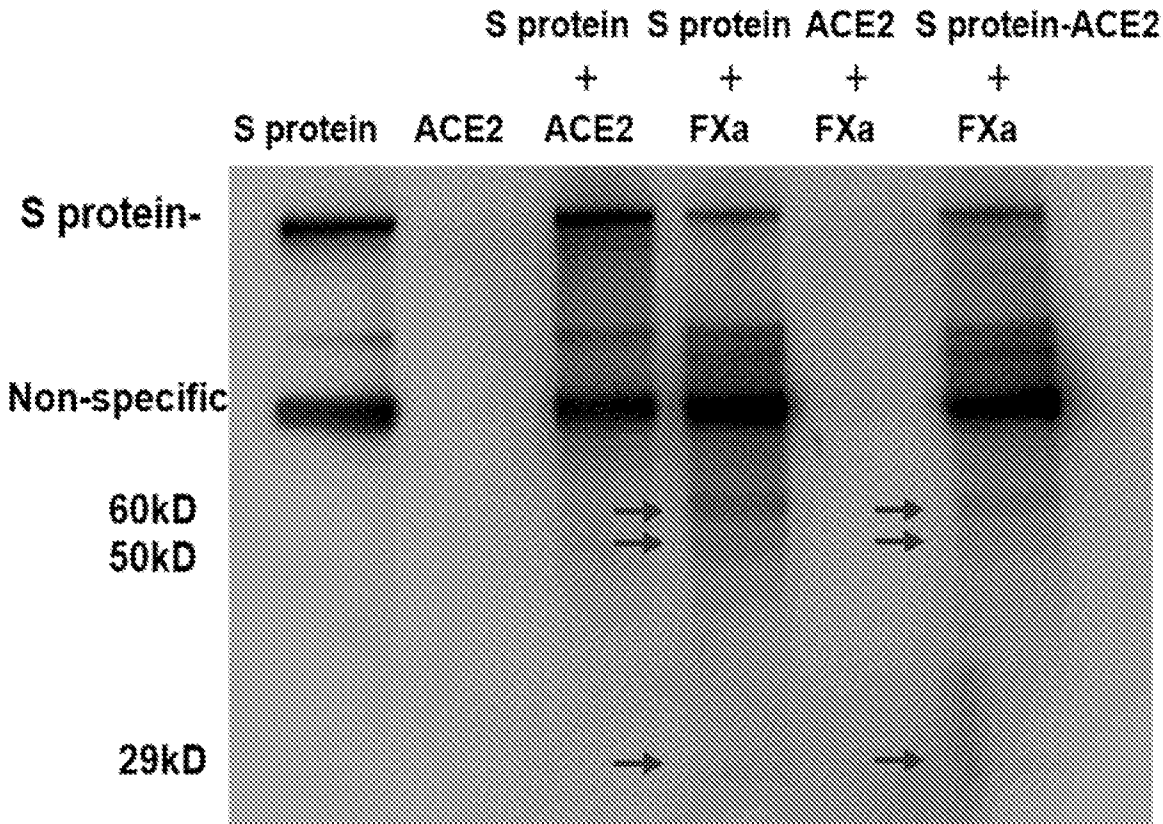


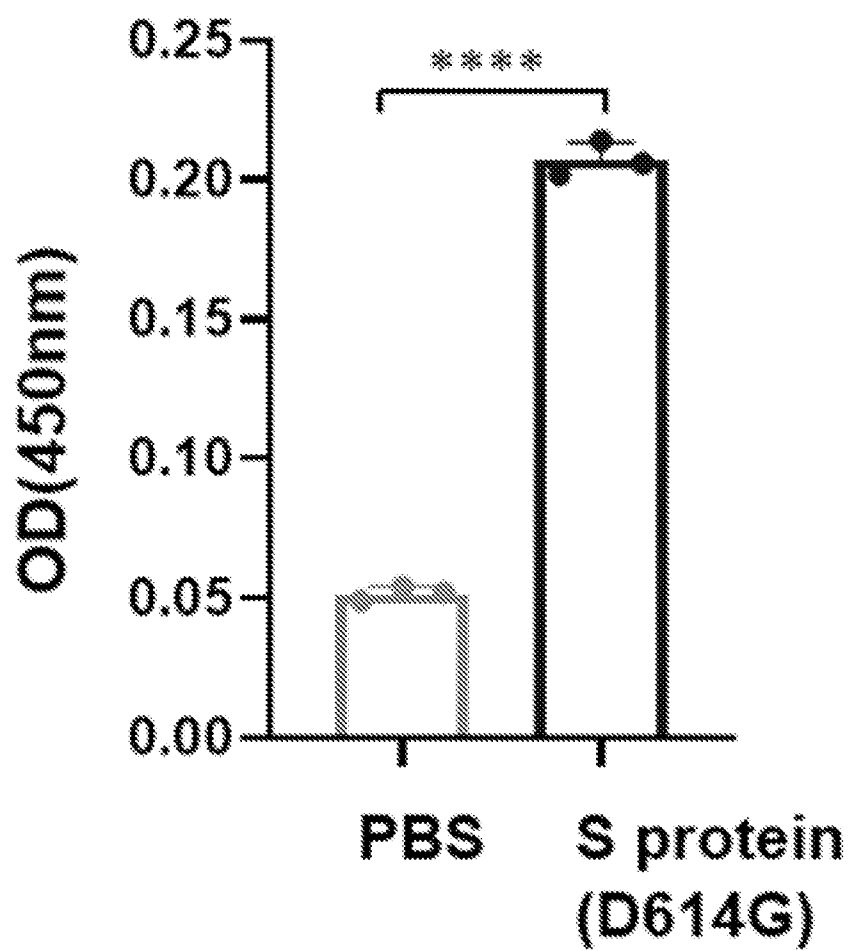
FIG. 11A

FIG. 11B

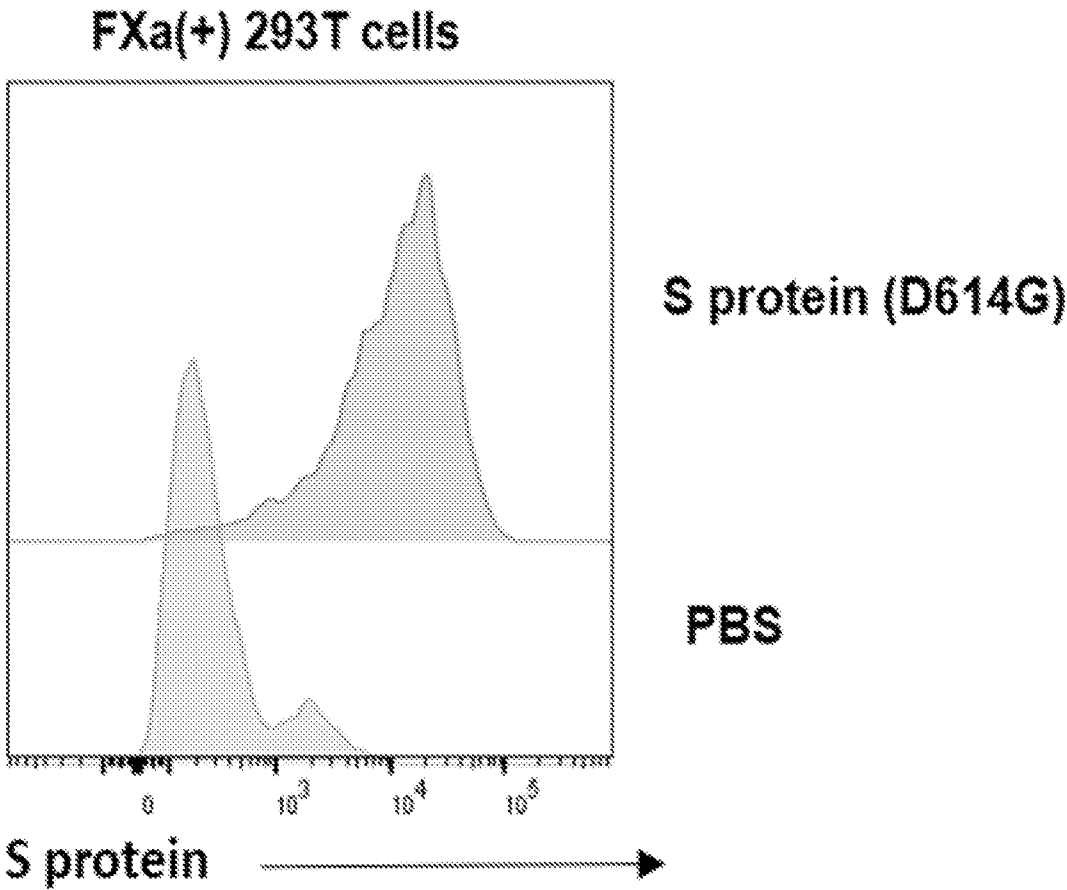


FIG. 11C

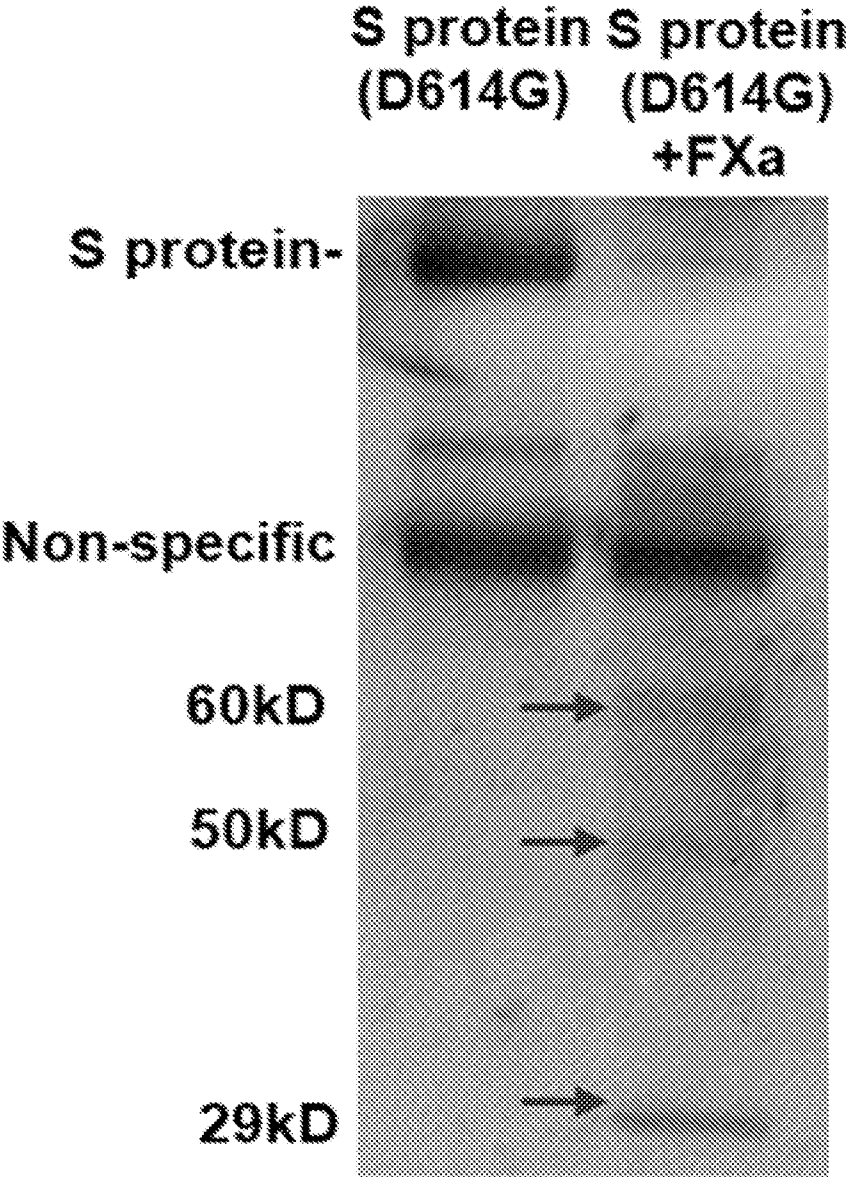


FIG. 11D

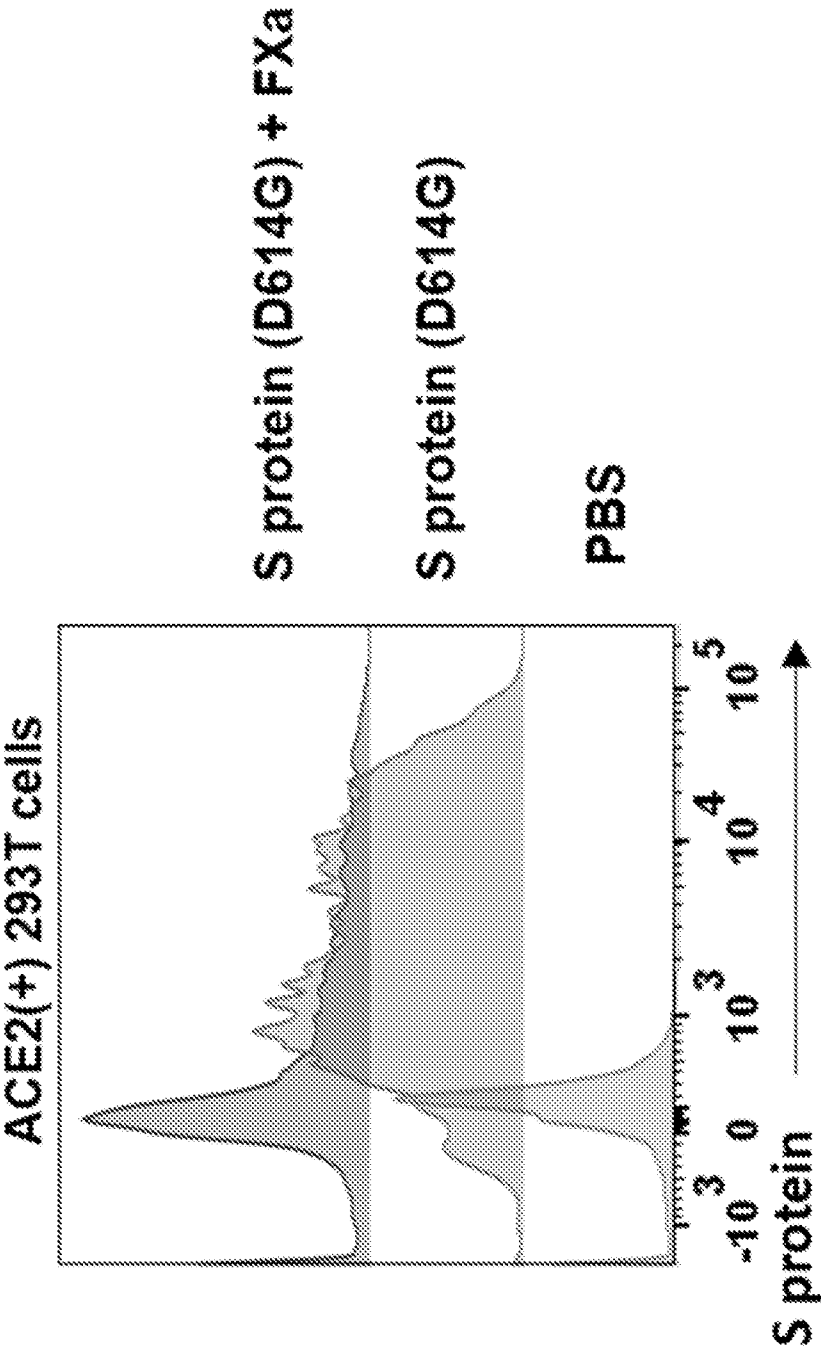


FIG. 11E

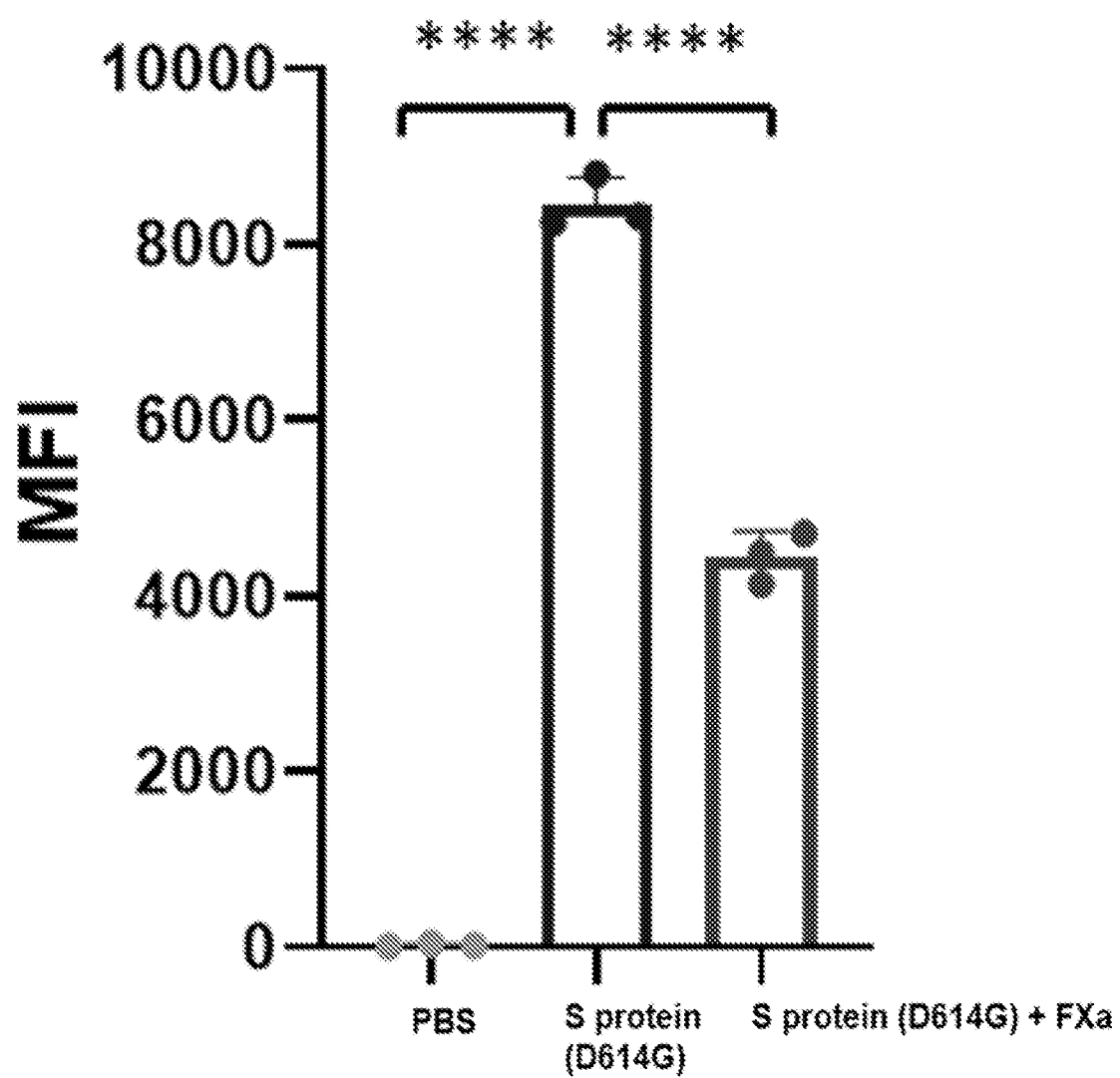


FIG. 12A

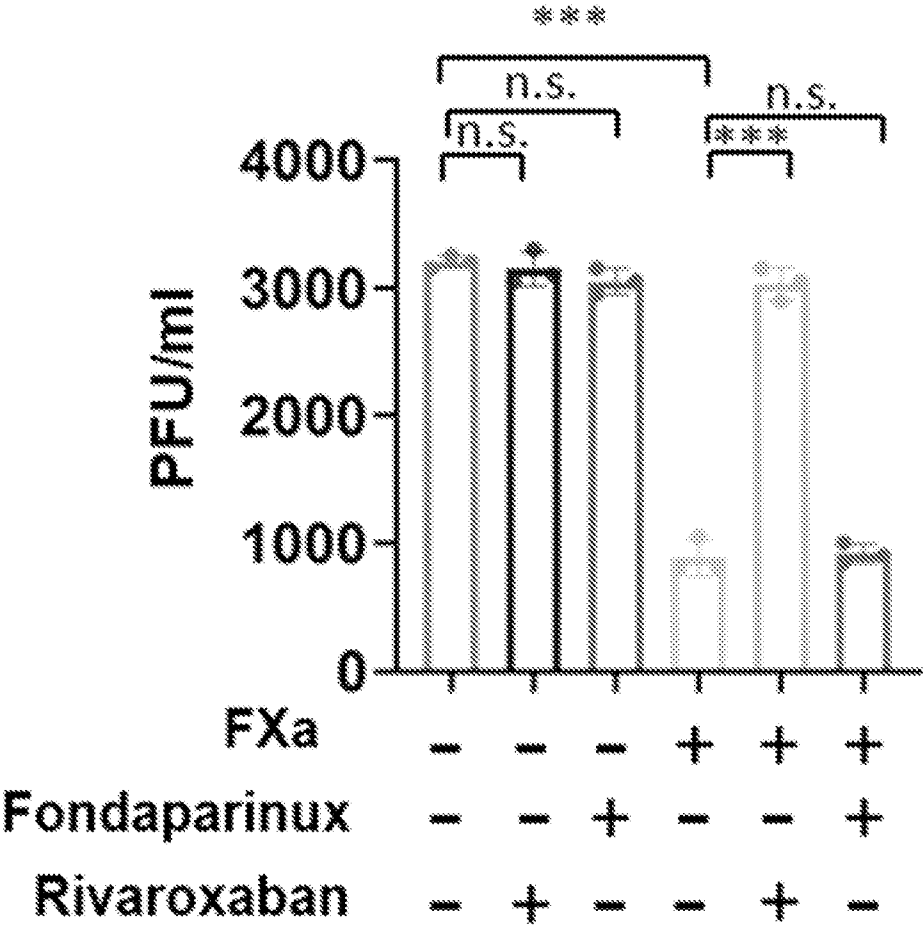


FIG. 12B

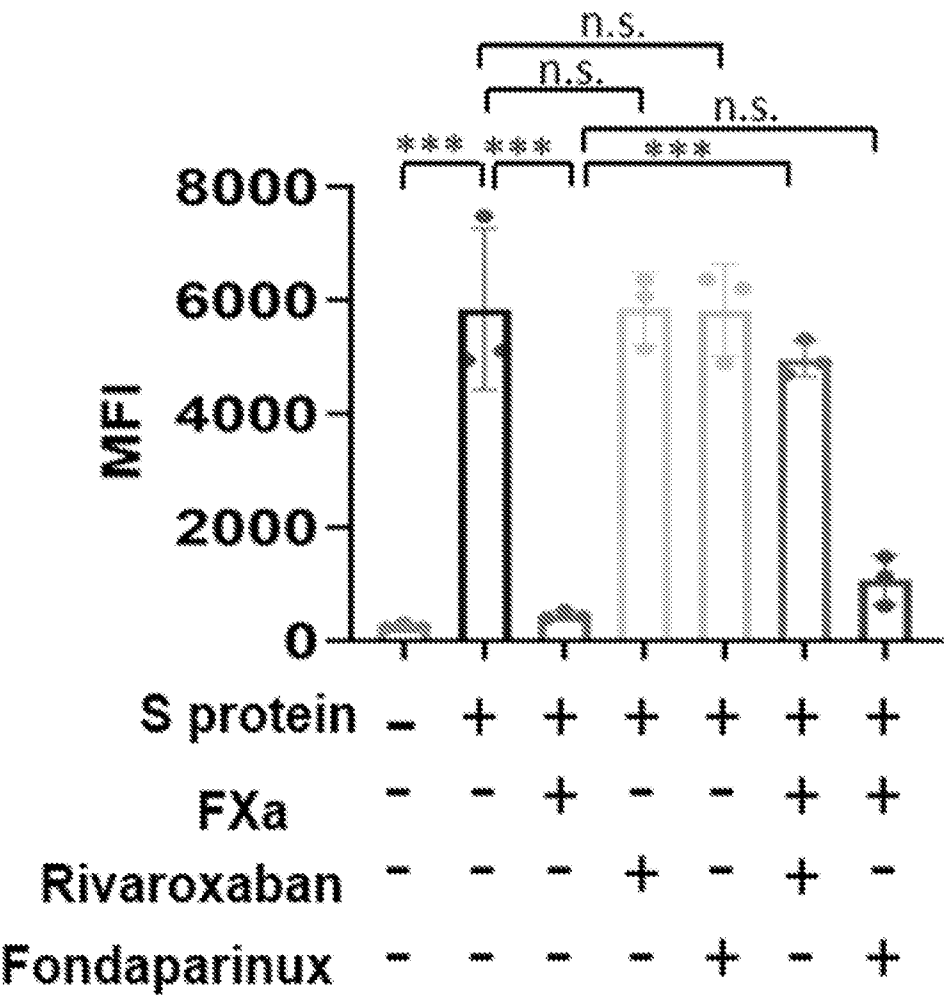


FIG. 12C

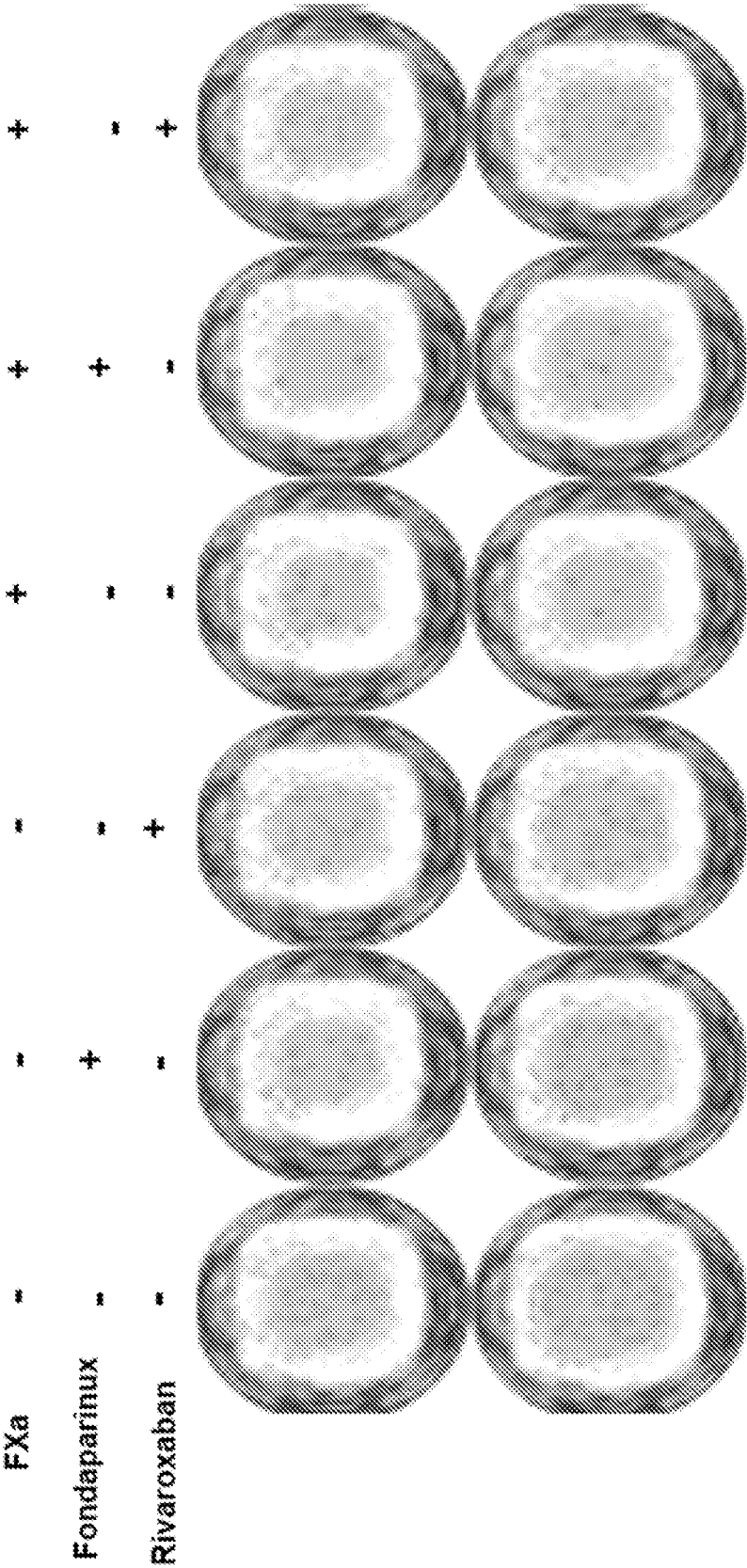


FIG. 13A

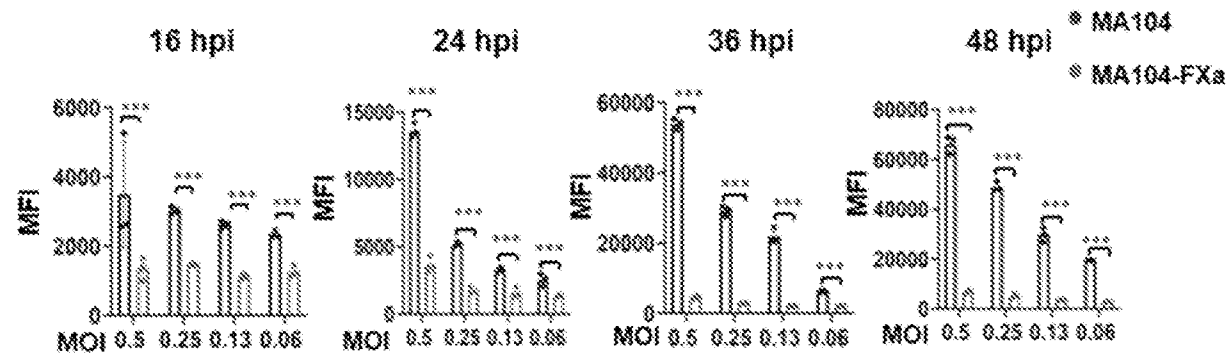


FIG. 13B

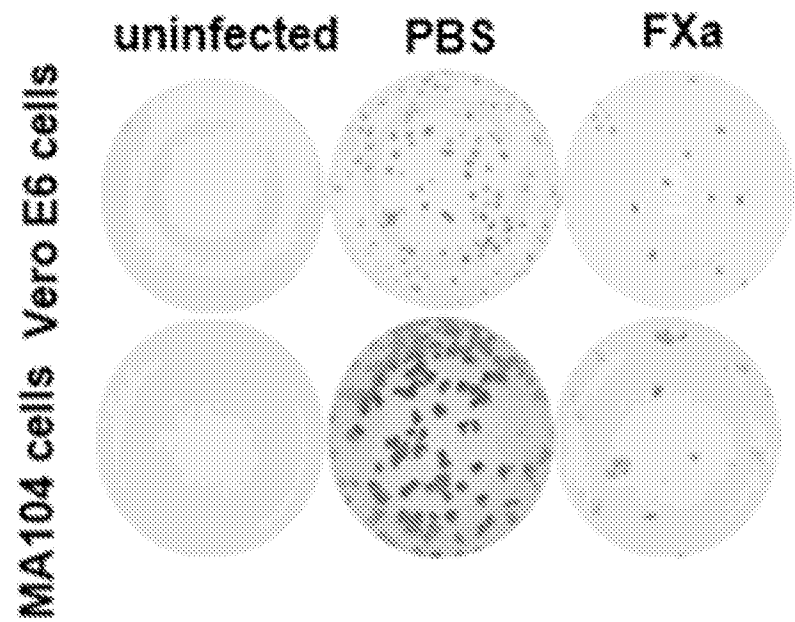


FIG. 13C

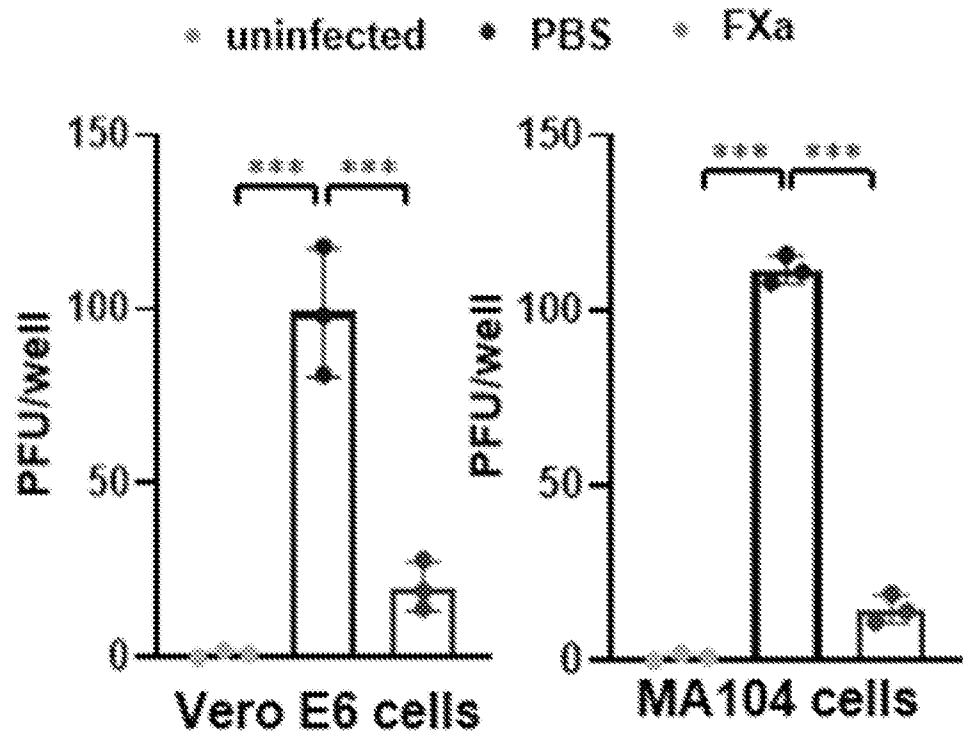


FIG. 13D

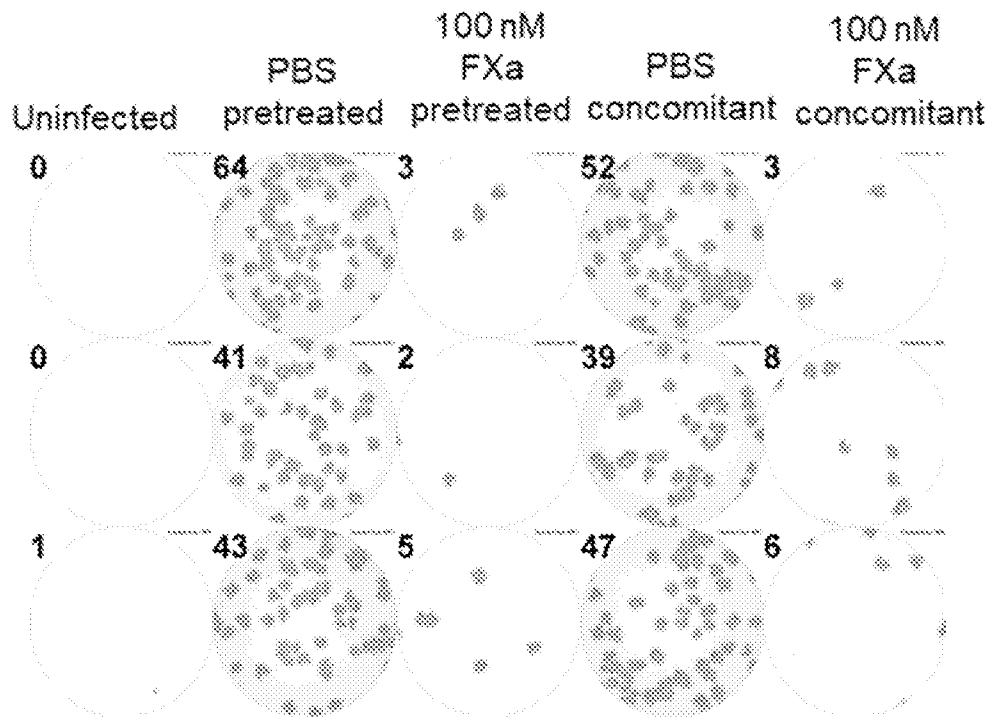


FIG. 14A

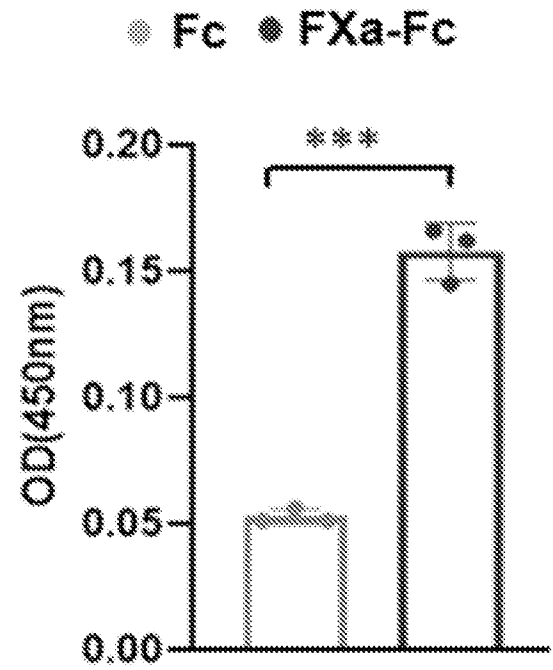


FIG. 14B

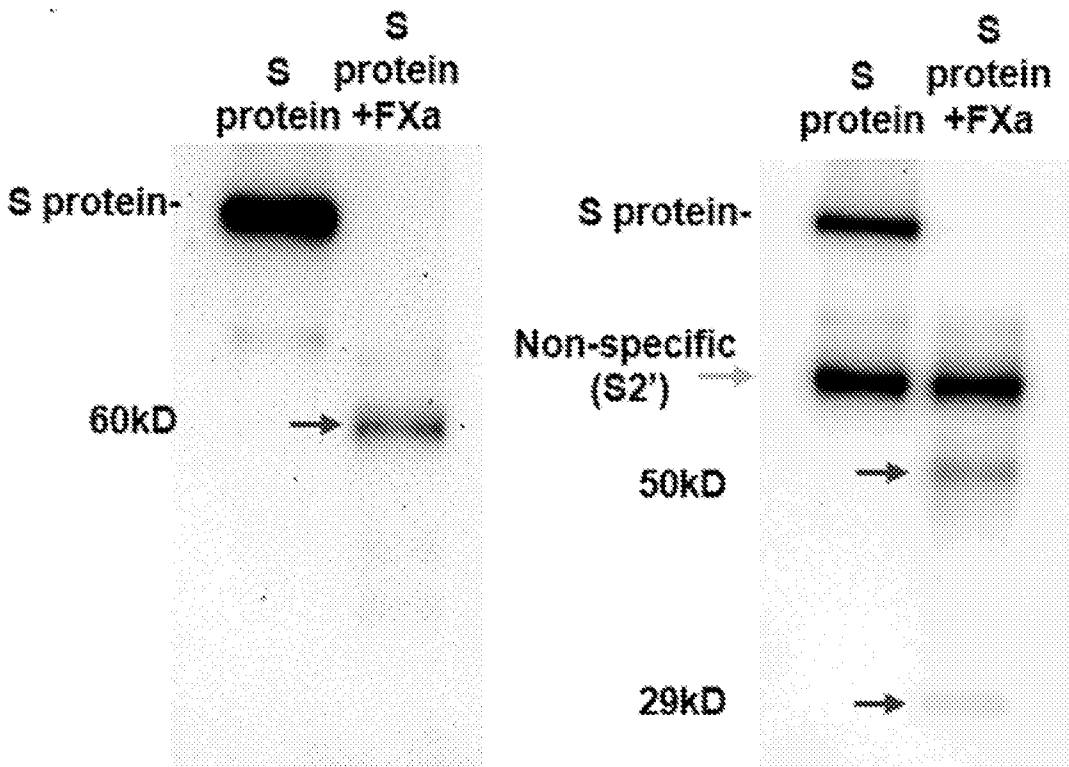


FIG. 14C

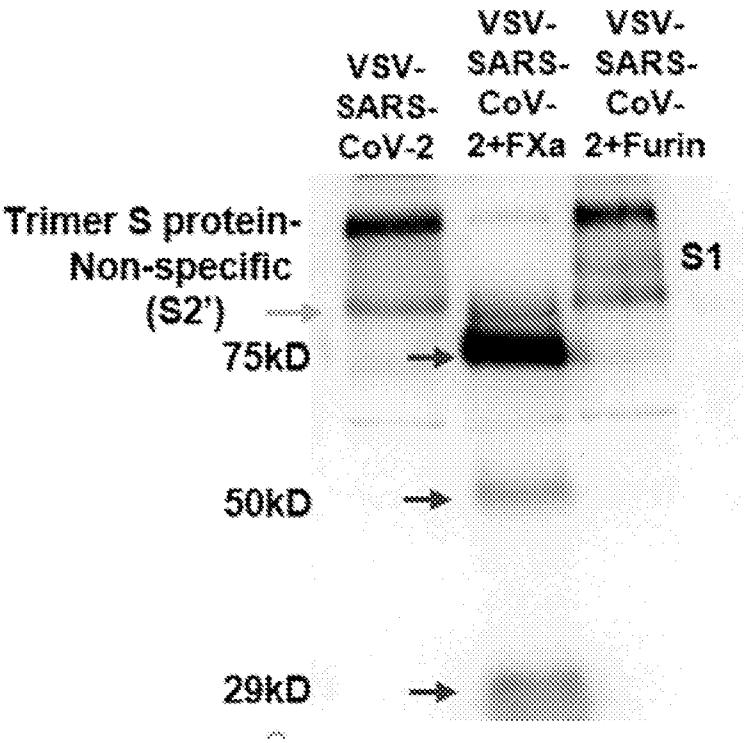


FIG. 15A

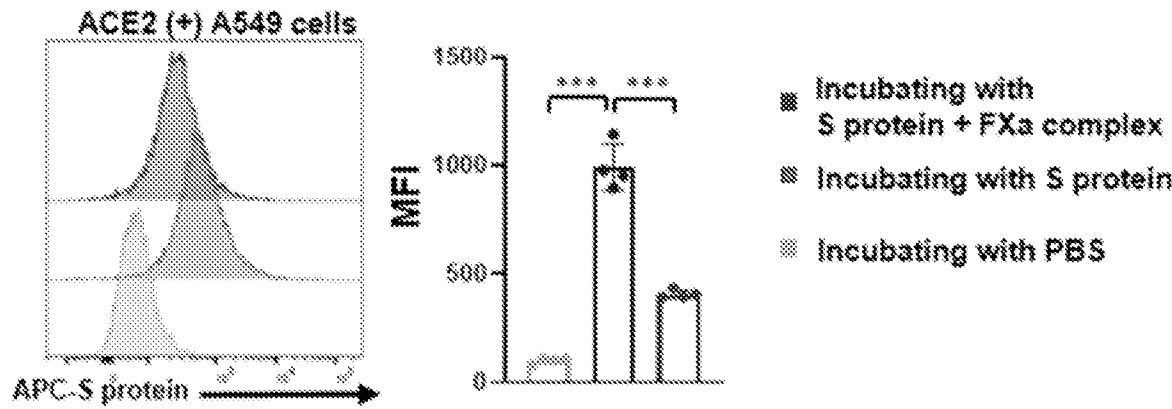


FIG. 15B

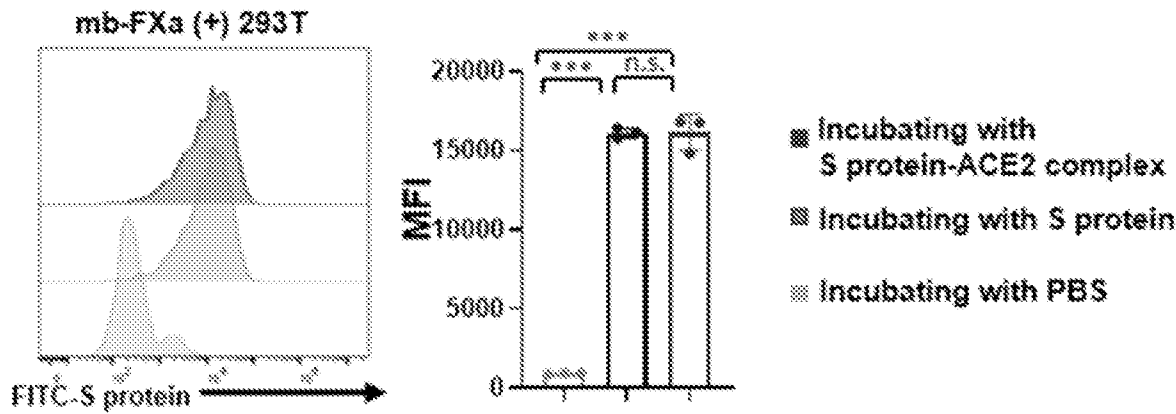


FIG. 16A

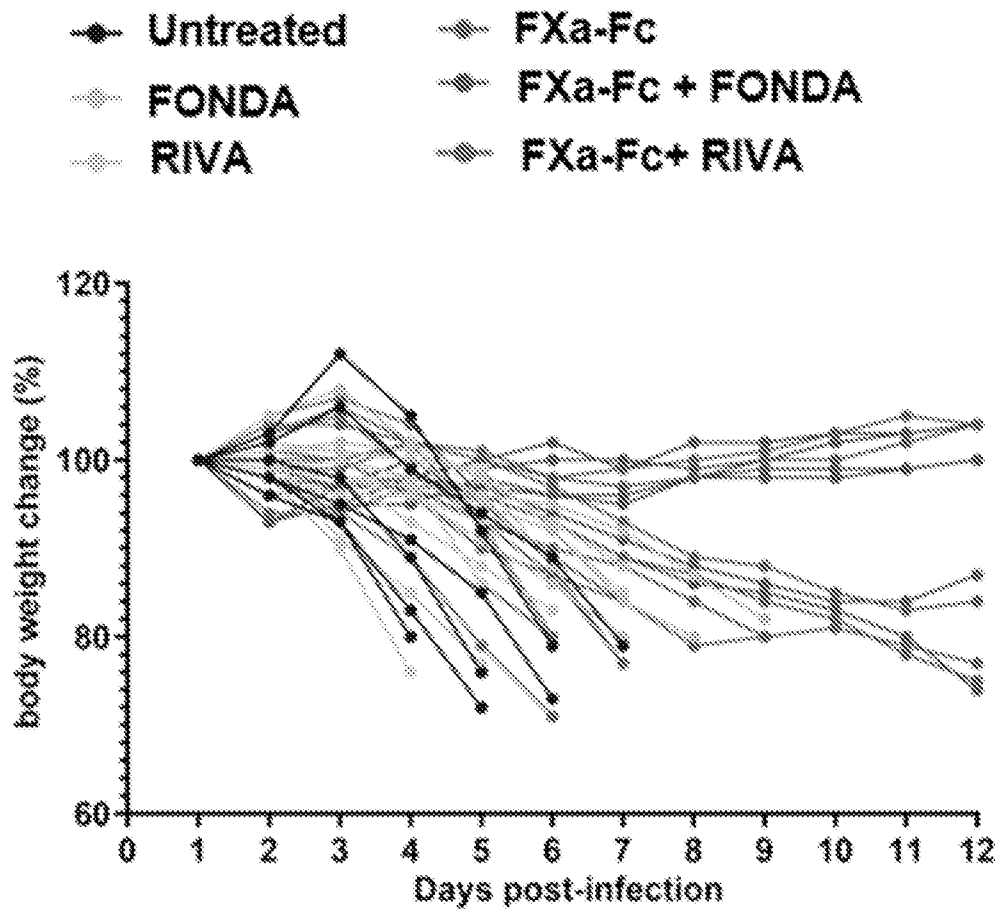


FIG. 16B

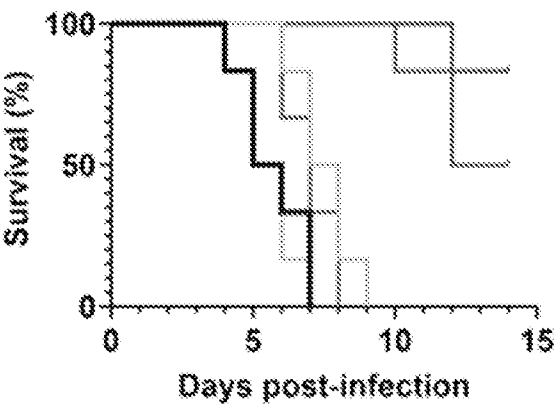
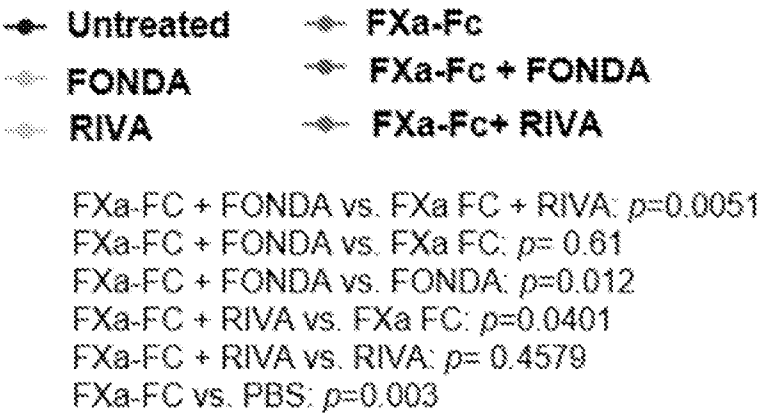


FIG. 17A

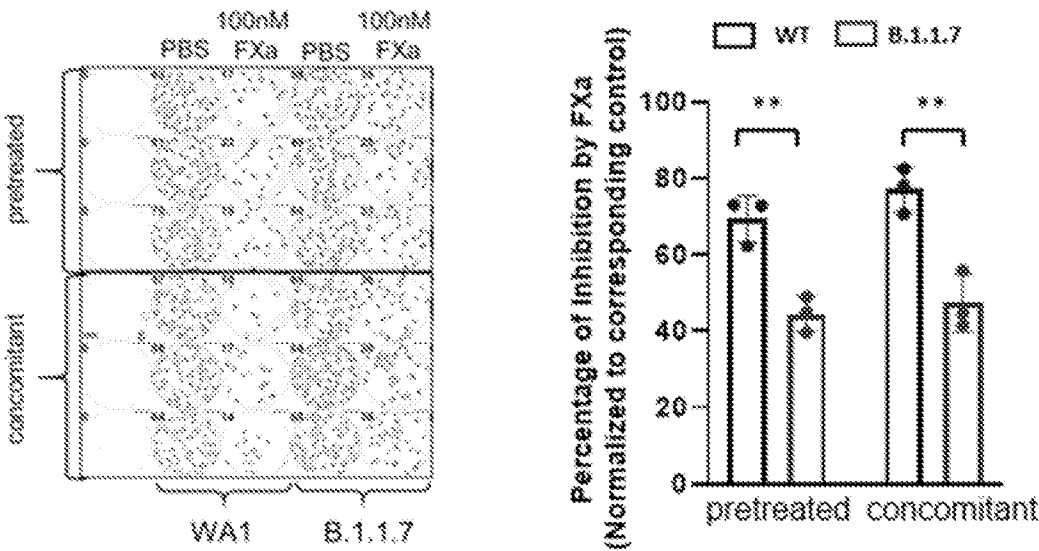


FIG. 17B

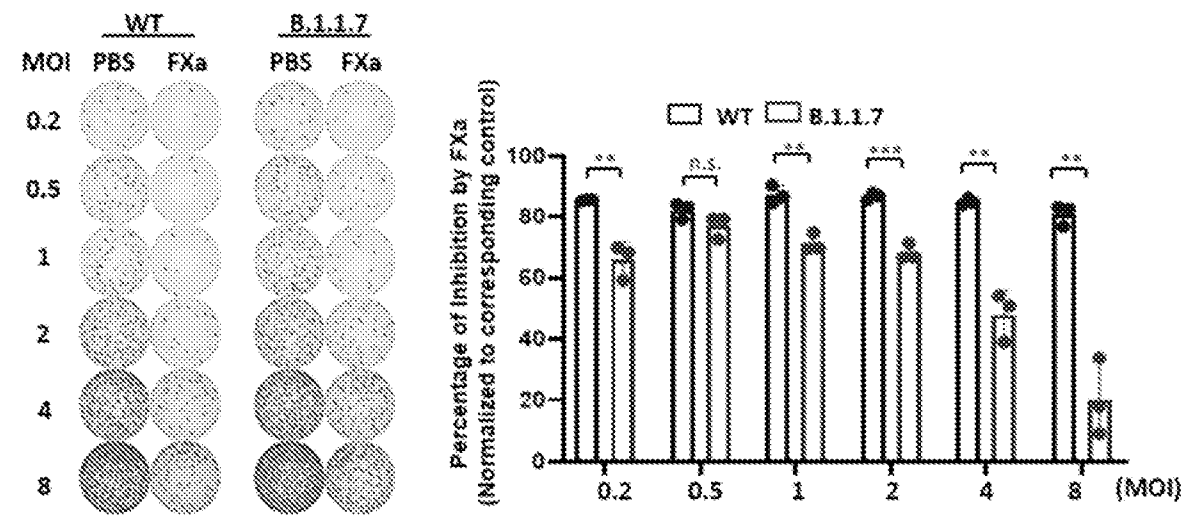


FIG. 17C

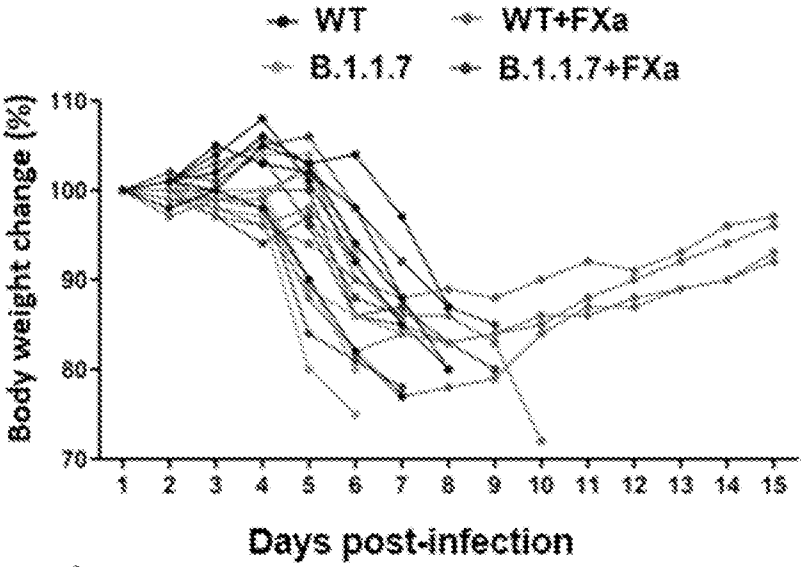


FIG. 17D

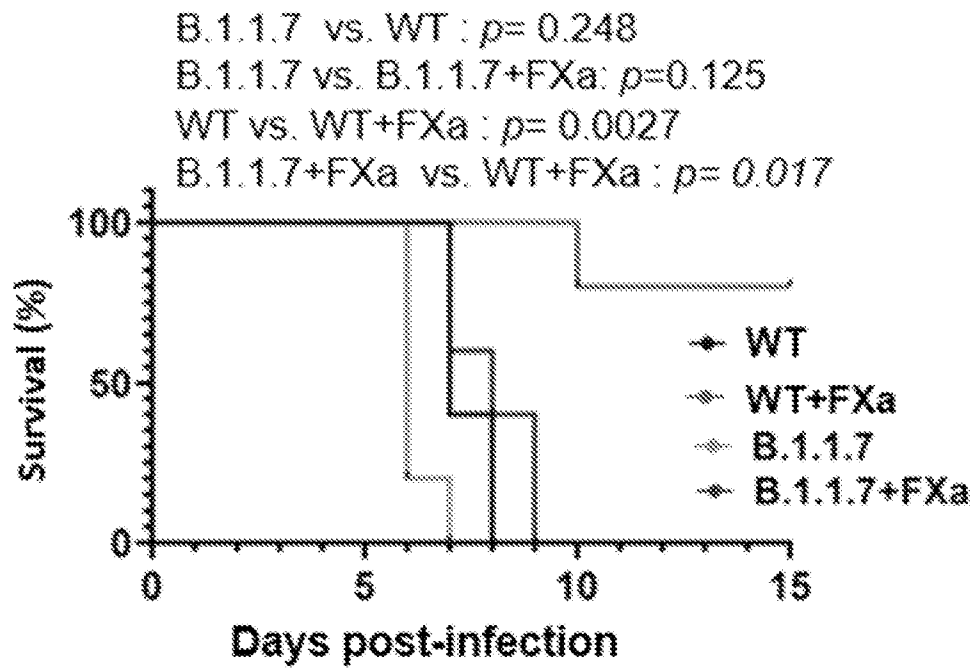


FIG. 17E

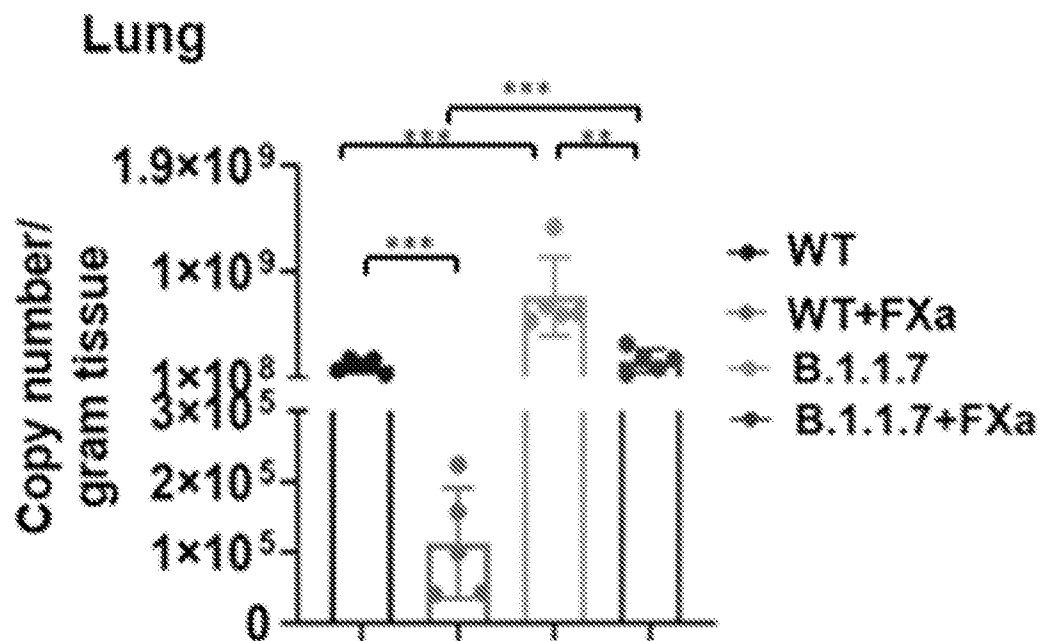


FIG. 17F

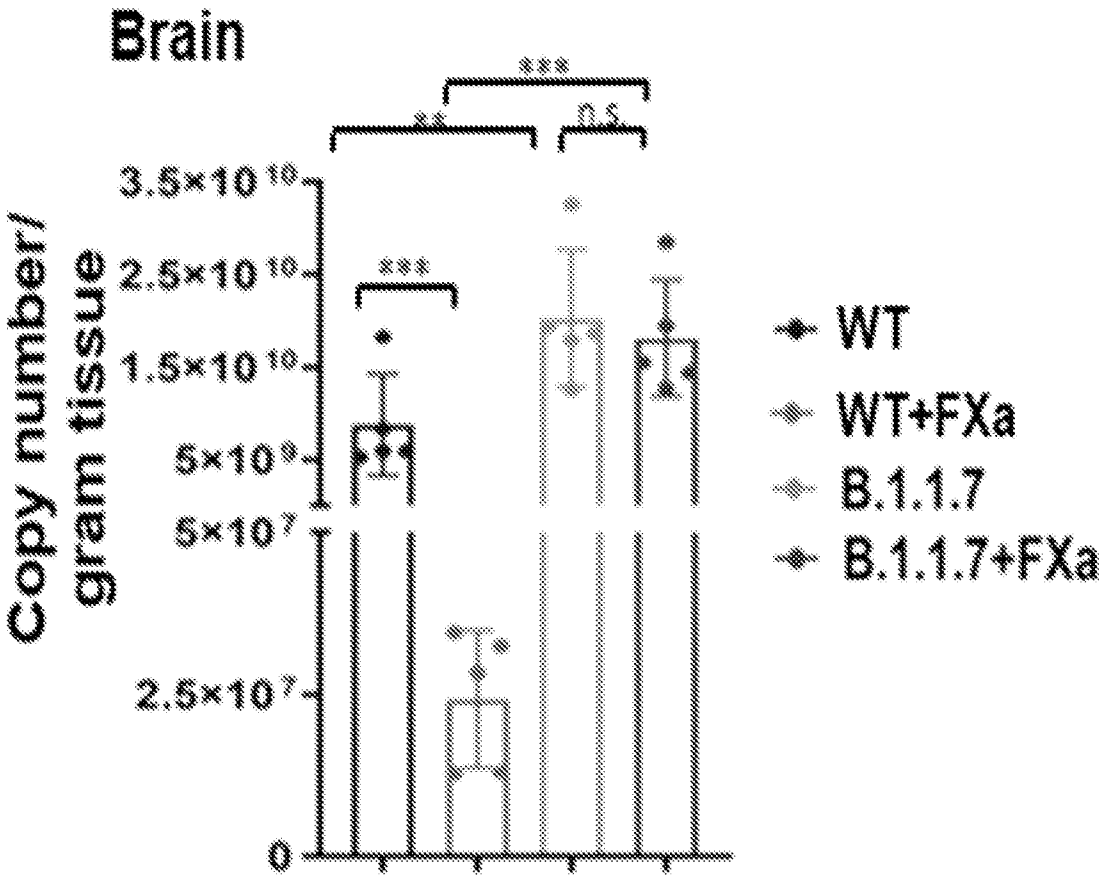


FIG. 18

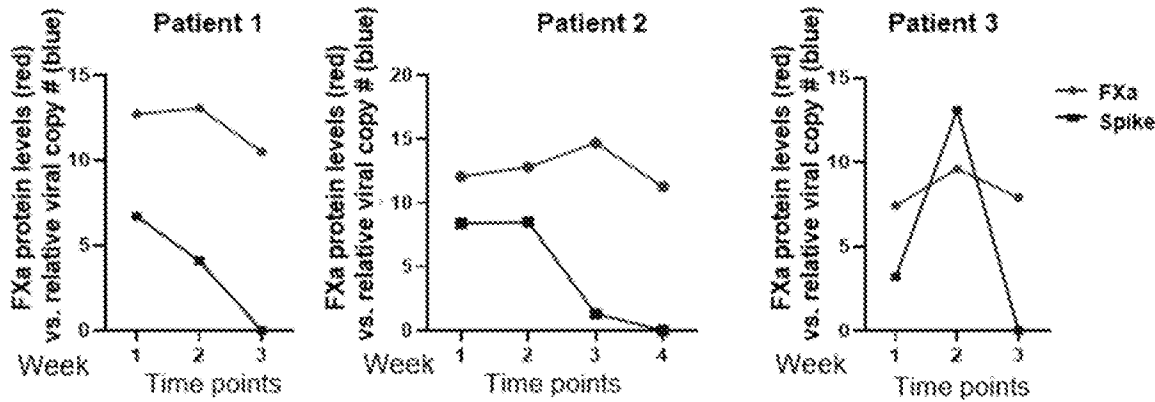


FIG. 19A

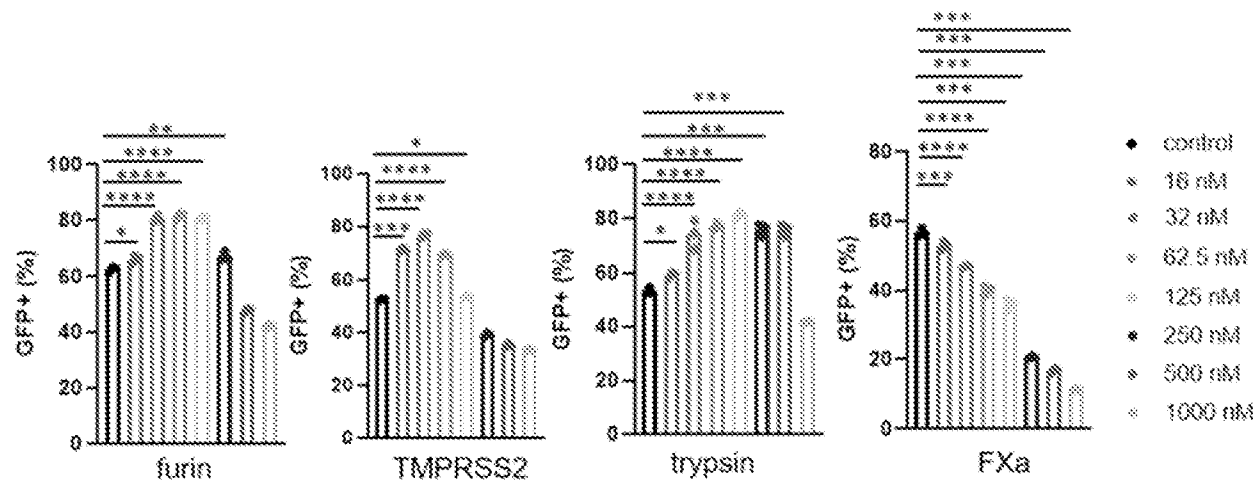


FIG. 19B

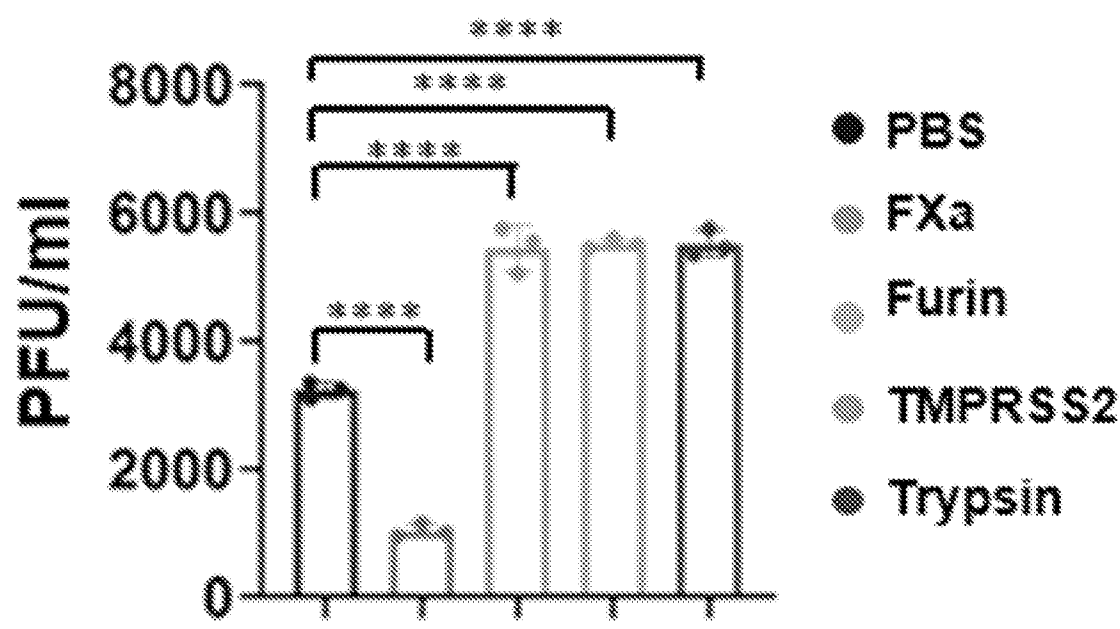


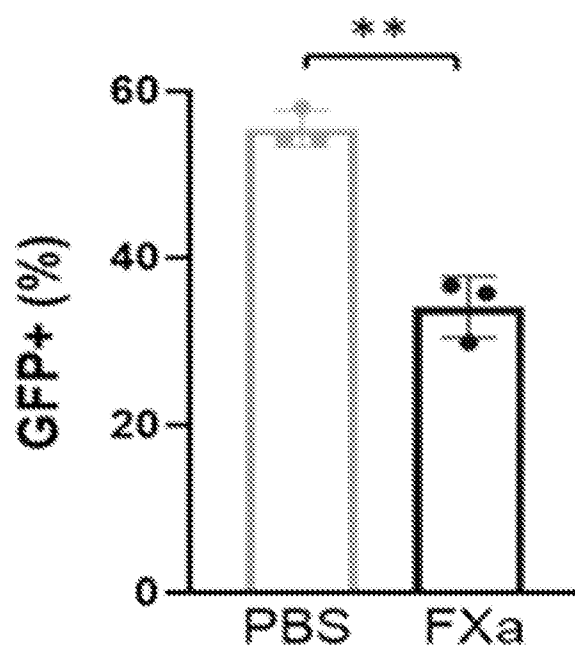
FIG. 19C

FIG. 20B

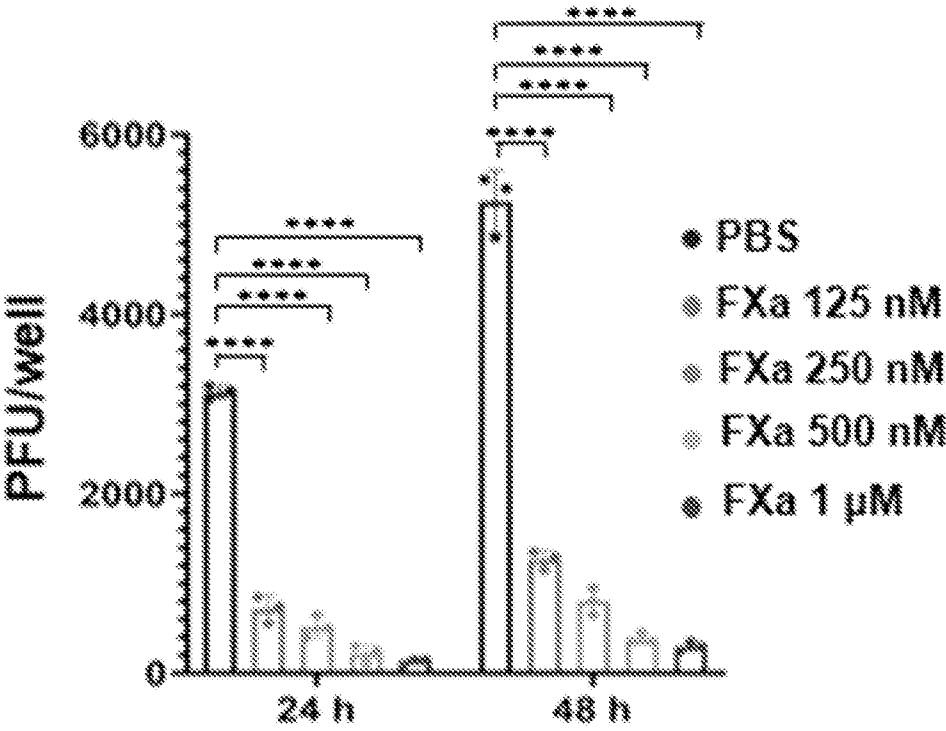


FIG. 20C

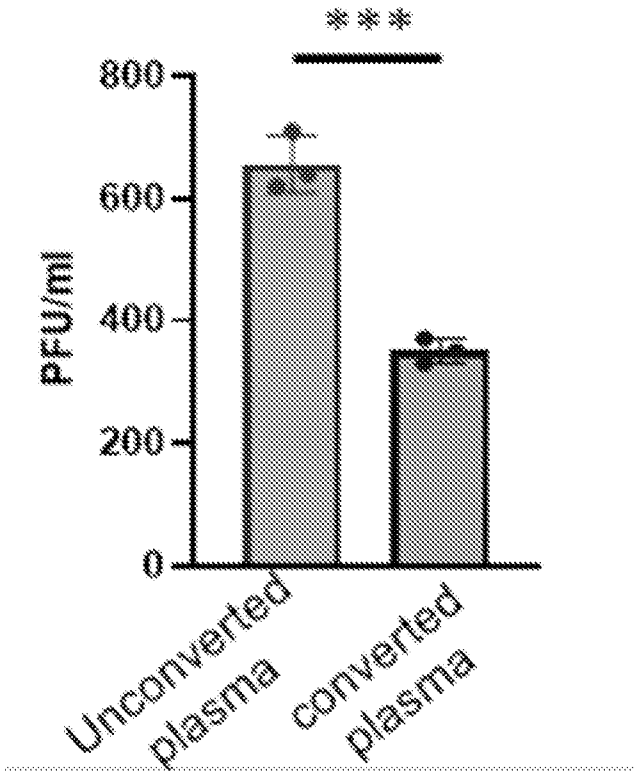


FIG. 20D

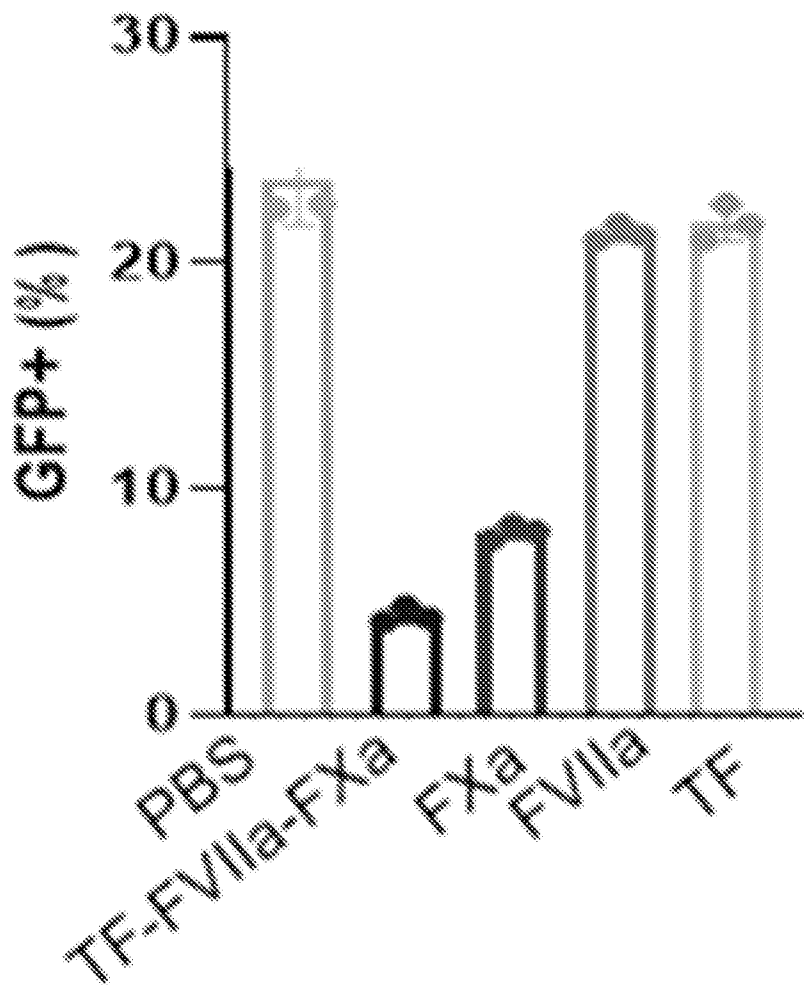


FIG. 21A

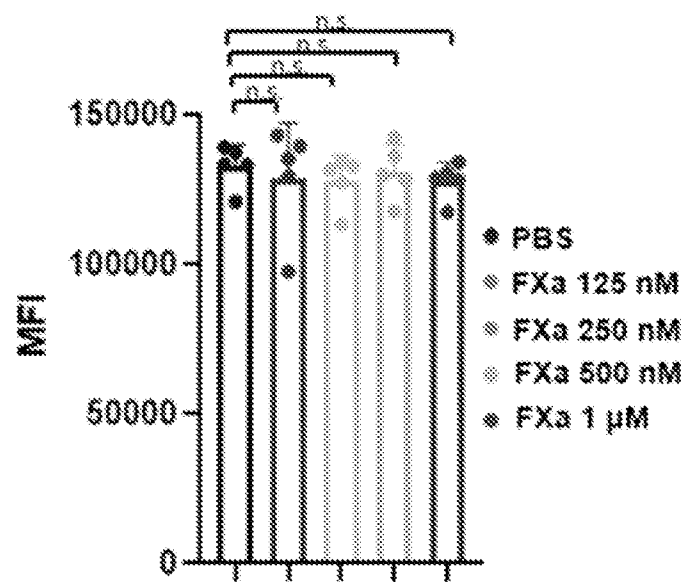


FIG. 21B

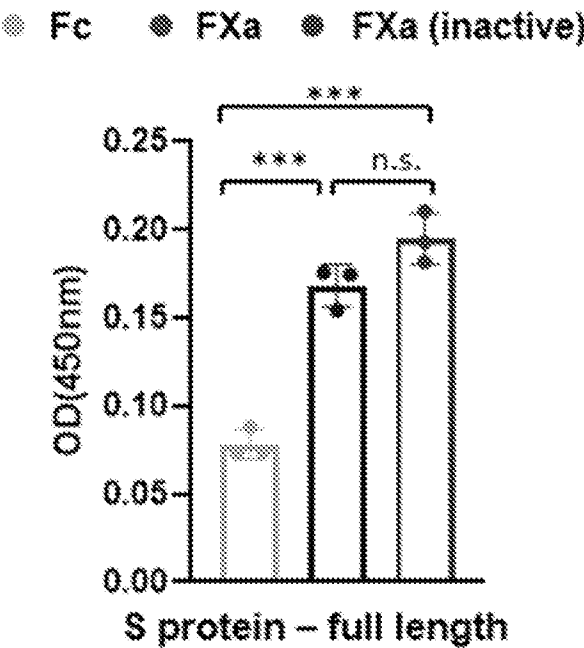


FIG. 21C

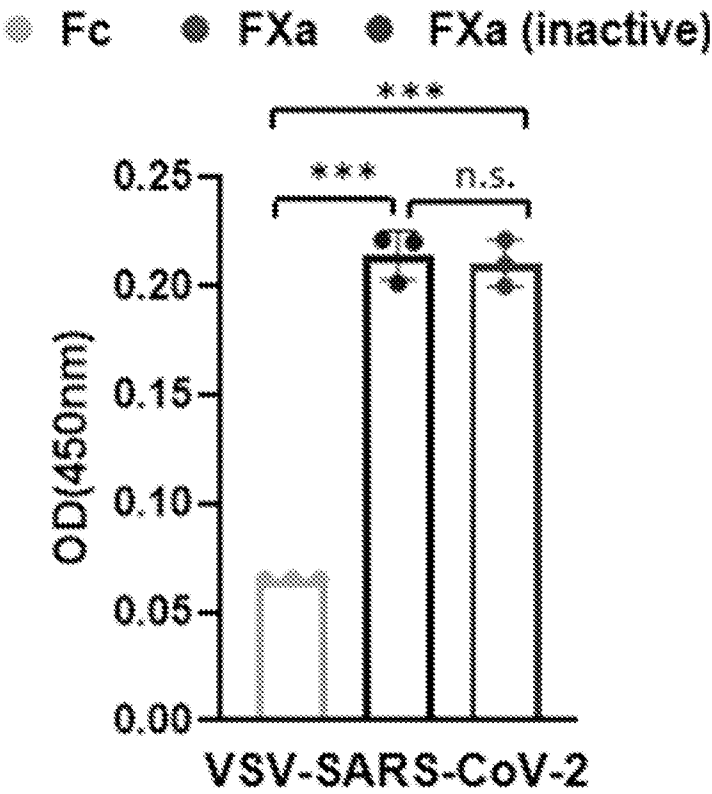


FIG. 21D

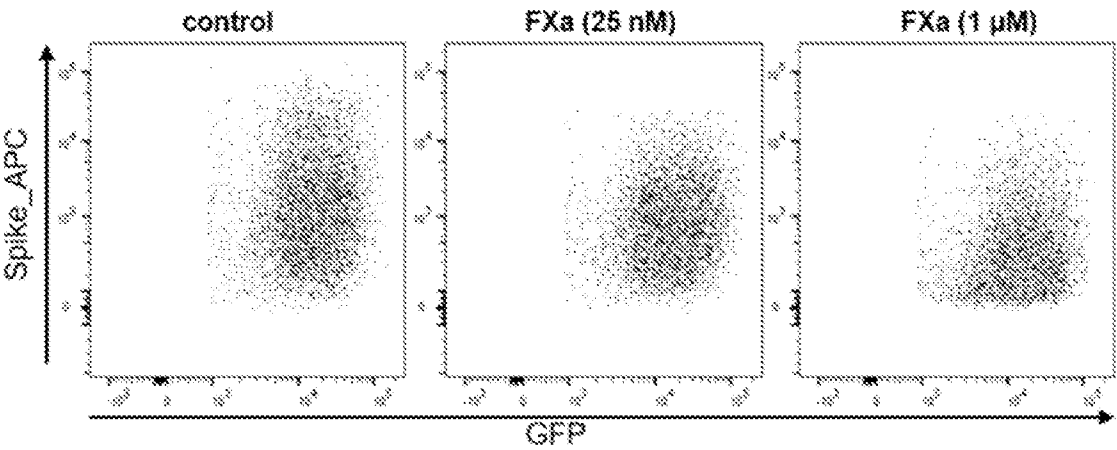


FIG. 21E

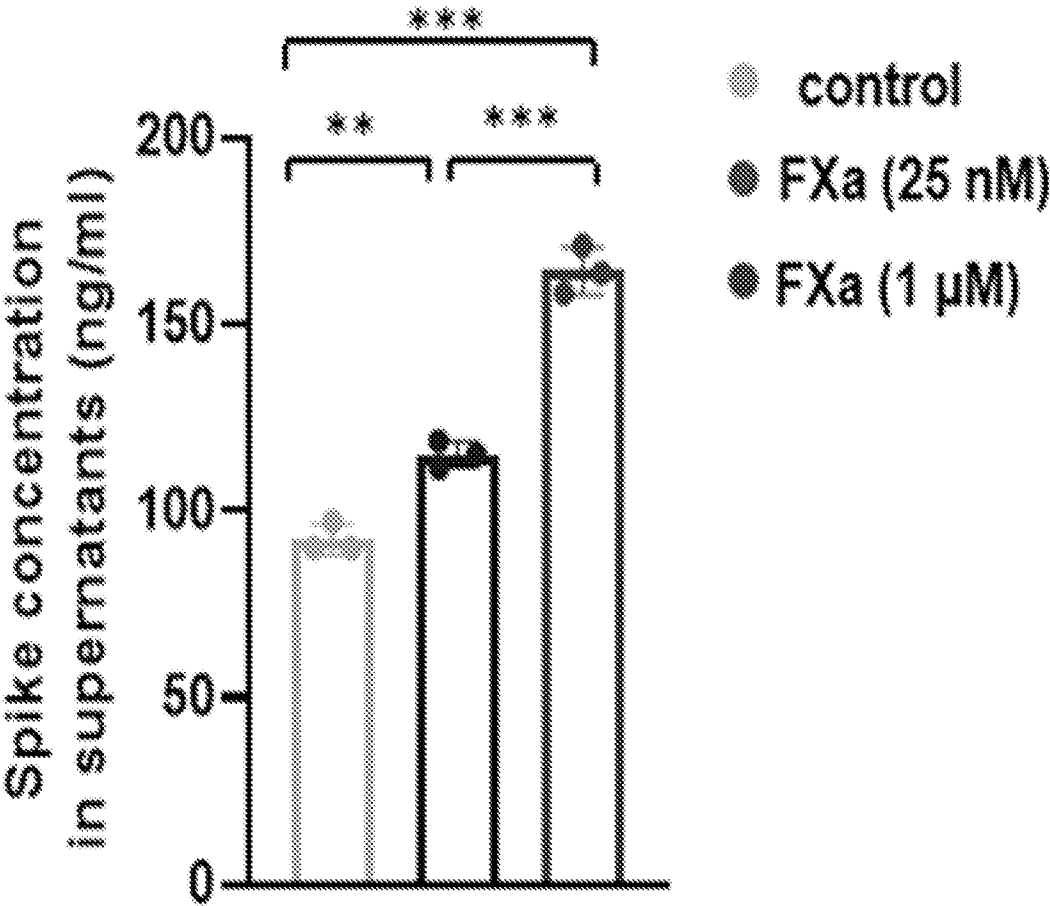


FIG. 21F

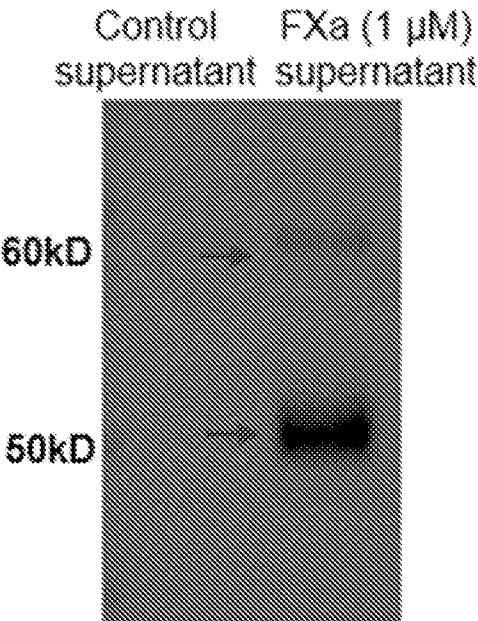


FIG. 22A

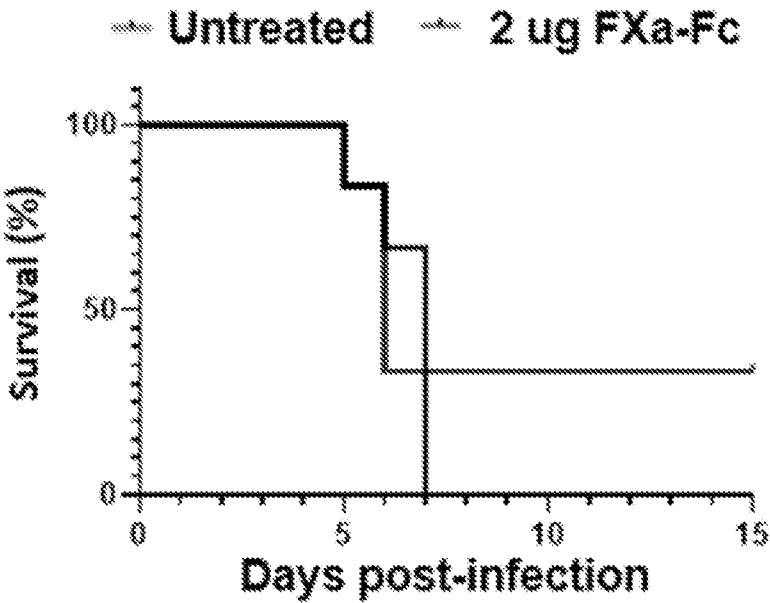


FIG. 22B

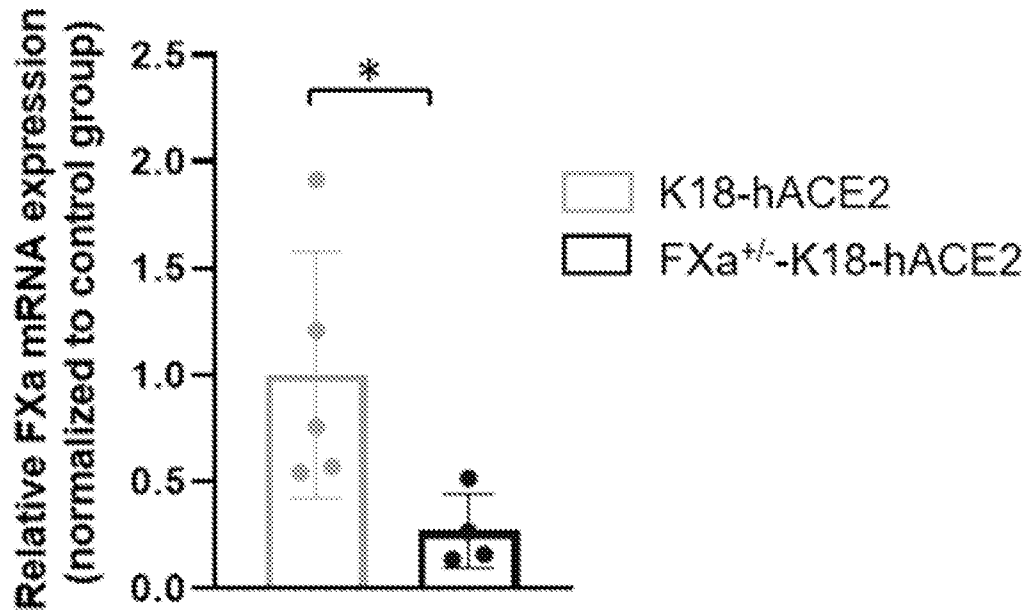


FIG. 22C

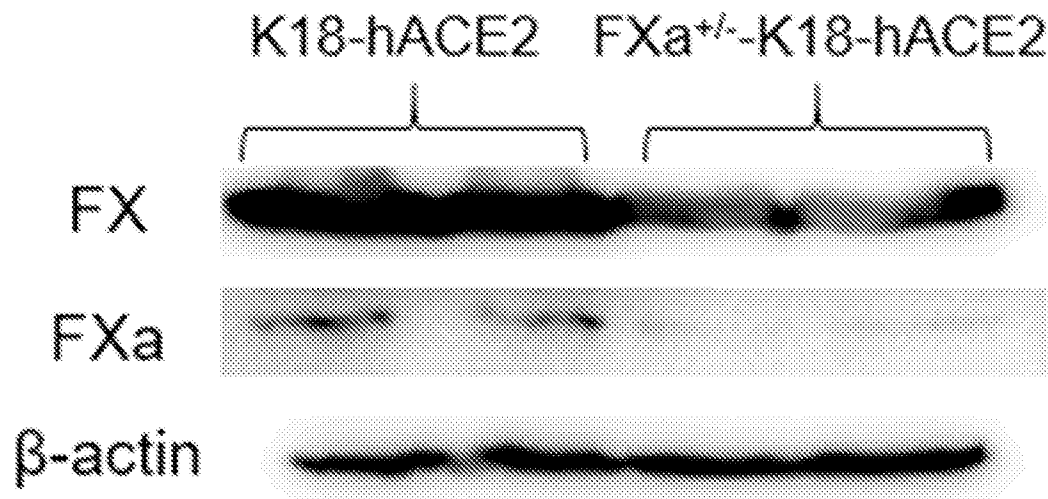


FIG. 22D

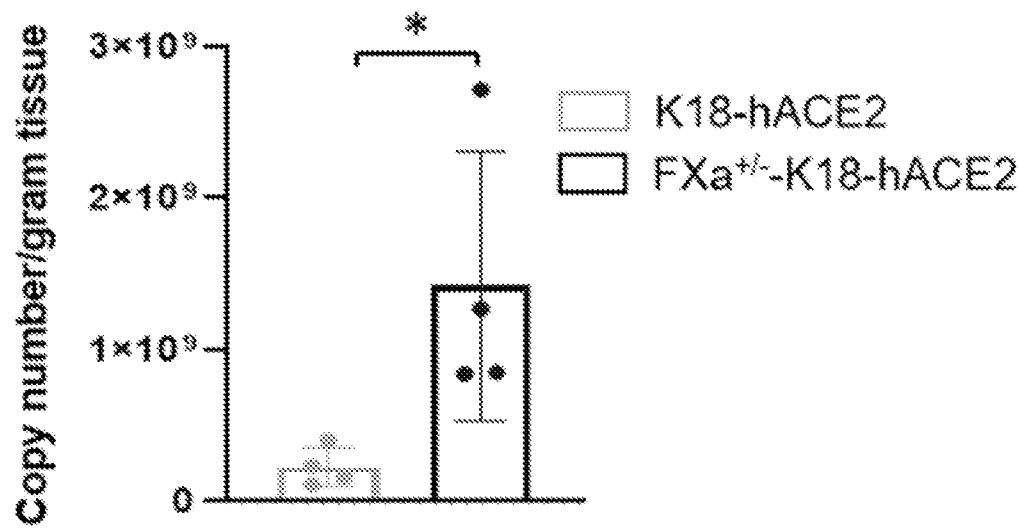


FIG. 23

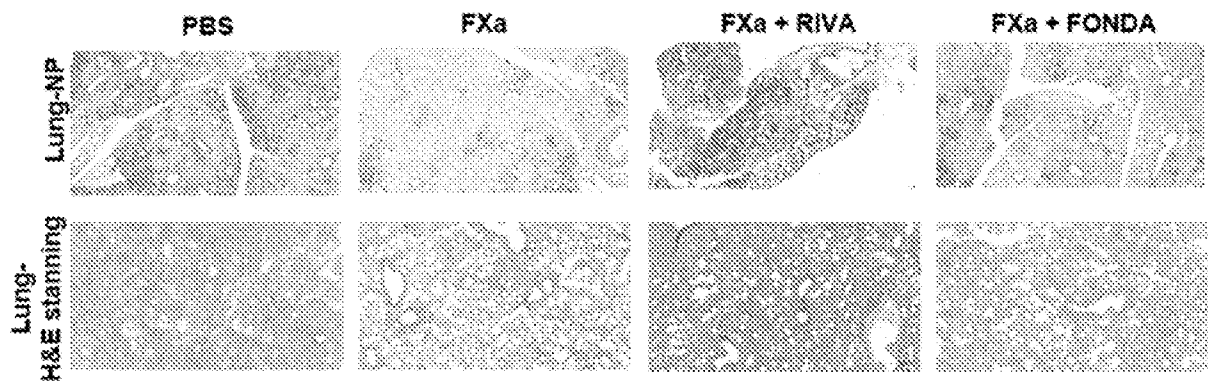


FIG. 24A

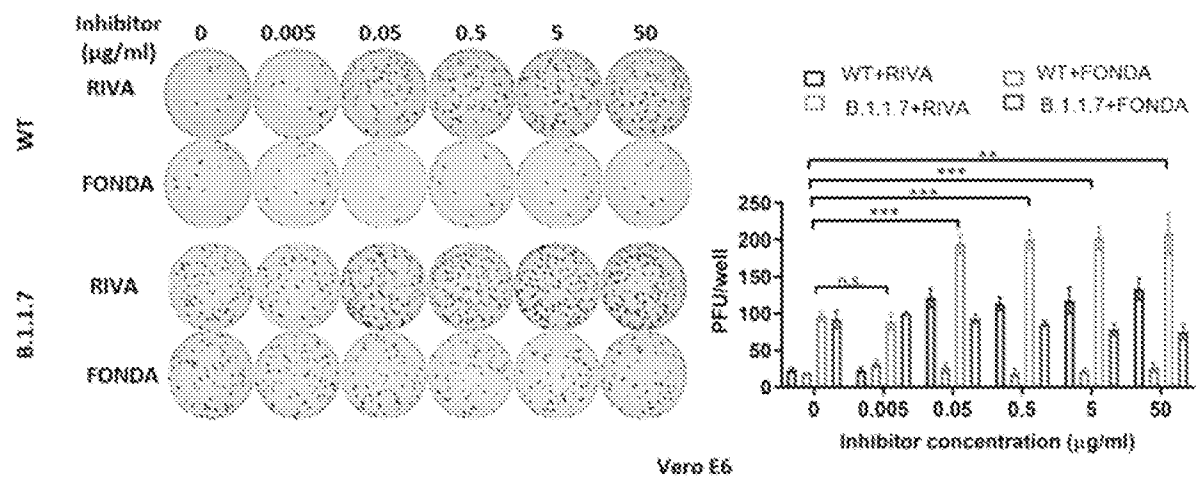


FIG. 24B

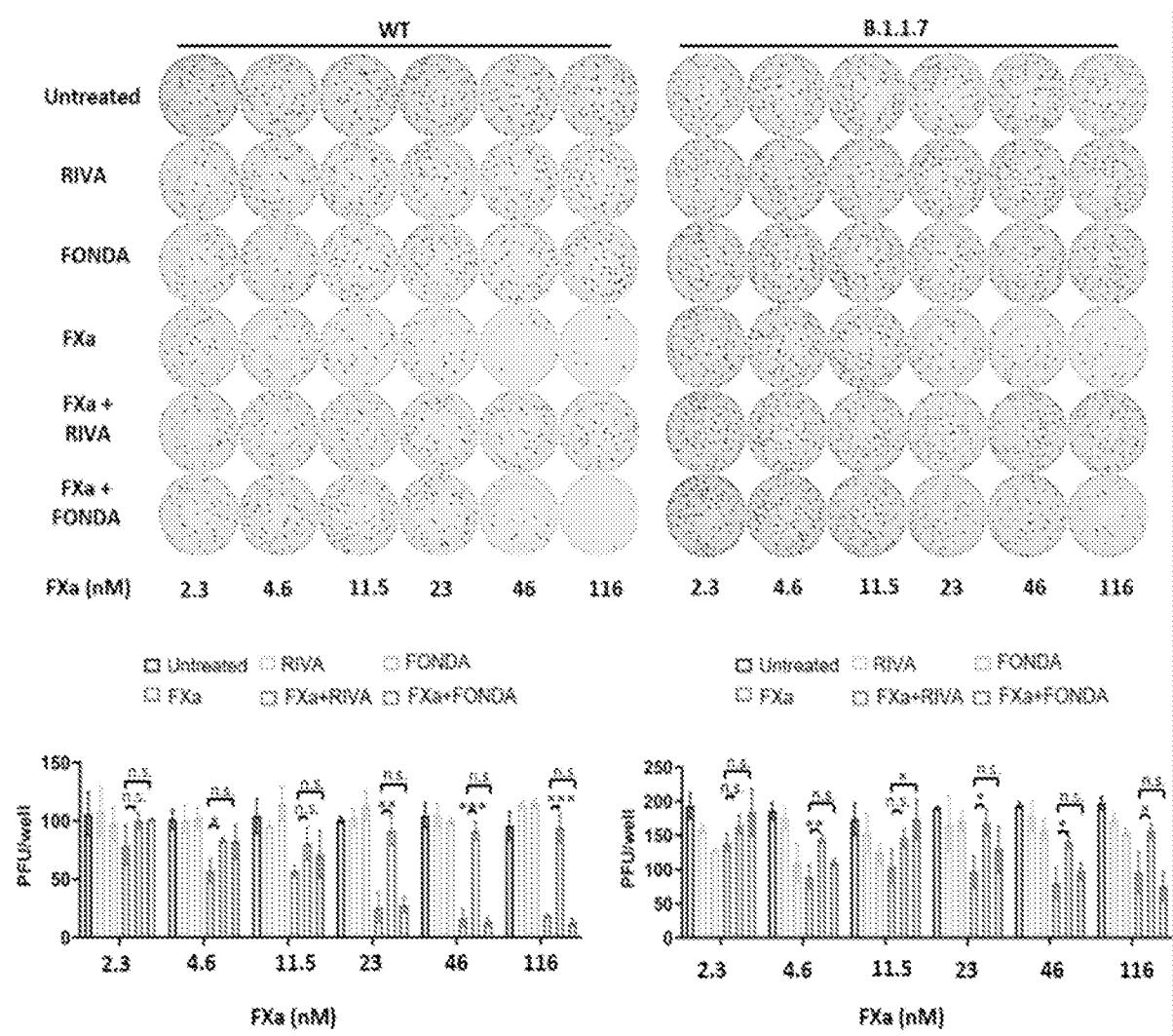


FIG. 25A

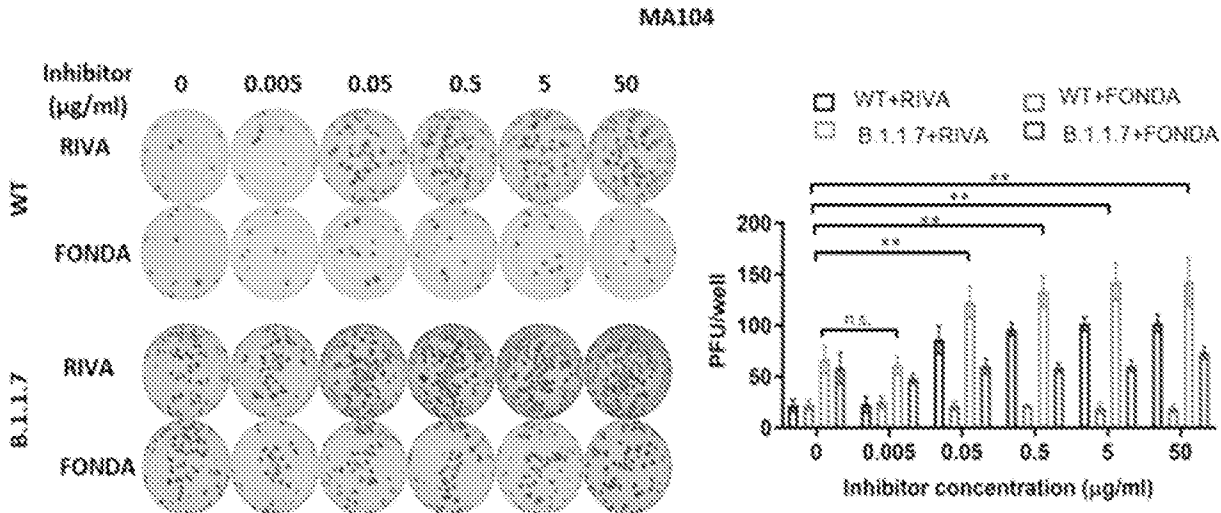


FIG. 25B

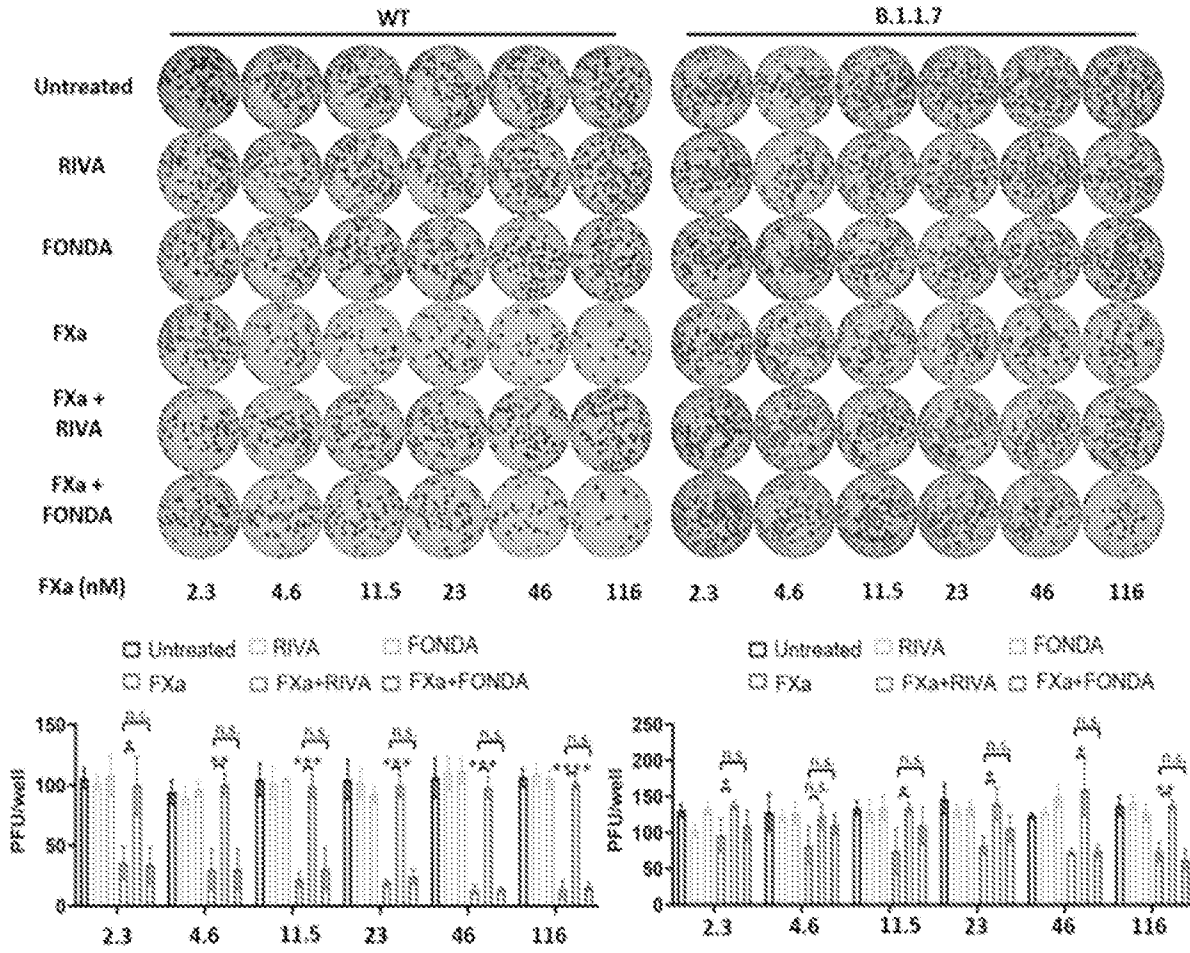


FIG. 26A

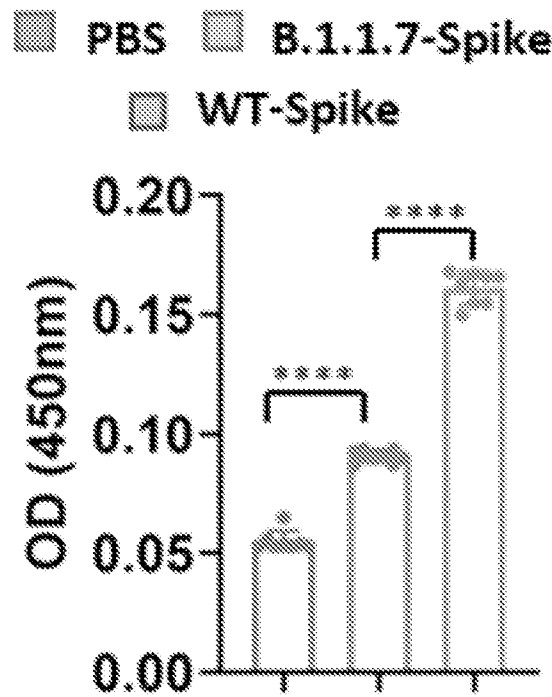


FIG. 26B

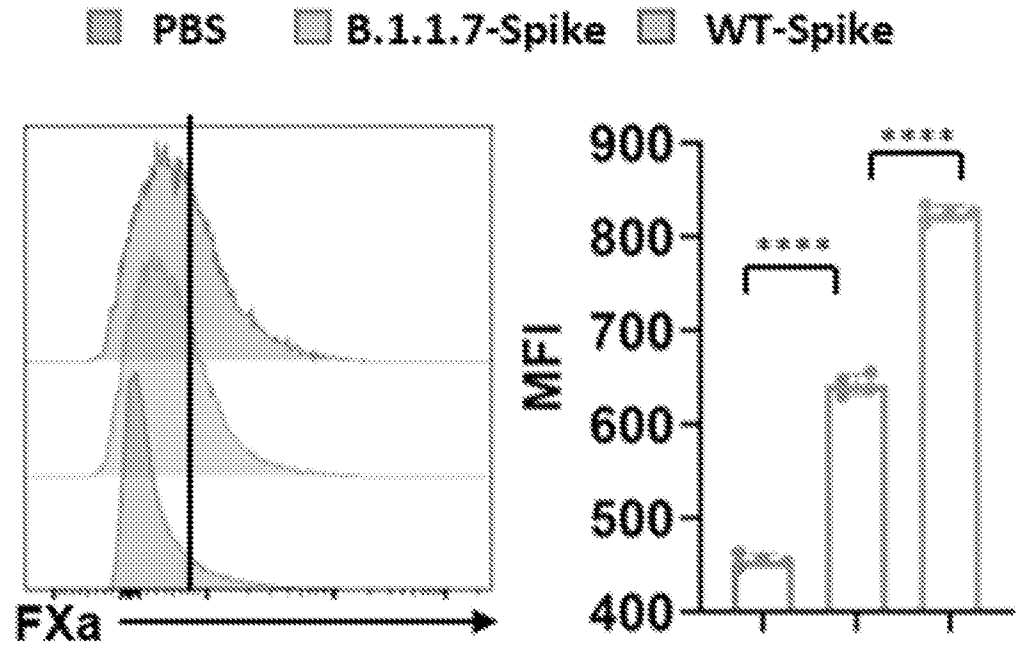


FIG. 26C

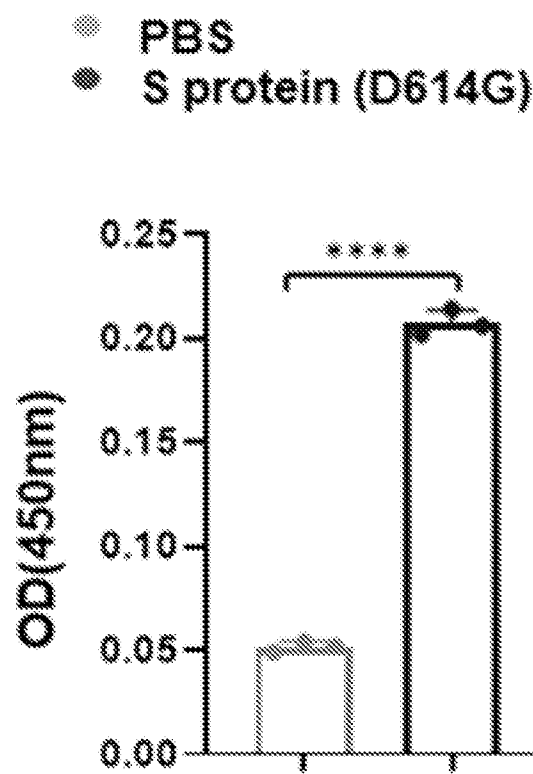


FIG. 26D

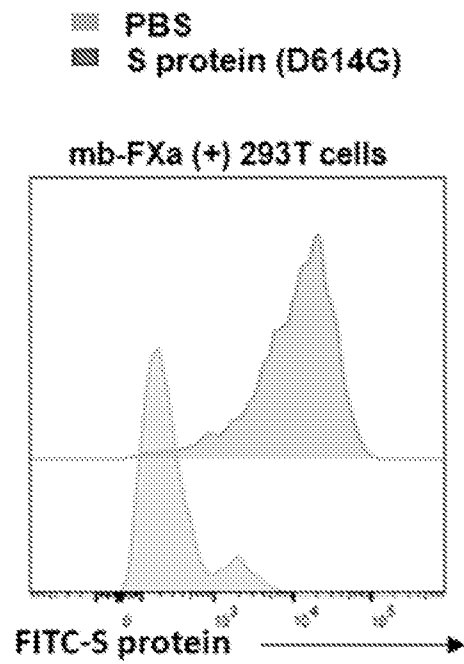


FIG. 26E

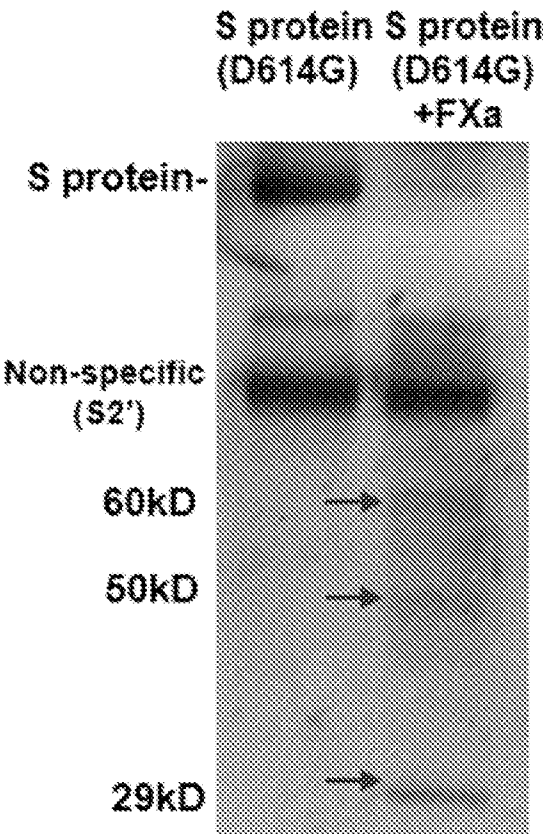


FIG. 26F

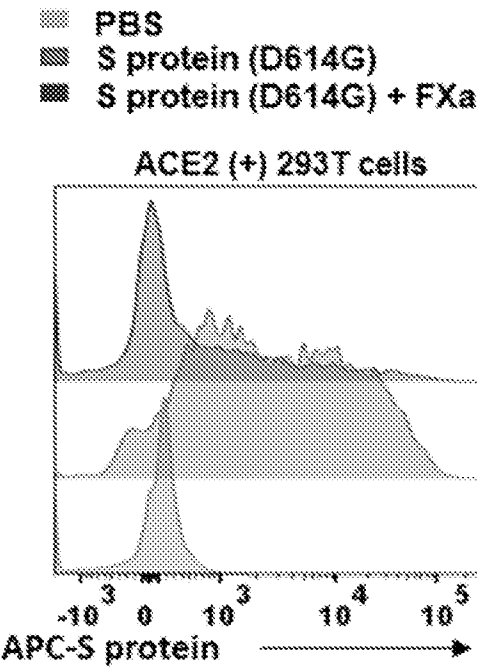


FIG. 26G

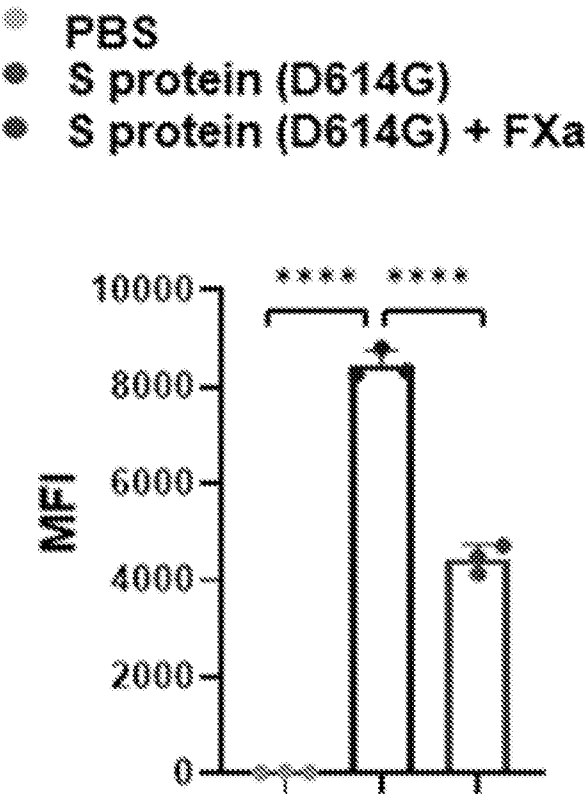


FIG. 26H

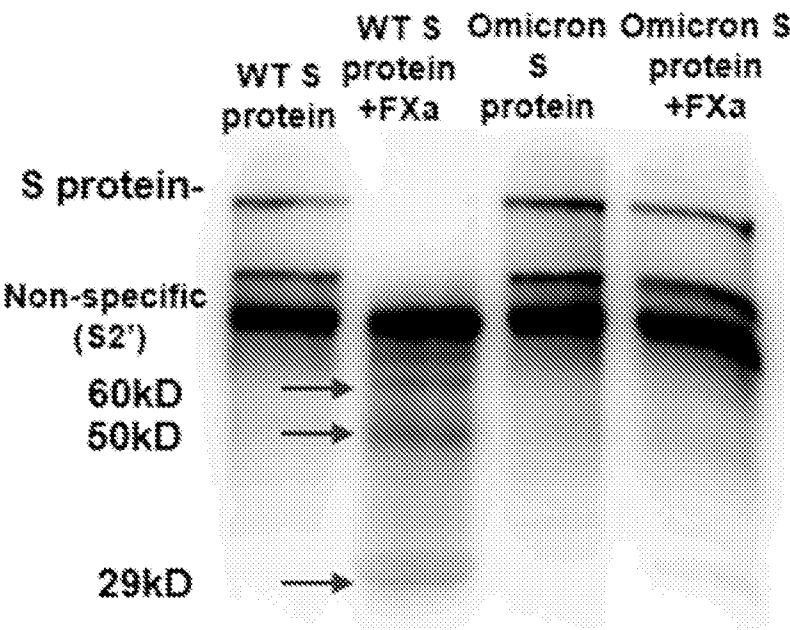


FIG. 27

Patient ID	age	sex	hospitalized	Treatment
COH001	60	M	no	none
COH003	58	M	no	none
COH004	68	F	no	none
COH006	45	M	yes	Leflunomide + Tocilizumab
COH007	64	F	no	oral steroids and inhalers
COH009	68	F	yes	Remdesivir
COH010	34	M	yes	Remdesivir
COH014	73	M	yes	Remdesivir, Toci, Plasma