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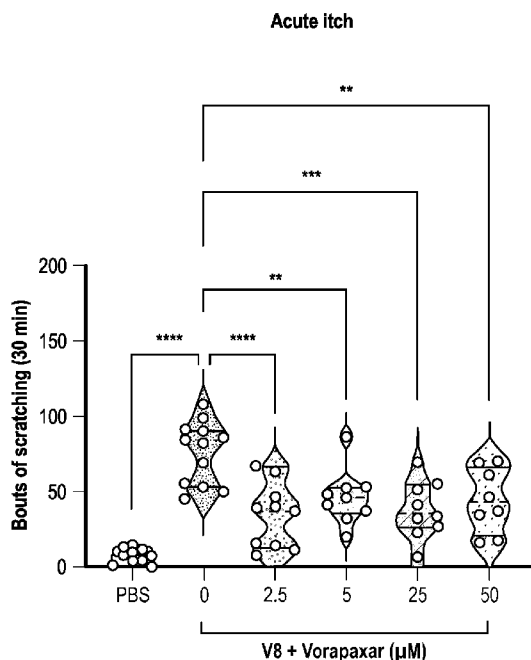


FIG. 7A

(57) Abstract: Described herein are methods and compositions for treating and/or preventing itch caused by microbial exposure. Aspects of the invention relate to administering to a subject an agent that inhibits PAR1 or serine protease V8. In some embodiments of any of the aspects, a subject has been diagnosed with having, or is at risk of having, a microbial exposure.

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METHODS AND COMPOSITIONS FOR TREATING OR PREVENTING ITCH

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This Application claims benefit under 35 U.S.C. § 119(e) of the U.S. Provisional Application No 63/438,668 filed January 12, 2023, the contents of each of which are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] The field of the invention relates to methods for treating and/or preventing itch caused by microbial exposure.

GOVERNMENT SUPPORT

[0003] This invention was made with Government support under Grant number AI168005 awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

[0004] Itch is an unpleasant sensation that evokes a desire to scratch. The skin barrier is constantly exposed to microbes and their products. However, the role of microbes in itch generation is unknown. Here, the inventors show that *Staphylococcus aureus*, a bacterial pathogen associated with itchy skin diseases, directly activates pruriceptor sensory neurons to drive itch. Epicutaneous *S. aureus* exposure causes robust itch and scratch-induced damage. By testing multiple isogenic bacterial mutants for virulence factors, *S. aureus* serine protease V8 was identified as a critical mediator in evoking spontaneous itch and alloknesis. V8 cleaves proteinase-activated receptor 1 (PAR1) on mouse and human sensory neurons. Targeting PAR1 for inhibition decreases itch and skin damage caused by V8 and *S. aureus* exposure. Thus, the invention described herein identifies a targetable mechanism of action for a pruritogenic bacterial factor and demonstrates that inhibiting V8-PAR1 signaling can identify therapeutics for treating or preventing itch.

SUMMARY

[0005] One aspect provided herein describes a method for treating or preventing itch caused by a microbial exposure in a subject, the method comprising; administering to a subject in need thereof an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch.

[0006] In one embodiment of this aspect or any aspect herein, the agent that inhibit PAR1 is selected from the group consisting of: an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule. In one embodiment of this aspect or any aspect herein, the small

molecule is selected from the group consisting of Vorapaxar, Atopaxar (E5555), Parmodulin 2 (PM2, ML161), SCH 79797, FR171113, and RWJ-56110, RWJ-58259.

[0007] In one embodiment of this aspect or any aspect herein, the inhibitory nucleic acid encodes an inhibitor of PAR1. In one embodiment of this aspect or any aspect herein, the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits PAR1.

[0008] In one embodiment of this aspect or any aspect herein, the administering occurs at the site of microbial exposure.

[0009] In one embodiment of this aspect or any aspect herein, the agent that inhibits PAR1 inhibits the expression of PAR1. In one embodiment of this aspect or any aspect herein, the agent inhibits the expression of PAR1 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.

[0010] In one embodiment of this aspect or any aspect herein, the agent that inhibits PAR1 inhibits the function of PAR1. In one embodiment of this aspect or any aspect herein, the agent inhibits the function of PAR1 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.

[0011] In one embodiment of this aspect or any aspect herein, the microbial exposure comprises a *Staphylococcus* exposure.

[0012] In one embodiment of this aspect or any aspect herein, the *Staphylococcus* exposure comprises *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus capitis* (*S. capitis*) and *Staphylococcus hominis* (*S. hominis*).

[0013] In one embodiment of this aspect or any aspect herein, *S. aureus* is methicillin resistant *S. aureus*.

[0014] In one embodiment of this aspect or any aspect herein, the microbial exposure comprises a bacterium which is resistant to at least one antibiotic. In one embodiment of this aspect or any aspect herein, the microbial exposure comprises a bacterium which is resistant to at least two antibiotics.

[0015] In one embodiment of this aspect or any aspect herein, the microbial exposure comprises *Streptococcus pyogenes* (*S. pyogenes*).

[0016] In one embodiment of this aspect or any aspect herein, the microbial exposure is colonization.

[0017] In one embodiment of this aspect or any aspect herein, the microbial exposure is epicutaneous colonization.

[0018] In one embodiment of this aspect or any aspect herein, colonization or epicutaneous colonization is not an infection.

[0019] In one embodiment of this aspect or any aspect herein, colonization or epicutaneous colonization does not elicit an immune response from the subject.

[0020] In one embodiment of this aspect or any aspect herein, colonization or epicutaneous colonization elicits a sub-clinical immune response from the subject.

[0021] In one embodiment of this aspect or any aspect herein, the microbial exposure is an infection.

[0022] In one embodiment of this aspect or any aspect herein, microbial exposure occurs in a lesion.

[0023] In one embodiment of this aspect or any aspect herein, the lesion is associated with a condition selected from a group consisting of: atopic dermatitis, impetigo, prurigo nodularis, psoriasis.

[0024] In one embodiment of this aspect or any aspect herein, the microbial exposure is acute or chronic.

[0025] In one embodiment of this aspect or any aspect herein, the microbial exposure is a reoccurring exposure.

[0026] In one embodiment of this aspect or any aspect herein, the method further comprises administering to a subject a second therapeutic agent. In one embodiment of this aspect or any aspect herein, the second therapeutic agent is an antibiotic, antifungal, or antimicrobial agent.

[0027] In one embodiment of this aspect or any aspect herein, the subject has previously been diagnosed with having a microbial exposure.

[0028] In one embodiment of this aspect or any aspect herein, the subject has not previously been diagnosed with having a microbial exposure.

[0029] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, diagnosing the subject of having or at risk of having itch associate with a microbial exposure.

[0030] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, receiving the results of an assay that diagnoses the subject of having or at risk of having itch associate with a microbial exposure.

[0031] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, diagnosing the subject of having or at risk of having a microbial exposure that can result in itch.

[0032] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, receiving the results of an assay that diagnoses the subject of having a microbial exposure that can result in itch.

[0033] In one embodiment of this aspect or any aspect herein, the administering is systemic or local administration. In one embodiment of this aspect or any aspect herein, local administration is topical administration. In one embodiment of this aspect or any aspect herein, systemic administration is intrathecal administration.

[0034] Another aspect provided herein describes a method for treating or preventing itch caused by a Staphylococcus exposure in a subject, the method comprising: administering to a subject in need thereof an agent that inhibits Staphylococcus serine protease V8 in an amount and for a duration sufficient to treat or prevent itch.

[0035] In one embodiment of this aspect or any aspect herein, the agent that inhibit serine protease V8 is selected from the group consisting of: an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule.

[0036] In one embodiment of this aspect or any aspect herein, the inhibitory nucleic acid encodes an inhibitor of serine protease V8. In one embodiment of this aspect or any aspect herein, the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits serine protease V8.

[0037] In one embodiment of this aspect or any aspect herein, the administering occurs at the site of *Staphylococcus* exposure.

[0038] In one embodiment of this aspect or any aspect herein, the agent that inhibits serine protease V8 inhibits the expression of serine protease V8. In one embodiment of this aspect or any aspect herein, the agent inhibits the expression of serine protease V8 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.

[0039] In one embodiment of this aspect or any aspect herein, the agent that inhibits serine protease V8 inhibits the function of serine protease V8. In one embodiment of this aspect or any aspect herein, the agent inhibits the function of serine protease V8 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.

[0040] In one embodiment of this aspect or any aspect herein, the function of serine protease V8 is cleaving PAR-1. In one embodiment of this aspect or any aspect herein, the *Staphylococcus* exposure comprises *S. aureus*, *S. epidermidis*, *Staphylococcus capitis* (*S. capitis*) and *Staphylococcus hominis* (*S. hominis*).

[0041] In one embodiment of this aspect or any aspect herein, *S. aureus* is methicillin resistant *S. aureus*.

[0042] In one embodiment of this aspect or any aspect herein, the *Staphylococcus* exposure is *Staphylococcus* colonization.

[0043] In one embodiment of this aspect or any aspect herein, the *Staphylococcus* exposure is an epicutaneous colonization.

[0044] In one embodiment of this aspect or any aspect herein, colonization or epicutaneous colonization is not an infection.

[0045] In one embodiment of this aspect or any aspect herein, colonization or epicutaneous colonization does not elicit an immune response from the subject.

[0046] In one embodiment of this aspect or any aspect herein, colonization or epicutaneous colonization elicits a sub-clinical immune response from the subject.

[0047] In one embodiment of this aspect or any aspect herein, the *Staphylococcus* exposure is an infection.

[0048] In one embodiment of this aspect or any aspect herein, the *Staphylococcus* exposure occurs in a lesion.

[0049] In one embodiment of this aspect or any aspect herein, the lesion is associated with a condition selected from a group consisting of: atopic dermatitis, impetigo, prurigo nodularis, psoriasis.

[0050] In one embodiment of this aspect or any aspect herein, the exposure is acute or chronic.

[0051] In one embodiment of this aspect or any aspect herein, the Staphylococcus exposure is a reoccurring exposure.

[0052] In one embodiment of this aspect or any aspect herein, the method further comprises administering to a subject a second therapeutic agent.

[0053] In one embodiment of this aspect or any aspect herein, the second therapeutic agent is an antibiotic, antifungal, or antimicrobial agent.

[0054] In one embodiment of this aspect or any aspect herein, the subject has previously been diagnosed with having a Staphylococcus exposure.

[0055] In one embodiment of this aspect or any aspect herein, the subject has not previously been diagnosed with having a Staphylococcus exposure.

[0056] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, diagnosing the subject of having or at risk of having itch associate with a Staphylococcus exposure.

[0057] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, receiving the results of an assay that diagnoses the subject of having or at risk of having itch associate with a Staphylococcus exposure.

[0058] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, diagnosing the subject of having or at risk of having a Staphylococcus exposure that can result in itch.

[0059] In one embodiment of this aspect or any aspect herein, the method further comprises the step, prior to administering, receiving the results of an assay that diagnoses the subject of having a Staphylococcus exposure that can result in itch.

[0060] In one embodiment of this aspect or any aspect herein, the administering is systemic or local administration. In one embodiment of this aspect or any aspect herein, local administration is topical administration. In one embodiment of this aspect or any aspect herein, systemic administration is intrathecal administration.

[0061] Another aspect provided herein describes a method for treating itch caused by a microbial exposure in a subject, the method comprising; topically administering to a subject having microbial exposure an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch, wherein administration occurs at the site of microbial exposure.

[0062] In one embodiment of this aspect or any aspect herein, the subject does not have a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.

[0063] In one embodiment of this aspect or any aspect herein, the subject is not being treated for a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.

[0064] Another aspect provided herein describes a composition for treating or preventing itch caused by a microbial exposure in a subject, the composition comprising; an amount of an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount sufficient to treat or prevent itch.

[0065] Another aspect provided herein describes a composition for treating or preventing itch caused by a Staphylococcus exposure in a subject, the composition comprising; an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch.

[0066] In one embodiment of this aspect or any aspect herein, the composition further comprises a pharmaceutically acceptable carrier.

[0067] In one embodiment of this aspect or any aspect herein, the composition is formulated for topical administration. In one embodiment of this aspect or any aspect herein, the composition is formulated for systemic administration.

[0068] In one embodiment of this aspect or any aspect herein, the composition further comprises a second therapeutic agent. In one embodiment of this aspect or any aspect herein, the second therapeutic agent is an antibiotic or antimicrobial agent.

[0069] Another aspect provided herein describes a use of a composition for treating or preventing itch caused by a microbial exposure in a subject, the composition comprising; an amount of an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount sufficient to treat or prevent itch.

[0070] Another aspect provided herein describes a use of a composition for treating or preventing itch caused by a Staphylococcus exposure in a subject, the composition comprising; an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch.

[0071] Definitions

[0072] As used herein, a “subject” means a human or animal. Usually the animal is a vertebrate such as a primate, rodent, domestic animal or game animal. Primates include, for example, chimpanzees, cynomolgous monkeys, spider monkeys, and macaques, e.g., Rhesus. Rodents include, for example, mice, rats, woodchucks, ferrets, rabbits and hamsters. Domestic and game animals include, for example, cows, horses, pigs, deer, bison, buffalo, feline species, e.g., domestic cat, canine species, e.g., dog, fox, wolf, avian species, e.g., chicken, emu, ostrich, and fish, e.g., trout, catfish and salmon. In some embodiments, the subject is a mammal, e.g., a primate, e.g., a human. The terms, “individual,” “patient” and “subject” are used interchangeably herein.

[0073] Preferably, the subject is a mammal. The mammal can be a human, non-human primate, mouse, rat, dog, cat, horse, or cow, but is not limited to these examples. Mammals other than humans can be advantageously used as subjects that represent animal models of microbial exposure or itch related to microbial exposure e.g., *Staphylococcus aureus* exposure. A subject can be male or female.

[0074] As used herein, an “exposure” refers to a presence of a microbe, i.e., bacterium, virus, and/or fungus, in or on a subject that does not result in illness or disease. The presence can be normal in that the microbe is found typically found in or on a healthy subject. The presence can be abnormal in that the microbe is a noncommensal species, e.g. one not typically found in or on a healthy subject, it can be

abnormal as in a localization that the bacteria does not normally colonize, or it can be abnormal in that the microbe is present at abnormally high levels, e.g. at least twice the level found in or on a healthy subject (e.g. twice the level, three times the level, four times the level, five times the level, or greater). Microbial exposure can refer to the invasion and propagation of the microbe in or on a subject that does not result in illness or disease.

[0075] As used herein, “microbial infection” refers to the presence of the microbe on a subject that causes or contributes to disease or symptoms thereof, e.g., necrosis, disfigurement, delayed wound healing, etc. An infection can refer to the invasion and propagation of the microbe in or on a subject that directly results in illness or disease.

[0076] As used herein, an “agent” refers to e.g., a molecule, protein, peptide, antibody, or nucleic acid, that inhibits expression of a polypeptide or polynucleotide, or binds to, partially or totally blocks stimulation, decreases, prevents, delays activation, inactivates, desensitizes, or down regulates the activity of the polypeptide or the polynucleotide. Agents that inhibit PAR1 or serine protease V8, e.g., inhibit expression, e.g., translation, post-translational processing, stability, degradation, or nuclear or cytoplasmic localization of a polypeptide, or transcription, post transcriptional processing, stability or degradation of a polynucleotide or bind to, partially or totally block stimulation, DNA binding, transcription factor activity or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide. An agent can act directly or indirectly.

[0077] The term “agent” as used herein means any compound or substance such as, but not limited to, a small molecule, nucleic acid, polypeptide, peptide, drug, ion, etc. An “agent” can be any chemical, entity or moiety, including without limitation synthetic and naturally-occurring proteinaceous and non-proteinaceous entities. In some embodiments, an agent is nucleic acid, nucleic acid analogues, proteins, antibodies, peptides, aptamers, oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNazymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof etc. In certain embodiments, agents are small molecule having a chemical moiety. For example, chemical moieties included unsubstituted or substituted alkyl, aromatic, or heterocyclyl moieties including macrolides, leptomycins and related natural products or analogues thereof. Compounds can be known to have a desired activity and/or property or can be selected from a library of diverse compounds.

[0078] The agent can be a molecule from one or more chemical classes, e.g., organic molecules, which may include organometallic molecules, inorganic molecules, genetic sequences, etc. Agents may also be fusion proteins from one or more proteins, chimeric proteins (for example domain switching or homologous recombination of functionally significant regions of related or different molecules), synthetic proteins or other protein variations including substitutions, deletions, insertion and other variants.

[0079] As used herein, the term “small molecule” refers to a chemical agent which can include, but is not limited to, a peptide, a peptidomimetic, an amino acid, an amino acid analog, a polynucleotide, a polynucleotide analog, an aptamer, a nucleotide, a nucleotide analog, an organic or inorganic compound (e.g., including heterorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

[0080] The term “RNAi” as used herein refers to interfering RNA or RNA interference. RNAi refers to a means of selective post-transcriptional gene silencing by destruction of specific mRNA by molecules that bind and inhibit the processing of mRNA, for example inhibit mRNA translation or result in mRNA degradation. As used herein, the term “RNAi” refers to any type of interfering RNA, including but are not limited to, siRNA, shRNA, endogenous microRNA and artificial microRNA. For instance, it includes sequences previously identified as siRNA, regardless of the mechanism of down-stream processing of the RNA (i.e. although siRNAs are believed to have a specific method of in vivo processing resulting in the cleavage of mRNA, such sequences can be incorporated into the vectors in the context of the flanking sequences described herein).

[0081] Methods and compositions described herein require that the PAR1 activity or expression is inhibited. As used herein, proteinase-activated receptor 1 “PAR1” refers to a 7-transmembrane receptor involved in the regulation of thrombotic response. Proteolytic cleavage leads to the activation of the receptor. Alternative splicing results in multiple transcript variants. PAR1 is also known in the art as coagulation factor II thrombin receptor (F2R), TR, HTR, CF2R, and PAR-1. PAR1 sequences are known for a number of species, e.g., human PAR1 (NCBI Gene ID: 2149) and mRNA (NCBI Ref Seq NM_001311313.2, and NCBI Ref Seq NP_001298242.1). PAR1 can refer to human PAR1, including naturally occurring variants and alleles thereof. In some embodiments of any of the aspects, e.g., in veterinary applications, PAR1 can refer to the PAR1 of, e.g., dog, cat, cow, horse, pig, and the like. Homologs and/or orthologs of human PAR1 are readily identified for such species by one of skill in the art, e.g., using the NCBI ortholog search function or searching available sequence data for a given species for sequence similar to a reference PAR1 sequence.

[0082] As used herein, “inhibition of PAR1” refers to the inhibition of PAR1 activity or PAR1 expression”.

[0083] Methods and compositions described herein require that the serine protease V8 activity or expression. As used herein, “serine protease V8” refers to *sspA*. Serine protease V8 sequences are known for a number of species, e.g., *S. aureus* serine protease V8 (NCBI Gene ID: 3913747) and protein (NCBI Ref Seq WP_000676548.1). Serine protease V8 can refer to naturally occurring variants and alleles thereof. Homologs and/or orthologs of serine protease V8 are readily identified for such species by one of skill in the art, e.g., using the NCBI ortholog search function or searching available sequence data for a

given species for sequence similar to a reference serine protease V8 sequence. In one embodiment, serine protease V8 is any known serine protease V8 isoform.

[0084] As used herein, “inhibition of serine protease V8” refers to the inhibition of serine protease V8 activity or expression.

[0085] The term “gene” used herein can be a genomic gene comprising transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (e.g., introns, 5'- and 3'- untranslated sequences and regulatory sequences). The coding region of a gene can be a nucleotide sequence coding for an amino acid sequence or a functional RNA, such as tRNA, rRNA, catalytic RNA, siRNA, miRNA and antisense RNA. A gene can also be an mRNA or cDNA corresponding to the coding regions (e.g., exons and miRNA) optionally comprising 5'- or 3' untranslated sequences linked thereto. A gene can also be an amplified nucleic acid molecule produced *in vitro* comprising all or a part of the coding region and/or 5'- or 3'- untranslated sequences linked thereto. The term “gene product(s)” as used herein refers to include RNA transcribed from a gene, or a polypeptide encoded by a gene or translated from RNA.

[0086] The term “decrease”, “reduced”, “reduction”, or “inhibit” are all used herein to mean a decrease by a statistically significant amount. In some embodiments, “decrease”, “reduced”, “reduction”, or “inhibit” typically means a decrease by at least 10% as compared to an appropriate control (e.g. the absence of a given treatment) and can include, for example, a decrease by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or more. As used herein, “reduction” or “inhibition” does not encompass a complete inhibition or reduction as compared to a reference level. “Complete inhibition” is a 100% inhibition as compared to an appropriate control.

[0087] The terms “increase”, “enhance”, or “activate” are all used herein to mean an increase by a reproducible statistically significant amount. In some embodiments, the terms “increase”, “enhance”, or “activate” can mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, or at least about a 2-fold, or at least about a 3-fold, or at least about a 4-fold, or at least about a 5-fold or at least about a 10-fold increase, a 20 fold increase, a 30 fold increase, a 40 fold increase, a 50 fold increase, a 60 fold increase, a 75 fold increase, a 100 fold increase, etc. or any increase between 2-fold and 10-fold or greater as compared to an appropriate control. In the context of a marker, an “increase” is a reproducible statistically significant increase in such level.

[0088] As used herein, an “appropriate control” refers to an untreated, otherwise identical cell or population (e.g., a patient who was not administered an agent described herein or was administered by only a subset of agents described herein, as compared to a non-control cell).

[0089] The terms “inhibitor” and “antagonist” refers to an agent that inhibits expression of a polypeptide or polynucleotide, or binds to, partially or totally blocks stimulation, decreases, prevents, delays activation, inactivates, desensitizes, or down regulates the activity of the polypeptide or the polynucleotide. Inhibitors are agents that, e.g., inhibit expression, e.g., translation, post-translational processing, stability, degradation, or nuclear or cytoplasmic localization of a polypeptide, or transcription, post transcriptional processing, stability or degradation of a polynucleotide or bind to, partially or totally block stimulation, DNA binding, transcription factor activity or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide. An inhibitor can act directly or indirectly. Inhibition is achieved when the activity value of a polypeptide or polynucleotide is about at least 10% less, at least 20% less, at least 30% less, at least 40% less, at least 50% less, at least 60% less, at least 70% less, at least 80% less, at least 90% less, or absent or undetectable in comparison to a reference or control level in the absence of the inhibitor.

[0090] The terms “significantly different than”, “statistically significant,” and similar phrases refer to comparisons between data or other measurements, wherein the differences between two compared individuals or groups are evidently or reasonably different to the trained observer, or statistically significant (if the phrase includes the term “statistically” or if there is some indication of statistical test, such as a p-value, or if the data, when analyzed, produce a statistical difference by standard statistical tests known in the art).

[0091] The term “effective amount” is used interchangeably with the terms “sufficient amount” and “therapeutically effective amount” and refers to the amount of at least one agent, e.g., an inhibitor of PAR1 or serine protease V8, at dosages and for periods of time necessary to achieve the desired therapeutic result, for example, to reduce or stop at least one symptom of itch, e.g., caused by microbial exposure in the subject. For example, an effective amount using the methods as disclosed herein would be considered as the amount sufficient to reduce a symptom of itch by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 90%, at least 99%, as measured by any standard technique. An effective amount as used herein would also include an amount sufficient to prevent or delay the development of a symptom of itch, alter the course of a symptom of itch, or reverse a symptom of itch. Accordingly, the term “effective amount” or “therapeutically effective amount” as used herein refers to the amount of therapeutic agent (e.g. at least one PAR1 or serine protease V8 inhibitory agent as disclosed herein) of pharmaceutical composition to alleviate at least one symptom of itch. Stated another way, “therapeutically effective amount” of a PAR1 or serine protease V8 inhibitory agent as disclosed herein is the amount of PAR1 or serine protease V8 inhibitory agent which exerts a beneficial effect on, for example, the symptoms of itch. The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties of the

PAR1 or serine protease V8 inhibitory agent, the route of administration, conditions and characteristics (sex, age, body weight, health, size) of subjects, extent of symptoms, concurrent treatments, frequency of treatment and the effect desired. A therapeutically effective amount is also one in which any toxic or detrimental effects of the PAR1 or serine protease V8 inhibitory agent are outweighed by the therapeutically beneficial effects. The effective amount in each individual case can be determined empirically by a skilled artisan according to established methods in the art and without undue experimentation. In general, the phrases "therapeutically-effective" and "effective for the treatment, prevention, or inhibition", are intended to qualify the PAR1 or serine protease V8 inhibitory agent as disclosed herein which will achieve the goal of reduction in the severity of at least one symptom of itch, e.g., microbial exposure.

[0092] As used herein, the terms "treat," "treatment," "treating," or "amelioration" refer to therapeutic treatments, wherein the object is to reverse, alleviate, ameliorate, inhibit, slow down or stop the progression or severity of a condition associated with microbial exposure, e.g., itch. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of itch, e.g., caused by microbial exposure. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of itch is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but can also include a cessation or at least slowing of progress or worsening of symptoms that would be expected in absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s) of itch, diminishment of extent of itch, stabilized (i.e., not worsening) state of itch, delay or slowing of progression of itch, amelioration or palliation of the itch, and remission (whether partial or total), whether detectable or undetectable. The term "treatment" of itch also includes providing relief from the symptoms or side-effects of itch (including palliative treatment).

[0093] As used herein, the terms "preventing" and "prevention" have their ordinary and customary meanings, and include one or more of: preventing an increase of itch, e.g., caused by microbial exposure; preventing development of itch, e.g., caused by microbial exposure in a subject; and preventing symptoms of itch, e.g., caused by microbial exposure in a subject. As used herein, the prevention lasts at least about 0.5 days, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 25 days, 30 days, 35 days, 40 days or more days after administration or application of the effective amount of the agent that inhibits PAR1 or serine protease V8, as described herein. In one embodiment, the prevention results in an at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 99%, or more, reduction in the presence, the severity of, and/or the risk of having itch, e.g., caused by microbial exposure, as compared to an appropriate control. Used herein, an appropriate control refers to a subject not administered any of the agents described herein.

[0094] The "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for

use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agents from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, for example the carrier does not decrease the impact of the agent on the treatment. In other words, a carrier is pharmaceutically inert. The terms "physiologically tolerable carriers" and "biocompatible delivery vehicles" are used interchangeably.

[0095] The term "administered" is used interchangeably in the context of treatment of a disease or disorder, e.g., itch. Both terms refer to a subject being treated with an effective dose of pharmaceutical composition comprising, e.g., at least an PAR1 or serine protease V8 inhibitory agent of the invention, by methods of administration, for example subcutaneous or systemic administration.

[0096] The phrases "systemic administration," "administered systemically", "peripheral administration" and "administered peripherally" as used herein mean the administration of a pharmaceutical composition comprising at least an PAR1 or serine protease V8 inhibitory agent as disclosed herein such that it enters the subject's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

[0097] The term "optional" or "optionally" means that the subsequent described event, circumstance or substituent may or may not occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

[0098] As used herein, the term "comprising" means that other elements can also be present in addition to the defined elements presented. The use of "comprising" indicates inclusion rather than limitation. The term "consisting of" refers to compositions, methods, and respective components thereof as described herein, which are exclusive of any element not recited in that description of the embodiment. The term permits the presence of elements that do not materially affect the basic and novel or functional characteristic(s) of that embodiment of the invention.

[0099] The term "statistically significant" or "significantly" refers to statistical significance and generally means a two standard deviation (2SD) or greater difference.

[00100] As used herein the term "comprising" or "comprises" is used in reference to compositions, methods, and respective component(s) thereof, that are essential to the method or composition, yet open to the inclusion of unspecified elements, whether essential or not.

[00101] The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of this disclosure, suitable methods and materials are described below.

The abbreviation, "e.g." is derived from the Latin exempli gratia, and is used herein to indicate a non-limiting example. Thus, the abbreviation "e.g." is synonymous with the term "for example."

BRIEF DESCRIPTION OF THE DRAWINGS

[00102] Figs 1A-1J show epicutaneous *S. aureus* induces itch and scratch-induced skin pathology. (Fig. 1A) Murine model of *S. aureus* exposure and itch analysis. (Fig. 1B-1E) 5-days after epicutaneous exposure, dermatitis (Fig. 1B), spontaneous itch (Fig. 1C-1D), and (Fig. 1E) alloknosis were measured (n=8-13 males, 6-8 females per group). (Fig. 1F) Analysis of total skin damage after scratching (n=8 males, 8 females per group). (Fig. 1G) Total skin damage in mice allowed to scratch or prevented from scratching (n=6 males, 5-6 females per group). (Fig. 1H-1J) Mice inoculated with *S. aureus* epicutaneously or infected subcutaneously; Representative images (Fig. 1H), spontaneous itch (Fig. 1I), and alloknosis (Fig. 1J) on day-5 (n=16 per group). For each panel, data combined from 2 independent experiments are shown. Data are represented as mean±SD. Statistical analysis: (Fig. 1B, 1D, 1E, 1F, 1G, 1I, 1J) Two-way ANOVA with Sidak's multiple comparisons. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

[00103] Figs 2A-2H show bacterial factors including Agr quorum sensing and proteases mediate itch. (Fig. 2A) Whole mount images of skin from Nav1.8-tdTomato mice treated with PBS or GFP-MRSA (scale bars, 50 or 20µm). (Fig. 2B) Agr quorum sensing regulates expression of phenol soluble modulins (Psms), alpha-toxin (Hla), and proteases. (Fig. 2C-2D) Spontaneous itch, alloknosis (Fig. 2C) and dermatitis scores (Fig. 2D) recorded for control mice (PBS) or mice inoculated with WT or Δ agr MRSA (n=10 males, 10 females per group). (Fig. 2E-2F) Spontaneous itch, alloknosis (Fig. 2E) and dermatitis scores (Fig. 2F) for control mice (PBS) or mice inoculated with WT, Δ hla or Δ Psms MRSA (n=8-15 males, 8-16 females per group). (Fig. 2G-2H) Spontaneous itch, alloknosis (Fig. 2G) and dermatitis scores (Fig. 2H) for control mice (PBS) or mice inoculated with WT or Δ Protease MRSA (n=12 males, 12 females per group). For each panel, data combined from 4-6 independent experiments are shown. Data are represented as mean±SD. Statistical analysis: (Fig. 2C-2H) One-way ANOVA. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

[00104] Figs 3A-3N show *S. aureus* V8 protease contributes to itch and inflammation. (Fig. 3A-3B) Spontaneous itch (Fig. 3A) and alloknosis (Fig. 3B) for control mice (PBS) or mice inoculated with WT, Δ sspA, or Δ sspA + sspA MRSA (n=11-12 males, 12 females per group). (Fig. 3C) Total skin damage for control (PBS) or mice inoculated with WT, Δ sspA, or Δ sspA + sspA MRSA (n=4-5 males, 4-5 females per group). (Fig. 3D) Representative skin images and dermatitis scores from control mice (PBS) or mice inoculated with WT, Δ sspA, or Δ sspA + sspA MRSA (n=11-12 males, 12 females per group). (Fig. 3E-3H) Skin collected from mice at 1-, 3-, and 5-days post-inoculation with MRSA quantified for sspA (F), psmA1 (Fig. 3G), and hla (Fig. 3H) transcripts (normalized to 1-day post-inoculation) (n=2 males, 2 females per group). (Fig. 3I) Quantification of sspA mRNA from skin swabs from healthy human subjects or non-lesional and lesional skin from AD patients (n=13-14 per group).

(Fig. 3J) Mouse acute itch and pain behavior. (Fig. 3K-3L) Acute itch (Fig. 3K) and pain (Fig. 3L) following intradermal injection with PBS, V8, histamine or capsaicin (n=4-5 males, 4-5 females per group). (Fig. 3M) Mouse intradermal injection and allodynia model. (Fig. 3N) Allodynia after injection with PBS, V8, or histamine (n=3-5 males, 3-5 females per group). For each panel, data combined from 2 independent experiments are shown. Data are represented as mean±SD. Statistical analysis: (Fig. 3A-3D, 3F-3I, 3K-3L) One-way ANOVA. (3N) Two-way ANOVA, Tukey's multiple comparisons. *V8 vs. Histamine; #Histamine vs. PBS; \$V8 vs. PBS; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

[00105] Figs 4A-4H show V8 protease cleaves PAR1, which is expressed by pruriceptors. (Fig. 4A) PAR cleavage assays using nLuc-PAR-eYFP-CHO cells. (Fig. 4B-4C) Cleavage data of human PAR1, 2, 4 by V8 protease, or thrombin (for PAR1, PAR4) or trypsin (for PAR2). (Fig. 4D) V8 cleavage sites (arrows) on N-terminus of human PAR1 identified by mass spectrometry. (Fig. 4E) Representative images of RNAscope hybridization of mouse DRG sections for *F2r* and *Tubb3*. (Fig. 4F) Quantification of *F2r* expression in *Tubb3*-positive mouse neurons averaged per mouse (n=3 males, 3 females). Data are represented as mean±SD. (Fig. 4G) Representative images of RNAscope hybridization of human DRG sections for *F2R*, *TRPV1*, and *NPPB*. Total of 1,328 neurons analyzed across 4 donors. (Fig. 4H) Quantification of *F2R* expression in human neurons, proportions and frequency by size and marker expression.

[00106] Figs 5A-5H show V8 protease directly activates pruriceptor neurons. (Fig. 5A) Representative Fura-2 ratiometric fields and calcium traces of mouse DRG neurons. Scale, 100µm. (Fig. 5B) Percentages of total neurons (responsive to KCl) (n=41 fields) histamine-responsive (n= 10 fields) chloroquine-responsive (n=14 fields), S1P-responsive (n=4 fields) and capsaicin-responsive (n=16 fields) neurons that also respond to V8. (Fig. 5C) Calcium traces of human DRG neurons from a representative dish treated with V8, capsaicin, KCl. (Fig. 5D) Pie chart showing human neuron populations responding to V8 and capsaicin (V8+/Cap+), V8 alone (V8+/Cap-), capsaicin alone (V8-/Cap+), and unresponsive to either (V8-/Cap-). (Fig. 5E) Calcium imaging analysis of DRG neurons from *F2r*^{+/+} and *F2r*^{-/-} mice treated with increasing doses of V8. (Fig. 5F) Representative calcium traces of DRG neurons treated with V8, capsaicin, KCl with no pre-treatment (left) or 5 min. post-treatment with TLCK (middle) or Vorapaxar (right). (Fig. 5G-5H) Percentage of untreated neurons and neurons pre-treated with TLCK or Vorapaxar responding to V8 (Fig. 5G) or capsaicin (Fig. 5H). For each panel, data combined from 5 independent experiments are shown. Data are represented as mean±SD. Statistical analysis: (Fig. 5B, 5E, 5G, 5H) One-way ANOVA. *P<0.05; **P<0.01; ***P<0.001; ns, not significant.

[00107] Figs 6A-6I show neuronal PAR1 (*F2r*) is required for V8 and *S. aureus*-induced itch. (Fig. 6A) PBS or V8 protease injected intradermally into cheek of wildtype (*F2r*^{+/+}) and *F2r*^{-/-} mice, spontaneous scratching over 30 min. (n=3-4 males, 2-4 females per group). (Fig. 6B) Acute itch behaviors measured for mice treated with vehicle, control siRNA, or *F2r* siRNA (n=4-6 males, 4-6 females per group). (Fig. 6C-6F) Mice receiving intrathecal siRNA injections were treated with PBS or

exposed to MRSA. Spontaneous itch (Fig. 6D), alloknosis, (Fig. 6E) and scratch-induced skin damage (Fig. 6F) measured 5-days post-exposure (n=4-6 males per group). (Fig. 6G) Generation of *Trpv1* ^{$\Delta F2r$} and *Trpv1*^{cre-} control mice. (Fig. 6H-6I) Spontaneous itch (Fig. 6H) and alloknosis (Fig. 6I) for *Trpv1* ^{$\Delta F2r$} and control mice treated with PBS or exposed to MRSA (n=3-8 males, 1-6 females per group). For each panel, data combined from 2-3 independent experiments are shown. Data are represented as mean $\pm$ SD. Statistical analysis: (Fig. 6A, 6D-6F, 6H, 6I) Two-way ANOVA, Sidak's multiple comparisons. (Fig. 6B, 6E) Mann-Whitney test. **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

[00108] Figs 7A-7J show treatment with PAR1 antagonist reduces itch and skin damage during *S. aureus* exposure. (Fig. 7A) Bouts of scratching following cheek injection with PBS or V8 with increasing doses of Vorapaxar (n=4-6 males, 4-6 females per group). (Fig. 7B-7C) Alloknosis measured every 10 min. for 1hr (Fig. 7B) and 3 hrs (Fig. 7C) after cheek injection with PBS, V8, or V8+Vorapaxar (n=3-5 males, 3-5 females per group). (Fig. 7D) Mice injected with PBS, V8, or V8+Vorapaxar were allowed to scratch; TEWL measured 3 hrs post-injection. One group of V8-injected mice were wrapped in bandages to prevent scratching. (Fig. 7E-7J) Mice gavaged daily with vehicle or Vorapaxar from 2-days before exposure to PBS or MRSA. Dermatitis scores (Fig. 7F), spontaneous itch (Fig. 7G), alloknosis (Fig. 7H), scratch-induced skin damage (Fig. 7I-7J) measured for control and MRSA-exposed mice treated with vehicle or Vorapaxar (n=7-8 males, 8 females per group). For each panel, data combined from 2 independent experiments are shown. Data are represented as mean $\pm$ SD. Statistical analysis: (Fig. 7A, 7C, 7D) Mann-Whitney test (Fig. 7B) Two-way ANOVA with Tukey's multiple comparisons: *V8 vs. V8+Vorapaxar; #V8+Vorapaxar vs. PBS; \$V8 vs. PBS (Fig. 7F-7H, 7J) Two-way ANOVA with Sidak's multiple comparisons. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

[00109] Figs 8A-8G show epicutaneous *S. aureus* exposure induces inflammation, itch, and skin barrier damage. (Fig. 8A) Measurement of inflammation caused by bacterial exposure (skin score), skin barrier damage (TEWL), alloknosis, spontaneous itch, and total skin damage driven by scratching. (Fig. 8B) Histopathology of skin samples from mice 5-days after treatment with PBS or application of 10⁷ CFU MRSA. Scale bar, 50 μ m. E, epidermis; D, dermis; H, hypodermis; M, muscle. (Fig. 8C) Female and male transepidermal water loss (TEWL) 5-days post-exposure (n=8 males, 8 females per group). (Fig. 8D) Dermatitis measured 1-, 3-, and 5-days post-exposure with 10⁷ CFU MRSA (n=8 per group). (Fig. 8E) Spontaneous itch recorded 1-, 3-, and 5-days post-exposure with 10⁷ CFU MRSA (n=7-8 per group). (Fig. 8F) Mice treated with PBS or MRSA, and day-5 post-inoculation, nails of one group were trimmed; Mice were placed in home cages for 7 hr, and total damaged skin area measured. (Fig. 8G) Total skin damage in PBS, treated, MRSA (scratch), MRSA (nail trimmed mice) (n=2-4 males, 2-4 females per group). For each panel, data from 2 independent experiments are combined and shown. Data are represented as mean $\pm$ SD. Statistical analysis: (Fig. 8C, 8D, 8E, 8G) Two-way ANOVA, Sidak's multiple comparisons tests. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

[00110] Figs 9A-9Q show MYD88-mediated inflammation, mast cells, and basophils not required for itch. (Fig. 9A) Representative images of skin from PBS treated or MRSA treated wildtype or *Myd88*^{-/-} mice and dermatitis scores (n=3-6 males, 1-3 females per group). (Fig. 9B) TEWL for PBS treated or MRSA treated wildtype and *Myd88*^{-/-} mice (n=3-6 males, 1-3 females per group). (Fig. 9C) Skin bacterial load for PBS treated or MRSA treated wildtype and *Myd88*^{-/-} mice (n=3-6 males, 1-3 females per group). (Fig. 9D) Spontaneous itch for PBS treated or MRSA treated wildtype and *Myd88*^{-/-} mice (n=3-6 males, 1-3 females per group). (Fig. 9E) Alloknesis for PBS treated or MRSA treated wildtype and *Myd88*^{-/-} mice (n=3-6 males, 1-3 females per group). (Fig. 9F) Representative images of skin from PBS treated or MRSA treated wildtype or *Kit*^{W-sh} mice and dermatitis scores (n=8 per group). (Fig. 9G) TEWL for PBS treated or MRSA treated wildtype and *Kit*^{W-sh} mice (n=8 per group). (Fig. 9H) Skin bacterial load for PBS treated or MRSA treated wildtype and *Kit*^{W-sh} mice (n=8 per group). (Fig. 9I) Spontaneous itch for PBS treated or MRSA treated wildtype and *Kit*^{W-sh} mice (n=8 per group). (Fig. 9J) Alloknesis for PBS treated or MRSA treated wildtype and *Kit*^{W-sh} mice (n=8 per group). (Fig. 9K) Control IgG or Ba103 antibody treatment and flow cytometry gating strategy. (Fig. 9L) Quantification of skin basophils (n=3-4 mice per group). (Fig. 9M) Representative images of back skin and exposure site dermatitis for mice injected with control IgG and treated with PBS or MRSA, and mice injected with Ba103 antibody and exposed to MRSA (n=3-4 males, 4 females per group). (Fig. 9N) TEWL for mice injected with control IgG and treated with PBS or MRSA, and mice injected with Ba103 antibody and exposed to MRSA (n=3-4 males, 4 females per group). (Fig. 9O) Skin bacterial load for mice injected with control IgG and exposed to MRSA, and mice injected with Ba103 antibody and exposed to MRSA (n=4 males, 4 females per group). (Fig. 9P) Spontaneous itch for mice injected with control IgG and treated with PBS or MRSA, and mice injected with Ba103 antibody and exposed to MRSA (n=3-4 males, 4 females per group). (Fig. 9Q) Alloknesis for mice injected with control IgG and treated with PBS or MRSA, and mice injected with Ba103 antibody and exposed to MRSA (n=3-4 males, 4 females per group). For each panel, data from 2 independent experiments are combined and shown. Data are represented as mean±SD. Statistical analysis: (Fig. 9A, 9B, 9D, 9F, 9G, 9H, 9I, 9J) Two-way ANOVA, Sidak's multiple comparisons tests. (Fig. 9C, 9F, 9L-9Q) Mann-Whitney test. *P<0.05; **P<0.01; ***P<0.001; **** P<0.0001; ns, not significant.

[00111] Figs 10A-10Q show *Il31ra*, *Il4ra*, and lymphocytes not required for itch and inflammation. (Fig. 10A) ELISA to quantify IL-31 levels in the skin 5 days after treatment with PBS or MRSA exposure. (n=3-5 males, 3-5 females per group). (Fig. 10B) Mice were injected intrathecally with *Il31ra* siRNA then treated with PBS or exposed to MRSA. *Il31ra* expression in DRG tissue was confirmed by RT-qPCR (n=4-5 males per group). (Fig. 10C) Representative images of back skin and exposure site dermatitis for mice injected with control or *Il31ra* siRNA and treated with PBS or exposed to MRSA (n=3-4 males, 4 females per group). (Fig. 10D) TEWL for mice injected with control or *Il31ra* siRNA and treated with PBS or exposed to MRSA (n=3-4 males, 4 females per group). (Fig. 10E) Skin bacterial load for mice injected with control or *Il31ra* siRNA and treated with PBS or exposed to MRSA

(n=4 males, 4 females per group). (Fig. 10F) Spontaneous itch for mice injected with control or *Il31ra* siRNA and treated with PBS or exposed to MRSA (n=4 males, 3-4 females per group). (Fig. 10G) Alloeknesis for mice injected with control or *Il31ra* siRNA and treated with PBS or exposed to MRSA (n=4 males, 4 females per group). (Fig. 10H) Representative images of skin from PBS treated or MRSA treated wildtype BALBc/J or *Il4ra*^{-/-} mice and dermatitis scores (n=2 males, 3-4 females per group). (Fig. 10I) TEWL for PBS treated or MRSA treated wildtype BALBc/J or *Il4ra*^{-/-} mice (n=2 males, 3-4 females per group). (Fig. 10J) Skin bacterial load for PBS treated or MRSA treated wildtype BALBc/J or *Il4ra*^{-/-} mice (n=2 males, 3-4 females per group). (Fig. 10K) Spontaneous itch for PBS treated or MRSA treated wildtype BALBc/J or *Il4ra*^{-/-} mice (n=2 males, 3-4 females per group). (Fig. 10L) Alloeknesis for PBS treated or MRSA treated wildtype BALBc/J or *Il4ra*^{-/-} mice (n=2 males, 3-4 females per group). (Fig. 10M) Representative images of skin from PBS treated or MRSA treated wildtype C57BL/6NTac or *Rag2*^{-/-}*Il2rg*^{-/-} mice and dermatitis scores (n=3-5 males, 3-5 females per group). (Fig. 10N) TEWL for PBS treated or MRSA treated wildtype C57BL/6NTac or *Rag2*^{-/-}*Il2rg*^{-/-} mice (n=3-5 males, 3-5 females per group). (Fig. 10O) Skin bacterial load for PBS treated or MRSA treated wildtype C57BL/6NTac or *Rag2*^{-/-}*Il2rg*^{-/-} mice (n=3-5 males, 3-5 females per group). (Fig. 10P) Spontaneous itch for PBS treated or MRSA treated wildtype C57BL/6NTac or *Rag2*^{-/-}*Il2rg*^{-/-} mice (n=2-5 males, 3-5 females per group). (Fig. 10Q) Alloeknesis for PBS treated or MRSA treated wildtype C57BL/6NTac or *Rag2*^{-/-}*Il2rg*^{-/-} mice (n=3-5 males, 3-5 females per group). For each panel, data from 2 independent experiments are combined and shown. Data are represented as mean±SD. Statistical analysis: (Fig. 10A, 10E, 10J, 10O) Mann-Whitney test. (Fig. 10B, 10C, 10D, 10F, 10G, 10M, 10N, 10P) One way ANOVA. (Fig. 10H, 10I, 10K, 10L) Two-way ANOVA. *P<0.05; ***P<0.001; ****P<0.0001; ns, not significant.

[00112] Figs 11A-11D show roles of proteases, Hla, and Psms in epicutaneous *S. aureus* exposure. (Fig. 11A) Skin bacterial load from mice inoculated with WT, Δagr , Δhla or $\Delta Psms$, $\Delta Protease$, Δaur or $\Delta scpA\Delta sspB$, and *spl::erm* MRSA strains (n=7-12 males, 7-12 females per group). (Fig. 11B) Spontaneous itch for mice exposed to protease knockout MRSA strains (n=3-5 males, 4 females per group). (Fig. 11C) Alloeknesis for mice exposed to protease knockout MRSA strains (n=3-5 males, 4 females per group). (Fig. 11D) Representative images of back skin and dermatitis scores for mice infected with protease knockout MRSA strains (n=3-5 males, 4 females per group). For each panel, data from 2-6 independent experiments are combined and shown. Data are represented as mean±SD. Statistical analysis: (Fig. 11A, 11B, 11C, 11D,) One-way ANOVA. (Fig. 11A) Mann-Whitney test. *P<0.05; **P<0.01; ****P<0.0001; ns, not significant.

[00113] Figs 12A-12L role of V8 protease in inflammation and itch. (Fig. 12A) Diagram of *ssp* gene locus. *sspA* (V8), *sspB* (staphopain B), *sspC* (staphostatin B) and V8 protease activity assay with WT, $\Delta sspA$, or $\Delta sspA + sspA$ MRSA strains. (Fig. 12B) Transepidermal water loss for control mice (PBS) or mice inoculated with WT, $\Delta sspA$, or $\Delta sspA + sspA$ MRSA strains (n=4-9 males, 3-9 females per group). (Fig. 12C) WT, $\Delta sspA$, or $\Delta sspA + sspA$ MRSA adherence to KERTr cells *in vitro* (n=4 per group). (Fig. 12D) Skin bacterial load for or mice inoculated with WT, $\Delta sspA$, or $\Delta sspA + sspA$ MRSA

strains (n= 8 males, 8 females per group). (Fig. 12E) Acute itch following intradermal cheek injection with PBS or increasing doses of V8 protease (0.4 to 100U) (n=3-4 males, 4 females per group). (Fig. 12F) Acute itch following intradermal cheek injection with PBS, V8 (40U), or heat-inactivated V8 (n=3 males, 4 females per group). (Fig. 12G-12H) Acute itch (hindpaw scratching) and pain (forepaw wiping) behaviors following cheek intradermal injection with PBS or V8 (40U or 200U) (n=2-4 males, 2-4 females per group). (Fig. 12I) Alloknesis 3 hrs. after injection with PBS, V8 (40U), or histamine (100μg) (n=3-5 males, 3-5 females per group). (Fig. 12J) Mice were intradermally injected with PBS or V8 protease, followed by TEWL measurement. One group was wrapped with a bandage to prevent scratching. (Fig. 12K-12L) TEWL measured at 3h post-injection and at 6h post-injection in PBS, V8 (scratch), and V8-treated (no scratch) (n=2-4 males, 2-4 females per group). For each panel, data from 2 independent experiments are combined and shown. Data are represented as mean±SD. Statistical analysis: (Fig. 12A-12I, Fig. 12K- Fig. 12L) One-way ANOVA. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

[00114] Figs 13A-13F show V8 protease cleaves PAR1. (Fig. 13A) HEK cells expressing double brilliant PAR1 were exposed to HBSS or V8 (2U/mL) for 3 min. Scale bar, 10μm. (Fig. 13B) Left: time course of N-terminal PAR1 cleavage by V8 protease, the canonical PAR1 protease thrombin was included as a control. Right: peptides at 30 minutes identified by mass spec with their total spectral counts in the soluble and tethered fractions. Amino acids in red are a result of the His6-tagged construct and not native to PAR1 sequence. (Fig. 13C) Calcium signaling measured in HEK cells expressing human PAR1 were incubated with thrombin (3U/mL) and/or V8 (2U/mL). (Fig. 13D) Calcium signal measured in HEK cells expressing human PAR1 were incubated with thrombin (3U/mL) and/or V8 (20U/mL). (Fig. 13E) Calcium signal measured in HEK cells expressing human PAR1 were incubated with TFLLR-NH₂ (20μM) and/or V8 (2U/mL). (Fig. 13F) Calcium signal measured in HEK cells expressing human PAR1 were incubated with TFLLR-NH₂ (20μM) and/or V8 (20U/mL).

[00115] Figs 14A-14G show PAR1 expression in DRG neurons and calcium response to V8 protease. (Fig. 14A) Expression of *F2r* and select itch related transcripts by mouse DRG neuron populations based on published single-cell RNA-seq datasets⁶¹. *Scn10a*: Nav1.8 voltage gated sodium channel, *Trpv1*: transient receptor potential cation channel subfamily V member 1, *Trpa1*: transient receptor potential cation channel subfamily A member 1, *Mrgprd*: MAS-related GPR member D, *Mrgpra3*: MAS-related GPR member A3, *Mrgprx1*: MAS-related GPR member X1, *Nppb*: natriuretic peptide B, *Hrh1*: histamine receptor H1, *Slpr3*: sphingosine-1-phosphate receptor 3, *F2rl1*: coagulation factor II receptor-like 1 (PAR2), *F2rl3*: coagulation factor II receptor-like 3 (PAR4), *F2r*: coagulation factor II receptor (PAR1). (Fig. 14B) Spatial transcriptomic RNA sequencing data from⁶⁵ demonstrates that *F2R* is predominantly expressed in pruriceptors which also highly express *GFRA2*, *IL31RA*, and *NPPB*. Data are presented as estimated counts from Seurat analysis. *GFRA2*: GDNF family receptor alpha 2, *IL31RA*: interleukin 31 receptor alpha, *NPPB*: natriuretic peptide B, *F2R*: coagulation factor II receptor (PAR1). (Fig. 14C) Mouse DRG neurons were loaded with Fura-2AM and treated with

increasing doses of V8 protease for calcium imaging analysis (n= 6-11 fields per group). (Fig. 14D) Cumulative distributions of peak amplitudes after stimulation with increasing doses of V8. (Fig. 14E) Venn diagrams showing numbers of mouse DRG neurons responding to V8 and to histamine, chloroquine, S1P, or capsaicin. (Fig. 14F) Percentages of total mouse DRG neurons (responsive to KCl) that respond to V8 (69.2 μ M), Hla (10 μ g/mL), or fMLF (1 μ M); overlap in V8-responsive neurons that also respond to Hla or fMLF; Venn diagrams showing neuron numbers responding to V8 and Hla or fMLF. (Fig. 14G) Acute itch (bouts of scratching) and pain (wipes) behavior following intradermal injection with PBS, Hla (330 μ g), fMLF (1.3 μ g), or capsaicin (40 μ g) (n=2-6 males, 2-6 females per group). For each panel, data from 2-8 independent experiments are combined and shown. Data are represented as mean $\pm$ SD.

[00116] Figs 15A and 15B show characterization of skin immune cells after V8 injection. (Fig. 15A) Gating strategy for flow cytometric analysis of skin immune cells. (Fig. 15B) Skin immune populations in WT B6 and *F2r*^{-/-} mice treated with PBS or V8 protease (n=4 males per group). Data from 2 independent experiments are combined and shown. Data are represented as mean $\pm$ SD. Statistical analysis: (Fig. 15B) Two-way ANOVA; (Fig. 15B) Mann-Whitney test. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

[00117] Figs 16A-16M show targeting PAR1 reduces itch. (Fig. 16A) WT (n=4-5 males, 4 females per group), *Mrgpr*^{-/-} (n=4 males, 4 females per group), and *F2rl1*^{-/-} (n=5 males, 4 females per group) mice were treated with PBS or V8 (40U) intradermal cheek injections and monitored for acute itch behaviors. (Fig. 16B) RT-qPCR quantification of *F2r* expression by mouse DRGs following injections with vehicle, control siRNA, or *F2r* siRNA (n=2 males, 2-3 females per group). (Fig. 16C) Representative images of back skin and dermatitis scores for control and MRSA exposed mice injected with either control siRNA or *F2r* siRNA (n=4-6 males per group). (Fig. 16D) Transepidermal water loss measurements for control and MRSA exposed mice injected with either control siRNA or *F2r* siRNA (n=4-5 males per group). (Fig. 16E) Skin bacterial load for MRSA exposed mice injected with either control siRNA or *F2r* siRNA (n=4-5 males per group). (Fig. 16F) Representative images of skin from *Trpv1* ^{Δ F2r} mice and WT controls treated with PBS or MRSA and exposure-site dermatitis scores (n=3-8 males, 1-6 females per group). (Fig. 16G) TEWL for *Trpv1* ^{Δ F2r} mice and WT controls treated with PBS or MRSA (n=3-8 males, 1-6 females per group). (Fig. 16H) Skin bacterial load for *Trpv1* ^{Δ F2r} mice and WT controls inoculated with MRSA (n=5-6 males, 4-5 females per group). (Fig. 16I) Bouts of hindpaw scratching following intradermal cheek injection with PBS or V8 (40U) mixed with increasing doses of the PAR1 antagonist SCH79797 (0-50 μ M). (n=4-6 males, 4-6 females per group). (Fig. 16J) Bouts of hindpaw scratching following intradermal cheek injection with PBS or V8 (40U) mixed with increasing doses of the PAR4 antagonist BMS986120 (0-50 μ M). (n=2-3 males, 2-3 females per group). (Fig. 16K) Representative images of back skin for control and MRSA exposed mice gavaged with either vehicle or Vorapaxar. (Fig. 16L) Transepidermal water loss for control and MRSA exposed mice treated with either vehicle or Vorapaxar (n=8 males, 8 females per group). (Fig. 16M) Skin bacterial load for mice inoculated with

MRSA and treated with either vehicle or Vorapaxar (n=8 males, 8 females per group). For each panel, data from 2 independent experiments are combined and shown. Data are represented as mean±SD. Statistical analysis: (Fig. 16A, 16C, 16D, 16H, 16I, 16L) Two-way ANOVA with Sidak's multiple comparisons test. (Fig. 16B, 16E, 16F, 16G, 16J, 16M) Mann-Whitney test. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001; ns, not significant.

DETAILED DESCRIPTION

Method of treating and preventing

[00118] In one aspect of the invention is a method for treating or preventing itch caused by a microbial exposure in a subject, the method comprising; administering to a subject in need thereof an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch.

[00119] Also provided herein is a method for treating or preventing itch caused by a *Staphylococcus* exposure in a subject, the method comprising; administering to a subject in need thereof an agent that inhibits *Staphylococcus* serine protease V8 in an amount and for a duration sufficient to treat or prevent itch.

[00120] Further provided herein is a method for treating itch caused by a microbial exposure in a subject, the method comprising; topically administering to a subject having microbial exposure an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch, wherein administration occurs at the site of microbial exposure. In one embodiment, the agent is a small molecule. In one embodiment, the agent is Vorapaxar.

[00121] In one embodiment, the method further comprises administering to a subject a second therapeutic agent.

[00122] In one embodiment, the subject has previously been diagnosed with having a microbial exposure, e.g., *Staphylococcus* exposure.

[00123] In one embodiment, the subject has not previously been diagnosed with having a microbial exposure, e.g., *Staphylococcus* exposure.

[00124] In one embodiment, the method further comprises the step, prior to administering, diagnosing the subject of having or at risk of having itch associate with a microbial exposure, e.g., *Staphylococcus* exposure.

[00125] In one embodiment, the method further comprises the step, prior to administering, receiving the results of an assay that diagnoses the subject of having or at risk of having itch associate with a microbial exposure, e.g., *Staphylococcus* exposure.

[00126] In one embodiment, the method further comprises the step, prior to administering, diagnosing the subject of having or at risk of having a microbial exposure, e.g., *Staphylococcus* exposure, that can result in itch.

[00127] In one embodiment, the method further comprises the step, prior to administering, receiving the results of an assay that diagnoses the subject of having a microbial exposure, e.g., *Staphylococcus* exposure, that can result in itch.

[00128] In one embodiment, e.g., when the agent is Vorapaxar, the subject does not have a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.

[00129] In one embodiment, e.g., when the agent is Vorapaxar, the subject is not being treated for a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.

[00130] In one embodiment, itch is reduced by at least 5% as compared to an appropriate control following administration. For example, itch is reduced by at least 6%, by at least 7%, by at least 8%, by at least 9%, by at least 10%, by at least 11%, by at least 12%, by at least 13%, by at least 14%, by at least 15%, by at least 16%, by at least 17%, by at least 18%, by at least 19%, by at least 20%, by at least 21%, by at least 22%, by at least 23%, by at least 24%, by at least 25%, by at least 26%, by at least 27%, by at least 28%, by at least 29%, by at least 30%, by at least 31%, by at least 32%, by at least 33%, by at least 34%, by at least 35%, by at least 36%, by at least 37%, by at least 38%, by at least 39%, by at least 40%, by at least 41%, by at least 42%, by at least 43%, by at least 44%, by at least 45%, by at least 46%, by at least 47%, by at least 48%, by at least 49%, by at least 50%, by at least 51%, by at least 52%, by at least 53%, by at least 54%, by at least 55%, by at least 56%, by at least 57%, by at least 58%, by at least 59%, by at least 60%, by at least 61%, by at least 62%, by at least 63%, by at least 64%, by at least 65%, by at least 66%, by at least 67%, by at least 68%, by at least 69%, by at least 70%, by at least 71%, by at least 72%, by at least 73%, by at least 74%, by at least 75%, by at least 76%, by at least 77%, by at least 78%, by at least 79%, by at least 80%, by at least 81%, by at least 82%, by at least 83%, by at least 84%, by at least 85%, by at least 86%, by at least 87%, by at least 88%, by at least 89%, by at least 90%, by at least 91%, by at least 92%, by at least 93%, by at least 94%, by at least 95%, by at least 96%, by at least 97%, by at least 98%, by at least 99% as compared to an appropriate control following administration. An appropriate control can refer to, for example, a an otherwise identical biological sample that is not administered the agent(s) or the same dosage of the agent(s). For example, the identical biological sample can be administered only one agent, wherein the subject is administered is more than one agent.

Microbial Exposure

[00131] Microbial exposure refers to the presence of a microbe, e.g., colonization, on the subject that does not result in a illness or disease, for example, an infection. For example, the microbe can be present on the skin of the subject, in a lesion on the subject, or in an active infection on the subject. As used herein, an “exposure” refers to an presence of a microbe, i.e., bacterium, virus, and/or fungus, in or on a subject that does not result in illness or disease. The presence can be normal in that the microbe is found typically found in or on a healthy subject. The presence can be abnormal in that the microbe is a

noncommensal species, e.g. one not typically found in or on a healthy subject, it can be abnormal as in a localization that the bacteria does not normally colonize, or it can be abnormal in that the microbe is present at abnormally high levels, e.g. at least twice the level found in or on a healthy subject (e.g. twice the level, three times the level, four times the level, five times the level, or greater). Microbial exposure can refer to the invasion and propagation of the microbe in or on a subject that does not result in illness or disease.

[00132] As used herein, “microbial infection” refers to the presence of the microbe on a subject that causes or contributes to disease or symptoms thereof, e.g., necrosis, disfigurement, delayed wound healing, etc. An infection can refer to the invasion and propagation of the microbe in or on a subject that directly results in illness or disease.

[00133] In one embodiment, microbial exposure occurs at an infection on the subject. For example, the subject has infectious atopic dermatitis that is colonized with *S. aureus*. In this scenario, colonization of *S. aureus* does not cause or contribute to the infectious atopic dermatitis.

[00134] In one embodiment, the microbial exposure is colonization of the microbe. In one embodiment, the microbial exposure is epicutaneous colonization. In one embodiment, the microbial exposure is colonization or epicutaneous colonization of the microbe that does not cause illness or disease.

[00135] In one embodiment, colonization or epicutaneous colonization does not elicit an immune response from the subject. An immune response refers to a physiological reaction which occurs within an organism in the context of inflammation for the purpose of defending against exogenous factors, e.g., a microbe. An immune response can be assessed and diagnosed by a skilled practitioner using standard methods in the art.

[00136] In one embodiment, colonization or epicutaneous colonization elicits a sub-clinical immune response from the subject. A sub-clinical immune response, also known as a preinfection or inapparent infection, refers to an infection by a microbe that causes few or no signs or symptoms of infection in the host.

[00137] In one embodiment, the microbial exposure does cause illness or disease, or elicits an immune response in the subject.

[00138] In one embodiment, microbial exposure occurs in a lesion. In one embodiment, microbial exposure can occur in a lesion caused by any infection caused by bacteria, viruses, or fungus. For example, the lesion is associated with atopic dermatitis, impetigo, prurigo nodularis, psoriasis.

[00139] In one embodiment, the microbial exposure comprises a *Staphylococcus* bacteria. Exemplary *Staphylococcus* exposure comprises *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus capitis* (*S. capitis*) and *Staphylococcus hominis* (*S. hominis*).

[00140] *Staphylococcus* is a genus of gram-positive bacteria. They are round bacteria (cocci) and form “grape-like” clusters. The *Staphylococcus* genus includes at least 40 species, with nine having two subspecies, one having three subspecies, and one having four subspecies. *Staphylococci* are often

harmless and reside on skin and mucous membranes, in addition to being a small component of soil microbial flora. *Staphylococcus* species are facultative anaerobes. One important feature used to classify *Staphylococci* is their ability to produce coagulase. *Staphylococci* frequently colonize in the skin and upper respiratory tract, and are found often in mammals and birds.

[00141] In one embodiment, the *Staphylococcus* exposure is a *Staphylococcus aureus* exposure. *Staphylococcus aureus* (*S. aureus*) is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although *S. aureus* is not always pathogenic, it is a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies.

[00142] In one embodiment, the *Staphylococcus aureus* exposure is a methicillin resistant *Staphylococcus aureus* exposure.

[00143] In one embodiment, the microbial exposure comprises *Streptococcus* bacteria. *Streptococci* are spherical, chain-forming bacteria. Most *Streptococci* are oxidase-negative and catalase-negative. *Streptococci* are often facultative anaerobes, meaning they are capable of growing both aerobically and anaerobically. Over 50 species have been identified in the *Streptococcus* genus and are often found in the salivary microbiome. *Streptococci* are classified based on their hemolytic properties. Alpha-hemolytic species, such as *S. pneumococci*, cause oxidization of iron in hemoglobin molecules within red blood cells, giving it a greenish color on blood agar. Beta-hemolytic, such as *S. pyogenes*, cause complete rupture of red blood cells. On blood agar, this appears as wide areas clear of blood cells surrounding bacterial colonies. Gamma-hemolytic species cause no hemolysis. Beta-hemolytic *streptococci* are further classified by Lancefield grouping, a serotype describing specific carbohydrates present on the bacterial cell wall. The 20 described serotypes are named Lancefield groups A to V (excluding I and J).

[00144] In one embodiment, the *Streptococcus* exposure comprises Group A *Streptococcus* bacteria. In one embodiment, the *Streptococcus* exposure comprises *S. pyogenes* bacteria. In one embodiment, the *Streptococcus* exposure comprises *Streptococcus pneumoniae* bacteria. In one embodiment, the *Streptococcus* exposure comprises Group B *Streptococcus* bacteria.

[00145] In one embodiment, the microbial exposure comprises gram-positive bacteria. Gram-positive bacteria display a cytoplasmic lipid membrane, a thick peptidoglycan layer forming its cell wall, and a smaller volume or periplasm than that of gram-negative bacteria. Gram-positive bacteria have teichoic acids and lipoids which serve as chelating agents and allow for certain types of adherence. Gram positive bacteria can be identified by one skilled in the art, for example using a gram stain test. Optionally, gram-positive bacteria can have a capsule formed by polysaccharides, or contain flagella. Gram-positive bacteria are divided into two major groups: bacilli, which contain for example *Corynebacterium*, *Clostridium*, *Listeria*, and *Bacillus*; and Cocci, which are further divided into two groups: 1) *Staphylococcus* and 2) *Streptococcus*.

[00146] In one embodiment, the microbial exposure is localized to the skin, soft tissue, or subcutaneous infection.

[00147] In one embodiment, the microbial exposure occurs at a microbial infection, or lesion thereof, localized to the skin, soft tissue, or subcutaneous infection. Non-limiting examples of skin, soft tissue, or subcutaneous infections include but are not limited to impetigo, bullous impetigo, scalded skin syndrome, folliculitis, furuncles, carbuncles, cellulitis, myositis, necrotizing fasciitis, streptococcal toxic shock, toxic shock syndrome, acne, and gangrene.

[00148] In one embodiment, the subject further has a burn. In one embodiment, the burn comprises a microbial exposure.

[00149] In one embodiment, the microbial exposure occurs at a bacterial infection. Non-limiting examples of bacterial infections includes but is not limited to *Aeromonas* infection, African tick bite fever, American tick bite fever (*Rickettsia parkeri* infection), *Arcanobacterium haemolyticum* infection, Bacillary angiomatosis, Bejel (endemic syphilis), Blastomycosis-like pyoderma (*pyoderma vegetans*), Blistering distal dactylitis, Botryomycosis, Briei-Zinsser disease, Brucellosis (Bang's disease, Malta fever, undulant fever), Bubonic plague, Bullous impetigo, Cat scratch disease (cat scratch fever, English-Wear infection, inoculation lymphoreticulosis, subacute regional lymphadenitis), Cellulitis, Chancre, Chancroid (soft chancre, *ulcus molle*), Chlamydia infection, Chronic lymphangitis, Chronic recurrent erysipelas, Chronic undermining burrowing ulcers (Meleney gangrene), Chromobacteriosis infection, Condylomata lata, Cutaneous actinomycosis, Cutaneous anthrax infection, Cutaneous *C. diphtheriae* infection (Barcoo rot, diphtheric desert sore, septic sore, Veldt sore), Cutaneous group B streptococcal infection, Cutaneous *Pasteurella hemolytica* infection, Cutaneous *Streptococcus* *iniae* infection, Dermatitis gangrenosa (gangrene of the skin), Ecthyma, Ecthyma gangrenosum, Ehrlichiosis *ewingii* infection, Elephantiasis nostras, Endemic typhus (murine typhus), Epidemic typhus (epidemic louse-borne typhus), Erysipelas (*ignis sacer*, Saint Anthony's fire), Erysipeloid of Rosenbach, Erythema marginatum, Erythrasma, External otitis (otitis externa, swimmer's ear), Felon, Flea-borne spotted fever, Flinders Island spotted fever, Flying squirrel typhus, Folliculitis, Fournier gangrene (Fournier gangrene of the penis or scrotum), Furunculosis (boil), Gas gangrene (clostridial myonecrosis, myonecrosis), Glanders (equinia, farcy, malleus), Gonococcemia (arthritis-dermatosis syndrome, disseminated gonococcal infection), Gonorrhea (clap) Gram-negative folliculitis, Gram-negative toe web infection, Granuloma inguinale (Donovanosis, granuloma genitoinguinale, granuloma inguinale tropicum, granuloma venereum, granuloma venereum genitoinguinale, lupoid form of groin ulceration, serpiginous ulceration of the groin, ulcerating granuloma of the pudendum, ulcerating sclerosing granuloma), Green nail syndrome, Group JK *Corynebacterium* sepsis, *Haemophilus influenzae* cellulitis, *Helicobacter* cellulitis, Hospital furunculosis, Hot tub folliculitis (*Pseudomonas aeruginosa* folliculitis), Human granulocytotropic anaplasmosis, Human monocytotropic ehrlichiosis, Impetigo contagiosa, Japanese spotted fever, Leptospirosis (Fort Bragg fever, pretibial fever, Weil's disease), Listeriosis, Ludwig's angina, Lupoid sycosis, Lyme disease (Afzelius' disease, Lyme borreliosis),

Lymphogranuloma venereum (climatic bubo, Durand-Nicolas-Favre disease, lymphogranuloma inguinale, paradenitis inguinale, strumous bubo), Malakoplakia (malacoplakia), Mediterranean spotted fever (Boutonneuse fever), Melioidosis (Whitmore's disease), Meningococcemia, Missouri Lyme disease, Mycoplasma infection, Necrotizing fasciitis (flesh-eating bacteria syndrome), Neonatal toxic shock-like exanthematous disease, Nocardiosis, Noma neonatorum, North Asian tick typhus, Ophthalmia neonatorum, Oroya fever (Carrion's disease), Pasteurellosis, Perianal cellulitis (perineal dermatitis, streptococcal perianal disease), Periapical abscess, Pinta, Pitted keratolysis (keratolysis plantare sulcatum, keratoma plantare sulcatum, ringed keratolysis), Plague, Primary gonococcal dermatitis, Pseudomonal pyoderma, Pseudomonas hot-foot syndrome, Pyogenic paronychia, Pyomyositis, Q fever, Queensland tick typhus, Rat-bite fever, Recurrent toxin-mediated perineal erythema, Rhinoscleroma, Rickettsia aeschlimannii infection, Rickettsialpox, Rocky Mountain spotted fever, Saber shin (anterior tibial bowing), Saddle nose, Salmonellosis, Scarlet fever, Scrub typhus (Tsutsugamushi fever), Shigellosis, Staphylococcal scalded skin syndrome (pemphigus neonatorum, Ritter's disease), Streptococcal intertrigo, Superficial pustular folliculitis (impetigo of Bockhart, superficial folliculitis), Sycosis vulgaris (barber's itch, sycosis barbae), Syphilid, Syphilis (lues) Tick-borne lymphadenopathy, Toxic shock syndrome (streptococcal toxic shock syndrome, streptococcal toxic shock-like syndrome, toxic streptococcal syndrome), Trench fever (five-day fever, quintan fever, urban trench fever), Tropical ulcer (Aden ulcer, jungle rot, Malabar ulcer, tropical phagedena), Tularemia (deer fly fever, Ohara's disease, Pahvant Valley plague, rabbit fever), Verruga peruana, Vibrio vulnificus infection, Yaws (bouba, framboise, parangi, pian), Aquarium granuloma (fish-tank granuloma, swimming-pool granuloma), Borderline lepromatous leprosy, Borderline leprosy, Borderline tuberculoid, leprosy, Buruli ulcer (Bairnsdale ulcer, Searl ulcer, Searle's ulcer), Erythema induratum (Bazin disease), Histoid leprosy, Lepromatous leprosy, Leprosy (Hansen's disease), Lichen scrofulosorum (tuberculosis cutis lichenoides), Lupus vulgaris (tuberculosis luposa), Miliary tuberculosis (disseminated tuberculosis, tuberculosis cutis acuta generalisata, tuberculosis cutis disseminata), Mycobacterium avium-intracellulare complex infection, Mycobacterium haemophilum infection, Mycobacterium kansasii infection, Papulonecrotic tuberculid, Primary inoculation tuberculosis (cutaneous primary complex, primary tuberculous complex, tuberculous chancre), Rapid-growing Mycobacterium infection, Scrofuloderma (tuberculosis cutis colliquativa), Tuberculosis cutis orificialis (acute tuberculous ulcer, orificial tuberculosis), Tuberculosis verrucosa cutis (lupus verrucosus, prosector's wart, warty tuberculosis), Tuberculous cellulitis, Tuberculous gumma (metastatic tuberculous abscess, metastatic tuberculous ulcer), Tuberculoid leprosy, and sexually transmitted diseases caused by bacteria. In one embodiment, the microbial exposure occurs at a bacterial infection caused by any of the bacteria described herein, for example, *Staphylococcus* or *Streptococcus*.

[00150] In one embodiment, the microbial exposure occurs at a fungi infection. Non-limiting examples of infectious fungi causing fungal infections include, but are not limited to: *Candida* spp.; *Cryptococcus* spp.; *Aspergillus* spp.; *Microsporium* spp.; *Trichophyton* spp.; *Epidermophyton* spp.;

Trichosporon spp.; *Tinea versicolor*; *Tinea barbae*; *Tinea corporis*; *Tinea cruris*; *Tinea manuum*; *Tinea pedis*; *Tinea unguium*; *Tinea faciei*; *Tinea imbricate*; *Tinea incognito*; *Epidermophyton floccosum*; *Microsporum canis*; *Microsporum audouinii*; *Trichophyton interdigitale*; *Trichophyton mentagrophytes*; *Trichophyton tonsurans*; *Trichophyton schoenleini*; *Trichophyton rubrum*; *Hortaea werneckii*; *Piedraia hortae*; *Malasserzia furfur*; *Coccidioides immitis*; *Coccidioides posadasii*; *Histoplasma capsulatum*; *Histoplasma duboisii*; *Lacazia loboi*; *Paracoccidioides brasiliensis*; *Blastomyces dermatitidis*; *Sporothrix schenckii*; *Penicillium marneffeii*; *Candida albicans*; *Candida glabrata*; *Candida tropicalis*; *Candida lusitanae*; *Candida jirovecii*; *Exophiala jeanselmei*; *Fonsecaea pedrosoi*; *Fonsecasea compacta*; *Phialophora verrucosa*; *Geotrichum candidum*; *Pseudallescheria boydii*; *Rhizopus oryzae*; *Mucor indicus*; *Absidia corymbifera*; *Syncephalastrum racemosum*; *Basidiobolus ranarum*; *Conidiobolus coronatus*; *Conidiobolus incongruus*; *Cryptococcus neoformans*; *Enterocytozoan bienersi*; *Encephalitozoon intestinalis*; and *Rhinosporidium seeberi*.

[00151] In one embodiment, the microbial exposure is chronic. In one embodiment, the microbial exposure is acute. An acute exposure is a short term exposure, persisting less than 2 weeks, while a chronic exposure is long term, and persists longer than two weeks. The method for treating an acute exposure can be the same method used to treat a chronic exposure. In contrast, a different method can be used to treat an acute and chronic exposure.

[00152] In some embodiments of any of the aspects, the microbial exposure occurs at a microbial infection, or lesion thereof, that is a systemic infection. As described herein, “systemic infection” refers to an infection that has spread throughout the body, for example, an infection that is present in the blood. Non-limiting examples of systemic infections include bacterial sepsis and endotoxin shock.

[00153] In one embodiment, the microbial exposure is reoccurring. As used herein, “reoccurring” refers to the presence of the microbial exposure at once after clearance of an initial exposure. Clearance of the initial exposure can occur from treatment with an anti-microbial agent, such as an antibiotic, or could have occurred in a self-limiting manner. A reoccurring microbial exposure can occur at least 1, 2, 3, 4, 5, 6, 7, 8, or more times after an initial exposure. Reoccurring can refer to an at least a second exposure at a given lesion or location on the subject following an initial exposure at the same lesion or location, or can refer to an at least second exposure comprising a given microbe following an initial exposure comprising the same microbe (i.e., regardless of the exposure’s location).

Antibiotic resistance

[00154] The emergence of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine. In one embodiment, the microbial exposure comprises a bacterium which is resistant to at least one or more antibiotics. As used herein, “resistant” refers to a bacterium that is unaffected, e.g., can tolerate (e.g., continue to grow and divide) the presence of a given antibiotic. A “resistant” bacterium can continue to grow and divide at a slower rate in the presence of an antibiotic as compared to the growth in the division of the bacterium that is not in the

presence of a given antibiotic. In one embodiment, a microbial exposure comprises a bacterium which is resistant to at least one antibiotic. In one embodiment, the bacterium is resistant to at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more antibiotics.

[00155] In one embodiment, the exposure comprises *S. aureus* that is resistant to antibiotics. In one embodiment, *S. aureus* is resistant to methicillin. Methicillin-resistant *S. aureus* is commonly found in settings with large populations living within close proximity, for example a college dormitory, prison, and hospitals. A non-limiting example of another antibiotic *S. aureus* has developed resistance to is Vancomycin.

[00156] Non-limiting examples of bacteria that have adapted a resistance to current standard of care treatments include Methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci*, vancomycin-resistant *Staphylococcus aureus*, drug-resistant *Streptococcus pneumoniae*, Drug-Resistant *Mycobacterium tuberculosis*, carbapenem-resistant *Enterobacteriaceae*, Multiple drug-resistant *Pseudomonas Aeruginosa*, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, and drug-resistant *Neisseria gonorrhoeae*.

[00157] Antibiotic resistance can be assessed by a skilled practitioner using anti microbial susceptibility assays. Antimicrobial susceptibility testing is used to determine the effectiveness of particular antimicrobials against particular microbes, whether the microbes are resistant to selected antimicrobials, and/or to identify antimicrobial resistance patterns.

Identification of microbial exposure

[00158] In one embodiment, a subject is diagnosed with having a microbial exposure prior to administration of an agent described herein. There are various tests known to those skilled in the art that are performed in a laboratory to establish or confirm the diagnosis of microbial exposure, as well as to identify the microbial species. Culturing of the microbial species with antimicrobial sensitivity testing is considered the gold standard laboratory test. Skin samples can be collected in the following ways: 1) dry sterile cotton-tip swab rubbed on the suspicious skin site, e.g., blistered or dry skin lesions or pustules; 2) moist swab taken from a mucosal surface, such as inside the mouth; 3) aspiration of fluid/pus from a skin lesion using a needle and syringe; and 4) skin biopsy: a small sample of skin removed under local anesthetic. Culturing of, e.g., bacteria is most commonly done by brushing the skin swab on sheep blood agar plates and exposing them to different conditions. The species of microbe that grow depend on the medium used to culture the specimen, the temperature for incubation, and the amount of oxygen available. An obligate aerobe can only grow in the presence of oxygen, while an obligate anaerobe cannot grow at all in the presence of oxygen.

[00159] A gram stain uses a series of stains on a sample, followed by inspection under a light microscope to detect and identify bacteria as gram positive or gram negative. A gram stain can be done on the original sample, but it is usually done on cultured bacteria after transferring a colony of bacteria from the agar plate to a glass microscope slide. A gram-positive bacterium appears purple due to crystal

violet dye adhering to the cell wall. A gram-negative bacterium appears red due to the red dye used to counterstain. The gram stain also identifies the bacterium's shape and behavior; cocci are round in shape, bacilli are rod shaped, and some bacteria form clusters versus chains.

[00160] The coagulase test detects coagulase, which is an enzyme produced by certain bacteria that converts fibrinogen to fibrin and is observed as clumping of cells in plasma. The coagulase test differentiates coagulase-positive *S. aureus* from coagulase-negative staphylococci.

[00161] The catalase test detects catalase, an enzyme that degrades hydrogen peroxide into hydrogen and oxygen. The bacterial sample is added to a test tube of hydrogen peroxide. The production of bubbles (oxygen) indicates a positive result. The catalase test differentiates catalase-positive *staphylococci* and *micrococci* from catalase-negative *streptococci*.

[00162] Blood tests require a sample of blood accessed by a needle from a vein. Non-limiting examples of tests for bacterial infection include: 1) full blood count, bacterial infection often raises the white cell count with increased neutrophils (neutrophilia); 2) C-reactive protein (CRP), CRP is often elevated >50 in serious bacterial infections; 3) procalcitonin, a marker of generalized sepsis due to bacterial infection, 3) serology, tests 10 days apart to determine immune response to a particular organism; 4) Rapid Plasma Reagin (RPR) test, if syphilis is suspected; and 4) blood culture to detect bacteria if high fever >100.4°F.

[00163] Polymerase chain reaction (PCR) involves isolating and amplifying lengths of bacterial DNA from a sample of skin, blood or other tissue. The DNA is compared to bacterial DNA from known organisms, thus identifying the species. This test is useful for slow-growing bacteria such as anaerobic bacteria and mycobacteria (tuberculosis and atypical mycobacteria), or bacteria that cannot be cultured by standard methods.

Agents

[00164] In one embodiment, the agent that inhibits PAR1 is an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule.

[00165] In one embodiment, the agent that inhibit serine protease V8 is selected from the group consisting of: an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule.

[00166] In one embodiment, the small molecule is selected from the group consisting of Vorapaxar, Atopaxar (E5555), Parmodulin 2 (PM2, ML161), SCH 79797, FR171113, and RWJ-56110, RWJ-58259.

[00167] In one embodiment, the inhibitory nucleic acid encodes an inhibitor of PAR1. In one embodiment, the inhibitory nucleic acid encodes an inhibitor of serine protease V8.

[00168] In one embodiment, the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits PAR1. In one embodiment, the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits serine protease V8.

[00169] In one embodiment, the agent that inhibits PAR1 inhibits the expression of PAR1. In one embodiment, the agent inhibits the expression of PAR1 at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more as compared to an appropriate control. As used herein, an “appropriate control” refers to the level and/or activity of PAR1 prior to administration of the agent, or the level and/or activity of PAR1 in a population of cells that was not in contact with the agent.

[00170] In one embodiment, the agent that inhibits PAR1 inhibits the function of PAR1. In one embodiment, the agent inhibits the function of PAR1 at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more as compared to an appropriate control. As used herein, an “appropriate control” refers to the level and/or activity of PAR1 prior to administration of the agent, or the level and/or activity of PAR1 in a population of cells that was not in contact with the agent.

[00171] In one embodiment, the agent that inhibits serine protease V8 inhibits the expression of serine protease V8. In one embodiment, the agent inhibits the expression of serine protease V8 at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least

13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more as compared to an appropriate control. As used herein, an “appropriate control” refers to the level and/or activity of serine protease V8 prior to administration of the agent, or the level and/or activity of serine protease V8 in a population of cells that was not in contact with the agent.

[00172] In one embodiment, the agent that inhibits serine protease V8 inhibits the function of serine protease V8. For example, one function of serine protease V8 is cleaving PAR-1. In one embodiment, the agent inhibits the function of serine protease V8 at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more as compared to an appropriate control. As used herein, an “appropriate control” refers to the level and/or activity of serine protease V8 prior to administration of the agent, or the level and/or activity of serine protease V8 in a population of cells that was not in contact with the agent.

[00173] The agent may function directly in the form in which it is administered. Alternatively, the agent can be modified or utilized intracellularly to produce something which inhibits PAR1 or serine protease V8, such as introduction of a nucleic acid sequence into the cell and its transcription resulting in the production of the nucleic acid and/or protein inhibitor of PAR1 or serine protease V8 within the cell.

In some embodiments, the agent is any chemical, entity or moiety, including without limitation synthetic and naturally-occurring non-proteinaceous entities. In certain embodiments the agent is a small molecule having a chemical moiety. For example, chemical moieties included unsubstituted or substituted alkyl, aromatic, or heterocyclyl moieties including macrolides, leptomycins and related natural products or analogues thereof. Agents can be known to have a desired activity and/or property or can be identified from a library of diverse compounds.

[00174] In some embodiments of any of the aspects, an agent that inhibits PAR1 or serine protease V8 is a competitive inhibitor of PAR1 or serine protease V8, respectively. In some embodiments of any of the aspects, the agent is an antagonist of PAR1 or serine protease V8.

[00175] In some embodiments, the agent that inhibits PAR1 or serine protease V8 lowers expression of PAR1 or serine protease V8, respectively. One skilled in the art can determine if the levels of PAR1 or serine protease V8 are reduced, for example by detecting PAR1 or serine protease V8 levels via western blotting or PCR-based assays and comparing PAR1 or serine protease V8 protein or mRNA levels, respectively, prior to and after administration of the agent.

[00176] In some embodiments, the agent interferes with PAR1 or serine protease V8 function. One skilled in the art can assess PAR1 or serine protease V8 function, for example by assessing the activity of downstream targets.

[00177] *Antibodies*

[00178] In one embodiment, the agent that inhibits PAR1 or serine protease V8 is an anti- PAR1 or serine protease V8 antibody or antibody reagent, respectively.

[00179] As used herein, the term "antibody reagent" refers to a polypeptide that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence and which specifically binds a given antigen. An antibody reagent can comprise an antibody or a polypeptide comprising an antigen-binding domain of an antibody. In some embodiments of any of the aspects, an antibody reagent can comprise a monoclonal antibody or a polypeptide comprising an antigen-binding domain of a monoclonal antibody. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term "antibody reagent" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')₂, Fd fragments, Fv fragments, scFv, CDRs, and domain antibody (dAb) fragments (see, e.g., de Wildt et al., Eur J. Immunol. 1996; 26(3):629-39; which is incorporated by reference herein in its entirety)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, or IgM (as well as subtypes and combinations thereof). Antibodies can be from any source, including mouse, rabbit, pig, rat, and primate (human and non-human primate) and primatized antibodies. Antibodies also include midibodies, humanized antibodies, chimeric antibodies, and the like.

[00180] The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" ("FR"). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917; which are incorporated by reference herein in their entireties). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[00181] Exemplary anti-PAR1 antibodies can be obtained, e.g., commercially. Table 1 includes an exemplary list of commercially available anti-PAR1 antibodies.

Table 1: Exemplary anti-PAR1 antibodies		
Antibody Name	Type and Clone Name (if applicable)	Vendor and Product name
Anti-PAR1/Thrombin Receptor antibody	Monoclonal; [5E2E6]	Abcam, Cambridge, MA; (ab233741)
Anti-PAR1/Thrombin Receptor antibody	Polyclonal	Abcam, Cambridge, MA; (ab117749)
Anti-PAR1/Thrombin Receptor antibody	Polyclonal	Abcam, Cambridge, MA; (ab32611)
Anti-PAR1/Thrombin Receptor antibody	Polyclonal	Abcam, Cambridge, MA; (ab183083)
Anti-PAR1/Thrombin Receptor antibody	Polyclonal	Abcam, Cambridge, MA; (ab111976)
Anti-PAR1/Thrombin Receptor antibody - BSA and Azide free (Capture)	Monoclonal; [EPR23349-201]	Abcam, Cambridge, MA; (ab281275)
Anti-PAR1/Thrombin Receptor antibody - BSA and Azide free (Detector)	Monoclonal; [EPR23349-27]	Abcam, Cambridge, MA; (ab281125)
PAR1 Monoclonal Antibody	Monoclonal; (ATAP2)	Thermo Fisher Scientific, Waltham, MA; (Cat #35-2200)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PA5-19102)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PA5-94929)
F2R Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #26366-1-AP)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PA5-116040)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PA5-30590)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific,

		Waltham, MA; (Cat #PA5-34263)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PA5-34264)
F2R Monoclonal Antibody	Monoclonal; (2C5)	Thermo Fisher Scientific, Waltham, MA; (Cat #H00002149-M01)
PAR1 (F2R) (extracellular) Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #APR-031-200UL)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #HA500159)
Thrombin receptor Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #BS-0828R)
PAR1 (F2R) (extracellular) Polyclonal Antibody, FITC	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #APR-031-F-50UL)
PAR1 Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PAR1-101AP)
Phospho-PAR1 (Tyr420) Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PA5-105095)
PAR1 Polyclonal Antibody, Biotin	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PAR1-BIOTIN)
PAR1 Polyclonal Antibody, FITC	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #PAR1-FITC)
Thrombin Receptor Polyclonal Antibody	Polyclonal	Thermo Fisher Scientific, Waltham, MA; (Cat #TA590107)

[00182] In one embodiment, the antibody or antibody reagent binds to an amino acid sequence that corresponds to the amino acid sequence encoding PAR1 or serine protease V8 (SEQ ID NO: 1 or 2, respectively).

[00183] SEQ ID NO: 1 is an amino acid sequence encoding PAR1.

MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLDPRSFLLRNPNDKYEPFWEDEEKN
ESGLTEYRLVSINKSSPLQKQLPAFISEDASGYLTSSWLTFLVPSVYTGVFVVSLLPLNIMAIVV
FILKMKVKKPAVVYMLHLATADVLFVSVLPFKISYYFSGSDWQFGSELCRFVTAAFYCNY
ASILLMTVISIDRFLAVVYPMQSLSWRTLGRASFTCLAIWALAIAGVVPLLLKEQTIQVPGLNI
TTCHDVLNETLLEGYYAAYYFSAFSAVFFFVPLIISTVCYVSIIRCLSSSAVANRSKKSRAFLS
AAVFCIFIICFGPTNVLLIAHYSFLSHTSTTEAAYFAYLLCVCVSSISCCIDPLIYYYASSECQRY
VYSILCCKESSDPSSYNSSGQLMASKMDTCSSNLNNSIYKLLT (SEQ ID NO: 1)

[00184] SEQ ID NO: 2 is an amino acid sequence encoding serine protease V8.

MKGGKFLKVSSLFVATLTTATLVSSPAANALSSKAMDNHPQQTQSSKQQTTPKIQKGGNLKPL
EQREHANVILPNNDRHQITDITNGHYAPVTYIQVEAPTGTFTIASGVVVGKDTLLTNKHVVD
ATHGDPHALKAFPSAINQDNYPNGGFTAEEQITKYSGEGLAIVKFSNEQNKHIGEVVKPAT
MSNNAETQVNQNITVTGYPGDKPVATMWESKKGKITYLKGEAMQYDLSTTGGNSGSPVFNE
KNEVIGIHWGGVPNEFNNAVFINENVRNFLKQNIEDIHFANDDQPNPNPDNPNPNPNPN
PDEPNPNPDNPNPNPDNPDNGDNNNSDNPDA (SEQ ID NO: 2)

[00185] In another embodiment, the anti-PAR1 antibody or antibody reagent binds to an amino acid sequence that comprises the sequence of SEQ ID NO: 1; or binds to an amino acid sequence that comprises a sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or greater sequence identity to the sequence of SEQ ID NO: 1. In one embodiment, the anti-PAR1 antibody or antibody reagent binds to an amino acid sequence that comprises the entire sequence of SEQ ID NO: 1. In another embodiment, the antibody or antibody reagent binds to an amino acid sequence that comprises a fragment of the sequence of SEQ ID NO: 1, wherein the fragment is sufficient to bind its target, e.g., PAR1, and result in a reduction in the severity of the itch.

[00186] In another embodiment, the anti-serine protease V8 antibody or antibody reagent binds to an amino acid sequence that comprises the sequence of SEQ ID NO: 2; or binds to an amino acid sequence that comprises a sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or greater sequence identity to the sequence of SEQ ID NO: 2. In one embodiment, the anti-serine protease V8 antibody or antibody reagent binds to an amino acid sequence that comprises the entire sequence of SEQ ID NO: 2. In another embodiment, the antibody or antibody reagent binds to an amino acid sequence that comprises a fragment of the sequence of SEQ ID NO: 2, wherein the fragment is sufficient to bind its target, e.g., serine protease V8, and result in a reduction in the severity of the itch.

[00187] In one embodiment, the agent is a compound that inhibits PAR1. In some embodiments of any of the aspects, an agent can be a small molecule inhibitor of PAR1 receptors. Non-limiting examples of a small molecule inhibitor of PAR1 include Vorapaxar, Atopaxar (E5555), Parmodulin 2 (PM2, ML161), SCH 79797, FR171113, RWJ-56110, and RWJ-58259.

[00188] In one embodiment, the agent is a compound that inhibits serine protease V8. In some embodiments of any of the aspects, an agent can be a small molecule inhibitor of serine protease V8 receptors.

[00189] In one embodiment, the agent that inhibits PAR1 or serine protease V8 is an antisense oligonucleotide. As used herein, an “antisense oligonucleotide” refers to a synthesized nucleic acid sequence that is complementary to a DNA or mRNA sequence, such as that of a microRNA. Antisense oligonucleotides are typically designed to block expression of a DNA or RNA target by binding to the target and halting expression at the level of transcription, translation, or splicing. Antisense oligonucleotides of the present invention are complementary nucleic acid sequences designed to hybridize under cellular conditions to a gene, e.g., PAR1 or serine protease V8. Thus, oligonucleotides

are chosen that are sufficiently complementary to the target, i.e., that hybridize sufficiently well and with sufficient specificity in the context of the cellular environment, to give the desired effect. For example, an antisense oligonucleotide that inhibits PAR1 or serine protease V8 may comprise at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, or more bases complementary to a portion of the coding sequence of a human PAR1 or serine protease V8 (e.g., SEQ ID NO: 3 or 4), respectively.

[00190] SEQ ID NO: 3 is a nucleic acid encoding PAR1.

ATGAAAGGTAAATTTTTTAAAGTTAGTTCTTTATTCGTTGCAACTTTGACAACAGCGACACTTGTGAGTTCTCC
AGCAGCAAACGCGTTATCTTCAAAGGCTATGGACAATCATCCACAACAAACGCAGTCAAGCAAACAGCAAACAC
CTAAGATTCAAAAAGGCGGTAACCTTAAACCATTAGAACAACGTGAACACGCAAATGTTATATTACCAAATAAC
GATCGTCACCAAATCACAGATACAACGAATGGTCATTATGCACCCGTAACCTTATATTCAAGTTGAAGCACCTAC
TGGTACATTTATTGCTTCCGGTGTAGTTGTAGGTAAAGATACTCTTTTAAACAAATAAACACGTCGTAGATGCTA
CGCAGGTTGATCCTCATGCTTTAAAGCATTCCTTCTGCAATTAACCAAGACAATTATCCAAATGGTGGTTTC
ACTGCTGAACAAATCACTAAATATTCAAGGCGAAGGTGATTTAGCAATAGTTAAATTTCTCCCTAATGAGCAAAA
CAAACATATTGGTGAAGTAGTTAAACCAGCAACAATGAGTAATAATGCTGAAACACAAGTTAACCAAAATATTA
CTGTAAACAGGATATCCTGGTGATAAACCTGTAGCAACAATGTGGGAAAGTAAAGGAAAAATCACTTACCTCAA
GGCGAAGCTATGCAATATGATTTAAGTACAACCTGGTGGTAATTCAGGTTACCTGTATTTAATGAAAAAATGA
AGTGATCGGAATTCATTGGGGCGGTGTACCAAAATGAATTTAATGGTGCGGTATTTATTAATGAAAATGTACGCA
ACTTCTTAAACAAAATATTGAAGATATCCATTTTGCCAACGATGACCAACCTAATAACCCAGATAATCCTGAT
AACCCTAACAATCCTGATAACCCTAACAACCCAGATGAACCAAATAACCCTGACAACCCTAACAACCCTGATAA
TCCAGACAATGGCGATAACAATAATTGAGACAATCCAGATGCAGCTTAA (SEQ ID NO: 3)

[00191] SEQ ID NO: 4 is a nucleic acid encoding serine protease V8.

ATGAAAGGTAAATTTTTTAAAGTTAGTTCTTTATTCGTTGCAACTTTGACAACAGCGACACTTGTGAGTTCTCC
AGCAGCAAACGCGTTATCTTCAAAGGCTATGGACAATCATCCACAACAAACGCAGTCAAGCAAACAGCAAACAC
CTAAGATTCAAAAAGGCGGTAACCTTAAACCATTAGAACAACGTGAACACGCAAATGTTATATTACCAAATAAC
GATCGTCACCAAATCACAGATACAACGAATGGTCATTATGCACCCGTAACCTTATATTCAAGTTGAAGCACCTAC
TGGTACATTTATTGCTTCCGGTGTAGTTGTAGGTAAAGATACTCTTTTAAACAAATAAACACGTCGTAGATGCTA
CGCAGGTTGATCCTCATGCTTTAAAGCATTCCTTCTGCAATTAACCAAGACAATTATCCAAATGGTGGTTTC
ACTGCTGAACAAATCACTAAATATTCAAGGCGAAGGTGATTTAGCAATAGTTAAATTTCTCCCTAATGAGCAAAA
CAAACATATTGGTGAAGTAGTTAAACCAGCAACAATGAGTAATAATGCTGAAACACAAGTTAACCAAAATATTA
CTGTAAACAGGATATCCTGGTGATAAACCTGTAGCAACAATGTGGGAAAGTAAAGGAAAAATCACTTACCTCAA
GGCGAAGCTATGCAATATGATTTAAGTACAACCTGGTGGTAATTCAGGTTACCTGTATTTAATGAAAAAATGA
AGTGATCGGAATTCATTGGGGCGGTGTACCAAAATGAATTTAATGGTGCGGTATTTATTAATGAAAATGTACGCA
ACTTCTTAAACAAAATATTGAAGATATCCATTTTGCCAACGATGACCAACCTAATAACCCAGATAATCCTGAT
AACCCTAACAATCCTGATAACCCTAACAACCCAGATGAACCAAATAACCCTGACAACCCTAACAACCCTGATAA
TCCAGACAATGGCGATAACAATAATTGAGACAATCCAGATGCAGCTTAA (SEQ ID NO: 4)

[00192] In one embodiment, the agent is an inhibitory nucleic acid. Inhibitors of the expression of a given gene can be an inhibitory nucleic acid. In some embodiments of any of the aspects, the inhibitory nucleic acid is an inhibitory RNA (iRNA). Double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). The inhibitory nucleic acids described herein can include an RNA strand (the antisense strand) having a region which is 30 nucleotides or less in length, i.e., 15-30 nucleotides in length, generally 19-24 nucleotides in length, which region is substantially complementary to at least part the targeted mRNA transcript. The use of these iRNAs enables the targeted degradation of mRNA transcripts, resulting in decreased expression and/or activity of the target.

[00193] As used herein, the term “iRNA” refers to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript via an RNA-induced silencing

complex (RISC) pathway. In one embodiment, an iRNA as described herein effects inhibition of the expression and/or activity of a target, e.g., PAR1 or serine protease V8. In certain embodiments, contacting a cell with the inhibitor (e.g. an iRNA) results in a decrease in the target mRNA level in a cell by at least about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, up to and including 100% of the target mRNA level found in the cell without the presence of the iRNA.

[00194] In one embodiment, the agent is siRNA that inhibits PAR1 or serine protease V8. In one embodiment, the agent is shRNA that inhibits PAR1 or serine protease V8. In one embodiment, the agent is miRNA that inhibits PAR1 or serine protease V8.

[00195] In one embodiment, the iRNA can be a dsRNA. A dsRNA includes two RNA strands that are sufficiently complementary to hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of the target. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is between 15 and 30 inclusive, more generally between 18 and 25 inclusive, yet more generally between 19 and 24 inclusive, and most generally between 19 and 21 base pairs in length, inclusive. Similarly, the region of complementarity to the target sequence is between 15 and 30 inclusive, more generally between 18 and 25 inclusive, yet more generally between 19 and 24 inclusive, and most generally between 19 and 21 nucleotides in length, inclusive. In some embodiments of any of the aspects, the dsRNA is between 15 and 20 nucleotides in length, inclusive, and in other embodiments, the dsRNA is between 25 and 30 nucleotides in length, inclusive. As the ordinarily skilled person will recognize, the targeted region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a “part” of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to be a substrate for RNAi-directed cleavage (i.e., cleavage through a RISC pathway). dsRNAs having duplexes as short as 9 base pairs can, under some circumstances, mediate RNAi-directed RNA cleavage. Most often a target will be at least 15 nucleotides in length, preferably 15-30 nucleotides in length.

[00196] In one embodiment, PAR1 or serine protease V8 is depleted from the cell’s genome using any genome editing system including, but not limited to, zinc finger nucleases, TALENS, meganucleases, and CRISPR/Cas systems. In one embodiment, the genomic editing system used to incorporate the nucleic acid encoding one or more guide RNAs into the cell’s genome is not a CRISPR/Cas system; this can prevent undesirable cell death in cells that retain a small amount of Cas enzyme/protein. It is also contemplated herein that either the Cas enzyme or the sgRNAs are each expressed under the control of a different inducible promoter, thereby allowing temporal expression of each to prevent such interference.

[00197] When a nucleic acid encoding one or more sgRNAs and a nucleic acid encoding an RNA-guided endonuclease each need to be administered *in vivo*, the use of an adenovirus associated vector (AAV) is specifically contemplated. Other vectors for simultaneously delivering nucleic acids to both components of the genome editing/fragmentation system (e.g., sgRNAs, RNA-guided endonuclease) include lentiviral vectors, such as Epstein Barr, Human immunodeficiency virus (HIV), and hepatitis B virus (HBV). Each of the components of the RNA-guided genome editing system (e.g., sgRNA and endonuclease) can be delivered in a separate vector as known in the art or as described herein.

[00198] In one embodiment, the agent inhibits PAR1 or serine protease V8 by RNA inhibition. Inhibitors of the expression of a given gene can be an inhibitory nucleic acid. In some embodiments of any of the aspects, the inhibitory nucleic acid is an inhibitory RNA (iRNA). The RNAi can be single stranded or double stranded.

[00199] The iRNA can be siRNA, shRNA, endogenous microRNA (miRNA), or artificial miRNA. In one embodiment, an iRNA as described herein effects inhibition of the expression and/or activity of a target, e.g., PAR1 or serine protease V8. In some embodiments of any of the aspects, the agent is siRNA that inhibits PAR1 or serine protease V8. In some embodiments of any of the aspects, the agent is shRNA that inhibits PAR1 or serine protease V8.

[00200] One skilled in the art would be able to design siRNA, shRNA, or miRNA to target PAR1 or serine protease V8, e.g., using publicly available design tools. siRNA, shRNA, or miRNA is commonly made using companies such as Dharmacon (Lafayette, CO) or Sigma Aldrich (St. Louis, MO).

[00201] In some embodiments of any of the aspects, the iRNA can be a dsRNA. A dsRNA includes two RNA strands that are sufficiently complementary to hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of the target. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions.

[00202] The RNA of an iRNA can be chemically modified to enhance stability or other beneficial characteristics. The nucleic acids featured in the invention may be synthesized and/or modified by methods well established in the art, such as those described in “Current protocols in nucleic acid chemistry,” Beaucage, S.L. et al. (Eds.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference.

[00203] In one embodiment, the agent is miRNA that inhibits PAR1 or serine protease V8. microRNAs are small non-coding RNAs with an average length of 22 nucleotides. These molecules act by binding to complementary sequences within mRNA molecules, usually in the 3' untranslated (3'UTR) region, thereby promoting target mRNA degradation or inhibited mRNA translation. The interaction between microRNA and mRNAs is mediated by what is known as the “seed sequence”, a 6–8-nucleotide

region of the microRNA that directs sequence-specific binding to the mRNA through imperfect Watson–Crick base pairing. More than 900 microRNAs are known to be expressed in mammals. Many of these can be grouped into families on the basis of their seed sequence, thereby identifying a “cluster” of similar microRNAs. A miRNA can be expressed in a cell, e.g., as naked DNA. A miRNA can be encoded by a nucleic acid that is expressed in the cell, e.g., as naked DNA or can be encoded by a nucleic acid that is contained within a vector.

[00204] The agent may result in gene silencing of the target gene (e.g., PAR1 or serine protease V8), such as with an RNAi molecule (e.g. siRNA or miRNA). This entails a decrease in the mRNA level in a cell for a target by at least about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 99%, about 100% of the mRNA level found in the cell without the presence of the agent. In one preferred embodiment, the mRNA levels are decreased by at least about 70%, about 80%, about 90%, about 95%, about 99%, about 100%. One skilled in the art will be able to readily assess whether the siRNA, shRNA, or miRNA effectively targets e.g., PAR1 or serine protease V8, for its downregulation, for example by transfecting the siRNA, shRNA, or miRNA into cells and detecting the levels of a gene or protein (e.g., PAR1 or serine protease V8) found within the cell via PCR-based assay or western blotting, respectively.

[00205] An agent described herein may be contained in and thus further include a vector. Many such vectors useful for transferring exogenous genes into target mammalian cells are available. The vectors may be episomal, e.g., plasmids, virus-derived vectors such as cytomegalovirus, adenovirus, etc., or may be integrated into the target cell genome, through homologous recombination or random integration, e.g., retrovirus-derived vectors such as MMLV, HIV-1, ALV, etc. In some embodiments, combinations of retroviruses and an appropriate packaging cell line may also find use, where the capsid proteins will be functional for infecting the target cells. Usually, the cells and virus will be incubated for at least about 24 hours in the culture medium. The cells are then allowed to grow in the culture medium for short intervals in some applications, e.g., 24-72 hours, or for at least two weeks, and may be allowed to grow for five weeks or more, before analysis. Commonly used retroviral vectors are “defective”, i.e., unable to produce viral proteins required for productive infection. Replication of the vector requires growth in the packaging cell line.

[00206] The term “vector”, as used herein, refers to a nucleic acid construct designed for delivery to a host cell or for transfer between different host cells. As used herein, a vector can be viral or non-viral. The term “vector” encompasses any genetic element that is capable of replication when associated with the proper control elements and that can transfer gene sequences to cells. A vector can include, but is not limited to, a cloning vector, an expression vector, a plasmid, phage, transposon, cosmid, artificial chromosome, virus, virion, etc.

[00207] As used herein, the term “expression vector” refers to a vector that directs expression of an RNA or polypeptide (e.g., a PAR1 or serine protease V8 inhibitor) from nucleic acid sequences contained therein linked to transcriptional regulatory sequences on the vector. The sequences expressed will often,

but not necessarily, be heterologous to the cell. An expression vector may comprise additional elements, for example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in human cells for expression and in a prokaryotic host for cloning and amplification. The term "expression" refers to the cellular processes involved in producing RNA and proteins and as appropriate, secreting proteins, including where applicable, but not limited to, for example, transcription, transcript processing, translation and protein folding, modification and processing. "Expression products" include RNA transcribed from a gene, and polypeptides obtained by translation of mRNA transcribed from a gene. The term "gene" means the nucleic acid sequence which is transcribed (DNA) to RNA *in vitro* or *in vivo* when operably linked to appropriate regulatory sequences. The gene may or may not include regions preceding and following the coding region, e.g., 5' untranslated (5'UTR) or "leader" sequences and 3' UTR or "trailer" sequences, as well as intervening sequences (introns) between individual coding segments (exons).

[00208] Integrating vectors have their delivered RNA/DNA permanently incorporated into the host cell chromosomes. Non-integrating vectors remain episomal which means the nucleic acid contained therein is never integrated into the host cell chromosomes. Examples of integrating vectors include retroviral vectors, lentiviral vectors, hybrid adenoviral vectors, and herpes simplex viral vector.

[00209] One example of a non-integrative vector is a non-integrative viral vector. Non-integrative viral vectors eliminate the risks posed by integrative retroviruses, as they do not incorporate their genome into the host DNA. One example is the Epstein Barr oriP/Nuclear Antigen-1 ("EBNA1") vector, which is capable of limited self-replication and known to function in mammalian cells. As containing two elements from Epstein-Barr virus, oriP and EBNA1, binding of the EBNA1 protein to the virus replicon region oriP maintains a relatively long-term episomal presence of plasmids in mammalian cells. This particular feature of the oriP/EBNA1 vector makes it ideal for generation of integration-free iPSCs. Another non-integrative viral vector is adenoviral vector and the adeno-associated viral (AAV) vector.

[00210] Another non-integrative viral vector is RNA Sendai viral vector, which can produce protein without entering the nucleus of an infected cell. The F-deficient Sendai virus vector remains in the cytoplasm of infected cells for a few passages but is diluted out quickly and completely lost after several passages (e.g., 10 passages).

[00211] Another example of a non-integrative vector is a minicircle vector. Minicircle vectors are circularized vectors in which the plasmid backbone has been released leaving only the eukaryotic promoter and cDNA(s) that are to be expressed.

[00212] As used herein, the term "viral vector" refers to a nucleic acid vector construct that includes at least one element of viral origin and has the capacity to be packaged into a viral vector particle. The viral vector can contain a nucleic acid encoding a polypeptide as described herein in place of non-essential viral genes. The vector and/or particle may be utilized for the purpose of transferring nucleic acids into cells either *in vitro* or *in vivo*. Numerous forms of viral vectors are known in the art.

Compositions

[00213] One aspect of the invention is a composition for treating or preventing itch caused by a microbial exposure in a subject comprising an amount of an agent that inhibits PAR1 in an amount sufficient to treat or prevent itch. In one embodiment, any composition described herein further comprises a second therapeutic agent. In one embodiment, the second therapeutic agent is an antibiotic or antimicrobial agent.

[00214] One aspect of the invention is a composition for treating or preventing itch caused by a *Staphylococcus* exposure in a subject comprising an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch. In one embodiment, any composition described herein further comprises a second therapeutic agent. In one embodiment, the second therapeutic agent is an antibiotic or antimicrobial agent.

[00215] In one embodiment, the composition further comprises a pharmaceutically acceptable carrier. The pharmaceutical composition can be formulated with a pharmaceutically acceptable carrier or excipient. A pharmaceutically acceptable carrier or excipient refers to a carrier (e.g., carrier, media, diluent, solvent, vehicle, etc.) which does not significantly interfere with the biological activity or effectiveness of the active ingredient(s) of a pharmaceutical composition and which is not excessively toxic to the host at the concentrations at which it is used or administered. Other pharmaceutically acceptable ingredients can be present in the composition as well. Suitable substances and their use for the formulation of pharmaceutically active compounds are well-known in the art (see, for example, Remington: The Science and Practice of Pharmacy, 21st Edition, Philadelphia, PA, Lippincott Williams & Wilkins, 2005, for additional discussion of pharmaceutically acceptable substances and methods of preparing pharmaceutical compositions of various types).

[00216] A pharmaceutical composition is typically formulated to be compatible with its intended route of administration. For topical application, a pharmaceutical composition may be formulated in a suitable ointment, lotion, gel, or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers suitable for use in such compositions. For some applications, the composition is formulated as a solid (e.g., lyophilized), liquid, gel, or hydrogel and may contain additives such as surfactants, buffers (e.g., succinate), salts (e.g., sodium chloride), polymers (e.g., polysaccharides, hyaluronic acid), proteins (e.g., albumin, human serum albumin), or amino acids (e.g., methionine).

[00217] In one embodiment, the composition is formulated for topical administration. In one embodiment, the composition is formulated for systemic administration.

[00218] Also provided herein is a use of any composition described herein for treating or preventing itch caused by a microbial exposure in a subject, the composition comprising; an amount of an agent that inhibits PAR1 in an amount sufficient to treat or prevent itch.

[00219] Also provided herein is a use of any composition described herein for treating or preventing itch caused by a Staphylococcus exposure in a subject, the composition comprising: an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch.

Combination therapy

[00220] In one embodiment, the agent described herein is used as a monotherapy. In one embodiment, the agents described herein can be used in combination with other known agents and therapies for a microbial infection. Administered "in combination," as used herein, means that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the infection, e.g., the two or more treatments are delivered after the subject has been diagnosed with the infection and before the infection has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered. The agents described herein and the at least one additional therapy can be administered simultaneously, in the same or in separate compositions, or sequentially. For sequential administration, the agent described herein can be administered first, and the additional agent can be administered second, or the order of administration can be reversed. The agent and/or other therapeutic agents, procedures or modalities can be administered during periods of active disorder, or during a period of remission or less active disease. The agent can be administered before another treatment, concurrently with the treatment, post-treatment, or during remission of the disorder.

[00221] In some embodiments, the methods and compositions described herein further comprise administering a second therapeutic agent to a subject. In one embodiment, the second therapeutic agent is an antifungal. In one embodiment, the second therapeutic agent is an antibiotic. Examples of suitable antibiotics include, but are not limited to Aknilox, Ambisome, Amoxycillin, Ampicillin, Augmentin, Avelox, Azithromycin, Bactroban, Betadine, Betnovate, Blephamide, Cefaclor, Cefadroxil, Cefdinir, Cefepime, Cefix, Cefixime, Cefoxitin, Cefpodoxime, Cefprozil, Cefuroxime, Cefzil, Cephalixin, Cephazolin, Ceptaz, Chloramphenicol, Chlorhexidine, Chloromycetin, Chlorsig, Ciprofloxacin,

Clarithromycin, Clindagel, Clindamycin, Clindatech, Cloxacillin, Colistin, Co-trimoxazole, Demeclocycline, Diclocil, Dicloxacillin, Doxycycline, Duricef, Erythromycin, Flamazine, Floxin, Framycetin, Fucidin, Furadantin, Fusidic, Gatifloxacin, Gemifloxacin, Gemifloxacin, Ilosone, Iodine, Levaquin, Levofloxacin, Lomefloxacin, Maxaquin, Mefoxin, Meronem, Minocycline, Moxifloxacin, Myambutol, Mycostatin, Neosporin, Netromycin, Nitrofurantoin, Norfloxacin, Norilet, Ofloxacin, Omnicef, Ospamox, Oxytetracycline, Paraxin, Penicillin, Pneumovax, Polyfax, Povidone, Rifadin, Rifampin, Rifaximin, Rifinah, Rimactane, Rocephin, Roxithromycin, Seromycin, Soframycin, Sparfloxacin, Staphlex, Targocid, Tetracycline, Tetradox, Tetralysal, tobramycin, Tobramycin, Trecator, Tygacil, Vancocin, Velosef, Vibramycin, Xifaxan, Zagam, Zitrotek, Zoderm, Zymar, and Zyvox.

[00222] In one embodiment, the second therapeutic agent is an anti-bacterial agent. Examples of anti-bacterial agents include, but is not limited to aminoglycosides (e.g., amikacin (Amikin®), gentamicin (Garamycin®), kanamycin (Kantrex®), neomycin (Mycifradin®), netilmicin (Netromycin®), tobramycin (Nebcin®), Paromomycin (Humatin®)), ansamycins (e.g., geldanamycin, herbimycin), carbacephem (e.g., loracarbef (Lorabid®), Carbapenems (e.g., ertapenem (Invanz®), doripenem (Doribax®), imipenem/cilastatin (Primaxin®), meropenem (Merrem®), cephalosporins (first generation) (e.g., cefadroxil (Duricef®), cefazolin (Ancef®), cefalotin or cefalothin (Keflin®), cefalexin (Keflex®), cephalosporins (second generation) (e.g., cefaclor (Ceclor®), cefamandole (Mandol®), cefoxitin (Mefoxin®), cefprozil (Cefzil®), cefuroxime (Ceftin®, Zinnat®)), cephalosporins (third generation) (e.g., cefixime (Suprax®), cefdinir (Omnicef®, Cefdiel®), cefditoren (Spectracef®), cefoperazone (Cefobid®), cefotaxime (Ciaforan®), cefpodoxime (Vantin®), ceftazidime (Fortaz®), ceftibuten (Cedax®), ceftizoxime (Cefizox®), ceftriaxone (Rocephin®)), cephalosporins (fourth generation) (e.g., cefepime (Maxipime®)), cephalosporins (fifth generation) (e.g., ceftobiprole (Zeftera®)), glycopeptides (e.g., teicoplanin (Targocid®), vancomycin (Vancocin®), telavancin (Vibativ®)), lincosamides (e.g., clindamycin (Cieocin®), lincomycin (Lincocin®)), lipopeptide (e.g., daptomycin (Cubicin®)), macrolides (e.g., azithromycin (Zithromax®, Sumamed®, Zitrocin®), clarithromycin (Biaxin®), dirithromycin (Dynabac®), erythromycin (Erythocin®, Erythroped®), roxithromycin, troleandomycin (Tao®), telithromycin (Ketek®), spectinomycin (Trobicin®)), monobactams (e.g., aztreonam (Azactam®)), nitrofurans (e.g., furazolidone (Furoxone®), nitrofurantoin (Macrochantin®, Macrobid®)), penicillins (e.g., amoxicillin (Novamox®, Amoxil®), ampicillin (Principen®), azlocillin, carbenicillin (Geocillin®), cloxacillin (Tegopen®), dicloxacillin (Dynapen®), flucloxacillin (Fioxapen®), mezlocillin (Mezlin®), methicillin (Staphcillin®), nafcillin (Unipen®), oxacillin (Prostaphlin®), penicillin G (Pentids®), penicillin V (Pen-Vee-K®), piperacillin (Pipracil®), temocillin (Negaban®), ticarcillin (Ticar®)), penicillin combinations (e.g., amoxicillin/clavulanate (Augmentin®), ampicillin/sulbactam (Unasyn®), piperacillin/tazobactam (Zosyn®), ticarcillin/clavulanate (Timentin®)), polypeptides (e.g., bacitracin, colistin (Coly-Mycin-S®), polymyxin B, quinolones (e.g., ciprofloxacin (Cipro®, Ciproxin®, Ciprobay®), enoxacin (Penetrex®),

gatifloxacin (Tequin®), levofloxacin (Levaquin®), lomefloxacin (Maxaquin®), moxifloxacin (Avelox®), nalidixic acid (Neggram®), norfloxacin (Noroxin®), ofloxacin (Fioxin®, Ocuflor®), trovafloxacin (Trovan®), grepafloxacin (Raxar®), sparfloxacin (Zagam®), temafloxacin (Omniflox®), sulfonamides (e.g., mafenide (Sulfamylon®), sulfonamidochrysoidine (Prontosil®), sulfacetamide (Sulamyd®, Bleph-10®), sulfadiazine (Micro-Sulfon®), silver sulfadiazine (Silvadene®), sulfamethizole (Thiosulfil Forte®), sulfamethoxazole (Gantanol®), sulfanilimide, sulfasalazine (Azulfidine®), sulfisoxazole (Gantrisin®), trimethoprim (Proloprim®), Trimplex®), trimethoprim-sulfamethoxazole (co-trimoxazole) (TMP-SMX) (Bactrim®, Septra®), tetracyclines (e.g., demeclocycline (Declomycin®), doxycycline (Vibramycin®), minocycline (Minocin®), oxytetracycline (Terramycin®), tetracycline (Sumycin®, Achromycin® V, Steclin®)), drugs against mycobacteria (e.g., clofazimine (Lamprone®), dapsone (Avlosulfon®), capreomycin (Capastat®), cycloserine (Seromycin®), ethambutol (Myambutol®), ethionamide (Trecator®), isoniazid (I.N.H.®), pyrazinamide (Aidinamide®), rifampin (Rifadin®, Rimactane®), rifabutin (Mycobutin®), rifapentine (Priftin®), streptomycin), and others (e.g., arsphenamine (Salvarsan®), chloramphenicol (Chloromycetin®), fosfomycin (Monurol®), fusidic acid (Fucidin®), linezolid (Zyvox®), metronidazole (Flagyl®), mupirocin (Bactroban®), platensimycin, quinupristin/dalfopristin (Synercid®), rifaximin (Xifaxan®), thiamphenicol, tigecycline (Tigacyl®), and tinidazole (Tindamax®, Fasigyn®).

[00223] When administered in combination, the agent and the additional therapeutic (e.g., second or third therapeutic), or all, can be administered in an amount or dose that is higher, lower or the same as the amount or dosage of each agent used individually, e.g., as a monotherapy. In certain embodiments, the administered amount or dosage of the agent, the additional agent (e.g., second or third agent), or all, is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50%) than the amount or dosage of each agent used individually. In other embodiments, the amount or dosage of agent, the additional agent (e.g., second or third agent), or all, that results in a desired effect (e.g., treatment of an autoimmune disease) is lower (e.g., at least 20%, at least 30%, at least 40%, or at least 50% lower) than the amount or dosage of each agent individually required to achieve the same therapeutic effect.

[00224] A second or further therapeutic agent can be administered in an admixture with the inhibitor of PAR1 or serine protease V8 described herein. Alternatively, the second or further therapeutic agent can be administered separately, e.g., via a second route or site or at a second time, then the inhibitor of PAR1 or serine protease V8 described herein.

Dosages forms and administration

[00225] The dosages of compositions comprising an agent that inhibits PAR1 or serine protease V8 that treat and/or prevent itch associate with microbial exposure, e.g., *Staphylococcus* exposure, can be determined by one of ordinary skill in the art depending on the clinical severity of the disorder, the age and weight of the patient, and other pharmacokinetic factors generally understood in the art. The interrelationship of dosages for animals of various sizes and species and humans based on mg/m³ of

surface area is described by E. J. Freireich et al., "Quantitative Comparison of Toxicity of Anticancer Agents in Mouse, Rat, Hamster, Dog, Monkey and Man," Cancer Chemother. Rep. 50: 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the therapeutic response. Doses can be divided and administered on a daily basis or the dose can be reduced proportionally depending on the therapeutic situation.

[00226] The dosage range depends upon the potency and includes amounts large enough to produce the desired effect, *e.g.*, a decrease in microbial itch. The dosage should not be so large as to cause unacceptable adverse side effects. Generally, the dosage will vary with the type of agent (*e.g.*, an anti-PAR1 or serine protease V8 antibody, a small molecule inhibitor of PAR1 or serine protease V8, antibiotic, or antimicrobial), and with the age, and condition of the patient. The dosage can be determined by one of skill in the art and can also be adjusted by the individual physician in the event of any complication. Typically, the dosage will range from 0.001mg/kg body weight to 5 g/kg body weight. In some embodiments, the dosage range is from 0.001 mg/kg body weight to 1g/kg body weight, from 0.001 mg/kg body weight to 0.5 g/kg body weight, from 0.001 mg/kg body weight to 0.1 g/kg body weight, from 0.001 mg/kg body weight to 50 mg/kg body weight, from 0.001 mg/kg body weight to 25 mg/kg body weight, from 0.001 mg/kg body weight to 10 mg/kg body weight, from 0.001 mg/kg body weight to 5 mg/kg body weight, from 0.001 mg/kg body weight to 1 mg/kg body weight, from 0.001 mg/kg body weight to 0.1 mg/kg body weight, from 0.001 mg/kg body weight to 0.005 mg/kg body weight. Alternatively, in some embodiments the dosage range is from 0.1 g/kg body weight to 5 g/kg body weight, from 0.5 g/kg body weight to 5 g/kg body weight, from 1 g/kg body weight to 5 g/kg body weight, from 1.5 g/kg body weight to 5 g/kg body weight, from 2 g/kg body weight to 5 g/kg body weight, from 2.5 g/kg body weight to 5 g/kg body weight, from 3 g/kg body weight to 5 g/kg body weight, from 3.5 g/kg body weight to 5 g/kg body weight, from 4 g/kg body weight to 5 g/kg body weight, from 4.5 g/kg body weight to 5 g/kg body weight, from 4.8 g/kg body weight to 5 g/kg body weight. In some embodiments of any of the aspects, the dose range is from 5µg/kg body weight to 30µg/kg body weight. Alternatively, the dose range will be titrated to maintain serum levels between 5µg/mL and 30µg/mL.

[00227] Sub-lethal doses may be administered systemically (*e.g.*, intravenously).

[00228] These agents can be administered orally, and they can be administered in conventional pill or liquid form. If administered in pill form, they can be administered in conventional formulations with excipients, fillers, preservatives, and other typical ingredients used in pharmaceutical formations in pill form. Typically, the agents are administered in a conventional pharmaceutically acceptable formulation, typically including a carrier. Conventional pharmaceutically acceptable carriers known in the art can include alcohols, *e.g.*, ethyl alcohol, serum proteins, human serum albumin, liposomes, buffers such as phosphates, water, sterile saline or other salts, electrolytes, glycerol, hydroxymethylcellulose, propylene glycol, polyethylene glycol, polyoxyethylenesorbitan, other surface active agents, vegetable oils, and conventional anti-bacterial or anti-fungal agents, such as parabens, chlorobutanol, phenol, sorbic acid,

thimerosal, and the like. A pharmaceutically-acceptable carrier within the scope of the present invention meets industry standards for sterility, isotonicity, stability, and non-pyrogenicity.

[00229] The pharmaceutically acceptable formulation can also be in pill, tablet, or lozenge form as is known in the art, and can include excipients or other ingredients for greater stability or acceptability. For the tablets, the excipients can be inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc, along with the substance for autophagy modulation and other ingredients.

[00230] The agents can also be administered in liquid form in conventional formulations that can include preservatives, stabilizers, coloring, flavoring, and other generally accepted pharmaceutical ingredients. Typically, when the agents are administered in liquid form, they will be in aqueous solution. The aqueous solution can contain buffers, and can contain alcohols such as ethyl alcohol or other pharmaceutically tolerated compounds.

[00231] Alternatively, the agents can be administered by subcutaneous injection by one of several routes well known in the art. Agents can additionally be formulated for topical administration by one skilled in the art. Agents that inhibit PAR1 or serine protease V8 and additional therapeutic agents can be administered parenterally. The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intraventricular, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, sub capsular, subarachnoid, intraspinal, intracerebro spinal, and intrasternal injection, infusion and other injection or infusion techniques, without limitation.

[00232] In one embodiment, the agent is administered locally, e.g., at the site of the exposure, or the predicted site of a possible exposure. In one embodiment, the agent is administered systemically. For example, systemic administration is intrathecal administration.

[00233] In one embodiment, the agent is administered topically.

[00234] In some embodiments, a therapeutically effective amount of an agent that inhibits PAR1 or serine protease V8 is administered using intrapulmonary administration, such as an intranasal or intratracheal route. In one embodiment, a therapeutically effective amount of the agent or composition comprising an agent is administered using a systemic, such as an intraperitoneal or intravenous route. In one embodiment, a therapeutically effective amount of the agent is administered using both intrapulmonary and intraperitoneal administration. These methods are particularly aimed at therapeutic and prophylactic treatments of human subjects having, or at risk of having, a microbial exposure of the lung. As defined herein, "intrapulmonary" administration or delivery refers to all routes of administration whereby an agent is administered in a way that results in direct contact of the agent with the airways of a subject, including, but not limited to, transtracheal, intratracheal, and intranasal administration. In some such embodiments, the agent is injected into the nasal passages or trachea. In some embodiments, the

agent is directly inhaled by a subject. In some embodiments, intrapulmonary delivery of the agent includes administration methods whereby the agent is administered to an intubated subject via a tube placed in the trachea or “tracheal intubation.” As used herein, “tracheal intubation” refers to the placement of a flexible tube, such as a plastic tube, into the trachea. The most common tracheal intubation, termed herein as “orotracheal intubation” is where, with the assistance of a laryngoscope, an endotracheal tube is passed through the mouth, larynx, and vocal cords, into the trachea. A bulb is then inflated near the distal tip of the tube to help secure it in place and protect the airway from blood, vomit, and secretions. In some embodiments, an agent is administered to a subject having “nasotracheal intubation,” which is defined as a tracheal intubation where a tube is passed through the nose, larynx, vocal cords, and trachea.

[00235] The agents can be administered from once per day to up to at least five times per day, depending on the severity of the disease, the total dosage to be administered, and the judgment of the treating physician. The agent can be administered, for example, every minute, hour, day, week, month, or year. The composition can be administered for a specific duration (e.g., 1 minute, 1 hour, 1 day, 1 week, 1 month, or 1 year). In some cases, the agents need not be administered on a daily basis, but can be administered every other day, every third day, or on other such schedules. However, it is generally preferred to administer the agents daily.

[00236] In some embodiments of any of the aspects, the inhibitor of PAR1 or serine protease V8 described herein is administered as a monotherapy, e.g., another treatment for the microbial itch is not administered to the subject. In some embodiments of any of the aspects, the inhibitor of PAR1 or serine protease V8 is administered to the site of the microbial exposure and/or to the site of itch caused the microbial exposure.

[00237] In some embodiments, the technology described herein relates to a pharmaceutical composition comprising an inhibitor of PAR1 or serine protease V8 as described herein, and optionally a pharmaceutically acceptable carrier. In some embodiments, the active ingredients of the pharmaceutical composition comprise an inhibitor of PAR1 or serine protease V8 as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist essentially of an inhibitor of PAR1 or serine protease V8 as described herein. In some embodiments, the active ingredients of the pharmaceutical composition consist of PAR1 or serine protease V8 as described herein.

Controlled and Delayed Release Dosage Forms

[00238] In some embodiments of the aspects described herein, an agent is administered to a subject by controlled- or delayed-release means. Ideally, the use of an optimally designed controlled-release preparation in medical treatment is characterized by a minimum of drug substance being employed to cure or control the condition in a minimum amount of time. Advantages of controlled-release formulations include: 1) extended activity of the drug; 2) reduced dosage frequency; 3) increased patient compliance; 4) usage of less total drug; 5) reduction in local or systemic side effects; 6) minimization of

drug accumulation; 7) reduction in blood level fluctuations; 8) improvement in efficacy of treatment; 9) reduction of potentiation or loss of drug activity; and 10) improvement in speed of control of diseases or conditions. (Kim, Cherng-ju, *Controlled Release Dosage Form Design*, 2 (Technomic Publishing, Lancaster, Pa.: 2000)). Controlled-release formulations can be used to control a compound of formula (I)'s onset of action, duration of action, plasma levels within the therapeutic window, and peak blood levels. In particular, controlled- or extended-release dosage forms or formulations can be used to ensure that the maximum effectiveness of an agent is achieved while minimizing potential adverse effects and safety concerns, which can occur both from under-dosing a drug (i.e., going below the minimum therapeutic levels) as well as exceeding the toxicity level for the drug.

[00239] A variety of known controlled- or extended-release dosage forms, formulations, and devices can be adapted for use with any agent described herein. Examples include, but are not limited to, those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; 5,733,566; and 6,365,185, each of which is incorporated herein by reference in their entireties. These dosage forms can be used to provide slow or controlled-release of one or more active ingredients using, for example, hydroxypropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems (such as OROS® (Alza Corporation, Mountain View, Calif. USA)), multilayer coatings, microparticles, liposomes, or microspheres or a combination thereof to provide the desired release profile in varying proportions. Additionally, ion exchange materials can be used to prepare immobilized, adsorbed salt forms of the disclosed compounds and thus effect controlled delivery of the drug. Examples of specific anion exchangers include, but are not limited to, DUOLITE® A568 and DUOLITE® AP143 (Rohm&Haas, Spring House, Pa. USA).

Efficacy measurement

[00240] The efficacy of a given treatment or prevention of itch associated with microbial exposure can be determined by the skilled clinician. However, a treatment is considered “effective treatment,” as the term is used herein, if any one or all of the signs or symptoms of, as but one example, scratching, or other clinically accepted symptoms or markers of the itch are improved or ameliorated, *e.g.*, by at least 10% following treatment with a composition comprising an agent that inhibits PAR1 or serine protease V8 described herein. Efficacy can also be measured by failure of an individual to worsen as assessed by need for medical interventions (*e.g.*, progression of itch is halted or at least slowed). Methods of measuring these indicators are known to those of skill in the art and/or described herein. Example methods are described above herein. Treatment includes any treatment of the itch in an individual or an animal (some non-limiting examples include a human, or a mammal) and includes: (1) inhibiting the itch, *e.g.*, arresting, or slowing symptoms of the itch, for example scratching; or (2) relieving the itch, *e.g.*, causing regression of symptoms, reducing the symptoms by at least 10%; and (3) preventing future itch caused by microbial exposure.

[00241] An effective amount for the treatment of itch associated with microbial exposure means that amount which, when administered to a mammal in need thereof, is sufficient to result in effective treatment as that term is defined herein, for that itch. Efficacy of the composition can be determined by a physician by assessing physical indicators of itch.

[00242] The term “effective amount” as used herein refers to the amount of an agent that inhibits PAR1 or serine protease V8 described herein needed to alleviate at least one or more symptom of a itch caused by microbial exposure and relates to a sufficient amount of pharmacological composition to provide the desired effect. The term “therapeutically effective amount” therefore refers to an amount of a composition that is sufficient to provide a particular effect when administered to a typical subject. An effective amount as used herein, in various contexts, would also include an amount sufficient to delay the development of a symptom of itch, alter the course of a symptom, or reverse a symptom of itch. Thus, it is not generally practicable to specify an exact “effective amount.” However, for any given case, an appropriate “effective amount” can be determined by one of ordinary skill in the art using only routine experimentation. The term “effective amount” is used interchangeably with the term “therapeutically effective amount” and refers to the amount of at least one agent at dosages and for periods of time necessary to achieve the desired therapeutic result, for example, to reduce or stop at least one symptom of itch caused by microbial exposure, in the subject.

[00243] Effective amounts, toxicity, and therapeutic efficacy of drug agents, e.g., for formulations or treatments using antimicrobials in addition to PAR1 or serine protease V8 inhibitory agents, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dosage can vary depending upon the dosage form employed and the route of administration utilized. The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio LD₅₀/ED₅₀. Compositions and methods that exhibit large therapeutic indices are preferred. A therapeutically effective dose can be estimated initially from *in vivo* assays. Also, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the active ingredient, which achieves a half-maximal inhibition of symptoms). Levels in plasma can be measured, for example, by high performance liquid chromatography or other appropriate technique. The effects of any particular dosage can be monitored by a suitable bioassay. The dosage can be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment.

Kit

[00244] In one aspect is a kit comprising an agent that inhibits PAR1 or serine protease V8 as described herein in the amount sufficient to treat itch caused by microbial exposure.

[00245] In one aspect is a kit comprising an agent that inhibits PAR1 or serine protease V8 as described herein in the amount sufficient to prevent itch caused by microbial exposure.

[00246] A kit is any manufacture (*e.g.*, a package or container) comprising at least one reagent, *e.g.*, the agent being promoted, distributed, or sold as a unit for performing the methods described herein. The kits described herein can optionally comprise additional components useful for performing the methods described herein, *e.g.*, needles, tubing, etc. useful for administration by the desired route. By way of example, the kit can comprise fluids (*e.g.*, buffers) suitable for use with the agents described herein, an instructional material which describes performance of a method as described herein, and the like. A kit can further comprise devices and/or reagents for delivery of the composition as described herein. Additionally, the kit may comprise an instruction leaflet and/or may provide information as to dosages, administration frequency, etc.

[00247] The kits of the invention comprise one or more packages or containers containing the agent in combination with a set of instructions, generally written instructions, relating to the use and dosage of the emulsion. The kits may further comprise additional containers containing one or more second therapeutic agent that may be added to the agent prior to administration. The packages containing the agent may be in the form of unit doses or pharmacy bulk packages. The doses may be packaged in a format such that each dose is associated, for example, with a day of the week. There may also be associated with the kit a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of biological products, which notice reflects approval by the agency of manufacture, use or sale for human or animal administration.

[00248] One aspect of the invention is the use of any of the kits or compositions described herein for the treatment of itch caused by microbial exposure.

[00249] One aspect of the invention is the use of any of the kits or compositions described herein for the prevention of itch caused by microbial exposure.

[00250] Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00251] It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[00252] The invention disclosed herein can be further described in the following numbered paragraphs:

- 1) A method for treating or preventing itch caused by a microbial exposure in a subject, the method comprising; administering to a subject in need thereof an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch.

- 2) The method of paragraph 1, wherein the agent that inhibit PAR1 is selected from the group consisting of: an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule.
- 3) The method of any of the preceding paragraphs, wherein the small molecule is selected from the group consisting of Vorapaxar, Atopaxar (E5555), Parmodulin 2 (PM2, ML161), SCH 79797, FR171113, and RWJ-56110, RWJ-58259.
- 4) The method of any of the preceding paragraphs, wherein the inhibitory nucleic acid encodes an inhibitor of PAR1.
- 5) The method of any of the preceding paragraphs, wherein the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits PAR1.
- 6) The method of any of the preceding paragraphs, wherein the administering occurs at the site of microbial exposure.
- 7) The method of any of the preceding paragraphs, wherein the agent that inhibits PAR1 inhibits the expression of PAR1.
- 8) The method of any of the preceding paragraphs, wherein the agent inhibits the expression of PAR1 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.
- 9) The method of any of the preceding paragraphs, wherein the agent that inhibits PAR1 inhibits the function of PAR1.
- 10) The method of any of the preceding paragraphs, wherein the agent inhibits the function of PAR1 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.
- 11) The method of any of the preceding paragraphs, wherein the microbial exposure comprises a *Staphylococcus* exposure.
- 12) The method of any of the preceding paragraphs, wherein the *Staphylococcus* exposure comprises *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus capitis* (*S. capitis*) and *Staphylococcus hominis* (*S. hominis*).
- 13) The method of any of the preceding paragraphs, wherein *S. aureus* is methicillin resistant *S. aureus*.
- 14) The method of any of the preceding paragraphs, wherein the microbial exposure comprises a bacterium which is resistant to at least one antibiotic.
- 15) The method of any of the preceding paragraphs, wherein the microbial exposure comprises a bacterium which is resistant to at least two antibiotics.
- 16) The method of any of the preceding paragraphs, wherein the microbial exposure comprises *Streptococcus pyogenes* (*S. pyogenes*).
- 17) The method of any of the preceding paragraphs, wherein the microbial exposure is colonization.

- 18) The method of any of the preceding paragraphs, wherein the microbial exposure is epicutaneous colonization.
- 19) The method of any of the preceding paragraphs, wherein colonization or epicutaneous colonization is not an infection.
- 20) The method of any of the preceding paragraphs, wherein colonization or epicutaneous colonization does not elicit an immune response from the subject.
- 21) The method of any of the preceding paragraphs, wherein colonization or epicutaneous colonization elicits a sub-clinical immune response from the subject.
- 22) The method of any of the preceding paragraphs, wherein the microbial exposure is an infection.
- 23) The method of any of the preceding paragraphs, wherein microbial exposure occurs in a lesion.
- 24) The method of any of the preceding paragraphs, wherein the lesion is associated with a condition selected from a group consisting of: atopic dermatitis, impetigo, prurigo nodularis, psoriasis.
- 25) The method of any of the preceding paragraphs, wherein the microbial exposure is acute or chronic.
- 26) The method of any of the preceding paragraphs, wherein the microbial exposure is a reoccurring exposure.
- 27) The method of any of the preceding paragraphs, further comprising administering to a subject a second therapeutic agent.
- 28) The method of any of the preceding paragraphs, wherein the second therapeutic agent is an antibiotic, antifungal, or antimicrobial agent.
- 29) The method of any of the preceding paragraphs, wherein the subject has previously been diagnosed with having a microbial exposure.
- 30) The method of any of the preceding paragraphs, wherein the subject has not previously been diagnosed with having a microbial exposure.
- 31) The method of any of the preceding paragraphs, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having itch associate with a microbial exposure.
- 32) The method of any of the preceding paragraphs, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having or at risk of having itch associate with a microbial exposure.
- 33) The method of any of the preceding paragraphs, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having a microbial exposure that can result in itch.

34) The method of any of the preceding paragraphs, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having a microbial exposure that can result in itch.

35) The method of any of the preceding paragraphs, wherein the administering is systemic or local administration.

36) The method of any of the preceding paragraphs, wherein local administration is topical administration.

37) The method of any of the preceding paragraphs, wherein systemic administration is intrathecal administration.

38) A method for treating or preventing itch caused by a *Staphylococcus* exposure in a subject, the method comprising; administering to a subject in need thereof an agent that inhibits *Staphylococcus* serine protease V8 in an amount and for a duration sufficient to treat or prevent itch.

39) The method of any of the preceding paragraphs, wherein the agent that inhibit serine protease V8 is selected from the group consisting of: an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule.

40) The method of any of the preceding paragraphs, wherein the inhibitory nucleic acid encodes an inhibitor of serine protease V8.

41) The method of any of the preceding paragraphs, wherein the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits serine protease V8.

42) The method of any of the preceding paragraphs, wherein the administering occurs at the site of *Staphylococcus* exposure.

43) The method of any of the preceding paragraphs, wherein the agent that inhibits serine protease V8 inhibits the expression of serine protease V8.

44) The method of any of the preceding paragraphs, wherein the agent inhibits the expression of serine protease V8 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.

45) The method of any of the preceding paragraphs, wherein the agent that inhibits serine protease V8 inhibits the function of serine protease V8.

46) The method of any of the preceding paragraphs, wherein the agent inhibits the function of serine protease V8 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.

47) The method of any of the preceding paragraphs, wherein the function of serine protease V8 is cleaving PAR-1.

48) The method of any of the preceding paragraphs, wherein the *Staphylococcus* exposure comprises *S. aureus*, *S. epidermidis*, *Staphylococcus capitis* (*S. capitis*) and *Staphylococcus hominis* (*S. hominis*).

49) The method of any of the preceding paragraphs, wherein *S. aureus* is methicillin resistant *S. aureus*.

50) The method of any of the preceding paragraphs, wherein the *Staphylococcus* exposure is *Staphylococcus* colonization.

51) The method of any of the preceding paragraphs, wherein the *Staphylococcus* exposure is an epicutaneous colonization.

52) The method of any of the preceding paragraphs, wherein colonization or epicutaneous colonization is not an infection.

53) The method of any of the preceding paragraphs, wherein colonization or epicutaneous colonization does not elicit an immune response from the subject.

54) The method of any of the preceding paragraphs, wherein colonization or epicutaneous colonization elicits a sub-clinical immune response from the subject.

55) The method of any of the preceding paragraphs, wherein the *Staphylococcus* exposure is an infection.

56) The method of any of the preceding paragraphs, wherein the *Staphylococcus* exposure occurs in a lesion.

57) The method of any of the preceding paragraphs, wherein the lesion is associated with a condition selected from a group consisting of: atopic dermatitis, impetigo, prurigo nodularis, psoriasis.

58) The method of any of the preceding paragraphs, wherein the exposure is acute or chronic.

59) The method of any of the preceding paragraphs, wherein the *Staphylococcus* exposure is a reoccurring exposure.

60) The method of any of the preceding paragraphs, further comprising administering to a subject a second therapeutic agent.

61) The method of any of the preceding paragraphs, wherein the second therapeutic agent is an antibiotic, antifungal, or antimicrobial agent.

62) The method of any of the preceding paragraphs, wherein the subject has previously been diagnosed with having a *Staphylococcus* exposure.

63) The method of any of the preceding paragraphs, wherein the subject has not previously been diagnosed with having a *Staphylococcus* exposure.

64) The method of any of the preceding paragraphs, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having itch associate with a *Staphylococcus* exposure.

65) The method of any of the preceding paragraphs, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having or at risk of having itch associate with a *Staphylococcus* exposure.

66) The method of any of the preceding paragraphs, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having a *Staphylococcus* exposure that can result in itch.

67) The method of any of the preceding paragraphs, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having a *Staphylococcus* exposure that can result in itch.

68) The method of any of the preceding paragraphs, wherein the administering is systemic or local administration.

69) The method of any of the preceding paragraphs, wherein local administration is topical administration.

70) The method of any of the preceding paragraphs, wherein systemic administration is intrathecal administration.

71) A method for treating itch caused by a microbial exposure in a subject, the method comprising; topically administering to a subject having microbial exposure an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch, wherein administration occurs at the site of microbial exposure.

72) The method of any of the preceding paragraphs, wherein the subject does not have a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.

73) The method of any of the preceding paragraphs, wherein the subject is not being treated for a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.

74) A composition for treating or preventing itch caused by a microbial exposure in a subject, the composition comprising; an amount of an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount sufficient to treat or prevent itch.

75) A composition for treating or preventing itch caused by a *Staphylococcus* exposure in a subject, the composition comprising; an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch.

76) The composition of any of the preceding paragraphs, further comprising a pharmaceutically acceptable carrier.

77) The composition of any of the preceding paragraphs, formulated for topical administration.

78) The composition of any of the preceding paragraphs, formulated for systemic administration.

79) The composition of any of the preceding paragraphs, further comprising a second therapeutic agent.

80) The composition of any of the preceding paragraphs, wherein the second therapeutic agent is an antibiotic or antimicrobial agent.

81) Use of a composition for treating or preventing itch caused by a microbial exposure in a subject, the composition comprising; an amount of an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount sufficient to treat or prevent itch.

82) Use of a composition for treating or preventing itch caused by a *Staphylococcus* exposure in a subject, the composition comprising; an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch.

[00253] All patents, patent applications, and publications identified are expressly incorporated herein by reference for the purpose of describing and disclosing, for example, the methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.

EXAMPLES

INTRODUCTION

[00254] The skin is one of the most exposed barrier sites of the body, susceptible to both injury and pathogen invasion. It is innervated by dorsal root ganglia (DRG) sensory neurons which detect mechanical, thermal, and chemical stimuli, including noxious signals that cause itch or pain. Pruriceptors are sensory neurons that mediate itch and a desire to scratch¹⁻³. Microbes that colonize the skin play key roles in tissue homeostasis and physiology. However, a causative role for microbes in driving itch was unknown. It is specifically hypothesized herein that pruriceptors maybe activated following exposure to specific microbes, resulting in itch that drives skin damage.

[00255] *Staphylococcus aureus* is opportunistic bacterial pathogen and leading cause of human bacterial infections. Atopic dermatitis (AD) is a skin disease characterized by itchy, eczematous lesions. 90% of AD lesions are colonized with *S. aureus*, which is thought to be a trigger of inflammation⁴⁻⁷. *S. aureus* is also a leading cause of impetigo, a contagious skin infection characterized by itchy lesions⁸. Despite its association with these pruritic conditions, the contribution of *S. aureus* to itch is unclear. *S. aureus* encodes several virulence factors that promote colonization and tissue invasion, including α -hemolysin (Hla), phenol soluble modulins (PSMs), and proteases^{9,10}. Methicillin-resistant *S. aureus* (MRSA) continues to spread, necessitating an improved understanding of bacterial pathogenesis and host responses to this pathogen^{4,11}. The inventors previously found that nociceptors detect *S. aureus* and its

toxins to produce pain during subcutaneous infections¹²⁻¹⁴. Pruriceptor nerve endings are mainly found in the epidermis, unlike nociceptors, which innervate both skin and deeper tissues¹.

[00256] Itch provokes a desire to scratch, a behavioral reflex that could exacerbate skin damage. The importance of the itch-scratch cycle in skin pathology and negative impact on patient quality of life is well known for conditions including AD¹⁵, prurigo nodularis¹⁶, and psoriasis¹⁷. Scratching produces pain, which can temporarily suppress itch through spinal circuitry^{18,19}. Mechanical damage caused by scratching disrupts the skin barrier and can amplify inflammation. Therefore, understanding the triggers and factors that cause itch is critical for treatment of skin diseases.

[00257] Here the inventors find that *S. aureus* epicutaneous exposure induces robust itch and scratch-induced damage, which is mediated by the V8 protease. Pruriceptors are activated by V8 protease through PAR1. Targeting PAR1 abrogates itch, leading to improved skin pathology. Findings disclosed herein in the Examples uncover a role for bacterial proteases in itch and PAR1 as a candidate for therapeutic development.

RESULTS

[00258] Epicutaneous *S. aureus* exposure induces itch and alopecia

[00259] To investigate how *S. aureus* impacts itch, a murine model of epicutaneous exposure was adapted relevant to AD²⁰⁻²³. In this model, *S. aureus* is applied topically to depilated back skin under gauze, and mice wrapped with occlusive Tegaderm tape during bacterial exposure, resulting in epidermal breakdown at the inoculation site. At the experimental endpoint, tape and gauze are removed for inflammation and itch analysis (Fig. 1A, Fig. 8A).

[00260] USA300/LAC MRSA strain were utilized, which represents the leading cause of community-associated MRSA²⁴. Female and male mice were treated with MRSA, and inflammation scored at 5-days post-exposure. MRSA induced significant exposure-site dermatitis, quantified as a sum of edema, skin scale, erythema, thickness in both sexes (Fig. 1B). Histology showed hyperkeratosis, spongiosis, and inflammatory infiltrates (Fig. 8B). *S. aureus*-exposed mice showed higher transepidermal water-loss (TEWL) than controls, indicating disruption of skin barrier function (Fig. 8C).

[00261] Next, the role of *S. aureus* in itch was investigated. Spontaneous itch behaviors were assessed by placing mice in an infrared behavior observation box (iBOB) to record animals' activity over 90 min (Fig. 1C). Videos watched by blinded observers quantified scratching bouts (data not shown). Control mice produced minimal scratching while male and female animals treated with *S. aureus* exhibited significantly increased scratching behaviors (Fig. 1D). While dermatitis was observed by day 3, significantly increased scratching behaviors occurred by day 5 after *S. aureus* application (Fig. 8D-8E).

[00262] Alopecia is itch evoked by innocuous mechanical stimuli or touch^{1,25}. It is a form of dysesthesia driven by pruriceptor sensitization or spinal cord changes^{25,26} and regulated by Merkel cells²⁷. Alopecia can potentiate the itch-scratch cycle in AD patients. In mice, alopecia is measured by stimulation with a 0.07 g filament that normally does not elicit responses, but induces itch following

sensitization²⁸. Mice were stimulated with this filament 9 times and quantified scratching (data not shown). MRSA application induced significant alloeknesis compared to PBS-treated controls (Fig. 1E). While prior reports showed sex-dependent differences in itch^{29,30}, no differences in MRSA-induced itch and alloeknesis were found between female and male mice (Fig. 1D-1E).

[00263] Itch induced scratching exacerbates skin damage

[00264] Itch-evoked scratching exacerbates skin damage in AD patients. To quantify damage caused by scratching, *S. aureus* exposed mice were allowed to freely scratch the back for 7-hrs after Tegaderm/gauze removal. Total area of damaged skin was quantified using image analysis. Compared to controls, mice inoculated with MRSA had dramatically increased total damaged skin following scratching, resulting in areas of skin damage beyond bacterial exposure site (Fig. 1F). To confirm that scratching drives damage a cohort of infected mice were wrapped with bandages after Tegaderm/gauze removal. Wrapped mice prevented from scratching had significantly less skin damage than mice allowed to scratch (Fig. 1G). As a second way to prevent scratching, nails of mice were trimmed after Tegaderm/gauze removal (Fig. 8F). Nail trimming reduces itch/scratch-induced damage in mice³¹. Female and male mice that received nail trims had less skin damage than control mice after 7-hrs of recording (Fig. 8G).

[00265] Subcutaneous *S. aureus* infection does not induce itch

[00266] *S. aureus* is also a leading cause of human abscesses due to subcutaneous infections^{11,24}. However, dermonecrotic skin infections are often painful but not itchy. It was next determined whether *S. aureus* deeper infections caused itch. Mice were infected with MRSA by injecting subcutaneously in the back, inducing skin lesions by day 5 (Fig. 1H). Epicutaneous applications were performed in parallel, and itch behaviors measured. While spontaneous itch and alloeknesis occurred after epicutaneous MRSA exposure, mice infected subcutaneously did not show spontaneous itch or alloeknesis (Fig. 1I-1J). Therefore, while epicutaneous application causes itch, subcutaneous infection does not, indicating that bacterial localization affects neuronal phenotypes.

[00267] MYD88, mast cells and basophils do not mediate *S. aureus* itch

[00268] Given that *S. aureus* exposure mediates itch, determining underlying mechanisms could lead to therapies to limit itch-induced skin damage. Previous studies showed that *S. aureus* exposure induces IL-36 release, which activates IL-36R signaling through MYD88 to drive skin inflammation²⁰. Using *Myd88*^{-/-} mice, a significant reduction in dermatitis and TEWL was observed after *S. aureus* exposure compared to WT mice (Fig. 9A-9B). Bacterial load did not differ (Fig. 9C). However, differences in spontaneous itch behaviors or alloeknesis was not detected in *Myd88*^{-/-} mice compared to controls following *S. aureus* exposure (Fig. 9D-9E).

[00269] Mast cells are key drivers of itch by releasing pruritogens including histamine, serotonin, and tryptase³². *Kit*^{W-sh} mice, which lack mast cells, were utilized to determine their role in *S. aureus* itch. No difference in dermatitis or TEWL were found between *Kit*^{W-sh} and WT animals following *S. aureus* application (Fig. 9F-9G), but there was an increase in bacterial load in *Kit*^{W-sh} mice (Fig. 9H). Differences

between WT and *Kit^{W-sh}* mice in spontaneous itch and allodynia were not detected following MRSA exposure (Fig. 9I-9J). Basophils also drive itch in AD by release of leukotrienes, histamine and serotonin^{33,34}. To determine whether basophils mediate itch during *S. aureus* exposure, mice were treated with Ba103 antibody to deplete basophils³⁵ or control IgG (Fig. 9K). Flow cytometry revealed basophil recruitment in mouse skin following *S. aureus* exposure and that Ba103 antibody successfully eliminated basophils (Fig. 9L). After *S. aureus* exposure, no differences in dermatitis, TEWL, bacterial load, spontaneous itch, and allodynia were observed between mice injected with Ba103 and mice injected with control IgG (Fig. 9M-9Q). Taken together, mast cells and basophils are not required for *S. aureus*-induced itch or dermatitis.

[00270] IL31RA, IL4RA, and lymphocytes do not mediate *S. aureus* itch

[00271] Itch is associated with type 2 inflammation and can be driven by cytokines including IL4, IL13, and IL31³⁶. The role of IL31 in *S. aureus*-mediated itch was investigated. IL31 was elevated in skin on day-5 after *S. aureus* exposure (Fig. 10A). siRNA was administered via intrathecal injection³⁷ to knock down expression of the IL31 receptor, *Il31ra*, in DRG neurons. RT-qPCR analysis of thoracic DRGs confirmed that *Il31ra* siRNA reduced *Il31ra* expression compared to control siRNA injection (Fig. 10B). Mice treated with *Il31ra* siRNA showed no differences in *S. aureus* induced dermatitis, TEWL, bacterial load, spontaneous itch, and allodynia compared to control siRNA-treated mice (Fig. 10C-10G). Pruriceptors also express *Il4ra*, which mediates IL4 and IL13 signaling to drive itch³³. *Il4ra*^{-/-} and WT control mice were exposed to *S. aureus*. No differences in dermatitis, TEWL, bacterial load, spontaneous itch, and allodynia were observed between *Il4ra*^{-/-} and control mice (Fig. 10H-10L). Therefore, type 2 cytokines likely do not mediate *S. aureus* induced itch.

[00272] Roles for lymphocytes in itch was next investigated. *Rag2^{-/-}Il2rg^{-/-}* mice are deficient in T, B, NK cells and ILCs^{38,39}. Following MRSA exposure, no differences in dermatitis, TEWL, spontaneous itch, and allodynia were observed in *Rag2^{-/-}Il2rg^{-/-}* mice compared to WT controls (Fig. 10M-10N, 10P-10Q). More tissue bacteria load from *Rag2^{-/-}Il2rg^{-/-}* mice was obtained, indicating that lymphocytes affect bacterial clearance (Fig. 10O). Overall, a role for MYD88, mast cells, basophils, IL31RA, IL4RA, and lymphocytes in itch were ruled out (data not shown).

[00273] *S. aureus* localizes near epidermal sensory nerves

[00274] Pruriceptive nerve endings are mainly located in epidermis⁴⁰. It is specifically hypothesized herein that bacteria may localize to areas close to nerves during epicutaneous exposure to drive itch. Nav1.8 is a voltage-gated sodium channel expressed in C-fibers including pruriceptors^{41,42}. *Nav1.8-Cre* mice were bred with tdTomato reporter mice to label sensory neurons. Mice were topically treated with GFP-expressing MRSA or PBS. Whole mount imaging showed Nav1.8-TdTomato⁺ nerves in dermis and epidermis. Sensory innervation was maintained throughout the thickened dermis and epidermis below the *S. aureus* exposure site. In MRSA-exposed mice, GFP⁺ bacteria localized close to Nav1.8-TdTomato⁺ sensory nerves in the epidermis was observed (Fig. 2A).

[00275] *S. aureus* Agr is required for itch

[00276] Because bacteria were localized close to nerve endings, it is specifically hypothesized herein that secreted factors from *S. aureus* could activate neurons to drive itch. *S. aureus* virulence factors are regulated by its Agr quorum sensing system, including expression of multiple secreted cytolytic toxins and proteases (Fig. 2B)⁴³. Mice were exposed to WT MRSA or an Δagr isogenic mutant strain. Significant reductions were observed in spontaneous itch and allodynia in mice exposed to Δagr compared to WT MRSA (Fig. 2C). Δagr strain also induced less dermatitis (Fig. 2D). Fewer bacteria were recovered from skin of Δagr compared to WT MRSA-exposed mice (Fig. 11A). Thus, Agr mediates both itch and inflammation.

[00277] Bacterial toxins (Hla, PSMs) do not mediate *S. aureus* itch

[00278] Agr controls expression of phenol soluble modulins (PSMs) and α -hemolysin (Hla) (Fig. 2B). Requirement of these toxins in itch were tested by inoculating mice with WT MRSA or isogenic strains lacking Hla (Δhla) or PSMs ($\Delta psma$ $\Delta psmb$ Δhld ; ΔPsm s). Epicutaneous exposure to Δhla or ΔPsm s MRSA resulted in similar spontaneous itch and allodynia as WT MRSA (Fig. 2E). MRSA ΔPsm s caused less exposure-site dermatitis than WT MRSA, whereas MRSA Δhla induced similar inflammation as WT MRSA (Fig. 2F). These results are in line with previous reports demonstrating PSMs driving inflammation^{20,23}. No differences were observed in bacterial load for WT, Δhla , or ΔPsm s MRSA (Fig. 11A). Thus, *S. aureus* toxins are not required for itch. Furthermore, itch and inflammation can be decoupled, given that MRSA ΔPsm s induced itch despite absence of dermatitis (Fig. 2E-2F).

[00279] Proteases are necessary for *S. aureus* itch

[00280] Proteases from plants, allergens, and mammals have been shown to cause itch^{44,45}. *S. aureus* produces 10 proteases including cysteine, serine, and metalloproteases⁴⁶, and these proteases are under Agr control (Fig. 2B). The requirement for *S. aureus* proteases was tested in itch by inoculating mice with WT MRSA or isogenic mutant lacking genes for all 10 proteases (Δaur $\Delta sspA$ $\Delta scpA$; $spl::erm$; $\Delta Protease$)⁴⁷. Spontaneous itch behaviors and allodynia were significantly reduced in mice exposed to $\Delta protease$ compared to WT MRSA (Fig. 2G). MRSA $\Delta protease$ strain was shown previously to induce less inflammation⁴⁸. A similar reduction in dermatitis in animals inoculated with $\Delta protease$ was found compared to WT MRSA (Fig. 2H), and decreased bacterial load (Fig. 11A). Therefore, *S. aureus* proteases are necessary for itch and inflammation.

[00281] The inventors next sought to identify the role of specific *S. aureus* protease(s) in itch. Compared to WT MRSA, treatment with MRSA lacking aureolysin (Δaur) resulted in no difference in dermatitis, while a strain deficient in both staphopain A and staphopain B ($\Delta scpA$ $\Delta sspB$) caused a slight decrease in dermatitis (Fig. 11B). Mice inoculated with Δaur or $\Delta scpA$ $\Delta sspB$ had no differences in spontaneous itch, allodynia, or bacterial load compared to mice treated with WT MRSA (Fig. 11A, 11C-11D). MRSA secretes 6 serine protease-like proteins (Spl)s. MRSA lacking all serine protease-like proteins SplA-F ($spl::erm$) caused a similar degree of dermatitis, spontaneous itch, and allodynia as WT MRSA (Fig. 11B-11D). These data rule out 9/10 known proteases in itch, narrowing the search to serine protease V8, which is encoded by *sspA* gene⁴⁹.

[00282] *S. aureus* V8 protease contributes to itch and skin inflammation

[00283] To test the requirement of V8 protease in itch, a *sspA* deletion mutant (Δ *sspA*) that does not disrupt downstream *sspB* gene encoding Staphopain B was generated (Fig. 12A). Chromosomally complemented strain Δ *sspA*+*sspA* was also generated and confirmed loss of V8 protease activity in Δ *sspA* and restoration of protease activity in Δ *sspA*+*sspA* complement (Fig. 12A). Epicutaneous application of Δ *sspA* MRSA resulted in significantly less itch behaviors measured by spontaneous itch and alloknesis compared to WT bacteria (Fig. 3A-3B). Itch behaviors were restored in mice exposed to Δ *sspA*+*sspA* strain (Fig. 3A-3B). Reduction in scratching resulted in decreased total skin damage in animals exposed to MRSA Δ *sspA* (Fig. 3C). Epicutaneous application of Δ *sspA* MRSA resulted in reduction in dermatitis and lower TEWL measurements, indicating reduced skin barrier damage, compared to mice inoculated with WT or complemented MRSA strains (Fig. 3D, Fig. 12B). No differences were observed between WT and MRSA Δ *sspA* strains in adherence to KERTr keratinocyte cells *in vitro*, suggesting that reduction in itch and inflammation are independent of adherence defects (Fig. 12C). A decrease was observed in tissue bacterial load in mice infected with Δ *sspA* mutant (Fig. 12D). Therefore, V8 protease is a critical bacterial factor that drives itch.

[00284] V8 is upregulated in mouse skin and human AD skin lesions

[00285] Having identified V8 protease as a mediator of itch, *sspA* transcript was next quantified at different time points in *S. aureus* exposure model (Fig. 3E). *sspA* transcript increased over time, becoming significantly higher on day 5 compared to day 1 post-exposure (Fig. 3F). By contrast, *psmA1* transcript did not change and *hla* transcript from mouse skin samples during MRSA exposure was not detected (Fig. 3G-3H). The timing of increased *sspA* transcript coincides with when robust induction of itch was observed (Fig. 8E). The inventors next determined whether *S. aureus* V8 (*sspA*) is expressed in human skin samples relevant to disease. Skin swabs from were obtained healthy individuals, non-lesional and lesional skin from AD patients. Significantly higher amounts of *sspA* transcript were observed in lesional AD skin samples compared to healthy controls (Fig. 3I).

[00286] V8 injection induces itch and skin damage

[00287] The inventors next tested whether purified V8 protease causes spontaneous itch or pain behaviors. Following intradermal cheek injections, pruritogens induce mice to scratch with the hind-paw, whereas pain-inducing algogens cause mice to wipe with the forelimb (Fig. 3J)^{50,51}. The inventors found that injecting 40U V8 protease induced robust itch and not pain (Fig. 3K-3L). As a positive control and comparison, mice were injected with histamine, a pruritogen which produced itch (Fig. 3K-3L). By contrast, the TRPV1 ligand capsaicin caused pain behaviors (Fig. 3K-3L). V8 protease injection induced itch in a dose-dependent manner (Fig. 12E). Itch likely depends on protease activity, as mice injected with heat-inactivated V8 did not exhibit increased scratching compared to untreated V8 (Fig. 12F). While 40U of V8 protease induced itch specifically, 200U of V8 caused both itch and pain behaviors (Fig. 11G-11H).

[00288] V8 protease injection was also sufficient to cause allodynia. Mice injected with vehicle, histamine, or V8, followed by allodynia measurements (Fig. 3M). V8 protease resulted in significantly higher allodynia at every time point measured up to 1 hr compared to histamine and buffer alone (Fig. 3N), and remained elevated in V8-treated mice at 3 hrs post-injection (Fig. 12I).

[00289] The inventors next tested whether V8-induced scratching drives skin damage. Mice were injected intradermally into back skin with PBS or V8. One set of V8-injected mice were allowed to scratch while another group was prevented from scratching by wrapping with bandages (Fig. 12J). At 3- and 6-hrs post-injection, V8 protease-injected mice that could scratch exhibited higher TEWL than PBS-injected controls, indicating skin barrier damage (Fig. 12K-12L). In contrast, V8 protease did not induce higher TEWL in animals prevented from scratching compared to PBS-injected controls (Fig. 12K-12L).

[00290] *V8 protease cleaves PAR1*

[00291] The inventors specifically hypothesized herein that specific host receptors may mediate neuronal recognition of V8 protease to drive itch. Proteinase-activated receptors (PARs) are G protein-coupled receptors activated by proteolytic cleavage of an extracellular N-terminal domain, leading to exposure of a tethered ligand that induces activation^{52,53}. Humans and mice express four PAR family members, with PAR1, PAR2, and PAR4 having intracellular signaling capabilities^{54,55}. PARs are expressed in pruriceptive neurons and their activities linked to itch⁴⁵.

[00292] A luminescence-based PAR cleavage assay⁵⁶ was employed to determine whether V8 can proteolytically cleave PARs (Fig. 4A). V8 protease potently cleaved human PAR1 ($EC_{50} = 4$ Units of activity (U)/mL), but did not cleave PAR2, and had modest activity in cleaving PAR4 ($EC_{50} = 219$ U/mL) (Fig. 4B). As positive controls, PAR cleavage assays were performed with canonical PAR-ligand proteases thrombin (PAR1, PAR4) or trypsin (PAR2) (Fig. 4C). As a second assay, human embryonic kidney (HEK-293) cells expressing PAR1 tagged N-terminally with mRFP and C-terminally with eYFP were exposed to V8. Microscopy showed V8 treatment resulting in cleavage and removal of N-terminal mRFP tag. Only cleaved receptor (solely eYFP-positive) was detected at cell membrane after V8 exposure (Fig. 13A).

[00293] To map potential V8 cleavage sites in N-terminus of PAR1, a limiting concentration of V8 protease were incubated with C-terminally His₆-tagged ligand of human PAR1 (hPAR1²²⁻¹⁰²) attached to Ni-NTA beads. Mass spectrometry analysis of supernatant identified 10 cleavage sites (Fig. 4D, 13B, data not shown) including sites upstream and downstream of canonical thrombin cleavage site (R⁴¹/S⁴²), and several peptide fragments. V8 protease did not cleave at E/D|P, possibly due to steric hindrance documented with other proteases⁵⁷. The inventors tested whether V8 protease cleaves the tethered ligand and disarms PAR1 by monitoring thrombin evoked calcium signaling in HEK cells expressing hPAR1 after exposure to V8. No change in intracellular calcium was observed between cells treated with thrombin alone or pre-treated with 2 U/mL V8 protease and thrombin, indicating that, at a lower concentration, V8 cleaves upstream of the thrombin cleavage site (Fig. 13C). At 20 U/mL, V8 abrogated responses to thrombin, suggesting that V8 can cleave downstream of thrombin site at higher

concentrations (Fig. 13D). The inventors further tested whether V8 affected PAR1 activation by the synthetic peptide TFLLR-NH₂, observing no inhibition of TFLLR-NH₂ response with 2 U/mL and 20 U/mL V8 protease (Fig. 13E-13F). Intact TFLLR-NH₂ responses suggest that V8 does not cleave at receptor sites involved in tethered ligand binding such as extracellular loops and ligand binding pocket.

[00294] *PAR1 is expressed by DRG neurons*

[00295] PAR1 expression and activation has been demonstrated in human and mouse DRG neurons^{58,59}. The inventors determined whether PAR1 is expressed in DRG neurons linked to itch. PAR1 is encoded by *F2r* gene^{53,60}. RNAscope *in situ* hybridization (ISH) analysis was performed in mouse DRG to visualize *F2r* transcripts along with pan-neuronal marker *Tubb3* (Fig. 4E), finding *F2r* expression in 40% of mouse DRG neurons (Fig. 4F). Analysis of a scRNAseq dataset of mouse neurons⁶¹ showed *F2r* expression in several DRG subsets, including neurons previously linked to itch: NP2 neurons that express *mrgpra3* and *hrh1*^{2,32}, and peptidergic neurons that express *slpr3*⁶² (Fig. 14A). The inventors also performed RNAScope analysis of *F2R* expression in human DRG samples, observing *F2R* in 35% neurons, in accordance with previous studies⁵⁹. *F2R*⁺ neurons were small in diameter (average 53.1 μ m) and positive for *TRPV1* and *NPPB*, a marker of pruriceptive neurons^{63,64} (Fig. 4G-4H). Mining scRNAseq data of human DRG neurons, enrichment of *F2R* was detected in a subset of putative pruriceptors expressing *NPPB*, *IL31RA* and *GFRA2* (Fig. 14B)⁶⁵.

[00296] *V8 activates mouse and human DRG neurons*

[00297] Having confirmed that purified V8 protease induces itch and cleaves PAR1, the inventors next tested whether V8 could directly activate sensory neurons. Mouse DRG neurons were loaded with calcium indicator Fura-2, followed by application with vehicle or V8 protease. V8 protease induced DRG neuron calcium influx, including a concentration-dependent increase in the number of responsive neurons and the amplitude of calcium responses to V8 (Fig. 14C-14D). Neuronal responses were subsequently analyzed to 40 U/mL of V8, an amount that induced itch *in vivo* (Fig. 3A-3N) and at mid-dose range (Fig. 14C). At 40 U/mL, V8 protease induced intracellular calcium responses in ~10% of mouse DRG neurons (Fig. 5B). Neurons were subsequently exposed to pruritogens histamine, chloroquine, or sphingosine-1-phosphate (S1P), followed by capsaicin and KCl to mark ligand-responsive subsets (Fig. 5A). Many V8-responsive cells also responded to pruritogens: V8 activated 25% histamine-responsive, 38% chloroquine-responsive, 25% S1P-responsive neurons; V8 activated 22% capsaicin-responsive neurons (Fig. 5B, 14E).

[00298] The inventors previously showed that *S. aureus* Hla and N-formylated peptides can activate DRG neurons to mediate pain¹². Using calcium imaging, the inventors determined whether V8-responsive neurons responded to Hla or fMLF. Hla induced the highest proportion of DRG neuron responses, followed by V8, then fMLF (Fig. 14F). ~74% V8-responsive neurons responded to Hla, and ~26% V8-responsive neurons to fMLF (Fig. 14F). The inventors next performed intradermal cheek injections with Hla or fMLF. While fMLF did not induce itch or pain, Hla injection induced both itch and

pain (Fig. 14G). These data suggest that Hla is capable of inducing itch, though Δ Hla mutant MRSA did abrogate itch following *S. aureus* exposure (Fig. 2A-2H).

[00299] The inventors also tested whether human DRG neurons could respond to V8 with freshly dissociated DRG neurons dissected from organ donors. Human neurons were loaded with the calcium indicator Fluo-4, and intracellular calcium changes measured after treatment with V8 protease and capsaicin (Fig. 5C). 26.5% of human DRG neurons were activated by V8, and 95.5% of V8 responsive cells responded to capsaicin (Fig. 5D). These data show that V8 can induce calcium influx in both mouse and human DRG neurons.

[00300] *PAR1 mediates V8 induced neuronal activation and itch*

[00301] To test whether V8 activation of neurons is dependent on PAR1, calcium imaging of DRG neurons from wildtype ($F2r^{+/+}$) or $F2r^{-/-}$ mice was performed. Compared to neurons from wildtype animals, $F2r^{-/-}$ neurons were not responsive to treatment with V8 protease (Fig. 5E). The inventors asked if blocking protease activity or PAR1 signaling reduces neuron responses to V8. Mouse DRG neurons were pre-treated with serine protease inhibitor TLCK^{66,67} or PAR1 antagonist Vorapaxar⁶⁸ (Fig. 5F). Pre-treatment with TLCK or Vorapaxar eliminated V8-induced calcium influx in neurons (Fig. 5F-5G). In contrast, neither Vorapaxar nor TLCK affected responses to capsaicin (Fig. 5F, 5H). These results demonstrate that PAR1 is required for V8 activation of sensory neurons.

[00302] The inventors next tested the requirement of PAR1 for V8-induced itch *in vivo*. Intradermal cheek injections were performed with PBS or V8 protease into wildtype (WT) and $F2r^{-/-}$ mice. V8 elicited significantly less scratching in $F2r^{-/-}$ mice than WT mice, and no significant differences between V8 and PBS-treatment in $F2r^{-/-}$ mice (Fig. 6A). To determine whether V8 injection induces immune cell recruitment dependent on *F2r*, flow cytometry was performed to characterize skin immune cells in WT and $F2r^{-/-}$ mice after treatment with PBS or V8 (Fig. 15A). A baseline increase was observed in T cells in $F2r^{-/-}$ mice compared to WT, and increased T cells in WT mice treated with V8 compared to PBS. Macrophages decreased in $F2r^{-/-}$ mice at baseline, and further decreased in mice injected with V8. There were no changes in neutrophils, eosinophils, mast cells, basophils, and dendritic cells (Fig. 15B).

[00303] The inventors examined the contribution of other protease-activated receptors in V8-induced itch. DRG neurons express various members of the Mas-related G coupled receptors (MRGPRs) family, including MRGPRA3, MRGPRX1, and MRGPRD, which have been linked to itch⁶⁹. The inventors found that mice lacking the *Mrgpr* locus ($Mrgpr^{-/-}$) showed similar acute itch behaviors following V8 intradermal cheek injection as wild-type mice (Fig. 16A). PAR2, encoded by *F2rl1*, also mediates protease-induced itch responses^{45,70}. However, the inventors did not find PAR2 cleavage by V8 (Fig. 4B). $F2rl1^{-/-}$ mice also showed similar itch as WT mice when injected with V8 (Fig. 16A).

[00304] *F2r targeting in DRG neurons inhibits V8 and S. aureus itch*

[00305] The inventors next determined the role of *F2r* in DRG neurons in V8-induced itch using siRNA and conditional knockout approaches. Using intrathecal injections of siRNA to target sensory neurons³⁷, mice were injected with vehicle, control siRNA, or *F2r* siRNA (Fig. 6B). RT-qPCR of

thoracic DRGs confirmed efficient knockdown of *F2r* in animals treated with *F2r* siRNA but not control siRNA (Fig. 16B). The inventors next injected V8 protease intradermally at the upper back of mice to observe spontaneous scratching behaviors. *F2r* siRNA-treated animals had significantly reduced V8-induced itch compared to mice injected with vehicle or control siRNA (Fig. 6B). The inventors next determined if *F2r* knockdown in DRGs inhibits itch during epicutaneous *S. aureus* exposure. Following *F2r* or control siRNA injection, mice were inoculated with WT MRSA or treated with PBS (Fig. 6C). At 5-days post-exposure, no differences were observed in dermatitis, skin barrier damage (TEWL), and tissue bacterial load between control and *F2r* siRNA treated mice (Fig. 16C-16E). A significant reduction was observed in spontaneous itch behaviors and alloknesis for mice injected with *F2r* siRNA (Fig. 6D-6E). *F2r* knockdown mice also showed less total skin damage caused by scratching (Fig. 6F). Therefore, knockdown of *F2r* expression in DRG neurons leads to inhibition of itch caused by V8 protease and *S. aureus*.

[00306] To target *F2r* specifically in sensory neurons, *Trpv1*^{Δ*F2r*} conditional knockout mice were generated by crossing *Trpv1*^{cre} with *F2r*^{fl/fl} mice (Fig. 6G). *Trpv1*^{cre} lineage-based analysis has shown that it targets both peptidergic and non-peptidergic C-fibers⁷¹. *Trpv1*^{Δ*F2r*} mice or cre-negative control littermates were treated with PBS or exposed to MRSA. No differences were found in dermatitis, TEWL, and skin bacterial load between the two groups (Fig. 16F-16H). Similar to mice injected intrathecally with *F2r* siRNA, *Trpv1*^{Δ*F2r*} mice exhibited significantly less spontaneous itch and alloknesis following MRSA exposure compared to control mice (Fig. 6H-6I).

[00307] *PAR1 pharmacological inhibition reduces S. aureus itch and skin damage*

[00308] The inventors next investigated the therapeutic potential of PAR1 blockade in blocking itch and skin pathology. Vorapaxar is an FDA-approved PAR1 antagonist and drug used for reducing the risk of thrombotic cardiovascular events⁷². The inventors found that co-administration of V8 and Vorapaxar resulted in significantly reduced scratching for all doses of Vorapaxar tested (Fig. 7A). The inventors also tested the effect of another PAR1 antagonist, SCH79797, and found that all doses of this drug reduced scratching responses to V8 (Fig. 16I). In contrast, PAR4 antagonist BMS986120 minimally affected V8-induced itch, though it did have anti-pruritic effects at the highest dose (Fig. 16J). SCH79797 has direct antimicrobial effects, whereas Vorapaxar does not affect bacterial growth^{73,74}. Therefore, the inventors focused on Vorapaxar for further tests in mice.

[00309] Vorapaxar also significantly reduced V8 protease-induced alloknesis. The inventors found that vorapaxar treatment reduced alloknesis responses up to 3 hours after V8 injection (Fig. 7B-7C). Mice injected with V8 protease had higher TEWL measurements in back skin, which could be blocked by Vorapaxar treatment or wrapping with bandages to prevent scratching (Fig. 7D). Therefore, blocking PAR1 reduced itch and scratch-induced skin barrier damage after V8 injection.

[00310] The inventors next investigated whether Vorapaxar could treat itch during *S. aureus* epicutaneous exposure. Mice were gavaged daily with Vorapaxar or vehicle control (Fig. 7E). It was found that, similar to *F2r* siRNA, Vorapaxar treatment had no effect on dermatitis, TEWL, and bacterial

load (Fig. 7F, 16K-16M). Vorapaxar significantly reduced spontaneous itch behaviors and alloknesis following epicutaneous *S. aureus* application (Fig. 7G-7H). Less skin damage from scratching was also observed for the mice treated with Vorapaxar and MRSA (Fig. 7I-7J). In summary, pharmacological inhibition of PAR1 in mice significantly reduces itch behaviors that drives skin damage during bacterial exposure.

DISCUSSION

[00311] The underlying mechanisms of itch during microbial exposure were not previously understood. Here, the inventors established that human pathogen *S. aureus* induces robust itch and scratch-induced damage during epicutaneous exposure. By screening through bacterial genetic mutants *in vivo*, the inventors find V8 protease as necessary and sufficient for itch and alloknesis. *S. aureus* V8 induced mouse and human pruriceptor neuron activation, which was mediated by host receptor PAR1. PAR1 inhibition prevented neuronal activation, itching, and skin damage. Findings disclosed herein reveal a role for bacteria in causing itch and highlight the importance of the itch-scratch cycle in skin injury.

[00312] Given that *S. aureus* produces 10 proteases, it was striking that V8 protease specifically mediated itch. Staphopains A and B, Aureolysin, and SPLs did not contribute to itch. V8 is a serine protease with specificity to cleaving after glutamic acids, and in some conditions after aspartates⁷⁵. V8 protease has been shown to be a dominant *S. aureus* virulence factor causing damage to keratinocytes⁷⁶. Topical application of V8 increased TEWL and serum IgE levels in hairless mice⁷⁷. A recent study profiled skin from healthy adults and AD patients for *S. aureus* virulence factors, and *sspA* transcript was detected in samples from both healthy and AD skin⁷⁸. It was found that *sspA* transcript increased in AD compared to healthy skin. Reports have demonstrated that nearly all *S. aureus* isolates contain the *sspA* gene⁷⁹⁻⁸¹. Antibodies specific to V8 can be detected from humans who have previously been infected with *S. aureus* and in the general population^{82,83}.

[00313] In addition to V8, *S. aureus* Hla activated DRG neurons and induced itch and pain when injected. PSMs can also cause itch and pain when injected into the mouse cheek⁸⁴. While Hla and PSMs can induce itch, no difference was found in spontaneous itch or alloknesis in mice inoculated with *S. aureus* deficient in these toxins (Fig. 2A-2H). Levels of Hla and PSMs produced by *S. aureus* on the skin surface maybe insufficient to induce itch. Matching this point, while *sspA* transcripts were high at day-5 following MRSA inoculation, the inventors did not observe increased *psma1* or *hla* transcripts (Fig. 3A-3N). How *S. aureus* regulates expression of these factors on skin surface remains to be determined.

[00314] Non-microbial proteases have been linked to itch. The inventors' study adds a bacterial protease as a pruritogen that acts through PAR1. Many previous reports have focused on the action of proteases on PAR2 and PAR4 in itch⁴⁵. Plant proteases including cowhage mucanain, bromelain, and papain induce itch by acting via PAR2 and PAR4. Keratinocytes and immune cells express cathepsin S, which can induce itch through PAR2 and PAR4. Mast cell tryptase and chymase can also induce itch. A

recent report found that mast cell tryptase activates PAR1 to cause anaphylaxis⁸⁵. Keratinocytes produce kallikrein (KLK) proteases including KLK5 and KLK14 which can cleave PAR2⁸⁶ and drive itch^{87,88}. *S. aureus* can also induce keratinocyte expression of KLKs⁸⁹. The inventors' finding that PAR1 mediates itch during *S. aureus* exposure introduces this receptor as a driver of itch. PAR1 was expressed across several sensory neurons, including those expressing *Nppb*, *Mrpgra3*, and *Slpr3*^{2,61}. In humans, its expression was more linked to NPPB⁺ neurons⁶⁵. How protease activation of PAR1⁺ neuronal subsets induces signaling at the cellular level remains to be fully determined.

[00315] The inventors observed that dermatitis at the site of bacterial exposure can be decoupled from itch and scratching behavior. PSMs and MYD88 were previously shown to drive inflammation following MRSA exposure^{20,23}. It was found that bacteria lacking PSMs caused decreased dermatitis but still induced itch; similarly, *Myd88*^{-/-} mice showed decreased dermatitis, but still scratched. Therefore, itch and scratching behavior do not require pre-existing inflammation and skin barrier disruption. Results disclosed herein indicate that V8 acts directly through neuronal PAR1 to induce itch independent of inflammation.

[00316] The role of other microbial proteases in itch requires further investigation. Bacteria produce numerous proteases that have various roles in health and disease⁹⁰. *Staphylococcus epidermidis*, an opportunistic pathogen frequently found on healthy and AD skin, makes the protease EcpA which causes skin damage in AD patients⁹¹. *Streptococcus pyogenes*, another skin pathogen, produces proteases including SpeB which impact skin infection⁹². Beyond bacteria, how fungi, viruses and parasites contribute to itch are unknown.

[00317] Neuronal sensing of pathogens can mediate early defense responses to infection through neurogenic inflammation⁹³. Nociceptors release neuropeptides including calcitonin gene-related peptide (CGRP) or substance P (SP) to mediate vascular^{94,95} and immune changes⁹⁶. PAR1 activation on primary afferents can induce release of CGRP and SP, provoking neurogenic inflammation⁹⁷. Neuronal PAR1 activation could therefore mediate quick and sustained depletion of neuropeptides from primary afferents and downstream immune modulation.

[00318] Pathogens may hijack itch and other neural reflexes for their advantage. *Mycobacterium tuberculosis* (Mtb) directly activate vagal nociceptor neurons through a sulfolipid SL-1 to mediate coughing in guinea pigs, which could facilitate pathogen transmission⁹⁸. *S. aureus* induces itch and scratching behaviors which mediate skin damage. This may impact bacterial spread deeper in the skin or result in dissemination to distant body sites. Scratching could also facilitate bacterial spread to other hosts. Further investigation into how bacteria induce maladaptive behaviors to mediate invasion and dissemination are needed.

[00319] The inventors found that blocking PAR1 reduced itch in mice, and V8 activates and cleave human PAR1. Therefore, PAR1 could be an attractive candidate to target for itch therapies. Vorapaxar is currently FDA-approved for prevention of thrombotic cardiovascular events⁷². Future development of topical application of such PAR1 antagonists could avoid adverse events caused by systemic delivery.

There is interest in intrathecal injection as a method to deliver therapeutic siRNAs to modulate gene expression in neurons⁹⁹. Itch is a major cause of suffering for the many patients with pruritic diseases accompanied by microbial dysbiosis^{15,19,100,101}. Targeting PAR1 or bacterial proteases including V8 may be promising approaches. Therefore, the inventors' study disclosed herein reveals a distinct bacterial-driven itch mechanism that contributes to skin pathology and may be targeted for therapeutic treatment of itch.

METHODS

[00320] Mice

[00321] All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Harvard Medical School under protocol numbers IS000000543 and IS000000546 and were conducted in accordance with National Institutes of Health (NIH) animal research guidelines. Mice were bred and housed in individually ventilated micro-isolator cages within a full barrier, specific pathogen-free animal facility at Harvard Medical School under a 12h light/dark cycle with *ad libitum* access to food and water. Age-matched littermate male and female mice were used for experiments.

[00322] C57/BL6 mice that were free of rodent pathogens and *Staphylococcus aureus* were purchased from Taconic Biosciences. C57/BL6J mice, Ai14 strain B6.Rosa26-stop(flox)-tdTomato (#007914)¹⁰⁴, *Myd88* knockout strain B6.129P2(SJL)-*Myd88*^{tm1.1Defr/J} (#009088)¹⁰⁵, *F2r* knockout strain B6.Cg-*F2r*^{tm1Mslb/J} (#004993)¹⁰⁶, mast cell deficient B6.Cg-*Kit*^{W-sh} mice (#030764), and *Trpv1*-Cre^{+/+} B6.129-Trpv1^{tm1(cre)Bbm/J} strain (#017769) were obtained from the Jackson Laboratory (Bar Harbor, ME). *Rag2*^{-/-}/*Il2rg*^{-/-} strain C57BL/6NTac.Cg-*Rag2*^{tm1Fwa} *Il2rg*^{tm1Wjl} was obtained from Taconic Biosciences. *F2r*-flox mice were generated previously¹⁰⁷. *Trpv1*-Cre^{+/+} mice were crossed with *F2r*-flox mice to generate *Trpv1*^{ΔF2r} mice. Balbc/J (#00651) and *Il4ra*^{-/-} (#003514) strain BALB/c-*Il4ra*^{tm1Sz/J} were purchased from Jackson Laboratory (Bar Harbor, ME). *Mrgpr* knockout mice¹⁰⁸ were provided by Xinzhong Dong (Johns Hopkins University). *Nav1.8*-Cre mice¹⁰⁹ were provided by John Wood (University College London). *Nav1.8*-Cre^{+/+} mice were crossed with Ai14 mice to generate Nav1.8^{tdTomato} mice. *F2r* knockout strain B6.129S4-*F2r*^{tm1Ajc/J} mice were obtained by recovery from cryopreservation from Jackson Laboratories (#002862).

[00323] Human subjects for skin swab collection

[00324] Experiments involving human subjects were done according to protocols approved by University of California, San Diego IRB (Project#140144). Written informed consent was obtained from all subjects. Swabs of surface microbiota from a 5 cm² area of the antecubital fossa skin of both left and right arms were collected from 14 healthy subjects and 13 patients with AD as previously described⁹¹. For subjects with AD, swabs were collected from both lesional and non-lesional skin. Swabs were stored in tryptic soy broth (TSB) and 16.67% glycerol at -80°C with swab intact until follow-on analysis was performed.

[00325] Human DRG samples from organ donors

[00326] Human lumbar DRGs were obtained from organ donors in collaboration with Southwest Transplant Alliance, as previously described⁶⁵. Human DRGs were immediately frozen in pulverized dry-ice on-site for RNAScope analysis or immersed in N-methyl-D-glutamate-artificial cerebrospinal fluid (NMDG-aCSF) for subsequent calcium imaging experiments¹¹⁰. All tissue procurement procedures were approved by the institutional review board at University of Texas at Dallas.

METHOD DETAILS

[00327] Bacterial strains and culture

[00328] All procedures related to bacterial strains and infectious disease work were approved by the Committee on Microbiological Safety (COMS) at Harvard medical school and were conducted under Biosafety Level 2 protocols and guidelines. Bacteria were grown in tryptic soy broth (TSB) at 37°C and growth was monitored by measuring the optical density at 600 nm (OD₆₀₀). *S. aureus* CA-MRSA strains LAC/USA300 (wildtype, WT) and GFP-MRSA are previously described¹². Δagr and Δhla MRSA were obtained from Dr. Victor Torres¹¹¹. The $\Delta psma$ $\Delta psmb$ Δhld ($\Delta Psms$) MRSA strain was a gift from Dr. Michael Otto¹¹².

[00329] Deletions of aureolysin (Δaur), staphopain A and staphopain B ($\Delta scpA$ $\Delta sspB$) and SplA-F ($\Delta spl::erm$) strains were generated as previously described¹¹³. The V8 protease deletion ($\Delta sspA$) was generated using homologous recombination as previously described, resulting in an encoded small peptide MKGPR* in its place¹¹⁴. Complementation of $\Delta sspA$ was achieved by cloning *sspA* with 245 bp of the promoter sequence into a pLL29-derived vector where the tetracycline antibiotic resistance cassette was replaced with an erythromycin resistance cassette (pLL29erm)^{115,116}. The resulting plasmid was integrated at the $\phi 11 attP$ site in RN4220 using helper plasmid pLL2787 and moved into the $\Delta sspA$ strain by phage transduction. The resulting strains were PCR and sequence verified.

[00330] V8 protease activity assays

[00331] Activity assays for V8 protease were performed as previously described with some modifications¹¹⁷. Filtered supernatants were further concentrated with an Amicon Ultra-15 Centrifugal Filter (10 kDa cutoff), dialyzed in 20 mM Tris pH 7.4, and normalized to a protein concentration of 0.45 mg/mL by the Pierce BCA Protein Assay (ThermoFisher) before beginning the FRET assay.

[00332] Epicutaneous MRSA exposure and measurement of itch and inflammation

[00333] The murine model of epicutaneous *Staphylococcus aureus* exposure was adapted from^{20,23}. Prior to MRSA application, mice were shaved and treated with chemical depilation to remove back fur. Two days after fur removal, a 1 cm² gauze piece of soaked with 100uL of bacterial suspension was placed onto the skin just below the shoulder blades and the animals were covered with Tegaderm occlusive tape. Control animals were treated with gauze soaked with 100uL sterile PBS. Mice were monitored daily and the Tegaderm tape and gauze were removed at the endpoint so that inflammation and itch could be measured.

[00334] *S. aureus* exposure site dermatitis: Inflammation caused directly by bacterial exposure was measured immediately after tape and gauze removal (before mice were able to scratch the back skin). Four parameters (edema, skin scale, erythema, and thickness) were assigned a score from 0 (none) to 3 (severe) and these measurements were summed for a total score of between 0 to 12 per mouse (Fig. 8B).

[00335] Transepidermal water loss: Following skin score assessment, a Tewameter TM300 device (Courage and Khazaka Electronic GmbH) was used to record TEWL at the site of gauze placement on the skin.

[00336] Alloknesis: Mice were stimulated 9 times with a 0.07g von Frey filament on the back skin near the exposure site. Bouts of scratching that occurred immediately after stimulation were recorded as a response.

[00337] Spontaneous itch: Prior to MRSA exposure, mice were habituated to the infrared behavior observation box (iBOB). Following tape and gauze removal at the endpoint of exposure, mice were returned to their home cage for several hours and allowed to groom the skin/fur that was previously covered by tape. After grooming behaviors returned to baseline, mice were placed in iBOB for 90 minutes of video recording. Bouts of scratching were counted by observers blinded to the treatment groups.

[00338] Scratch-induced skin damage: Immediately after tape and gauze removal, mice were photographed from above, returned to their home cage, and allowed to freely scratch the back skin for 7 hours. After scratching, mice were anesthetized and photographed. Blinded observers analyzed the images to measure the total shaved skin area and the skin area that appeared inflamed (including the infected lesion site and the surrounding scratched areas) using ImageJ. The area of damaged skin was calculated as the percentage of inflamed skin area out of the total shaved area.

[00339] Bacterial load: Mice were euthanized according to approved veterinary protocols and the back skin was dissected and placed in 1 mL sterile PBS and bead beaten for 10 min to homogenize the tissue. The resulting tissue homogenate was serially diluted and plated on CHROMagar (Hardy Diagnostics) supplemented with 5.2 µg/mL cefoxitin to enumerate MRSA CFU.

[00340] Histology

[00341] Mice were euthanized by CO₂ inhalation and the back skin was dissected and fixed for 24h at 4°C in 4% paraformaldehyde. Fixed skin samples were embedded with paraffin, sectioned, and stained with hematoxylin and eosin (H&E) dyes by the Harvard Medical School Rodent Histopathology Core. Stained sections were imaged by light microscopy on an Eclipse Ti-S/L100 inverted microscope (Nikon) and images collected by NIS-Elements AR software.

[00342] Subcutaneous MRSA infection

[00343] Mice were injected subcutaneously with 50µL of 10⁷ CFU of MRSA in PBS. At 5-days post-infection, mice developed large dermonecrotic lesions at the infection site. Alloknesis was assessed by stimulating the skin close to the necrotic tissue. Spontaneous itch was measured by counting bouts of scratching to both the infected area and the healthy back skin surrounding the lesion.

[00344] Whole mount confocal microscopy

[00345] *Nav1.8^{tdTomato}* mice were treated epicutaneously with GFP-MRSA or sterile PBS. At 5 days post-treatment, mice were euthanized following approved veterinary protocols and the skin was dissected and fixed for 24h at 4°C in 4% paraformaldehyde. Following fixation, the skin was imaged using a Leica Stellaris 8 confocal microscope.

[00346] Mouse skin RNA isolation and quantitative real-time PCR

[00347] Mouse skin tissue was placed into TRIzol reagent (thermo Fisher) and homogenized by bead beating for 10 min. RNA was isolated using the Direct-zol RNA MiniPrep Plus kit according to manufacturer's instructions (Zymo Research). RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad). Primers (*sspA* primers *sspA-F* AND *sspA-R*, *psmA1* primers *psmA1-F* and *psmA1-R*, and *hla* primers *hla-F* and *hla-R*) and cDNA were mixed with Power SYBR green PCR master mix (Life Technologies) and qPCR was performed using a QuantStudio Real-Time PCR instrument (Thermo Fisher).

[00348] Human skin swab RNA isolation quantitative real-time PCR

[00349] RNA was isolated using the Purelink RNA isolation kit according to manufacturer's instructions (Thermo Fisher Scientific). For human swabs, 250µL of sample was removed from collection tubes and added to 500µL of RNA Protect Bacteria Reagent (Qiagen) for 10min at RT, then pelleted (13,000RPM, 10', RT). Pellet was resuspended with 700µl of RNA lysis buffer with 1% Beta-mercaptoethanol followed by column-based isolation of RNA. RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad). qPCR reactions were run on a CFX96 Real-Time Detection System (Bio-Rad) with cDNA, 2x SYBR Green qPCR Master Mix, and *sspA* specific primers *sspA-F* and *sspA-R* previously described⁷⁸.

[00350] Cheek injections and measurement of itch and pain

[00351] Mouse cheeks were shaved 2 days before experiments and were habituated for 30 minutes in iBOB chambers. Male and female mice were injected intradermally in the cheek with 20µL of PBS, V8 protease (40U), histamine (100µg), or capsaicin (40µg). For injections with antagonists, V8 protease was mixed with vorapaxar, SCH79797, or BMS986120 30 minutes prior to injection. Immediately after injection, mice were placed into iBOB chambers and recovered for 30 minutes. Itch and pain behaviors were scored by blinded observers.

[00352] Alloknesis

[00353] The napes of mouse necks were shaved 2 days before experiments and mice were habituated in alloknesis chambers for 1 hour. Mice were injected intradermally in the upper back with 50µL of PBS, V8 protease (40U), or histamine (100µg). The skin surrounding the injection site was mechanically stimulated for 1 second 3 times in a row with a 0.07g Von Frey filament, and this was repeated 3 times for a total of 9 stimulations.

[00354] KERTr cell culture and adherence assays

[00355] KERTr immortalized human keratinocytes were obtained from the American Type Culture Collection (#CRL-2309) and maintained in keratinocyte serum-free medium (Gibco #17005-042) with

added Keratinocytes Supplements (Gibco #37000-015) including bovine pituitary extract (BPE; Gibco 13028-014) and human recombinant epidermal growth factor (EGF, Gibco #10450-013) and further supplemented with 35 ng/mL human recombinant epidermal growth factor (EGF; BD #354052).

[00356] Assays to quantify cell surface-adherent bacteria were performed as previously described¹¹⁸. Briefly, MRSA strains were grown to mid-log phase to infect confluent cell monolayers (multiplicity of infection [MOI], 1). Following a 30 min incubation, cells were treated with trypsin and lysed with 0.025% Triton X-100. The lysates were then serially diluted and plated on tryptic soy agar (TSA) to enumerate bacterial CFU. Experiments were performed four times with four replicates per MRSA strain, and results from a representative experiment are shown in Fig. 12D.

[00357] Expression and purification of recombinant PAR1 N-terminus

[00358] A codon-optimized sequence of human PAR1A22-T102 with a starting methionine was cloned into vector pTEV20 at the BspQ1 site¹¹⁹. The construct was transformed into *E. coli* BL21-Rosetta cells (Novagen) and grown overnight in LB supplemented with ampicillin (100 µg/mL) and chloramphenicol (10 µg/mL) at 37 °C. Overnight was subcultured at a 1:8 dilution in Terrific Broth (TB, BD Difco) supplemented with ampicillin (100 µg/mL) and chloramphenicol (10 g/mL) and grown at 37 °C shaking at 220 RPM to a cell density of OD600 0.8-1.0 as measured in a cuvette with 1 cm pathlength. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added at a final concentration of 1 mM, and the culture was incubated at 25 °C for 2 hours. Cells were then centrifuged at 3,900 $\times$ g in an Eppendorf 5810R centrifuge set at 4 °C for 30 minutes. Centrifuged cell paste was stored at -80 °C. For purification, thawed cell paste was resuspended in Buffer A (0.1 M sodium phosphate buffer, pH 7.8, 0.2 M NaCl, 6 M urea) at a ratio of 5 mL buffer: 1 g cell mass, and cells were lysed by pushing lysate through a 28G needle attached to a syringe three times or until cells were lysed. Lysate was centrifuged at 3,900 $\times$ g in an Eppendorf 5810R centrifuge set at 4 °C for 30 minutes, and supernatant was filtered with a 0.45 µm filter. Filtered lysate was loaded onto a 5 mL HisTrap-FF column (Cytiva) equilibrated with Buffer A. A gradient of Buffer B (0.1 M sodium phosphate buffer, pH 7.8, 0.2 M NaCl, 0.5 M imidazole, 6 M urea) was applied from 0-100% over 10 column volumes (CV). PAR1^{A22-T102}-His₆ eluted at 41-56% Buffer B. Fractions were collected, dialyzed thrice in 1 L storage buffer (0.01 M sodium phosphate buffer pH 7.8, 6 M urea), and flash frozen in liquid nitrogen for storage at -80 °C.

[00359] Limited proteolysis of PAR1 N-terminus

[00360] Unless otherwise specified, tubes were incubated at room temperature for 10 min on a tube revolver (Thermo Scientific) set to 15 speed, and beads were separated from supernatant with a magnetic separation rack for 2 min (New England Biolabs). Per tube, 400 µL of Pierce™ Ni-NTA Magnetic Agarose Beads were equilibrated with storage buffer, and 1.5 mg of thawed PAR1 recombinant N-terminus was applied to the beads. Beads were washed thrice with storage buffer and thrice with reaction buffer (0.01 M sodium phosphate buffer, pH 7.8) to wash off unbound protein and renature N-terminus. V8 protease (E.C. 3.4.21.19, Sigma) was added at a concentration of 10 µg/mL V8 protease in 500 µL total volume, and reaction was incubated rotating for 30 minutes. Supernatant was removed and added to

formic acid for a final concentration of 0.5% formic acid and immediately flash frozen. Resin beads were washed thrice with reaction buffer and incubated for 10 minutes with 400 μ L elution buffer (0.01 M sodium phosphate buffer, pH 7.8, 500 mM imidazole). Supernatant was removed and added to formic acid for a final concentration of 2% formic acid and immediately flash frozen. Samples were submitted to Dr. Greg Sabat at the University of Wisconsin-Madison Mass Spectrometry Core for solid phase purification and LC-MS/MS analysis.

[00361] Mass Spectrometry

[00362] Samples were desalted using Pierce C-18 Tips, 100 μ L bed (ThermoFisher Scientific) per manufacturer protocol and eluted in 10 μ L of 70/30/0.1% ACN/H₂O/TFA. Dried to completion in the speed-vac and finally reconstituted in 20 μ L of 0.1% formic acid / 3% ACN. Peptides were analyzed by nanoLC-MS/MS using the Agilent 1100 nanoflow system (Agilent) connected to hybrid linear ion trap-orbitrap mass spectrometer (LTQ-Orbitrap Elite™, Thermo Fisher Scientific) equipped with an EASY-Spray™ electrospray source (held at constant 35°C). Chromatography of peptides prior to mass spectral analysis was accomplished using capillary emitter column (PepMap® C18, 3 μ M, 100Å, 150x0.075mm, Thermo Fisher Scientific) onto which 2 μ L of extracted peptides was automatically loaded. NanoHPLC system delivered solvents A: 0.1% (v/v) formic acid, and B: 99.9% (v/v) acetonitrile, 0.1% (v/v) formic acid at 0.50 μ L/min to load the peptides (over a 30 minute period) and 0.3 μ L/min to elute peptides directly into the nano-electrospray with gradual gradient from 0% (v/v) B to 30% (v/v) B over 80 minutes and concluded with 5 minute fast gradient from 30% (v/v) B to 50% (v/v) B at which time a 5 minute flash-out from 50-95% (v/v) B took place. As peptides eluted from the HPLC-column/electrospray source survey MS scans were acquired in the Orbitrap with a resolution of 120,000 followed by CID-type MS/MS fragmentation of 30 most intense peptides detected in the MS1 scan from 350 to 1800 m/z; redundancy was limited by dynamic exclusion. Elite acquired MS/MS data files were converted to mgf file format using MSConvert (ProteoWizard: Open Source Software for Rapid Proteomics Tools Development). Resulting mgf files were used to search against Uniprot Escherichia coli reference database (UP000000625, 4,520 total sequences) appended with PAR1 (1-102 aa) protein along with a cRAP common lab contaminant database (116 total entries) using in-house Mascot search engine 2.7.0 [Matrix Science], assuming the digestion enzyme GluC, with fixed Cysteine carbamidomethylation and variable Methionine oxidation plus Asparagine or Glutamine deamidation. Peptide mass tolerance was set at 10 ppm and fragment mass at 0.6 Da. Protein annotations, significance of identification and spectral based quantification was done with Scaffold software (version 4.11.0, Proteome Software Inc., Portland, OR). Peptide identifications were accepted if they could be established at greater than 60.0% probability to achieve an FDR less than 1.0% by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 98.0% probability to achieve an FDR less than 1.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii, Al et al Anal. Chem. 2003;75(17):4646-58). Proteins that contained similar peptides and could not be differentiated based on

MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins sharing significant peptide evidence were grouped into clusters.

[00363] Culturing and calcium imaging of mouse DRG neurons

[00364] One day prior to calcium imaging, DRGs from male and female mice were collected and digested using a mixture of 2.5mg/mL Collagenase A and 1mg/mL Dispase II for 40 min. A single cell suspension was obtained by triturating the samples through 18G, 21G, and 26G needles. Neurons were separated from other cells and debris using BSA gradient and plated onto laminin-coated 35mm dishes or 96-well plates in Neural Basal Medium (NBM) (Thermo Fisher) supplemented with B27 serum-free supplement (Invitrogen), L-Glutamine (Invitrogen), Pen/Strep (Cellgro), and 25ng/mL NGF (Invitrogen).

[00365] Neurons were cultured overnight at 37°C with 5% CO₂, then loaded with 5µM Fura-2-AM in NBM for 30 min. For experiments with histamine, chloroquine, S1P, and capsaicin, neurons were washed with Krebs-Ringer buffer (KR) (Boston BioProducts) and 100µL of KR, V8 (69.2µM), histamine (100µM), chloroquine (1mM), S1P (1µM), capsaicin (1µM), and KCl (100µM) were sequentially pipetted into the 35mm dish. Images were acquired with alternating 340/380 nm excitation wavelengths using a Nikon Eclipse Ti inverted microscope and Zyla sCMOS camera (Andor). For experiments with Hla, fMLF, and V8 protease, neurons were washed with KR and 100µL of KR, V8, Hla, fMLF, and KCl were sequentially pipetted onto the cells. Ratiometric analysis of 340/380 signal intensities was performed as described in¹⁴. For V8 dose-response experiments, calcium imaging of neurons in 96-well plates was performed as described in¹²⁰.

[00366] Culturing and calcium imaging of human neurons

[00367] Human lumbar DRGs were cleaned of any fat and connective tissue surrounding the ganglion. The protocol for the dissociation of DRGs for calcium imaging was inspired by¹²¹. The DRG was cut into roughly 1-2 mm chunks and immersed in pre-warmed 5 ml enzyme solution containing 2 mg/ml STEMxyme I (Worthington, LS004106) and 0.1 mg/ml DNase I (Worthington, LS002139) in HBSS without calcium and magnesium (ThermoFisher, 14170161). The tissue-enzyme solution was gently and constantly mixed at 37°C in a shaking water bath. The tissue was triturated every 20 min using fire-polished glass pipette until a roughly homogenous solution was obtained (about 40 min). This mixture was passed through a 100 µm cell strainer (Corning, 352360) and the flow-through was layered onto 3ml of 10% bovine serum albumin density gradient. The resulting solution was centrifuged for 900 x g for 5 min at room temperature. The supernatant was removed and the pellet was resuspended in BrainPhys media (STEMCell, 05790) containing 1% penicillin-streptomycin (ThermoFisher, 15070063), 1% N2-A (STEMCell, 07152), 2% SM1 (STEMCell, 05711) and 1% GlutaMax (ThermoFisher, 35050061). Cells were plated onto 35 mm dishes that were pre-coated with poly-D-lysine (>300,000, Sigma Aldrich, P7405) overnight and then coated with laminin from human placenta (Sigma Aldrich, L6274) for 2-3 hours at 37°C right before culturing. Cells were initially cultured for 2 hours in a 50µl droplet followed by immersion in 2 ml of pre-warmed BrainPhys media.

[00368] Calcium imaging with Fluo-4 AM was performed 24 hours after plating. Fluo-4 AM (ThermoFisher, F14201) was reconstituted in 2% of Pluronic F-127 (20% in DMSO, ThermoFisher, P3000MP) in BrainPhys Imaging media (STEMCell, 05796). Cells were loaded with Fluo-4 AM (1:100) for 30 min prior to imaging. The Fluo-4 AM solution was replaced with 2mL of pre-warmed BrainPhys Imaging media. Cells were imaged at 20X on an Olympus IX73 inverted microscope and data was acquired using the MetaFluor software (Olympus). A 120 second baseline was acquired prior to the addition of V8 protease (2 mg/ml, Worthington, LS003605), capsaicin (400nM) at 500 seconds, and KCl (50mM) at 700 seconds for a total imaging time of 840 seconds. Cells with an increase in the intracellular Fluo-4 signal over 10% of their baseline were deemed to be responsive to V8 administration. Only cells that responded to KCl (50 mM) were used in the analysis. Calcium imaging was performed on cells from DRGs from two donors (data not shown).

[00369] RNAscope of mouse DRGs

[00370] Mouse samples for RNAscope were prepared as previously described¹²². Briefly, mouse DRGs were embedded in OCT and sectioned at 16µm on a cryostat. Sections were stored in -80C for 24 hrs, then brought to room temperature and fixed with 4% paraformaldehyde. In situ hybridization with *F2r* and *Tubb3* probes was performed using the RNAscope Multiplex V2 kit (ASD) according to manufacturer's instructions. All tissues were also tested with negative and positive control probe cocktails (ACD). Sections were imaged on a Leica Stellaris 8 confocal microscope.

[00371] RNAscope of human DRGs

[00372] Human samples for RNAscope were prepared as previously described⁶⁵. Frozen human DRG samples were gradually embedded in OCT in a cryomold. Tissue was cryosectioned at 20µm, thawing momentarily in order to adhere to the slide. In situ hybridization using the RNAscope Multiplex V2 kit (ACD) was performed according to the manufacturer's recommendations and with Akoya fluorescein, Cy3, Cy5 dyes, as previously described⁶⁵. All tissues were tested against a positive control probe cocktail (ACD) containing mRNAs with high, medium, and low expressions. Negative control probe against the bacterial DapB gene (ACD) was also used.

[00373] DRG sections were imaged on Olympus FV3000 confocal microscope at 20X or 40X magnification as per previously published parameters⁶⁵. Background lipofuscin was identified as large globular intracellular structures that autofluoresced at 488, 550, and 647 wavelengths. Lipofuscin was not analyzed. Three 20X images were obtained from each human DRG section, and three sections were imaged per human donor. Images were analyzed using Olympus CellSens software (v1.18) as previously described⁶⁵. Probes used in this study: Hs-F2R-C2 (ACD, 471081-C2), Hs-TRPV1-C3 (ACD, 415381-C3), Hs-NPPB-C1 (ACD, 448511).

[00374] Luciferase-based PAR cleavage assays

[00375] CHO-K1 cells stably transfected with nLuc-PAR1-eYFP, nLuc-PAR2-eYFP, and nLuc-PAR4-eYFP pcDNA3.1(+) plasmids separately were seeded in a 96-well cell culture plate (flat-clear bottom, polystyrene, Nunc, ThermoFisher Scientific) at a density of 1×10^4 cells per well and cultured

for 48 h in Ham's F-12 (1×) Nutrient Mix (Gibco ThermoFisher Scientific) supplemented with 1 mM L-Glutamine, 100 U/ml penicillin, 100 µg/mL streptomycin, 1 mM sodium pyruvate, 10% v/v heat inactivated Fetal Bovine Serum (FBS), and 600 µg/mL geneticin selective antibiotic (G418 Sulfate, Gibco® ThermoFisher Scientific). The cells were rinsed with HBSS (100 µL × 3) and incubated with 100 µL HBSS at 37°C for 15 minutes. Cell supernatant (50 µL) was aliquoted into a white 96-well plate (polystyrene, Nunc, ThermoFisher Scientific) to measure the basal luminescence. The cells were further incubated with 50 µL V8 protease or controls in a half-log scale concentrations at 37°C for 15 minutes, and 50 µL of cell supernatant from each well was aliquoted as before. Furimazine (2 µl/mL, Promega) was added and the nLuc cleavage of the receptor was measured on Mithras LB 940 (Berthold Technologies, measurement time: 1 s per well) plate reader. The luminescence measurements of the samples were normalized by subtracting the basal luminescence. The concentration-effect curves were plotted and analyzed using the dose-response stimulation three parameters model and the $\log_{10}EC_{50} \pm SEM$ values were obtained on GraphPad Prism 8.

[00376] PAR1 calcium signaling in HEK-293 cells

[00377] HEK-293 cells (0.01×10^6) were plated in poly-d-lysine coated black walled clear bottom 96 well plates (Nunc) and cultured for 48 h in DMEM containing 10% FBS (Gibco). Media was removed and cells were incubated with the calcium indicator Fluo-4 NW (Thermo Fisher Scientific) at 37°C for 30 minutes and for a further 15 minutes at room temperature in the dark. Agonists were added and Ca^{2+} mobilization induced change in fluorescence ($\lambda_{Ex}/\lambda_{Em}$ 494/516 nm) was measured in real time using a FlexStation3 microplate reader (Molecular Devices). The total run time for each spectrum was 180 s with baseline recorded for 20 s prior to the addition of agonists. To measure disarming of PAR1, cells were incubated with V8 for 15 minutes before measuring thrombin and TFLLR-NH₂ stimulated responses. (A23187, 6 µM, Sigma-Aldrich) was used as a control to ensure equivalent levels of Fluo-4 NW loading in each well.

[00378] PAR1 cleavage at HEK cell membrane

[00379] Dually tagged PAR1 in HEK cells was used as previously described¹²³. Cells were placed in growth media containing E64 (5µM) and heparin (5 units/ml). After 1h stimulation, cells were fixed with 10% buffered formalin, and images were recorded with confocal microscope (Infinity image facility, Toulouse, France).

[00380] Skin cell preparation and flow cytometry

[00381] Skin from mice injected intradermally with PBS or V8 protease were obtained 30 minutes after injection. Cells were isolated and stained for flow cytometry as previously described⁴⁴. Cells were preincubated with the Fcγ receptor-specific blocking mAb (clone 2.4G2, BioLegend) and washed before staining mAbs and analysis on LSR Fortessa (BD Biosciences). Data were analyzed with FlowJo software.

[00382] Intrathecal siRNA injection

[00383] Intrathecal delivery of siRNA was performed as in ³⁷. Briefly, siRNA purchased from Thermo Scientific were mixed with In Vivo JetPEI (Polyplus Transfection) according to the manufacturer's protocol. The N:P ratio used was 6. Mice were injected intrathecally between L5 and L6 spinal levels with 5µL volume for 3 days in a row prior to itch experiments.

[00384] Mouse DRG RT-qPCR

[00385] One day after the last intrathecal siRNA injection, thoracic DRGs were dissected out of mice and placed in RNAprotect reagent (Qiagen). Tissues were homogenized by beadbeating with 0.1mm silica beads, RNA was extracted with the NucleoSpin RNA isolation kit (Macherey Nagel) and converted to cDNA with the iScript cDNA synthesis kit (Bio-Rad) following manufacturer's instructions. Primers (*il31ra* primers il31ra-F and il31ra-R, or *f2r* primers f2r-F, f2r-R) and cDNA were mixed with Power SYBR green PCR master mix (Life Technologies) and qPCR was performed using a QuantStudio Real-Time PCR instrument (Thermo Fisher).

[00386] Oral Vorapaxar treatment

[00387] Mice were gavaged daily with 100µL volume of either vehicle or Vorapaxar solution starting 2-days prior to *S. aureus* topical application and until the endpoint of 5 days.

QUANTIFICATION AND STATISTICAL ANALYSIS

[00388] Analysis of data was performed using GraphPad Prism version 9.5.1 (GraphPad Software). Comparison of data between two groups was performed using Mann-Whitney tests. Comparison of data between more than two groups with one independent variable was performed using one-way ANOVA with Tukey's multiple comparisons tests. Comparison of two independent variables was performed using two-way ANOVA with Tukey's multiple comparisons tests. P-values of less than 0.05 were considered statistically significant. Statistical details including statistical tests used, number of samples (n), and P-values are reported in description of the drawings.

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Claims

- 1) A method for treating or preventing itch caused by a microbial exposure in a subject, the method comprising; administering to a subject in need thereof an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch.
- 2) The method of claim 1, wherein the agent that inhibit PAR1 is selected from the group consisting of: an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule.
- 3) The method of claim 2, wherein the small molecule is selected from the group consisting of Vorapaxar, Atopaxar (E5555), Parmodulin 2 (PM2, ML161), SCH 79797, FR171113, and RWJ-56110, RWJ-58259.
- 4) The method of claim 2, wherein the inhibitory nucleic acid encodes an inhibitor of PAR1.
- 5) The method of claim 2, wherein the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits PAR1.
- 6) The method of claim 1, wherein the administering occurs at the site of microbial exposure.
- 7) The method of any of claims 1-5, wherein the agent that inhibits PAR1 inhibits the expression of PAR1.
- 8) The method of claim 7, wherein the agent inhibits the expression of PAR1 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.
- 9) The method of any of claims 1-6, wherein the agent that inhibits PAR1 inhibits the function of PAR1.
- 10) The method of claim 9, wherein the agent inhibits the function of PAR1 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.
- 11) The method of claim 1, wherein the microbial exposure comprises a *Staphylococcus* exposure.
- 12) The method of claim 11, wherein the *Staphylococcus* exposure comprises *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus capitis* (*S. capitis*) and *Staphylococcus hominis* (*S. hominis*).

- 13) The method of claim 12, wherein *S. aureus* is methicillin resistant *S. aureus*.
- 14) The method of claim 1, wherein the microbial exposure comprises a bacterium which is resistant to at least one antibiotic.
- 15) The method of claim 1, wherein the microbial exposure comprises a bacterium which is resistant to at least two antibiotics.
- 16) The method of claim 1, wherein the microbial exposure comprises *Streptococcus pyogenes* (*S. pyogenes*).
- 17) The method of any one of claims 1-16, wherein the microbial exposure is colonization.
- 18) The method of any one of claims 1-16, wherein the microbial exposure is epicutaneous colonization.
- 19) The method of claims 17 or 18, wherein colonization or epicutaneous colonization is not an infection.
- 20) The method of claims 17 or 18, wherein colonization or epicutaneous colonization does not elicit an immune response from the subject.
- 21) The method of claims 17 or 18, wherein colonization or epicutaneous colonization elicits a sub-clinical immune response from the subject.
- 22) The method of any one of claims 1-16, wherein the microbial exposure is an infection.
- 23) The method of any one of claims 1-23, wherein microbial exposure occurs in a lesion.
- 24) The method of claim 23, wherein the lesion is associated with a condition selected from a group consisting of: atopic dermatitis, impetigo, prurigo nodularis, psoriasis.
- 25) The method of any one of claims 1-24, wherein the microbial exposure is acute or chronic.
- 26) The method of any one of claims 1-25, wherein the microbial exposure is a reoccurring exposure.
- 27) The method of any one of claims 1-26, further comprising administering to a subject a second therapeutic agent.
- 28) The method of claim 27, wherein the second therapeutic agent is an antibiotic, antifungal, or antimicrobial agent.
- 29) The methods of any one of claims 1-28, wherein the subject has previously been diagnosed with having a microbial exposure.

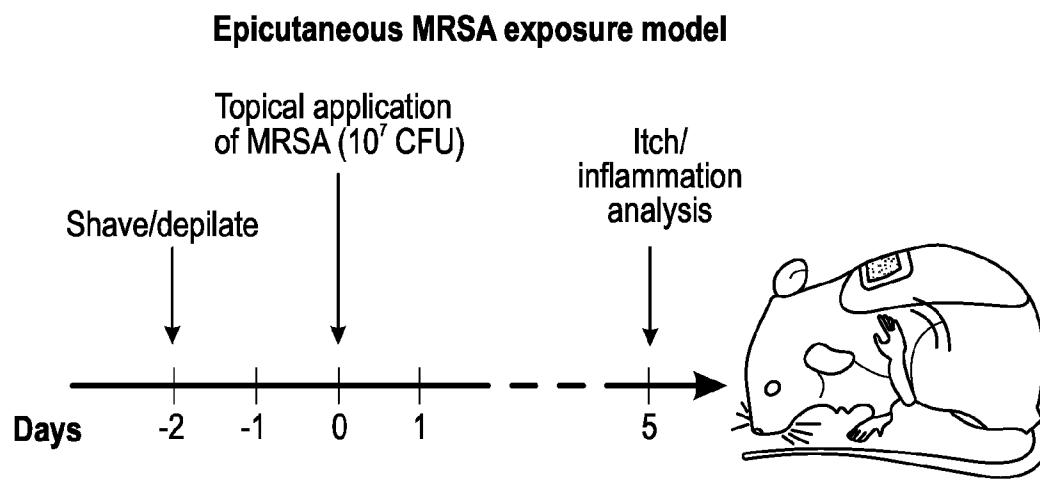
- 30) The methods of any one of claims 1-28, wherein the subject has not previously been diagnosed with having a microbial exposure.
- 31) The methods of any one of claims 1-28, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having itch associate with a microbial exposure.
- 32) The methods of any one of claims 1-28, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having or at risk of having itch associate with a microbial exposure.
- 33) The methods of any one of claims 1-28, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having a microbial exposure that can result in itch.
- 34) The methods of any one of claims 1-28, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having a microbial exposure that can result in itch.
- 35) The method of claim 1, wherein the administering is systemic or local administration.
- 36) The method of claim 35, wherein local administration is topical administration.
- 37) The method of claim 36, wherein systemic administration is intrathecal administration.
- 38) A method for treating or preventing itch caused by a *Staphylococcus* exposure in a subject, the method comprising; administering to a subject in need thereof an agent that inhibits *Staphylococcus* serine protease V8 in an amount and for a duration sufficient to treat or prevent itch.
- 39) The method of claim 38, wherein the agent that inhibit serine protease V8 is selected from the group consisting of: an antibody reagent, an inhibitory nucleic acid, peptide agonist, gene editing system, or a small molecule.
- 40) The method of claim 39, wherein the inhibitory nucleic acid encodes an inhibitor of serine protease V8.
- 41) The method of claim 39, wherein the inhibitory nucleic acid comprises siRNA, shRNA or miRNA that inhibits serine protease V8.
- 42) The method of claim 38, wherein the administering occurs at the site of *Staphylococcus* exposure.
- 43) The method of any of claims 38-41, wherein the agent that inhibits serine protease V8 inhibits the expression of serine protease V8.

- 44) The method of claim 43, wherein the agent inhibits the expression of serine protease V8 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.
- 45) The method of any of claims 38-42, wherein the agent that inhibits serine protease V8 inhibits the function of serine protease V8.
- 46) The method of claim 45, wherein the agent inhibits the function of serine protease V8 at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more as compared to an appropriate control.
- 47) The method of claim 46, wherein the function of serine protease V8 is cleaving PAR-1.
- 48) The method of claim 38, wherein the *Staphylococcus* exposure comprises *S. aureus*, *S. epidermidis*, *Staphylococcus capitis* (*S. capitis*) and *Staphylococcus hominis* (*S. hominis*).
- 49) The method of claim 38, wherein *S. aureus* is methicillin resistant *S. aureus*.
- 50) The method of any one of claims 38-49, wherein the *Staphylococcus* exposure is *Staphylococcus* colonization.
- 51) The method of any one of claims 38-49, wherein the *Staphylococcus* exposure is an epicutaneous colonization.
- 52) The method of claims 50 or 51, wherein colonization or epicutaneous colonization is not an infection.
- 53) The method of claims 50 or 51, wherein colonization or epicutaneous colonization does not elicit an immune response from the subject.
- 54) The method of claims 50 or 51, wherein colonization or epicutaneous colonization elicits a sub-clinical immune response from the subject.
- 55) The method of any one of claims 38-49, wherein the *Staphylococcus* exposure is an infection.
- 56) The method of any one of claims 38-49, wherein the *Staphylococcus* exposure occurs in a lesion.
- 57) The method of claim 56, wherein the lesion is associated with a condition selected from a group consisting of: atopic dermatitis, impetigo, prurigo nodularis, psoriasis.
- 58) The method of any one of claims 38-57, wherein the exposure is acute or chronic.

- 59) The method of any one of claims 38-58, wherein the *Staphylococcus* exposure is a reoccurring exposure.
- 60) The method of any one of claims 38-59, further comprising administering to a subject a second therapeutic agent.
- 61) The method of claim 60, wherein the second therapeutic agent is an antibiotic, antifungal, or antimicrobial agent.
- 62) The methods of any one of claims 38-61, wherein the subject has previously been diagnosed with having a *Staphylococcus* exposure.
- 63) The methods of any one of claims 38-61, wherein the subject has not previously been diagnosed with having a *Staphylococcus* exposure.
- 64) The methods of any one of claims 38-61, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having itch associate with a *Staphylococcus* exposure.
- 65) The methods of any one of claims 38-61, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having or at risk of having itch associate with a *Staphylococcus* exposure.
- 66) The methods of any one of claims 38-61, further comprising the step, prior to administering, diagnosing the subject of having or at risk of having a *Staphylococcus* exposure that can result in itch.
- 67) The methods of any one of claims 38-61, further comprising the step, prior to administering, receiving the results of an assay that diagnoses the subject of having a *Staphylococcus* exposure that can result in itch.
- 68) The method of claim 38, wherein the administering is systemic or local administration.
- 69) The method of claim 68, wherein local administration is topical administration.
- 70) The method of claim 68, wherein systemic administration is intrathecal administration.
- 71) A method for treating itch caused by a microbial exposure in a subject, the method comprising; topically administering to a subject having microbial exposure an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount and for a duration sufficient to treat or prevent itch, wherein administration occurs at the site of microbial exposure.

- 72) The method of any of the preceding claims, wherein the subject does not have a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.
- 73) The method of any of the preceding claims, wherein the subject is not being treated for a condition selected from the group consisting of Cerebral thromboembolism, Myocardial reinfarction, Peripheral arterial thromboembolism, and Thrombosis after PCI.
- 74) A composition for treating or preventing itch caused by a microbial exposure in a subject, the composition comprising; an amount of an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount sufficient to treat or prevent itch.
- 75) A composition for treating or preventing itch caused by a *Staphylococcus* exposure in a subject, the composition comprising; an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch.
- 76) The composition of claim 74 and 75, further comprising a pharmaceutically acceptable carrier.
- 77) The composition of claim 74 and 75, formulated for topical administration.
- 78) The composition of claim 74 and 75, formulated for systemic administration.
- 79) The composition of claim 74 and 75, further comprising a second therapeutic agent.
- 80) The composition of claim 79, wherein the second therapeutic agent is an antibiotic or antimicrobial agent.
- 81) Use of a composition for treating or preventing itch caused by a microbial exposure in a subject, the composition comprising; an amount of an agent that inhibits proteinase-activated receptor-1 (PAR1) in an amount sufficient to treat or prevent itch.
- 82) Use of a composition for treating or preventing itch caused by a *Staphylococcus* exposure in a subject, the composition comprising; an amount of an agent that inhibits serine protease V8 in an amount sufficient to treat or prevent itch.

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**FIG. 1A**

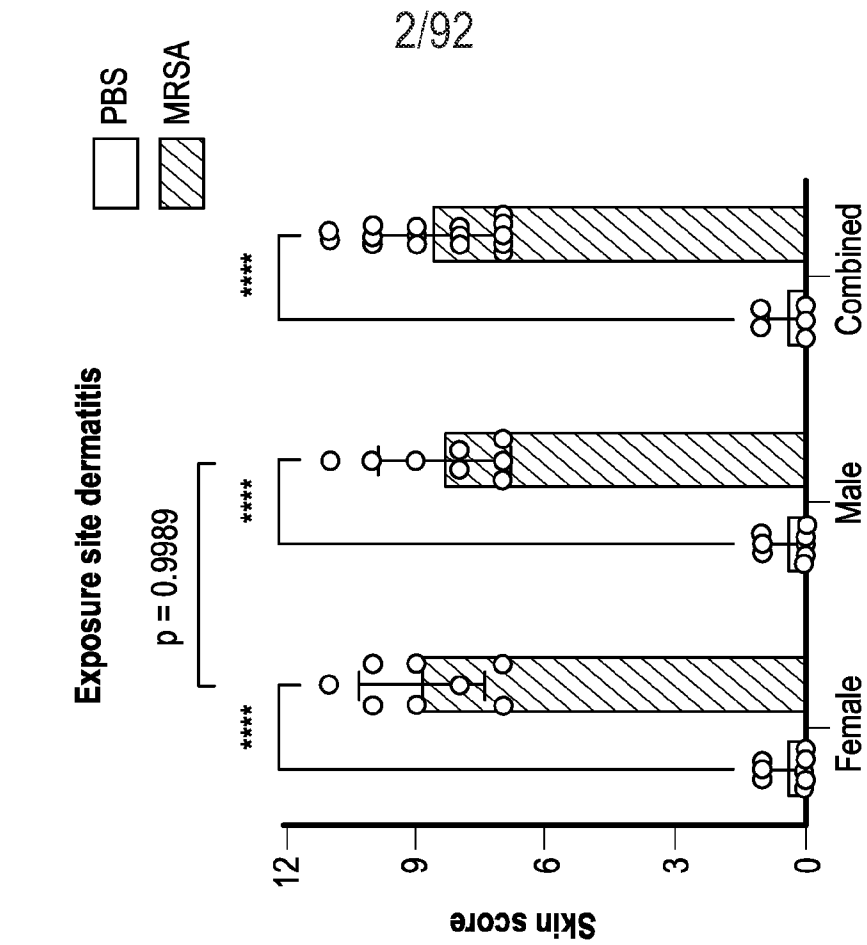
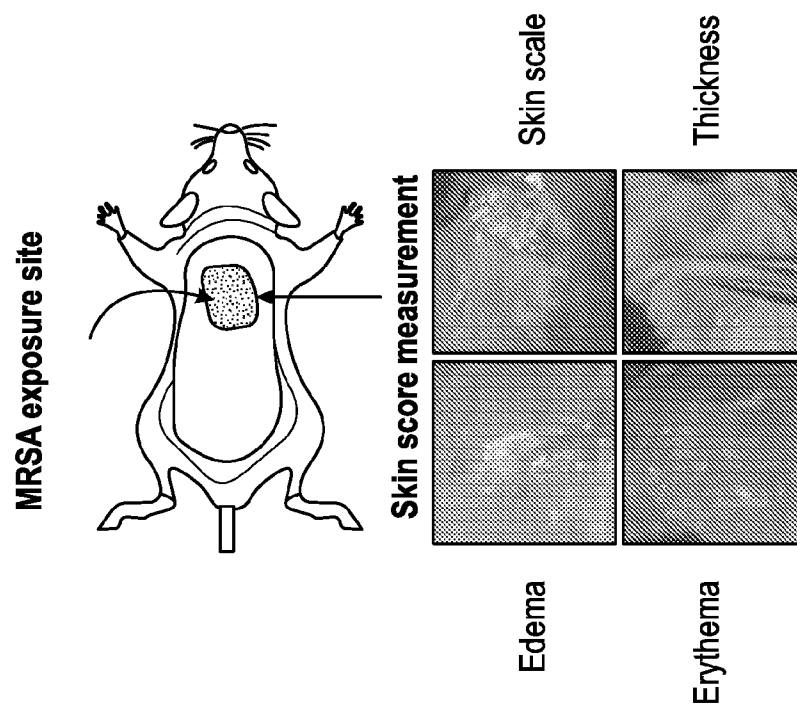
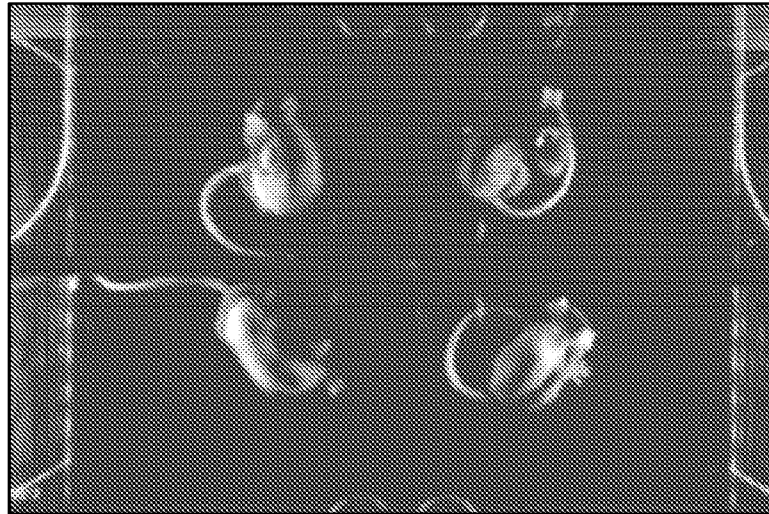


FIG. 1B



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Infrared behavior observation box
(iBOB)

FIG. 1C

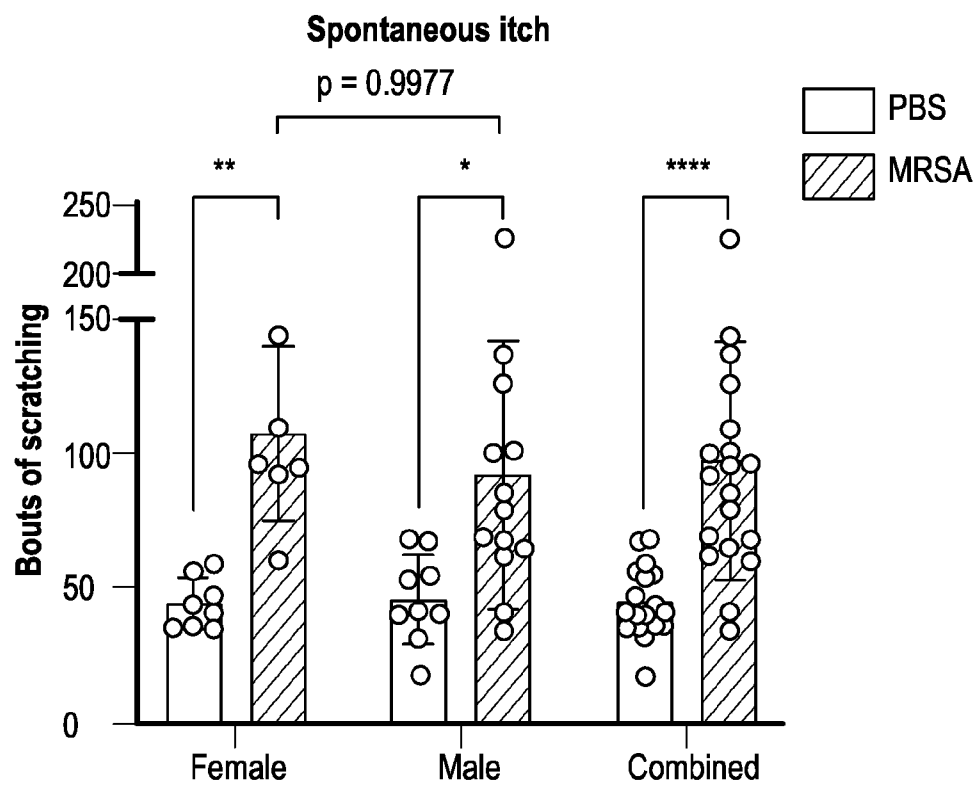


FIG. 1D

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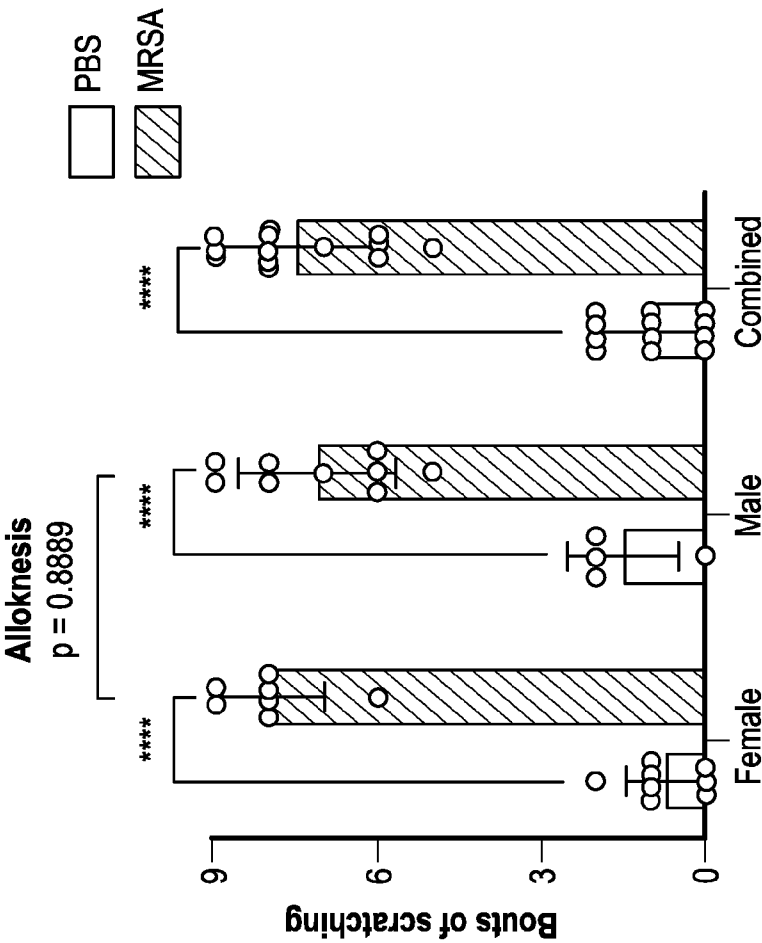
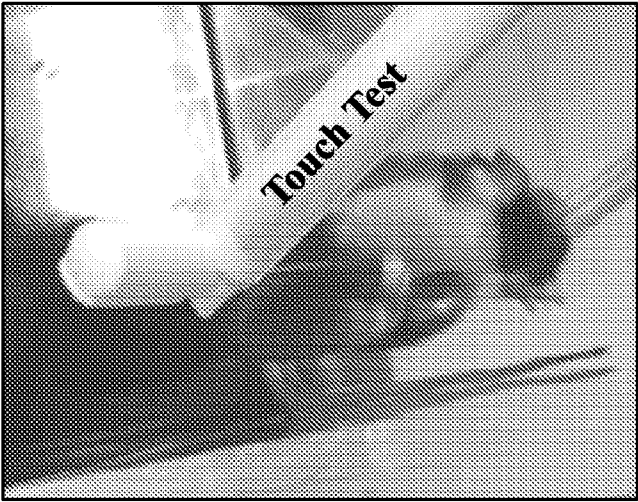


FIG. 1E



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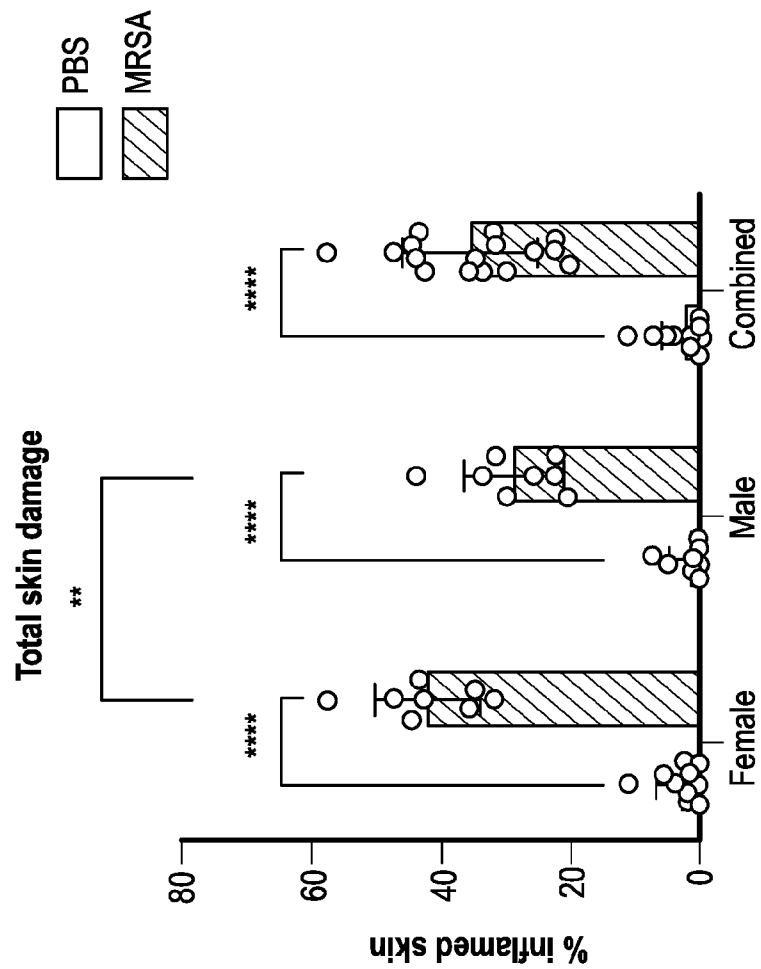
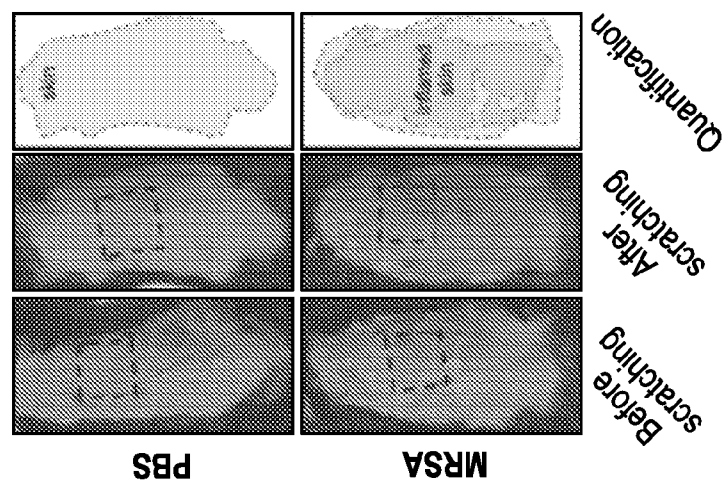


FIG. 1F



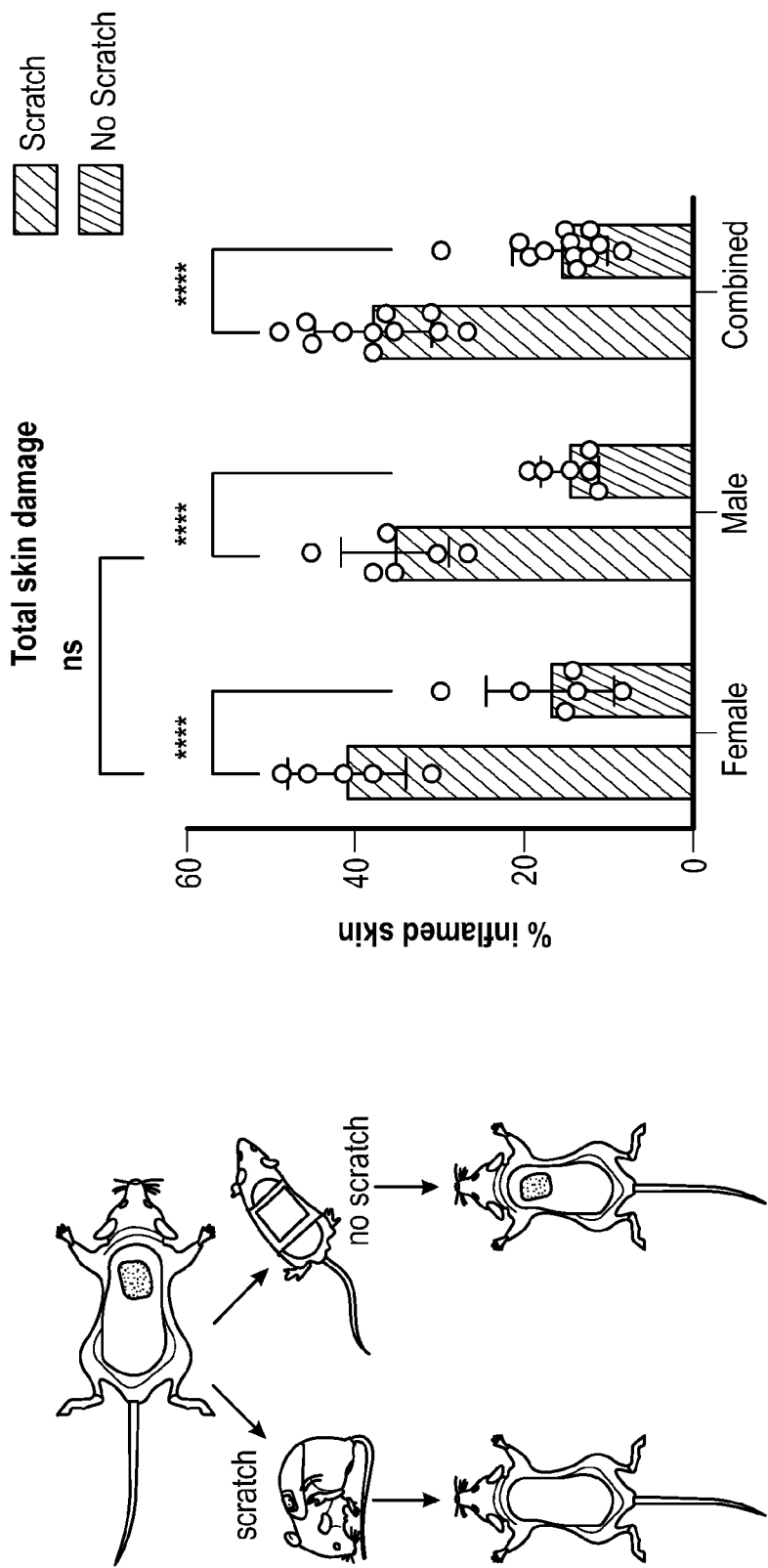


FIG. 1G

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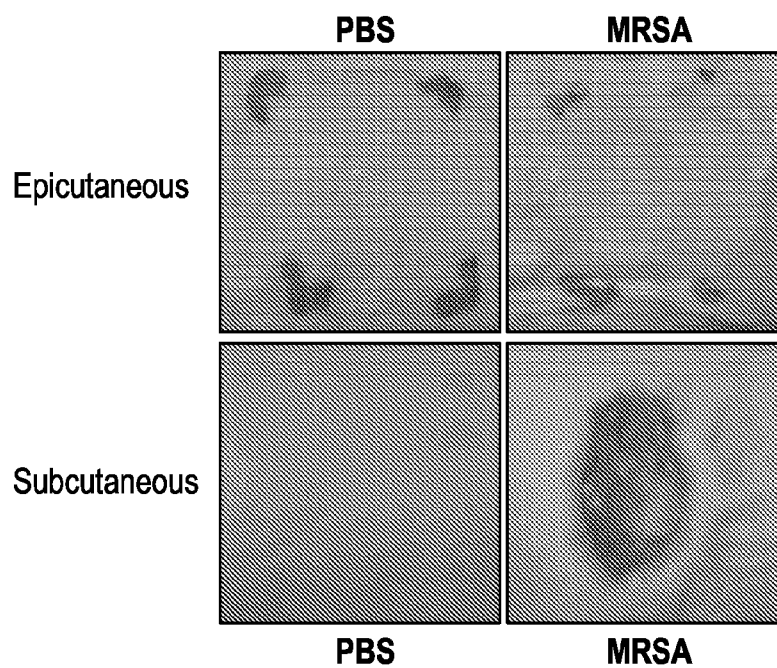


FIG. 1H

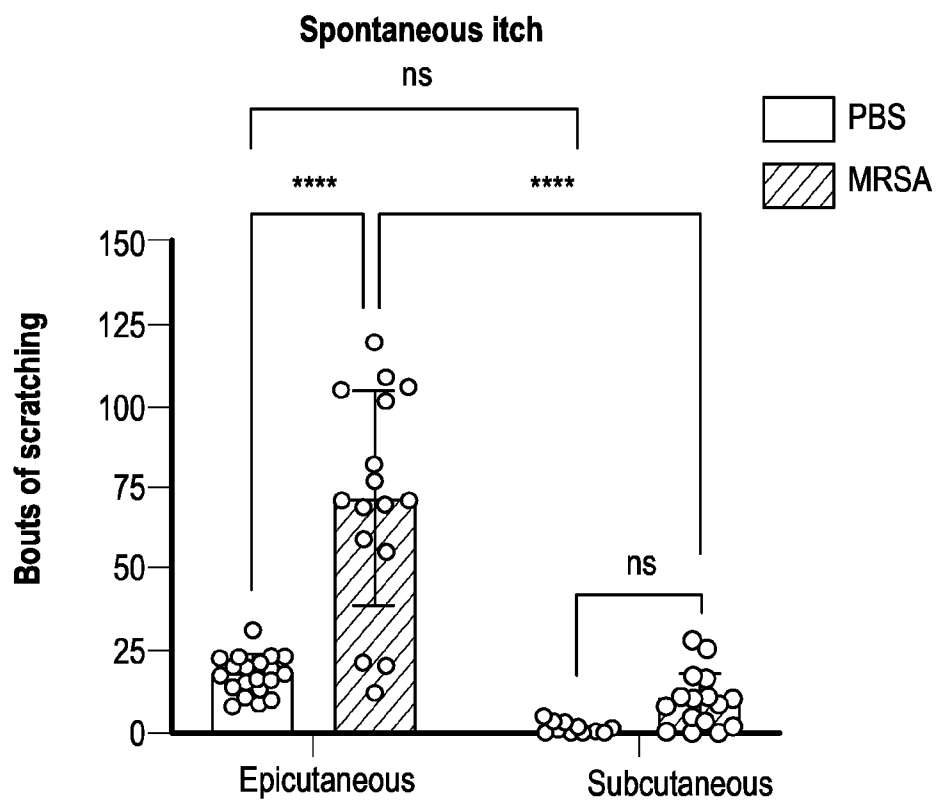


FIG. 1I

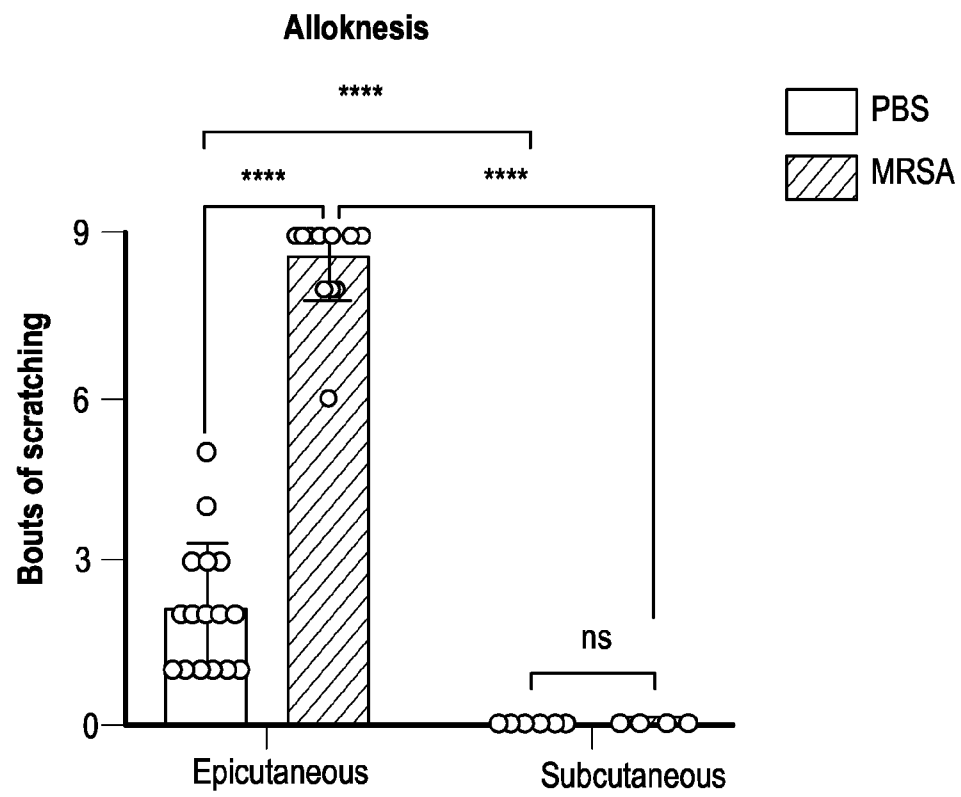


FIG. 1J

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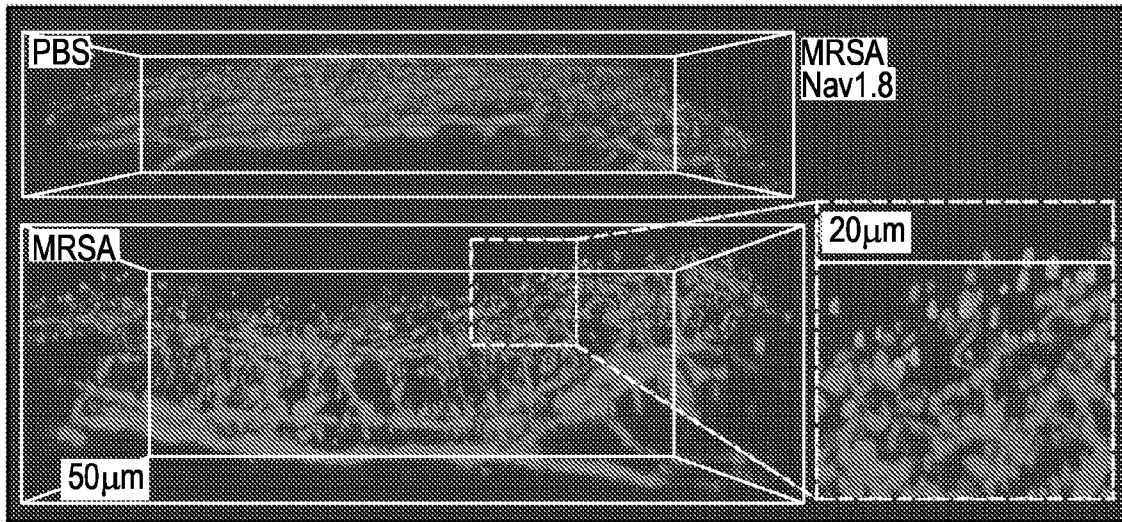


FIG. 2A

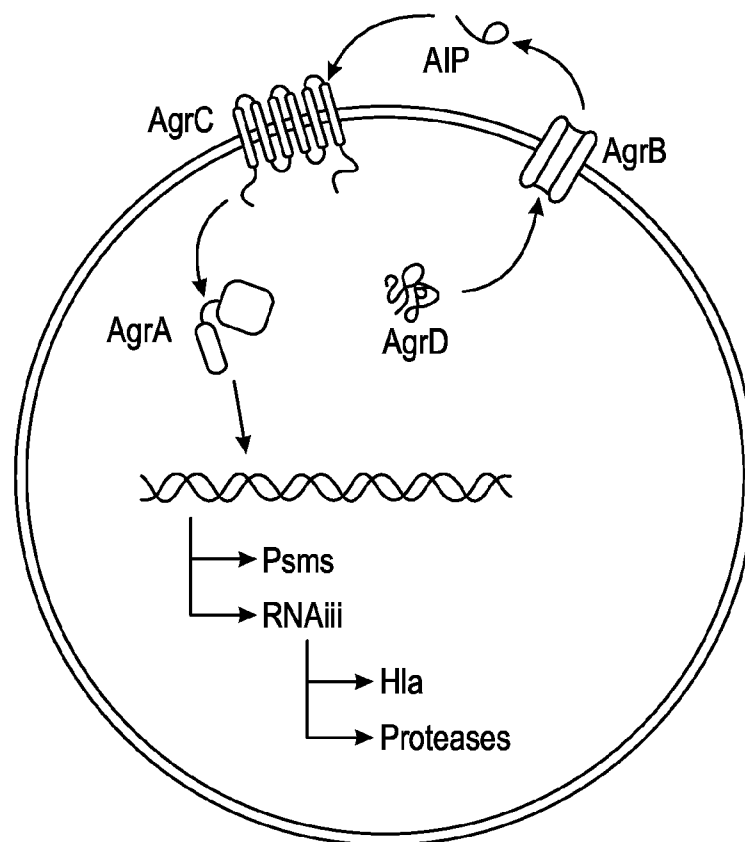


FIG. 2B

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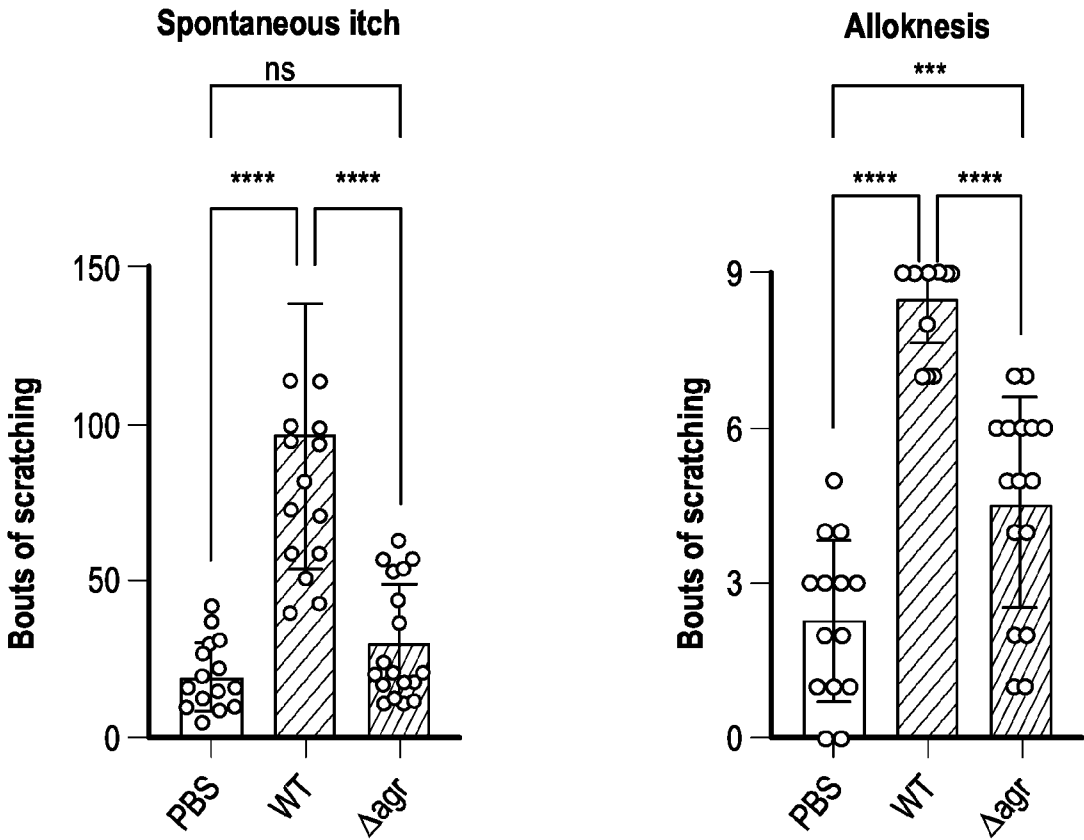


FIG. 2C

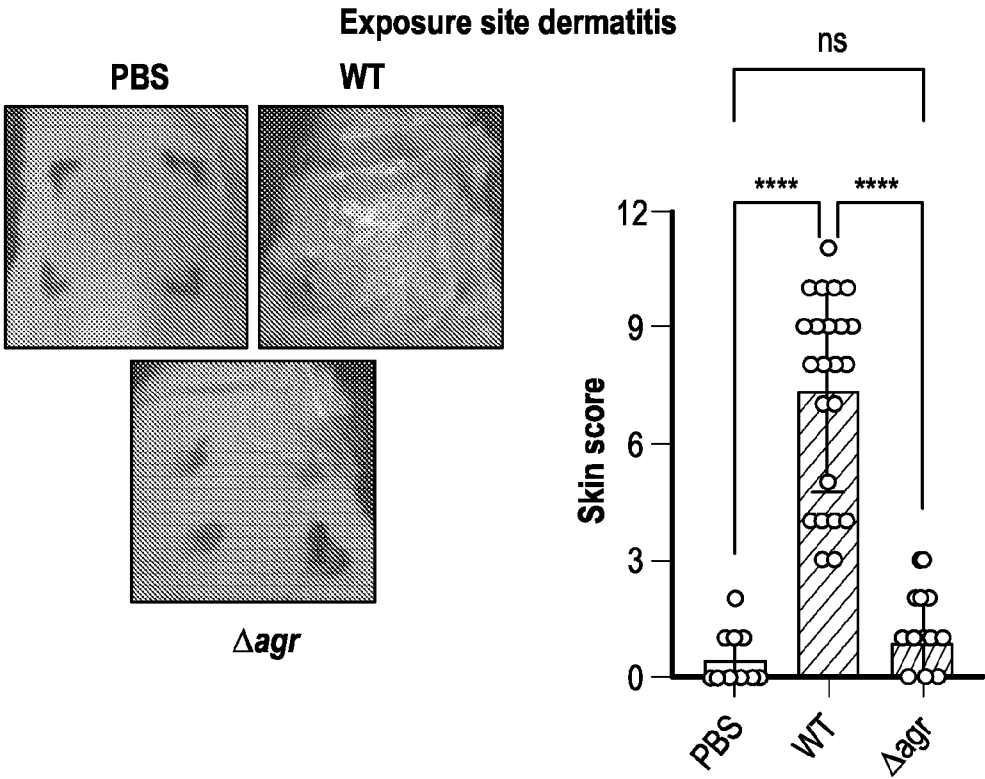


FIG. 2D

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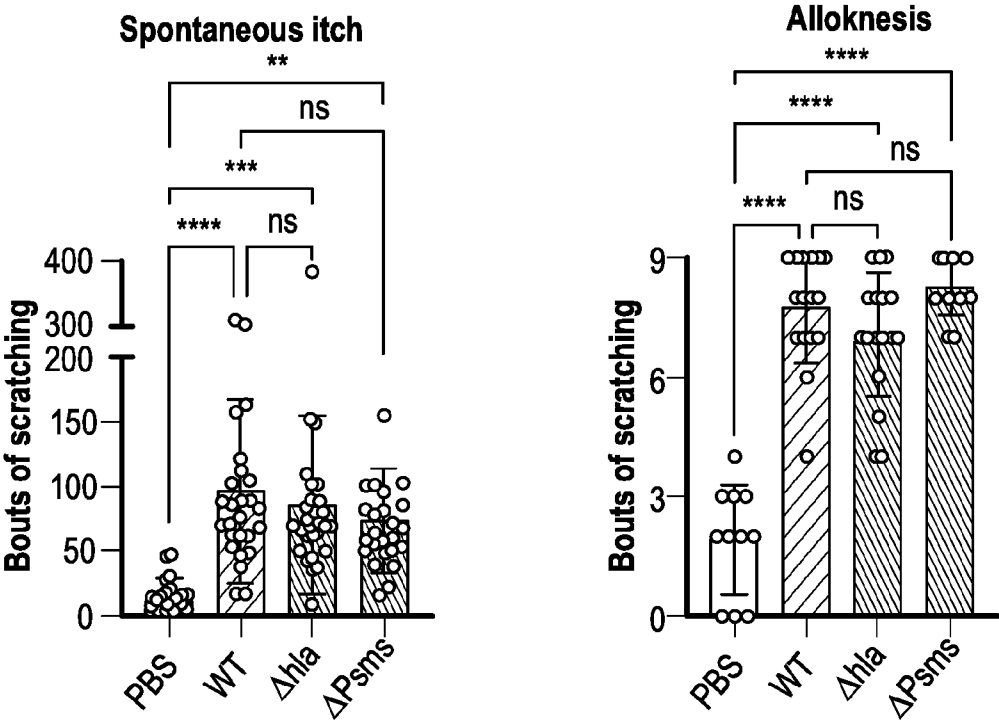


FIG. 2E

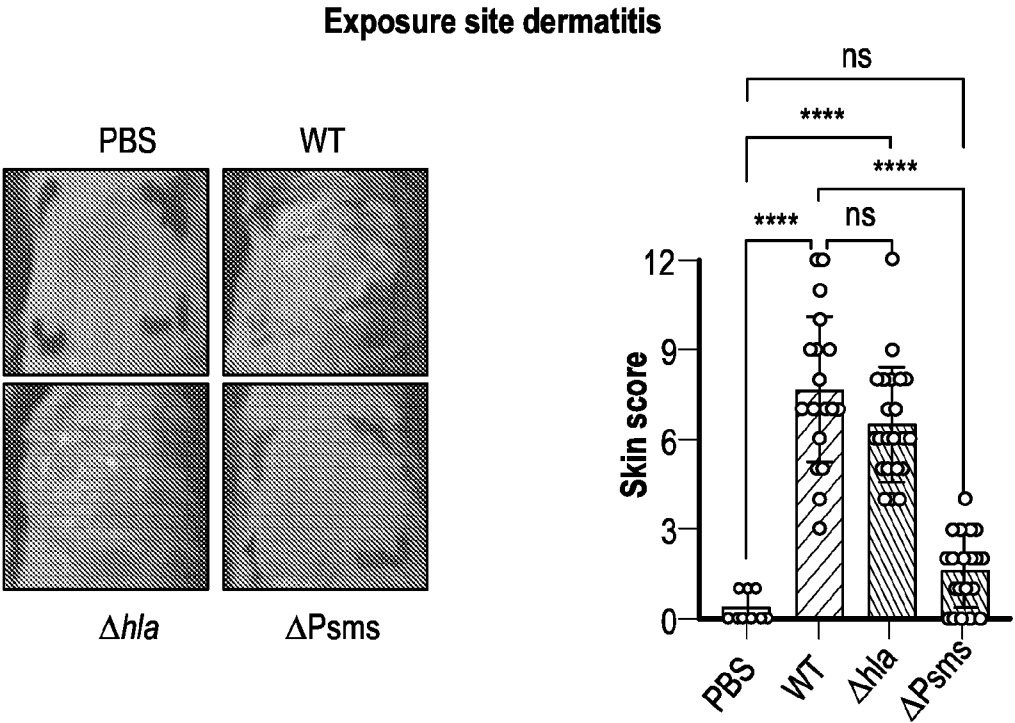


FIG. 2F

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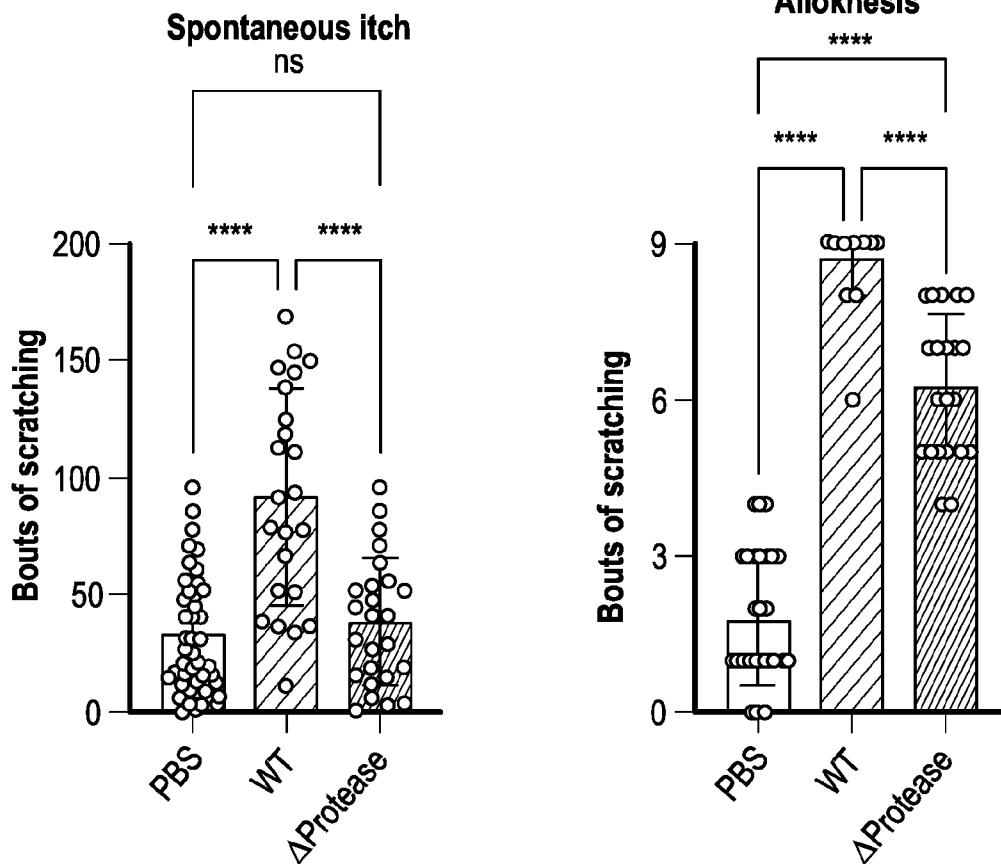


FIG. 2G

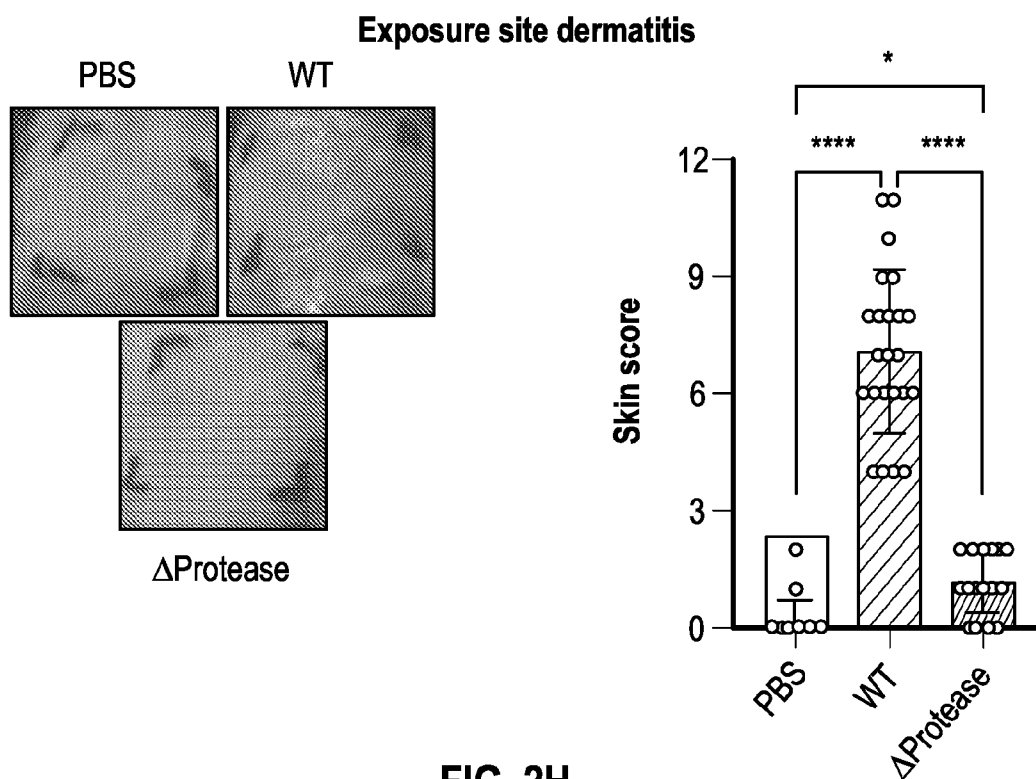


FIG. 2H

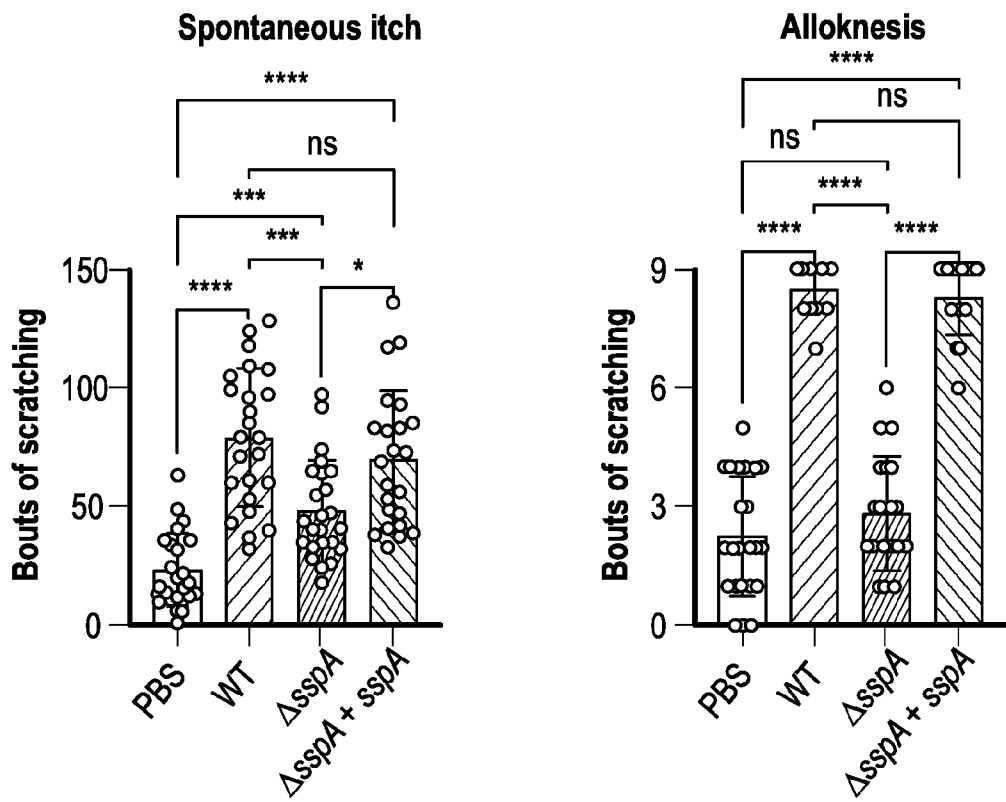


FIG. 3A

FIG. 3B

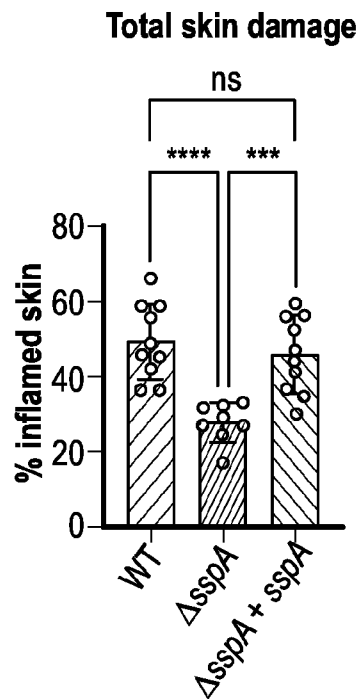


FIG. 3C

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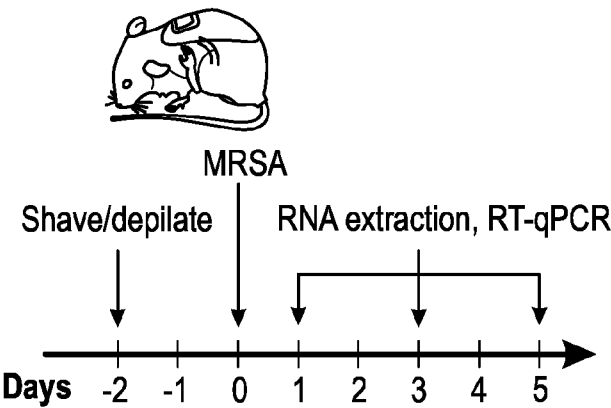


FIG. 3E

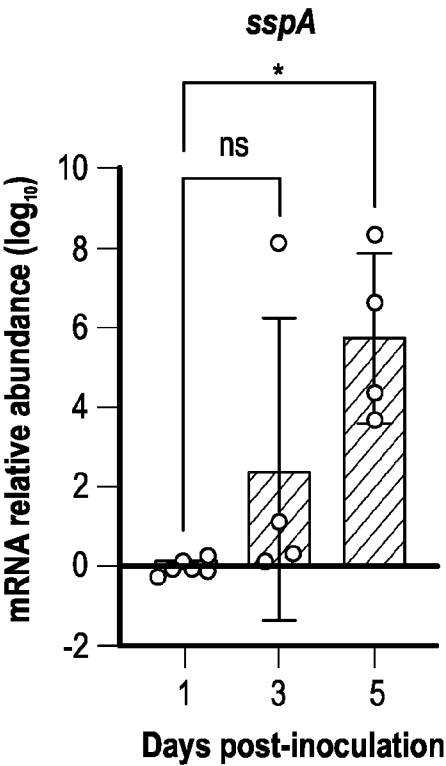


FIG. 3F

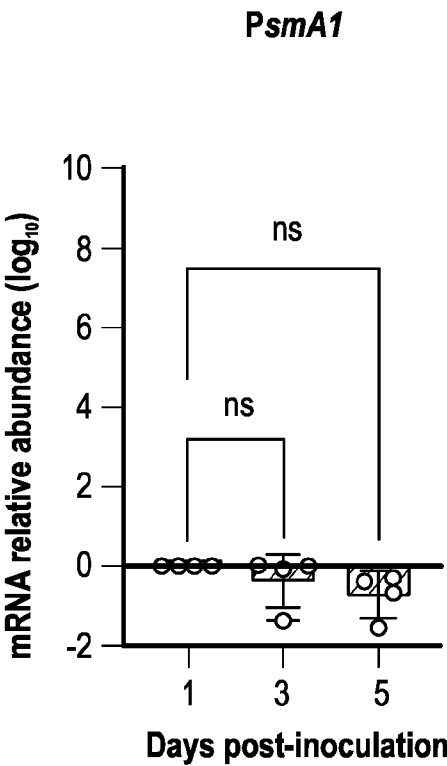


FIG. 3G

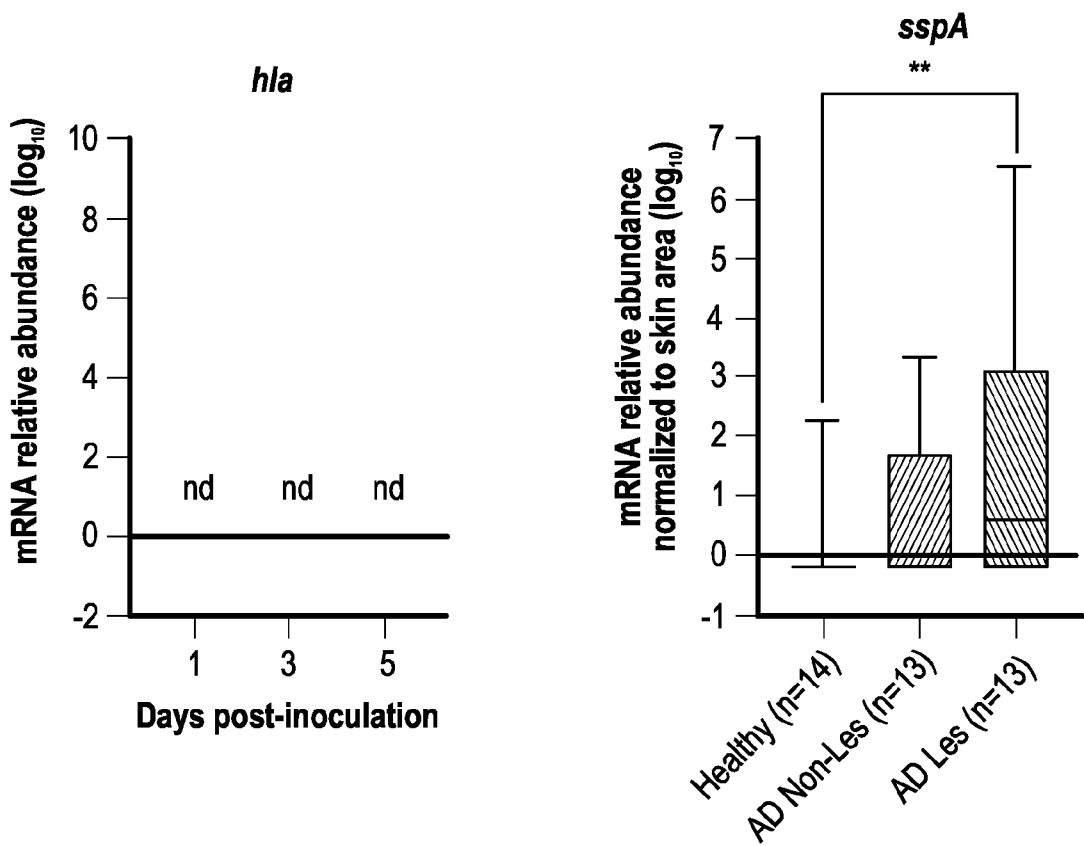


FIG. 3H

FIG. 3I

Acute spontaneous
itch and pain

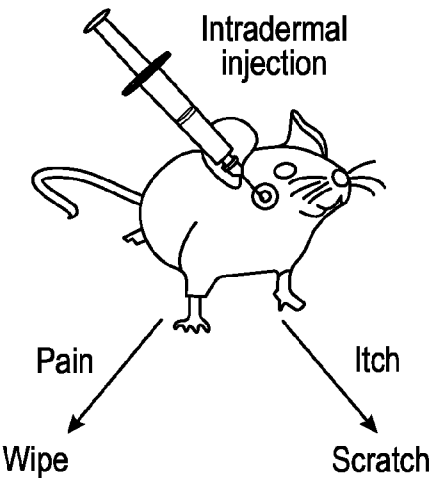


FIG. 3J

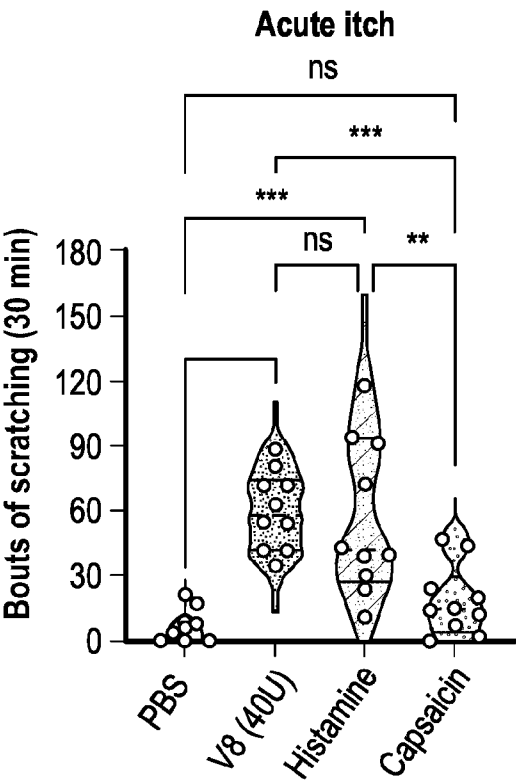


FIG. 3K

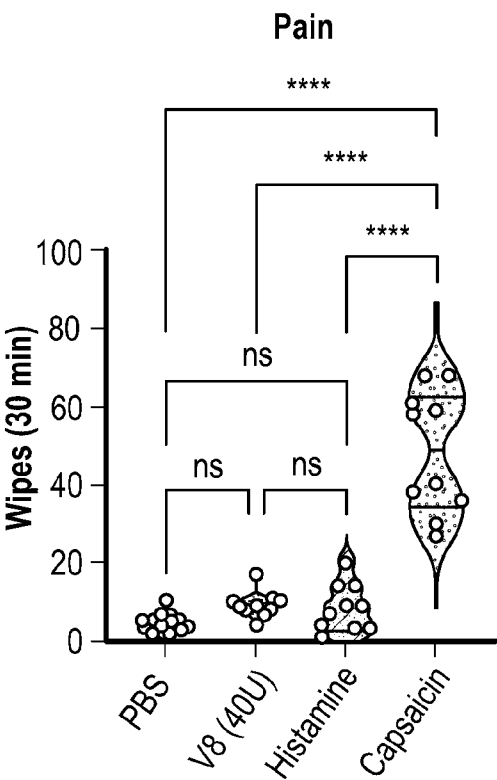


FIG. 3L

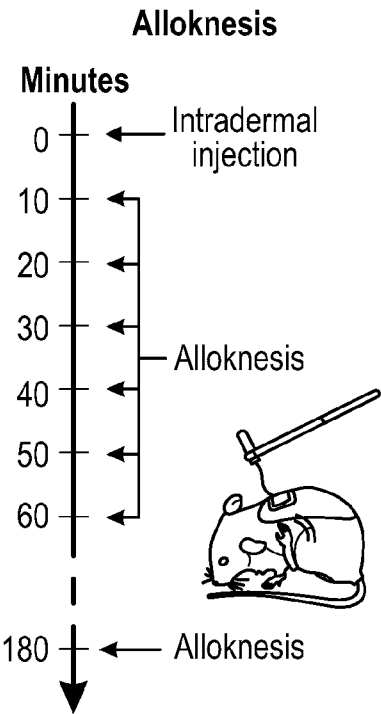


FIG. 3M

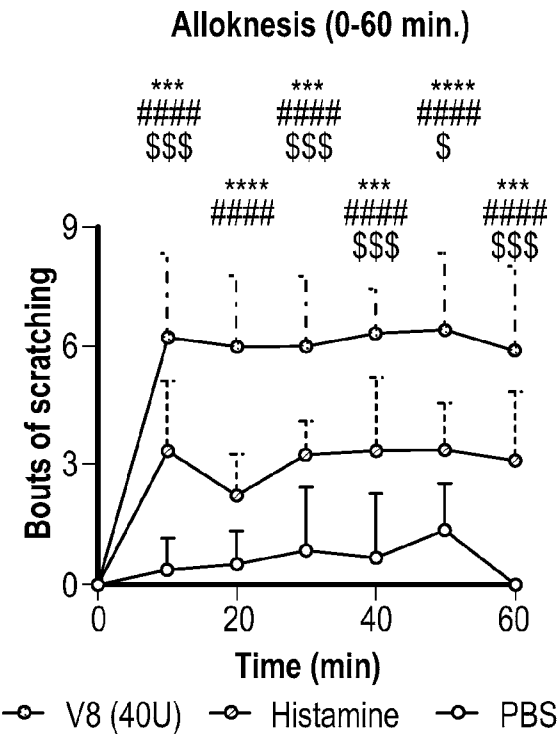
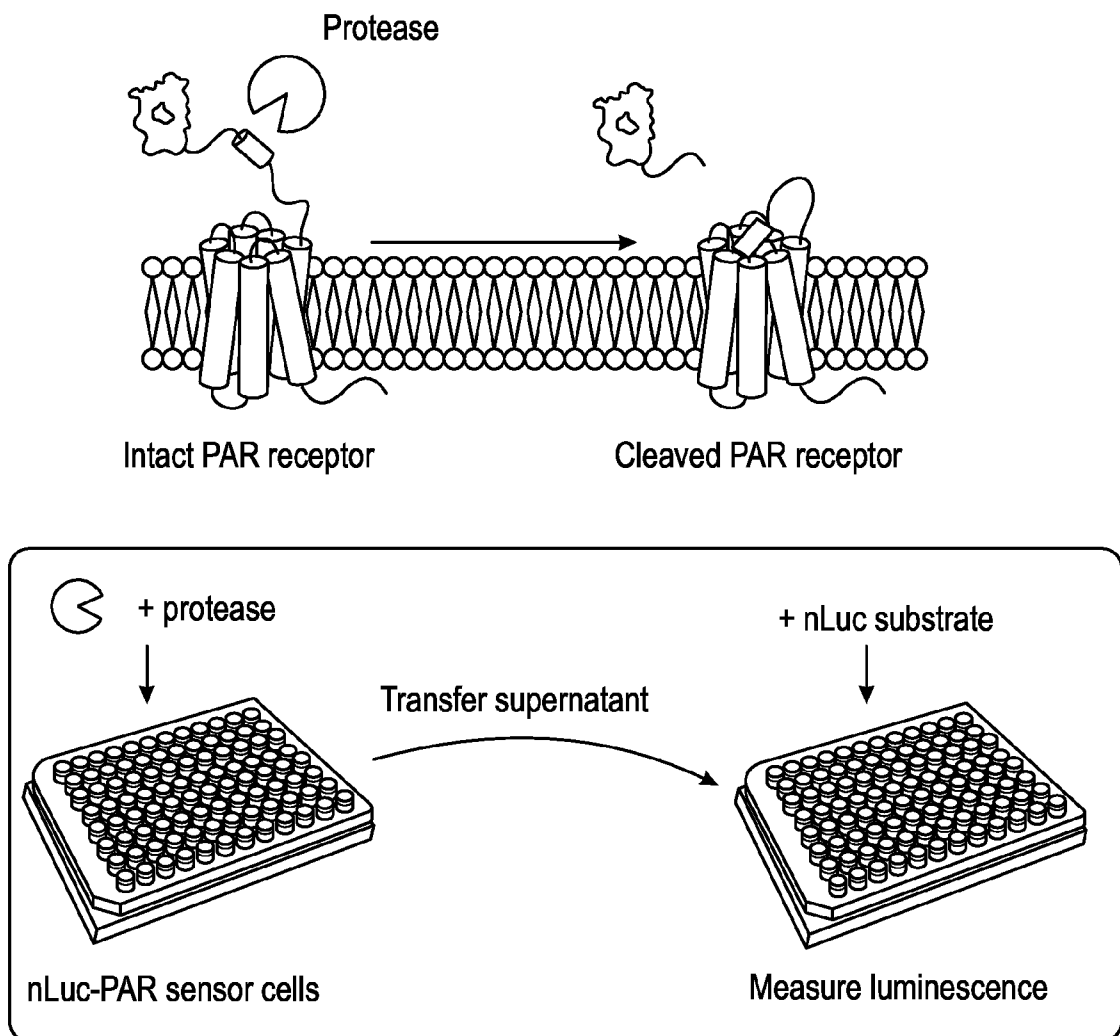
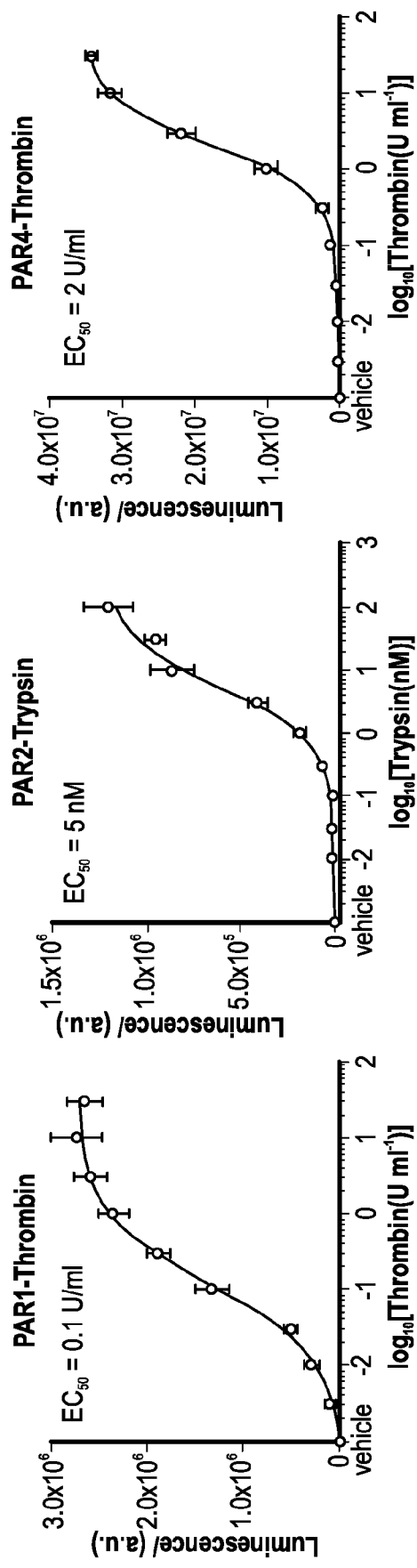
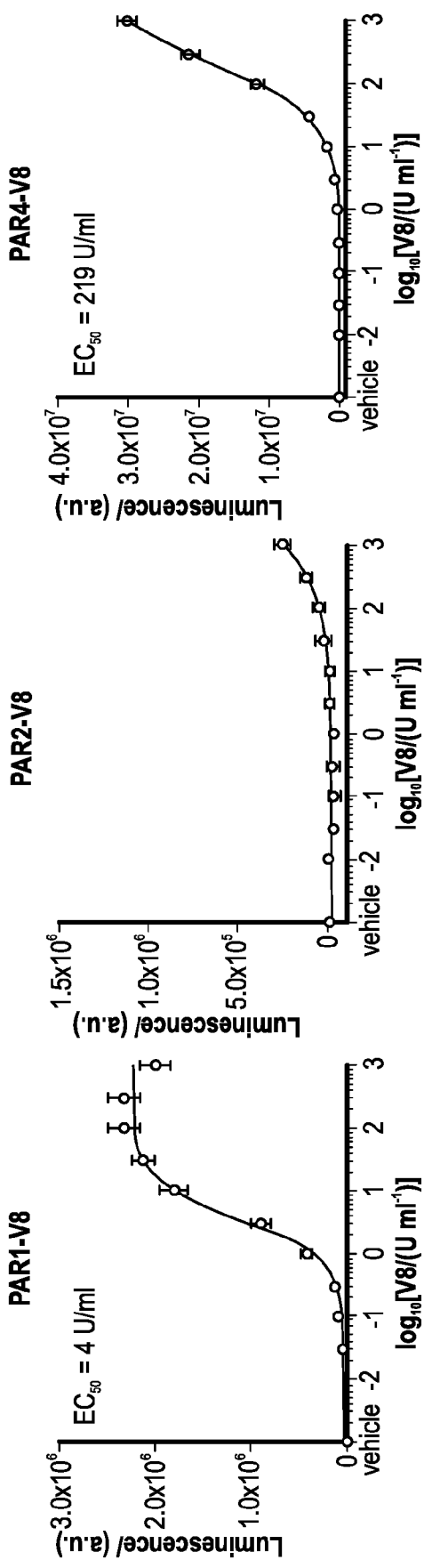


FIG. 3N

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PAR cleavage assay**FIG. 4A**



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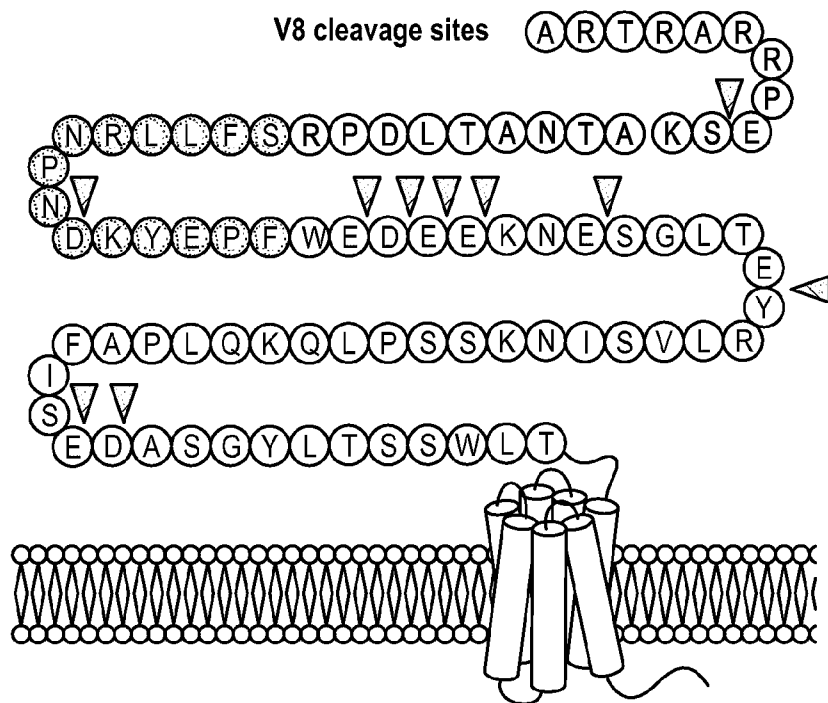


FIG. 4D

Mouse DRG

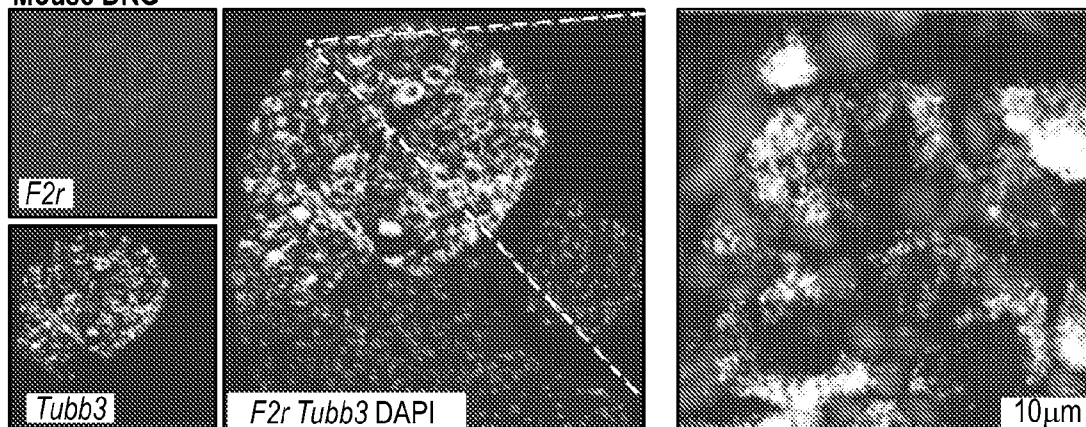
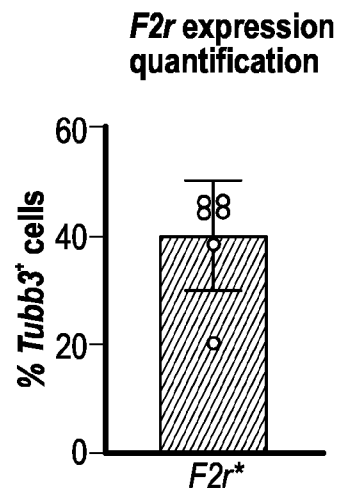
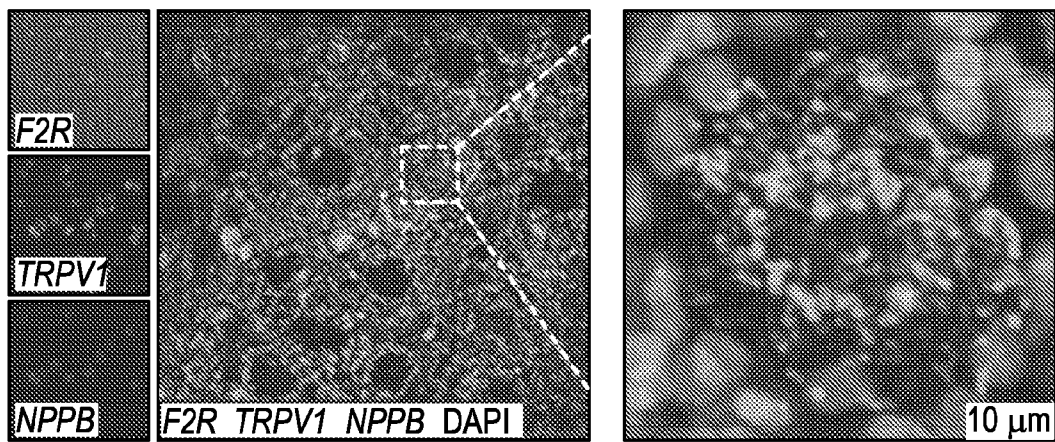


FIG. 4E

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**FIG. 4F****Human lumbar DRG****FIG. 4G**

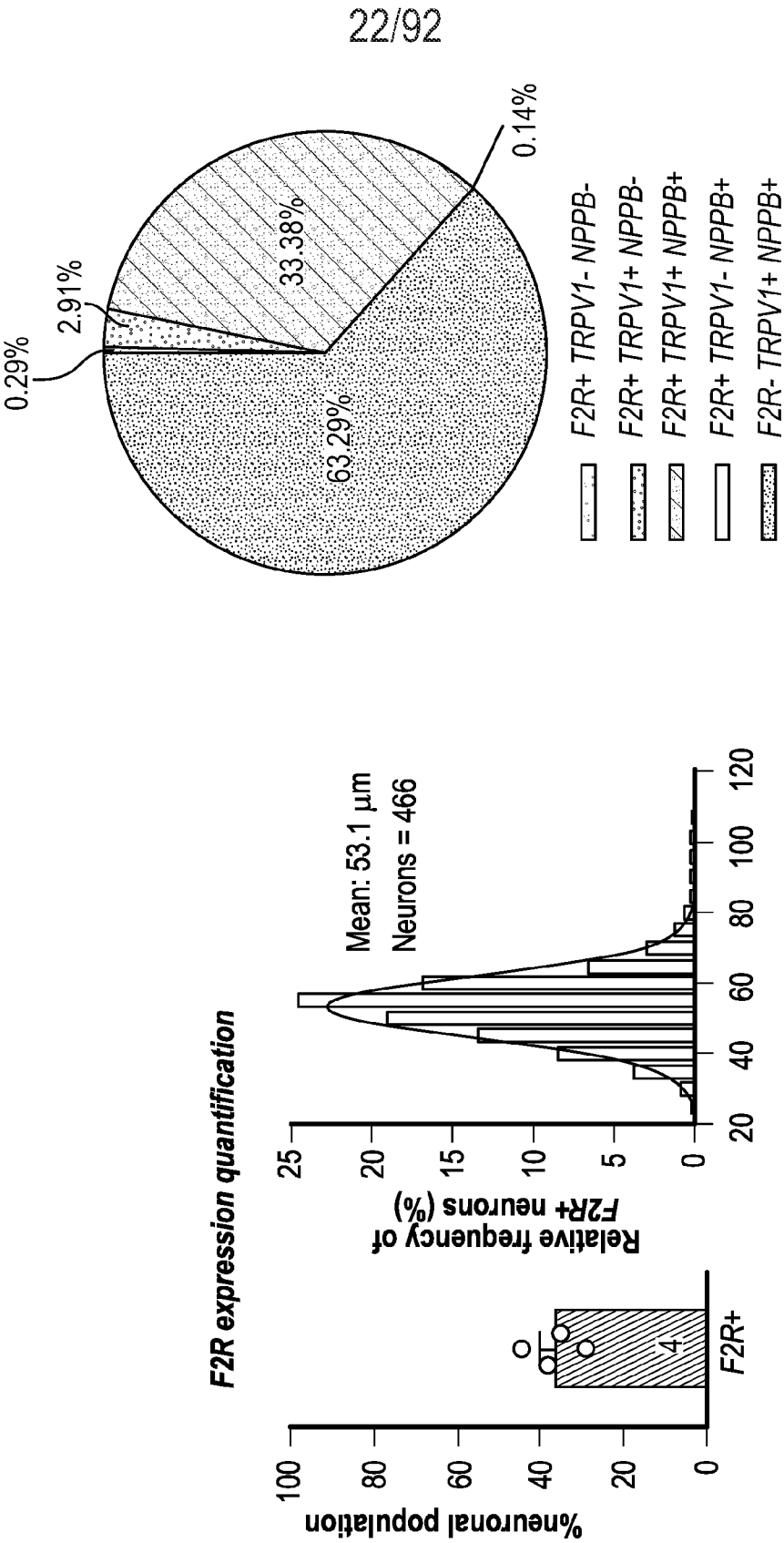


FIG. 4H

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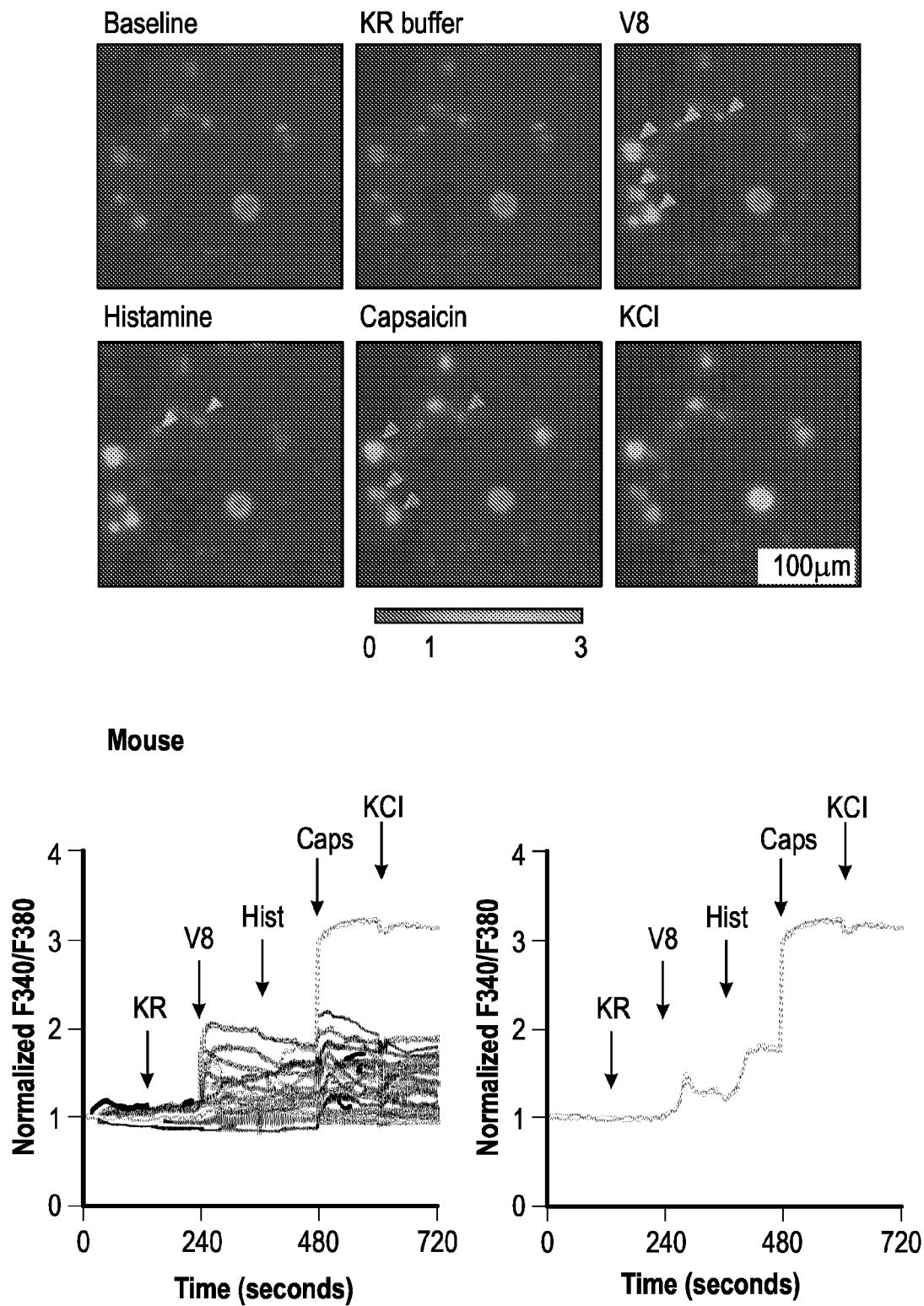


FIG. 5A

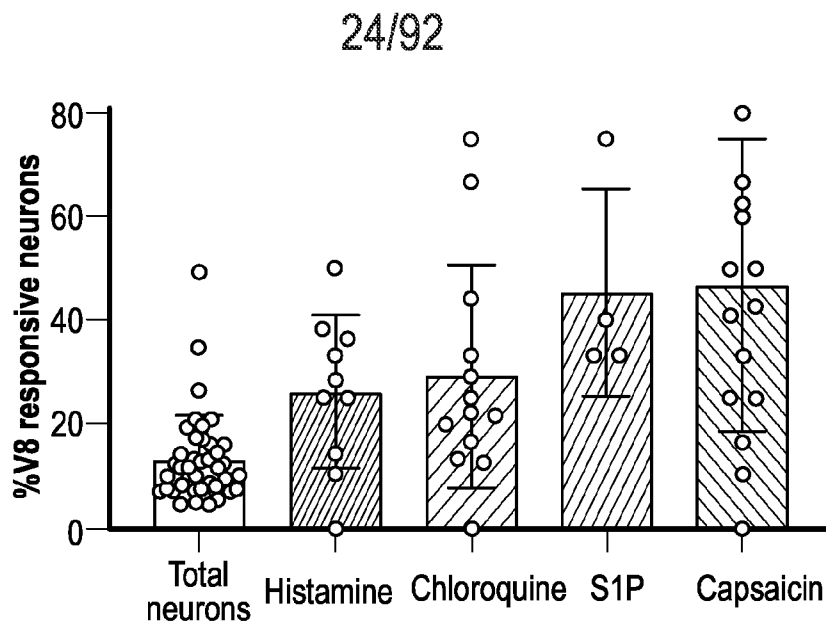


FIG. 5B

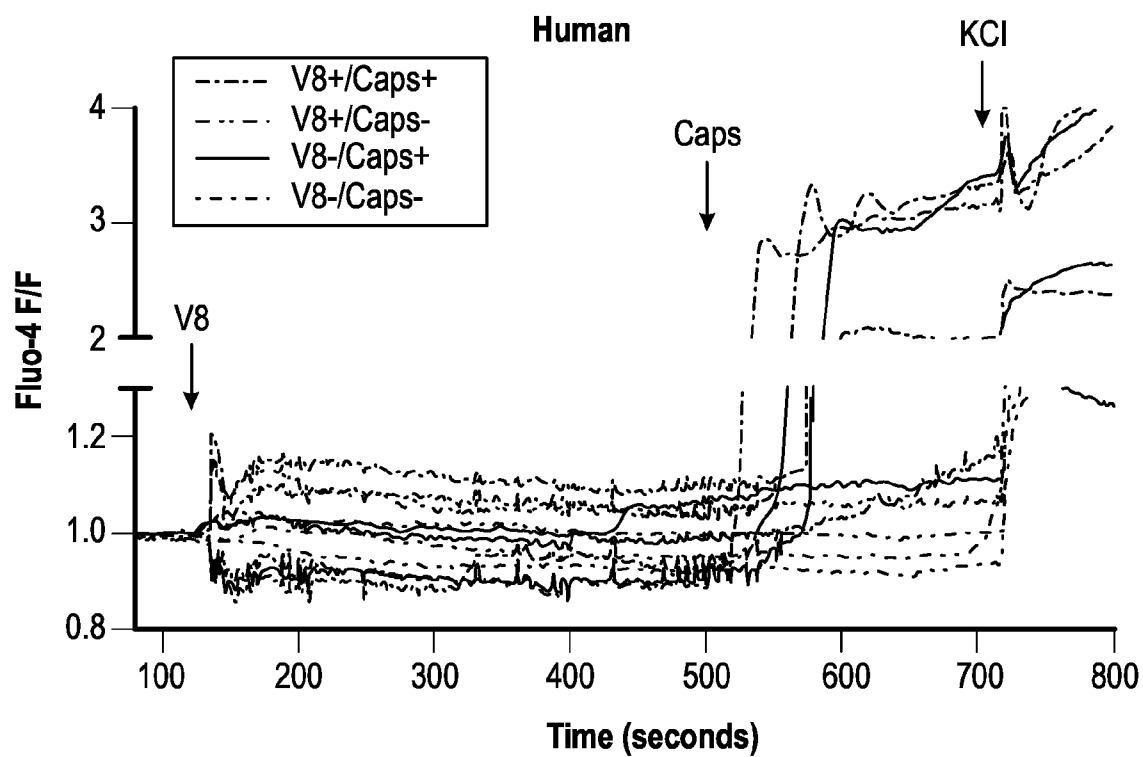
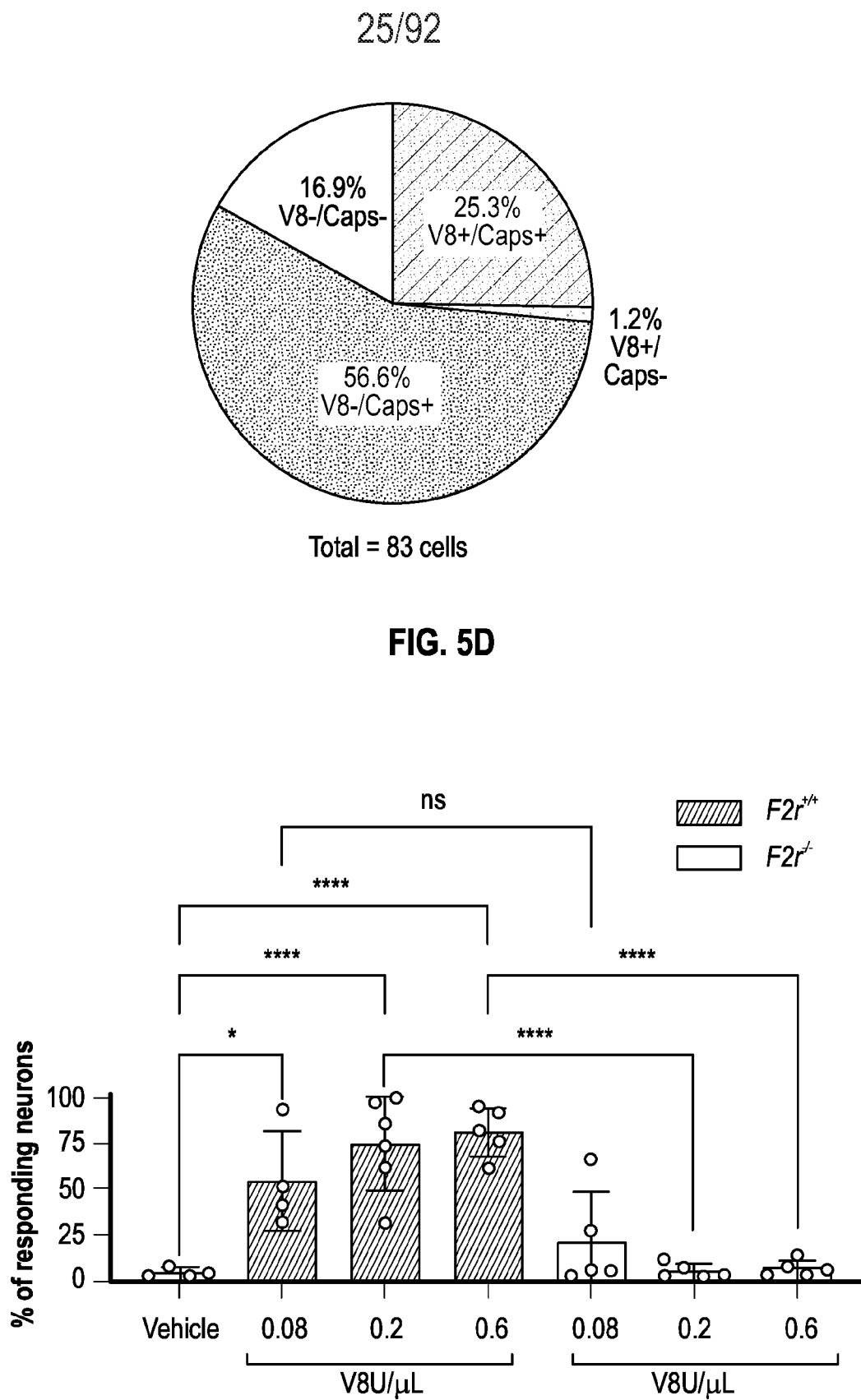


FIG. 5C

**FIG. 5E**

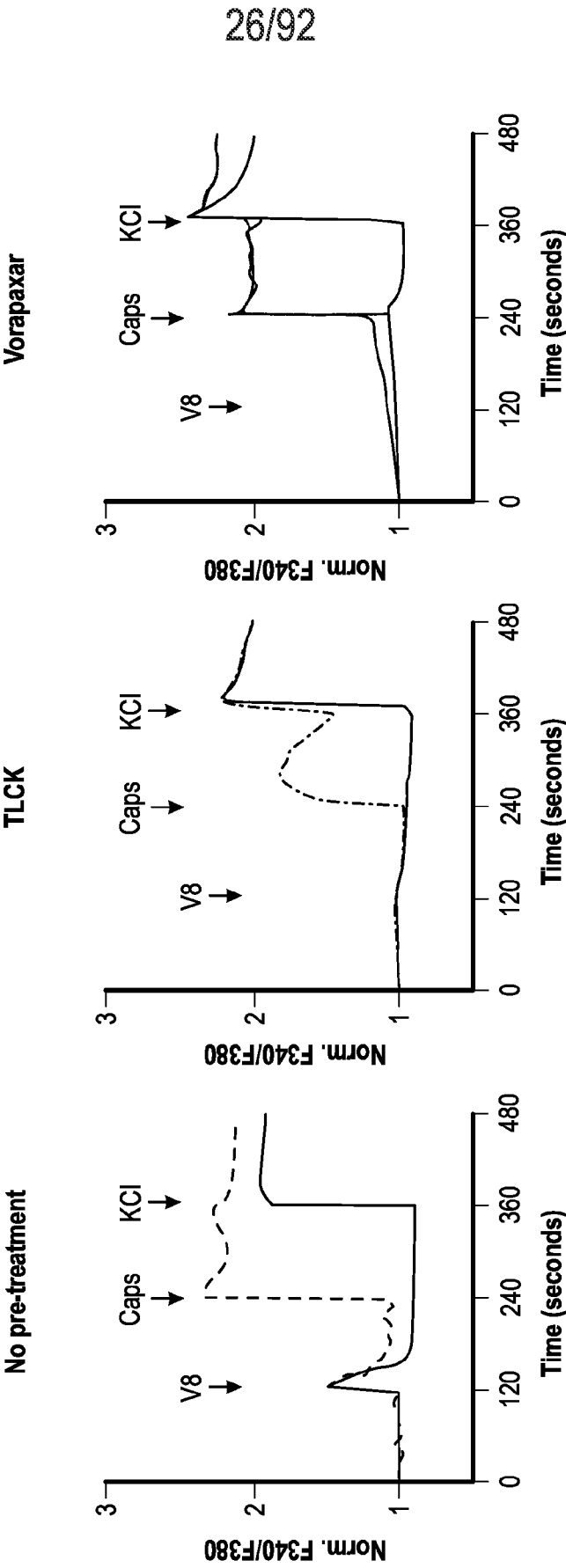


FIG. 5F

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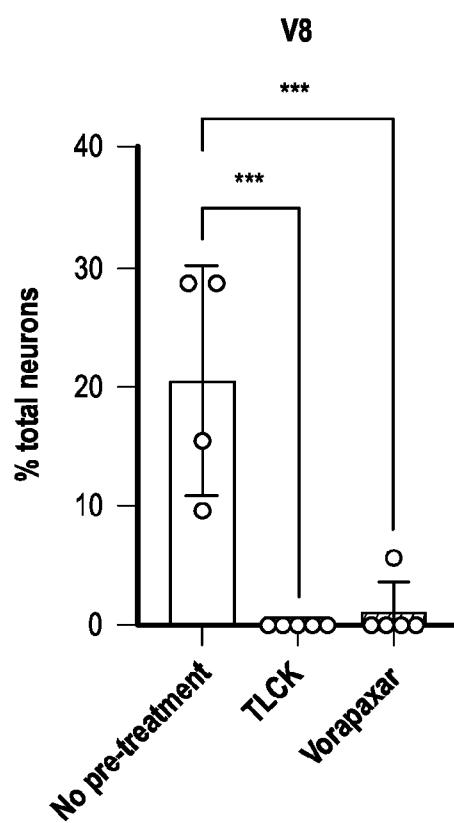


FIG. 5G

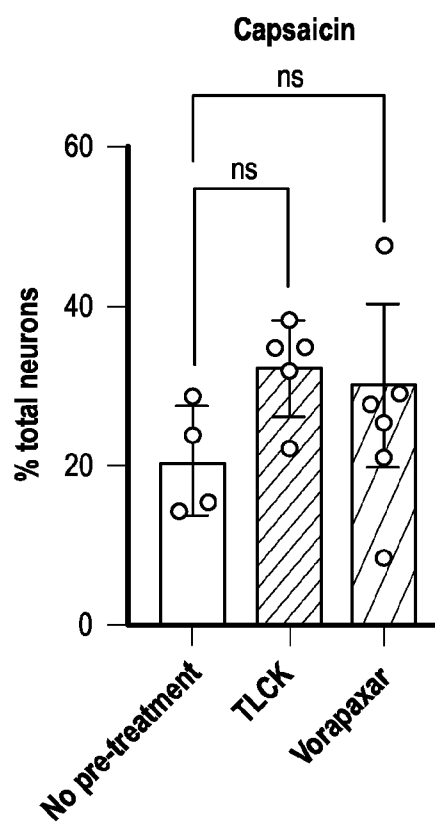


FIG. 5H

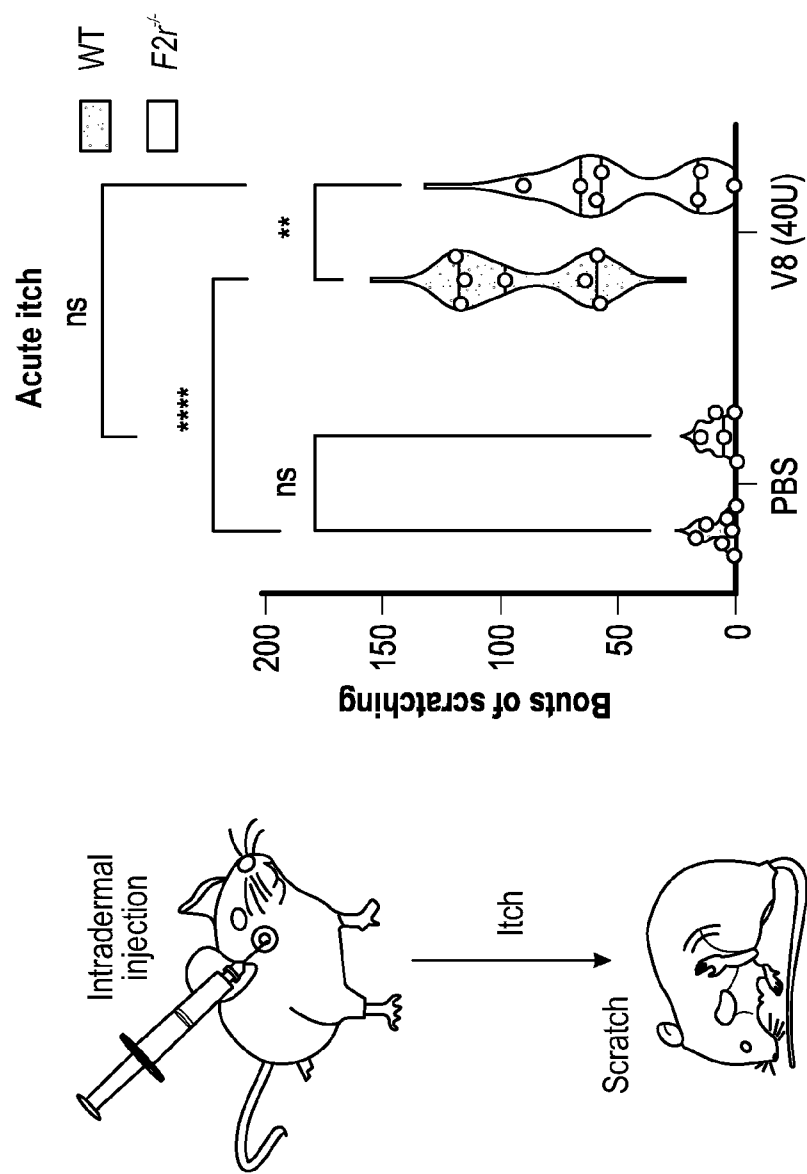


FIG. 6A

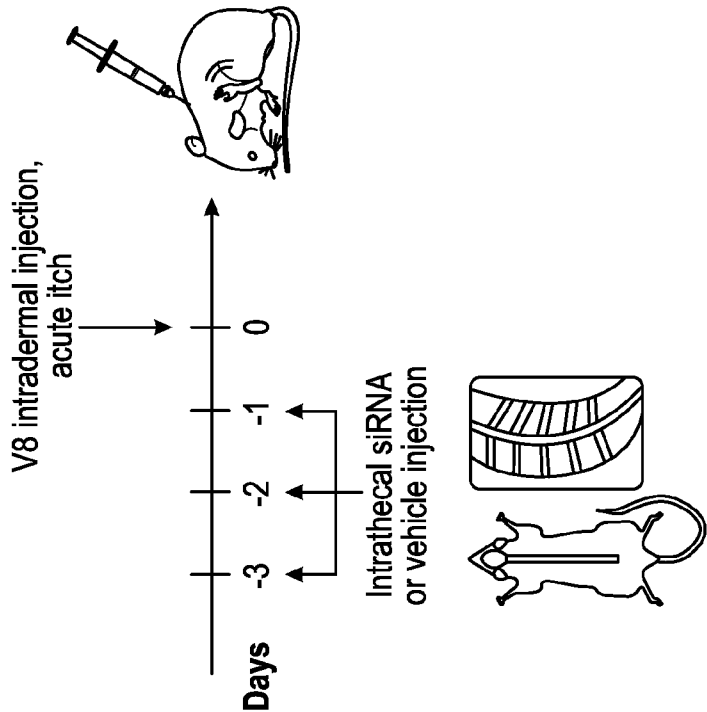
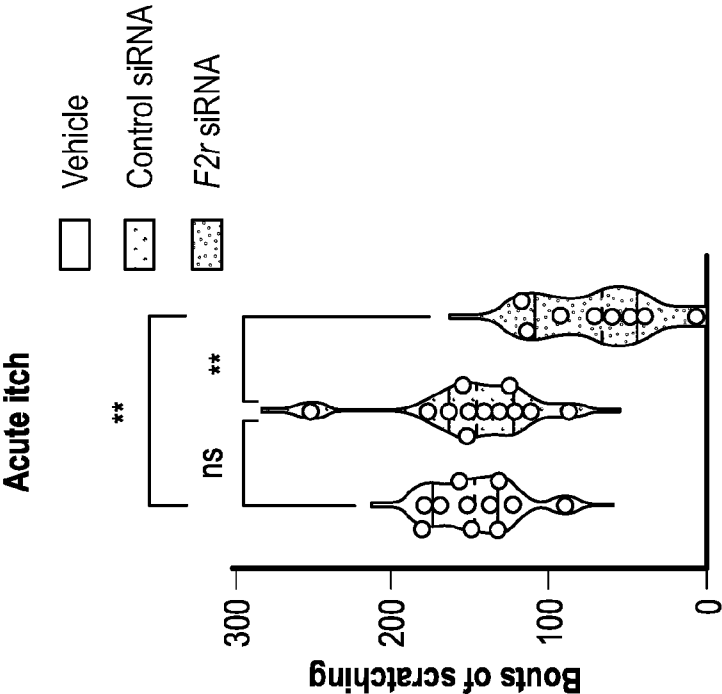


FIG. 6B

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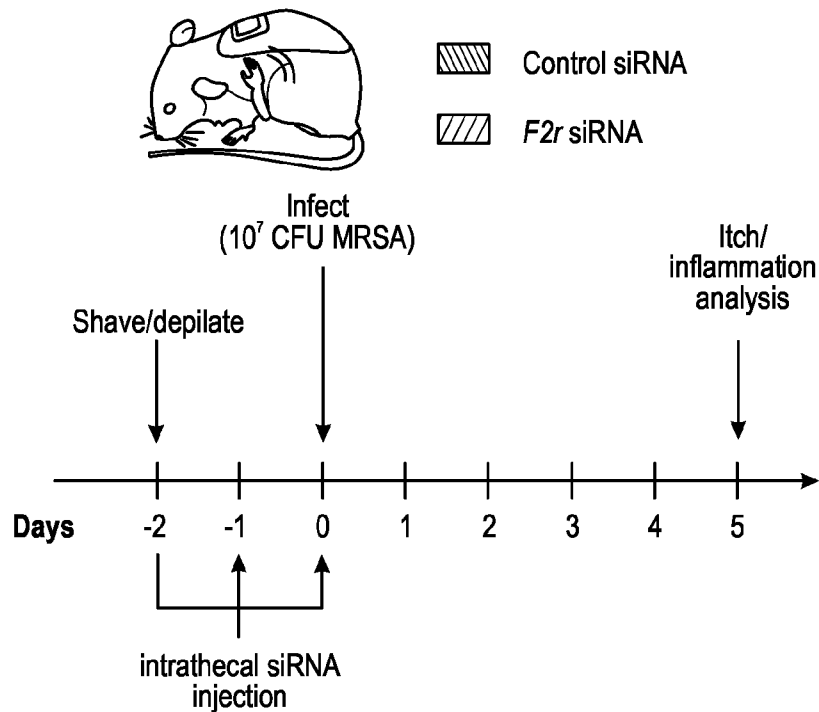


FIG. 6C

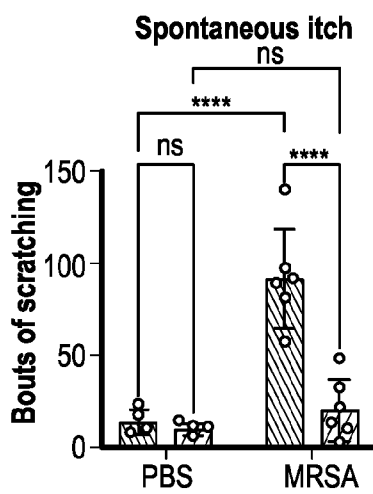


FIG. 6D

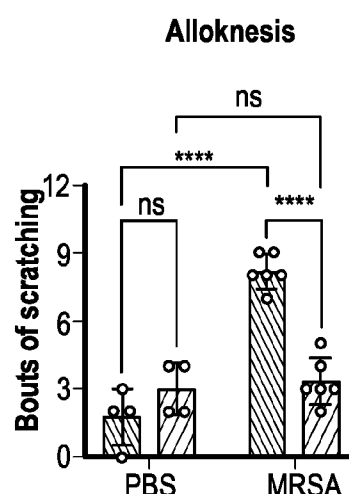


FIG. 6E

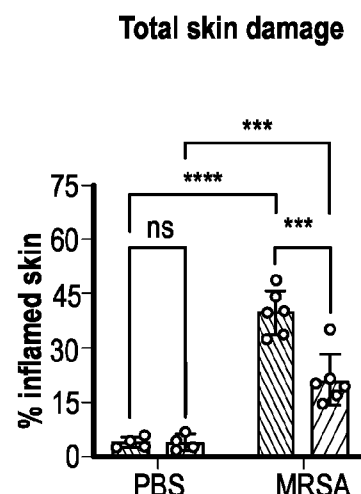


FIG. 6F

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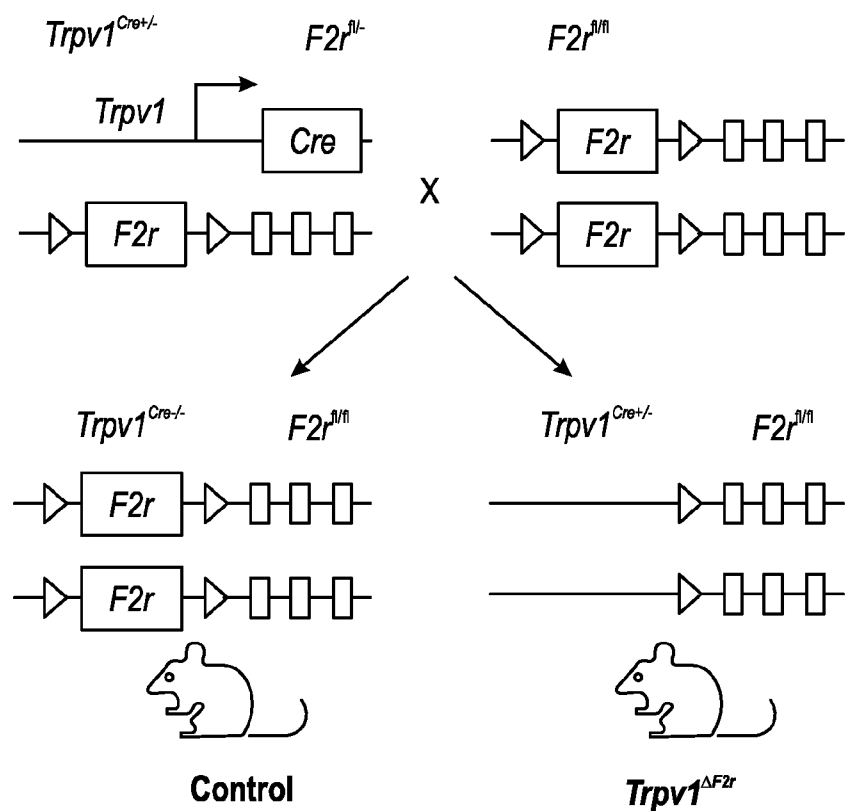


FIG. 6G

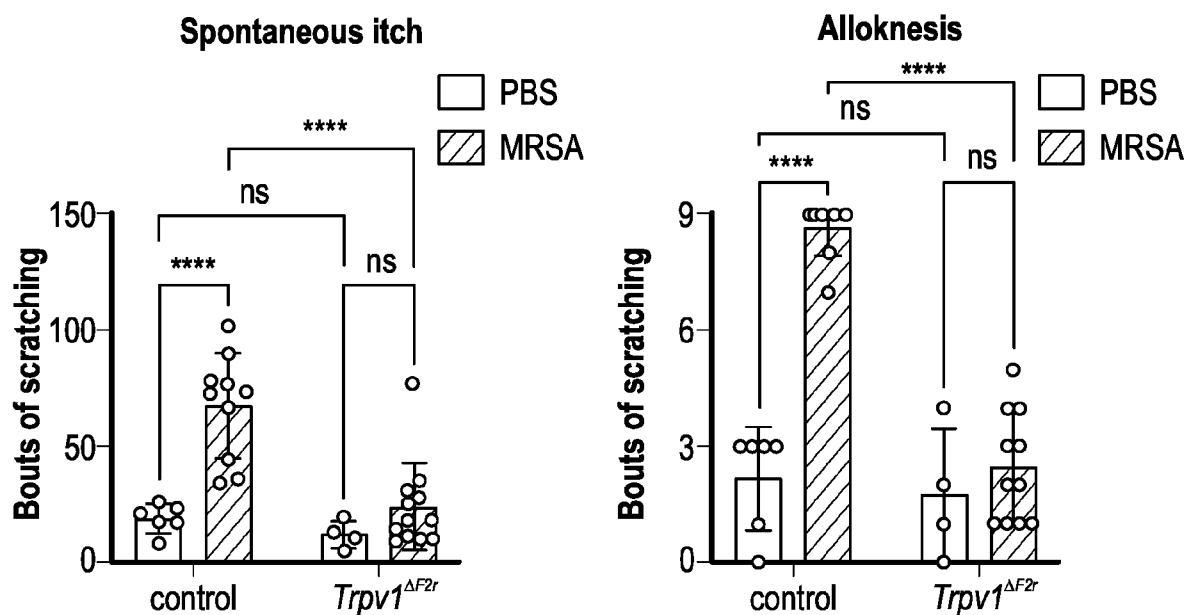
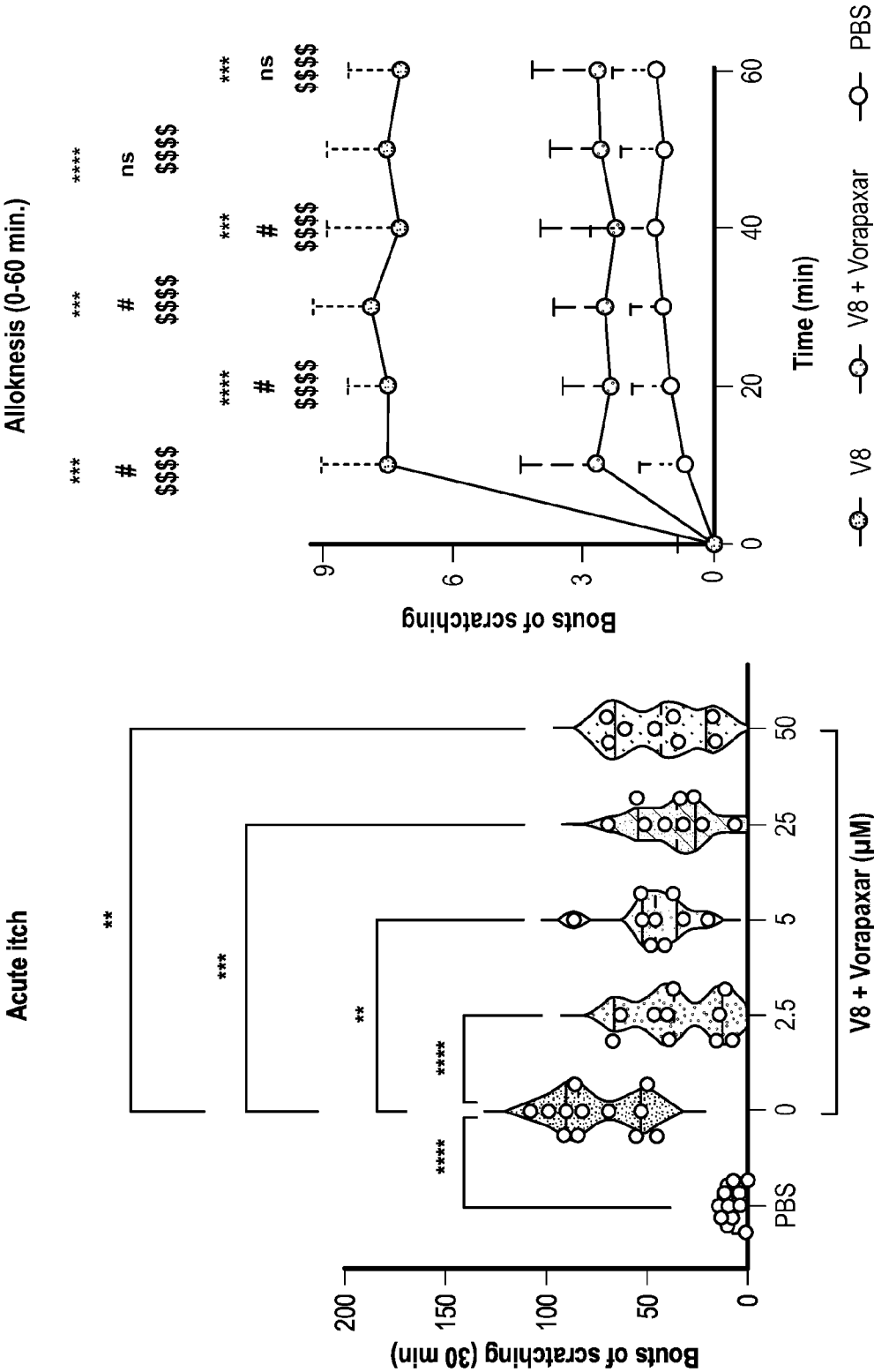


FIG. 6H

FIG. 6I



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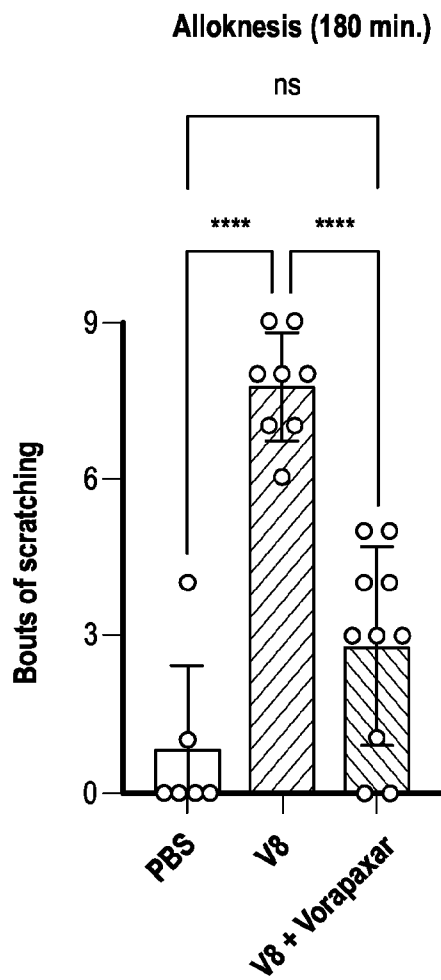


FIG. 7C

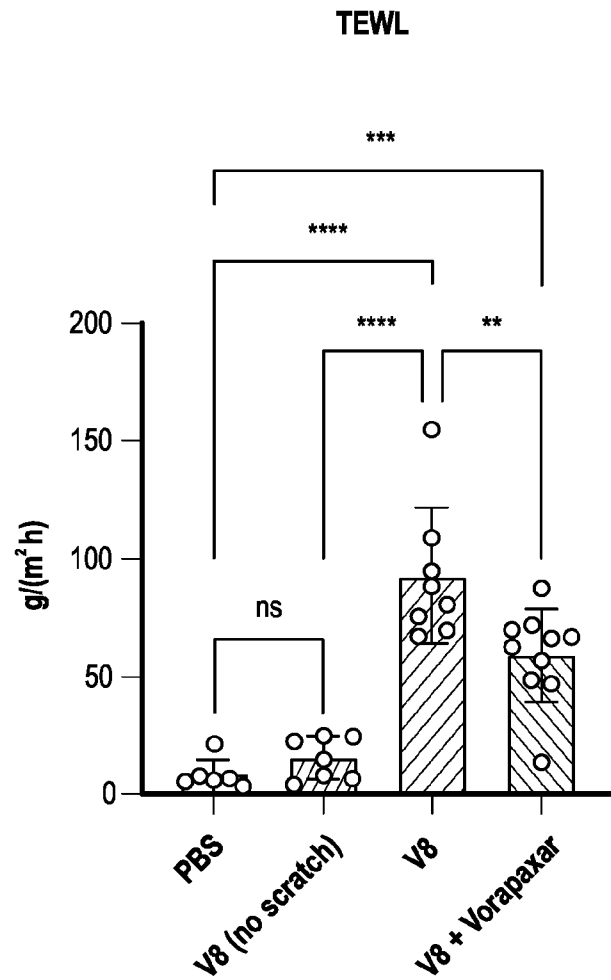


FIG. 7D

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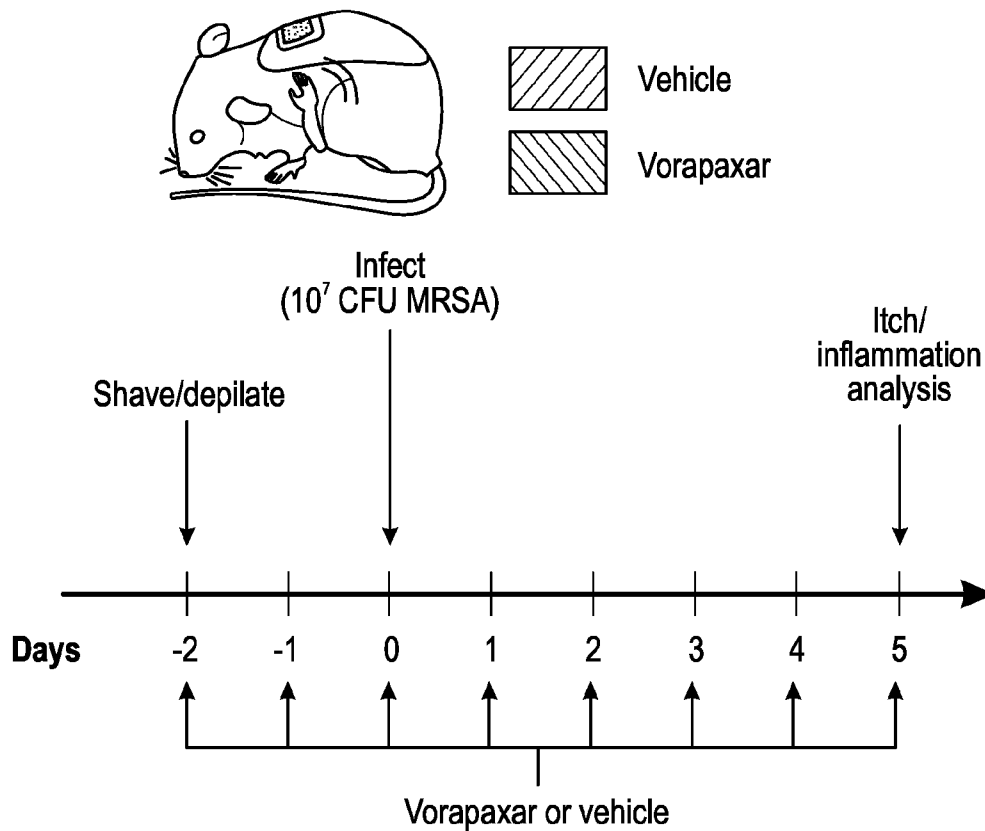


FIG. 7E

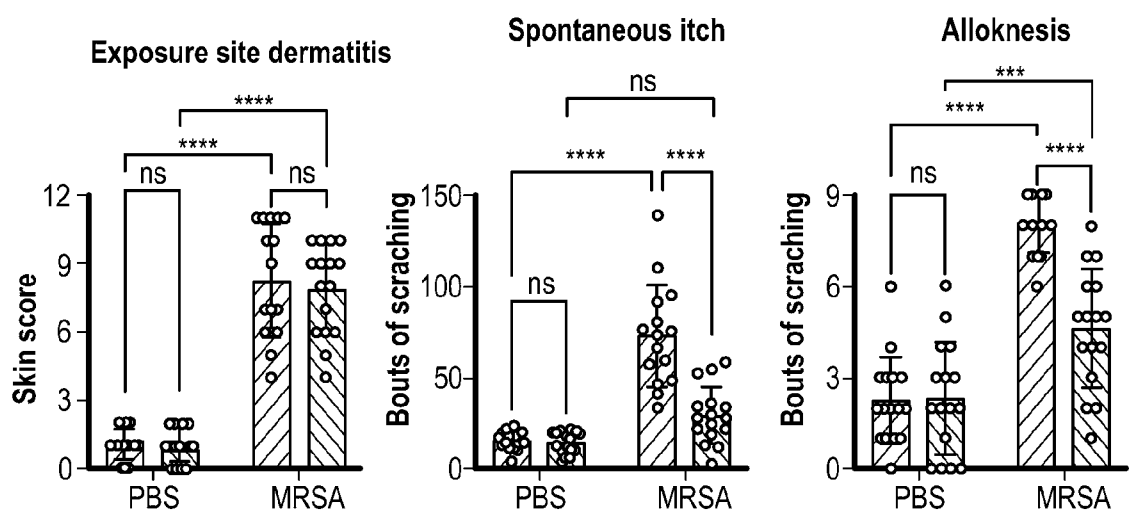
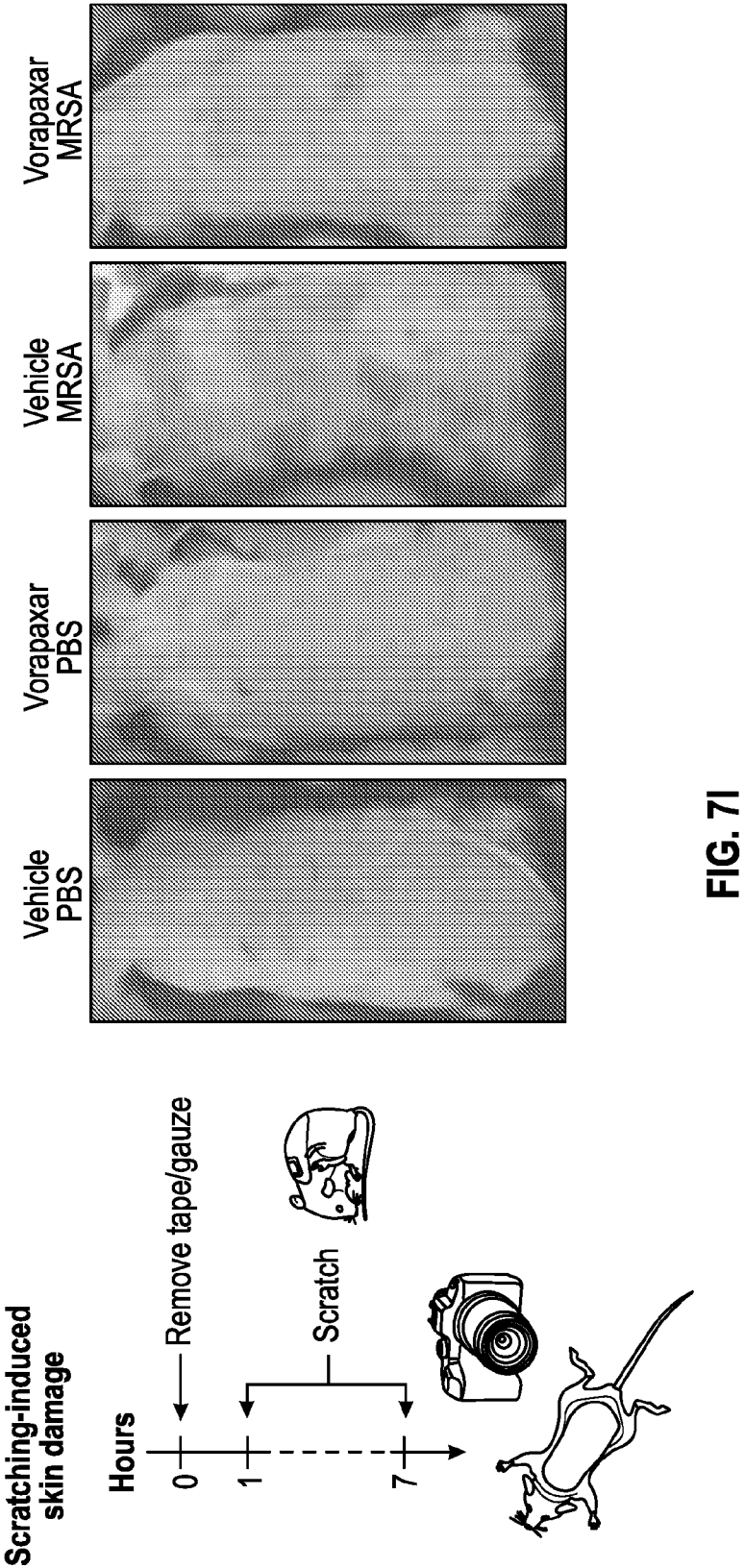


FIG. 7F

FIG. 7G

FIG. 7H



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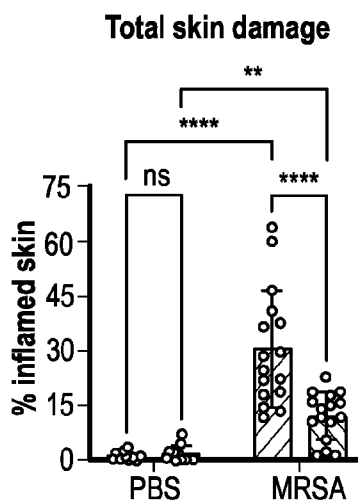


FIG. 7J

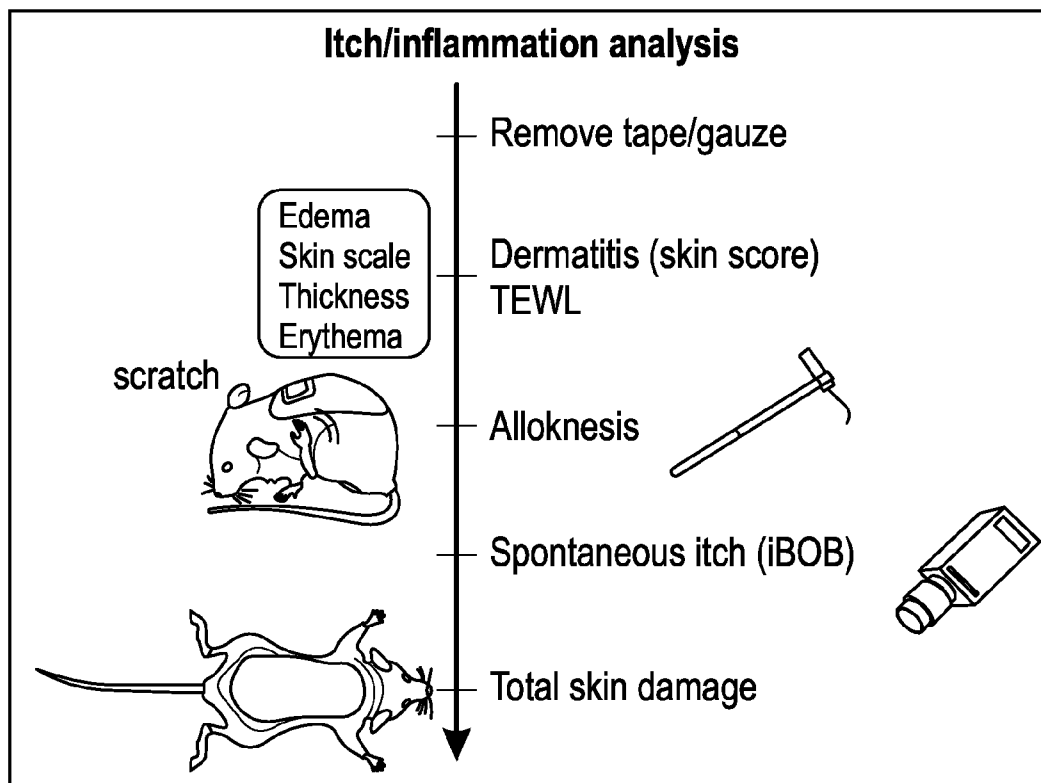


FIG. 8A

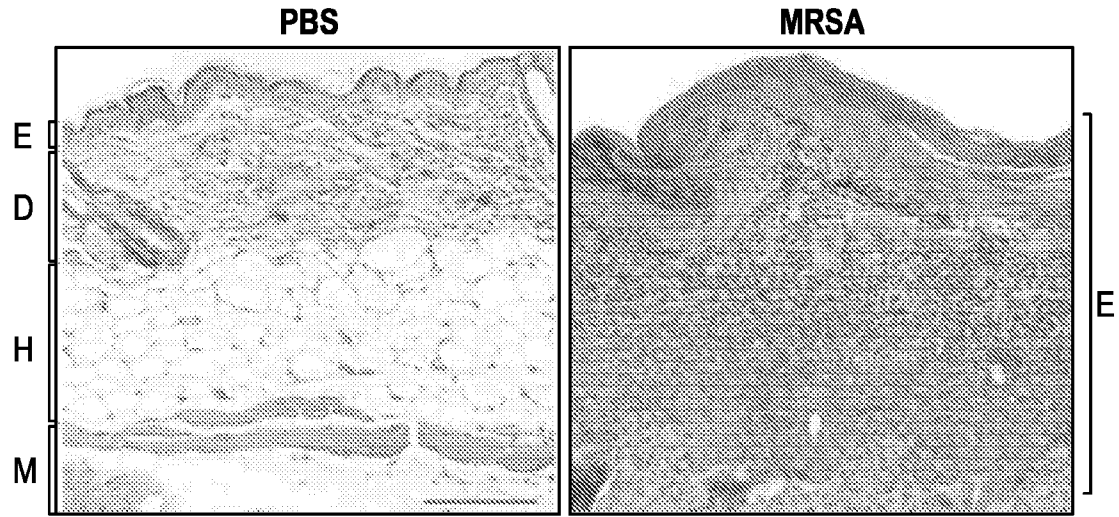


FIG. 8B

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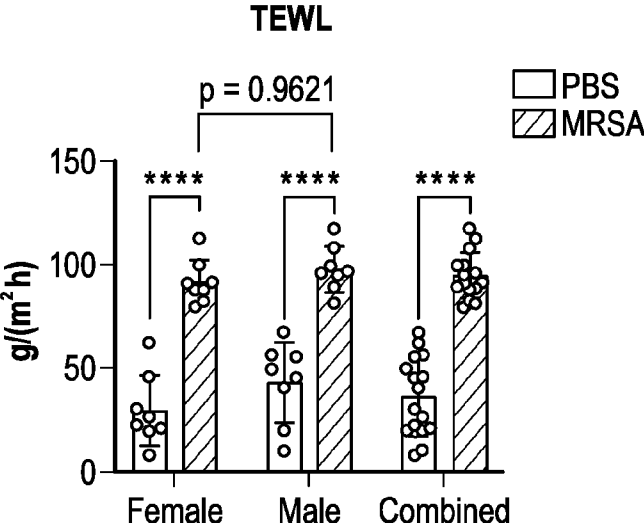


FIG. 8C

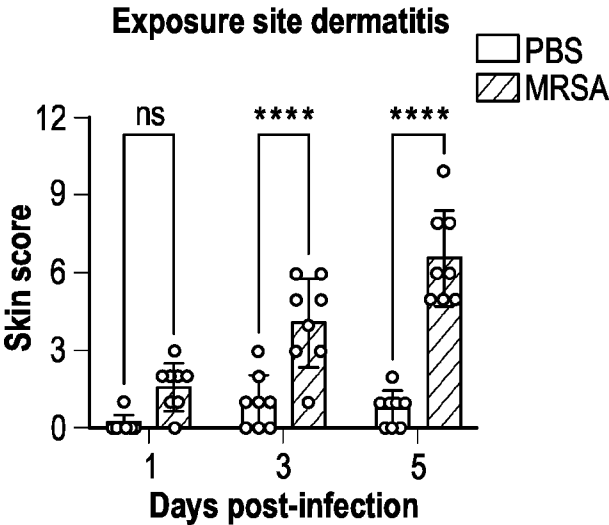


FIG. 8D

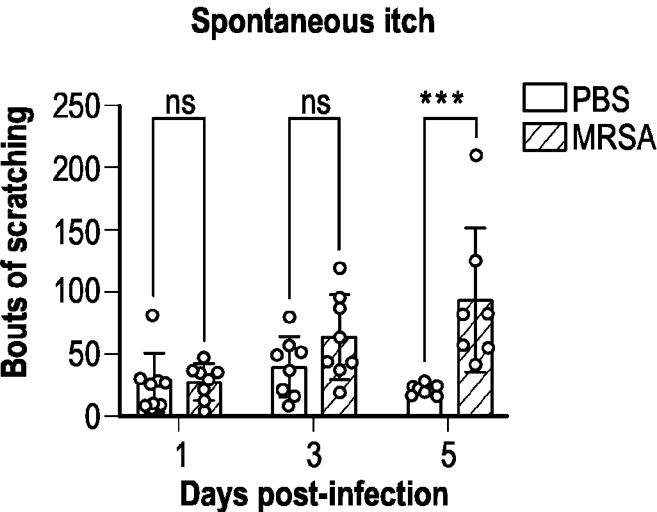


FIG. 8E

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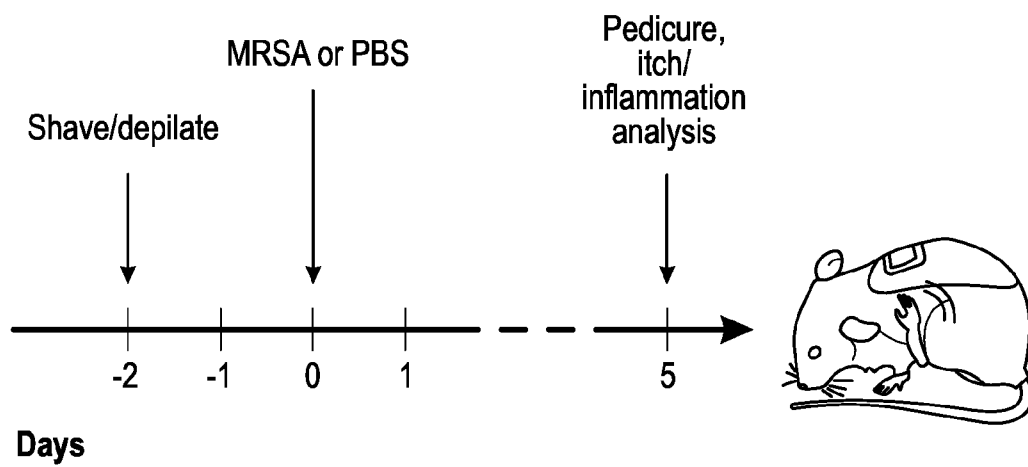


FIG. 8F

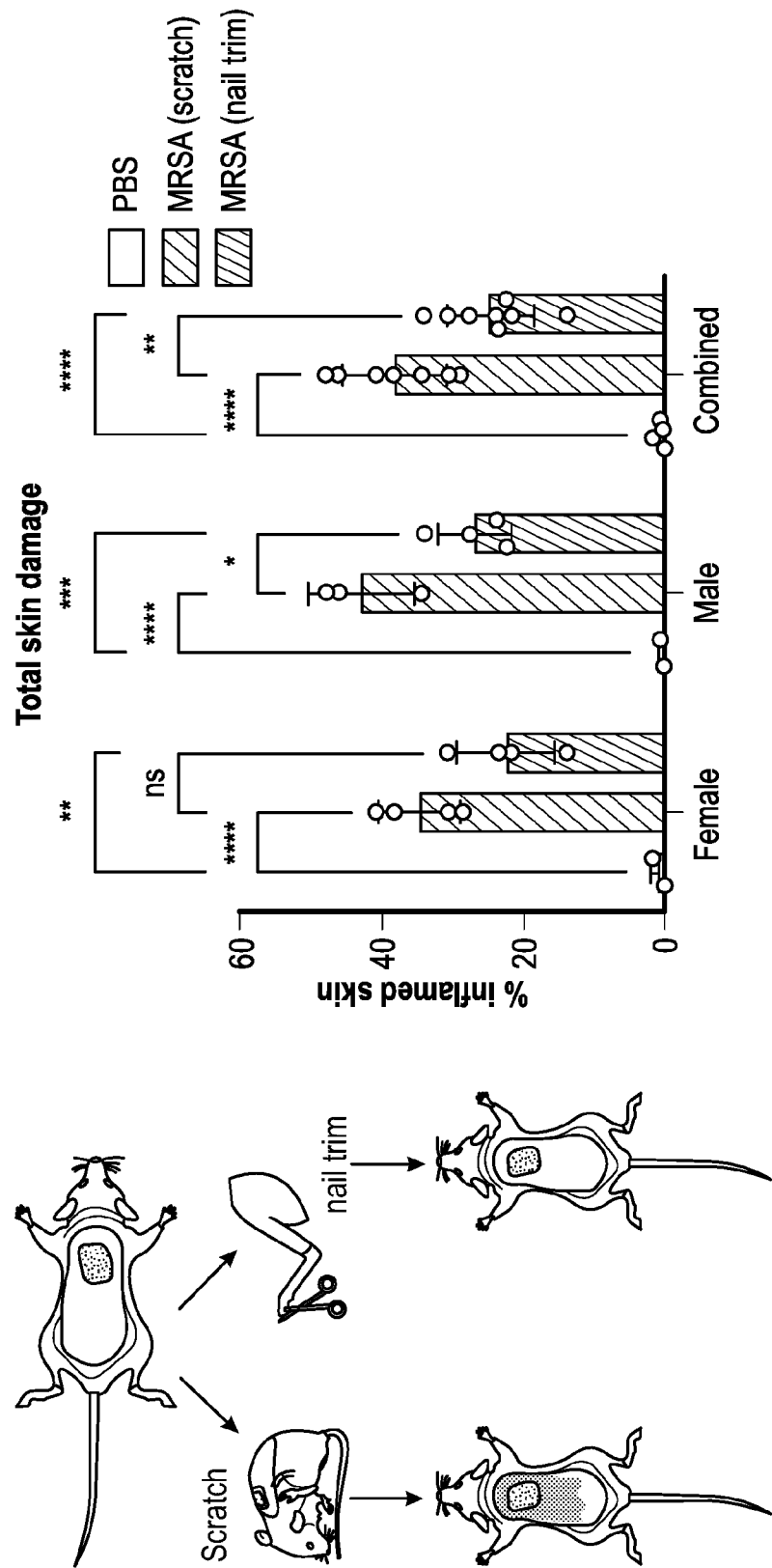


FIG. 8G

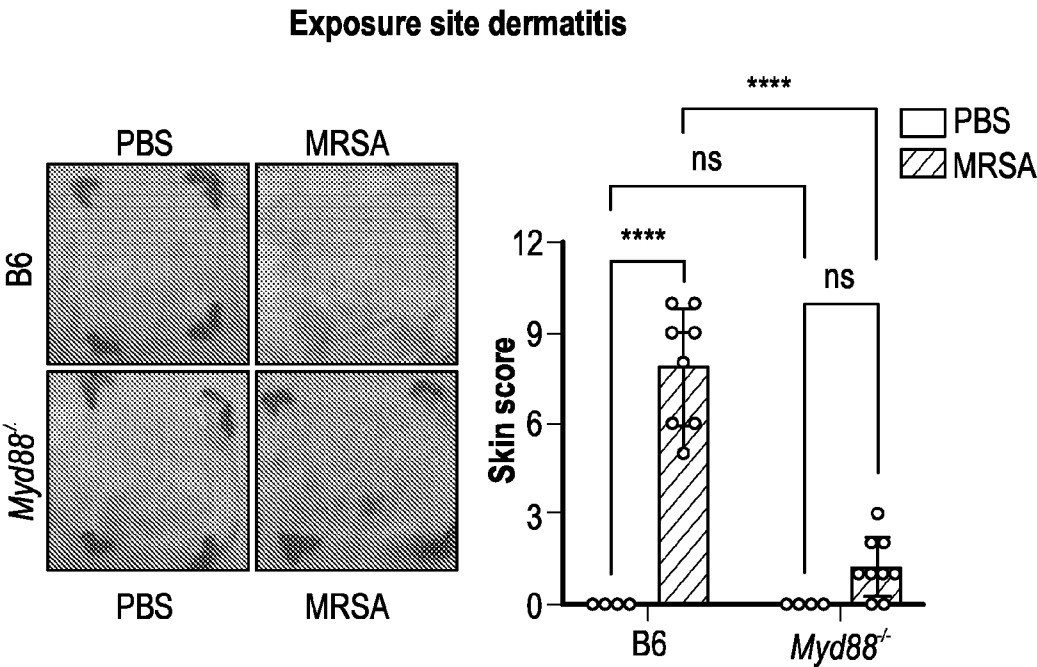


FIG.9A

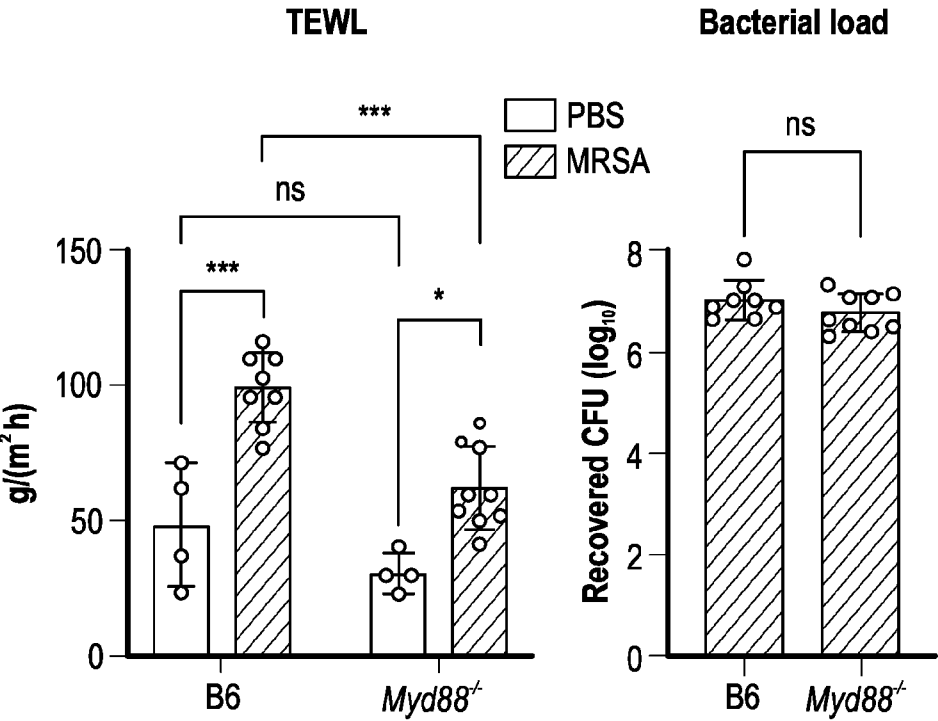


FIG.9B

FIG.9C

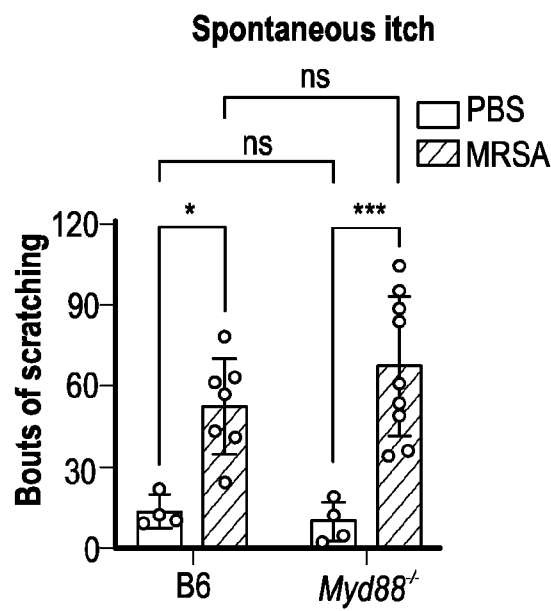


FIG.9D

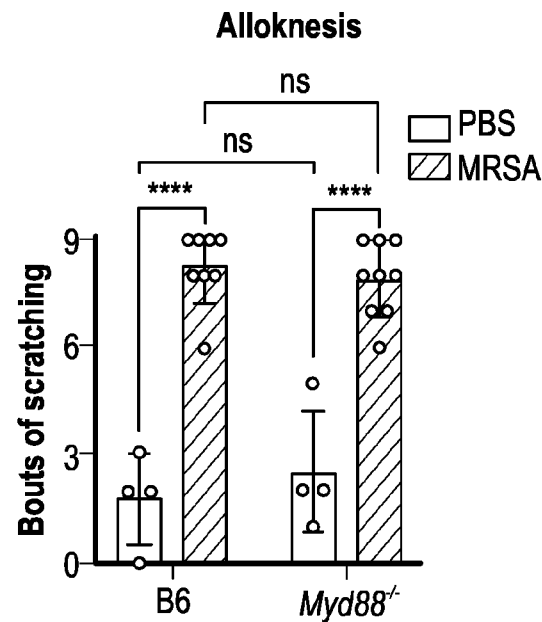


FIG.9E

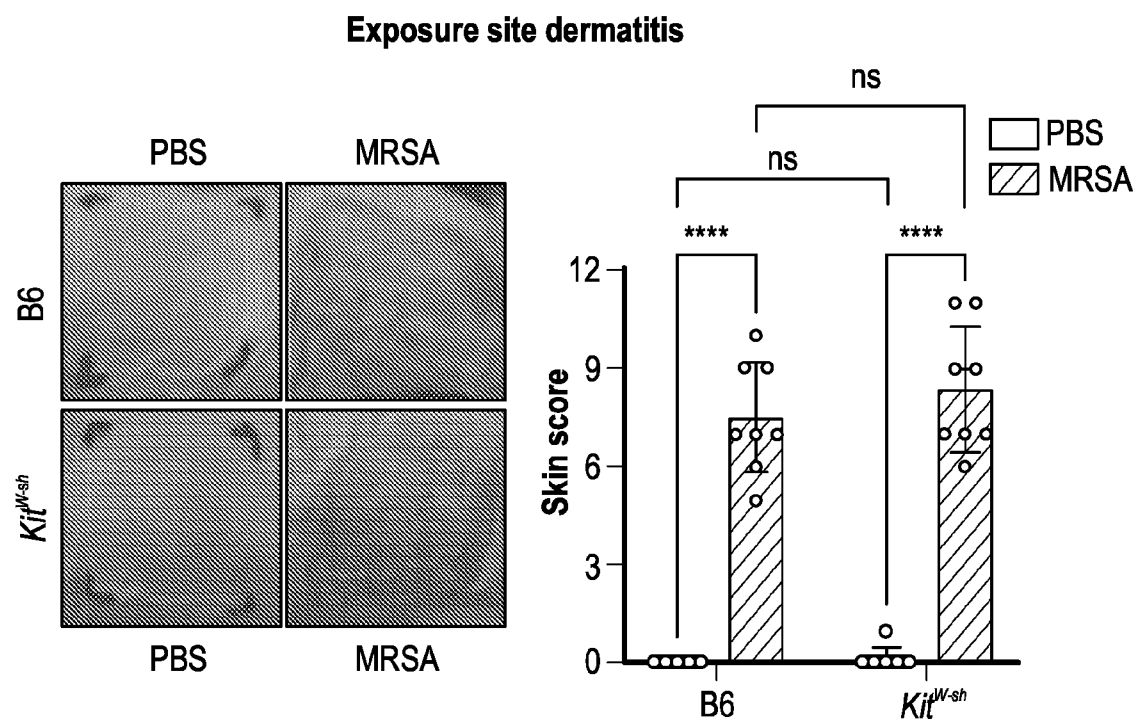


FIG.9F

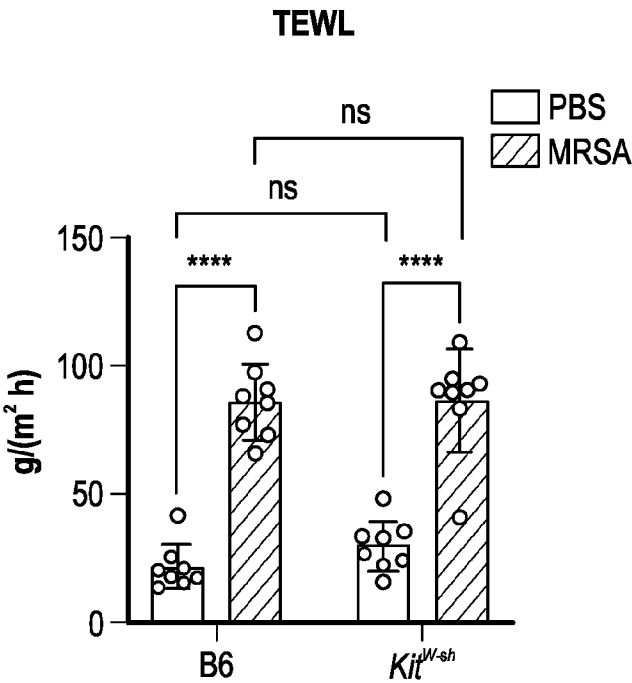


FIG.9G

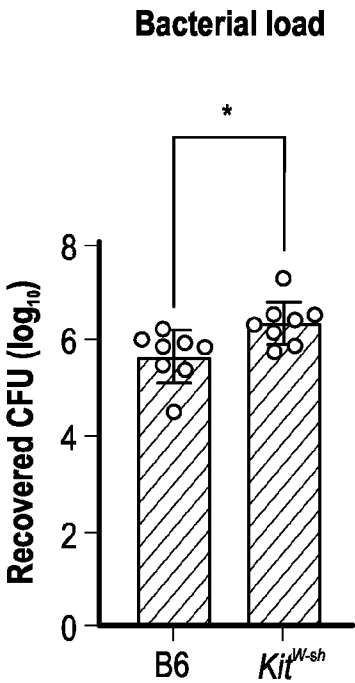


FIG.9H

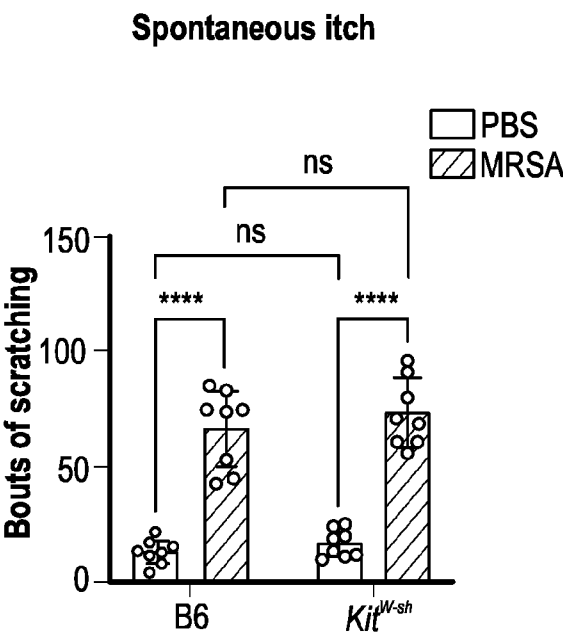


FIG.9I

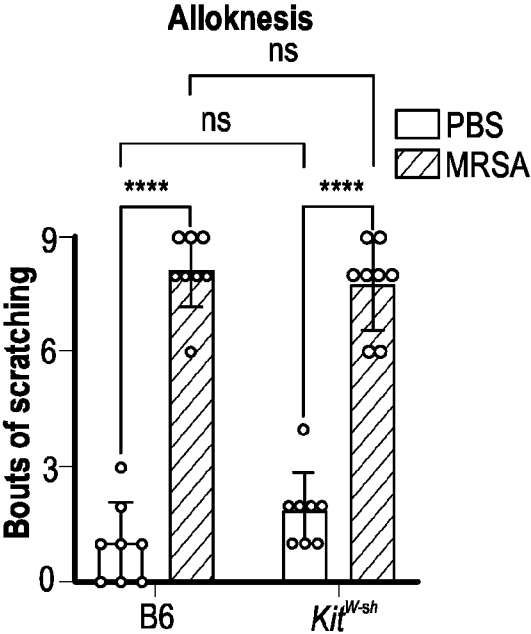


FIG.9J

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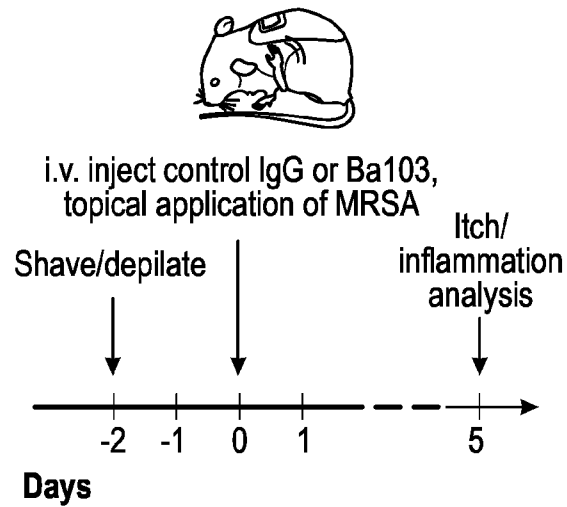


FIG.9K(Cont...)

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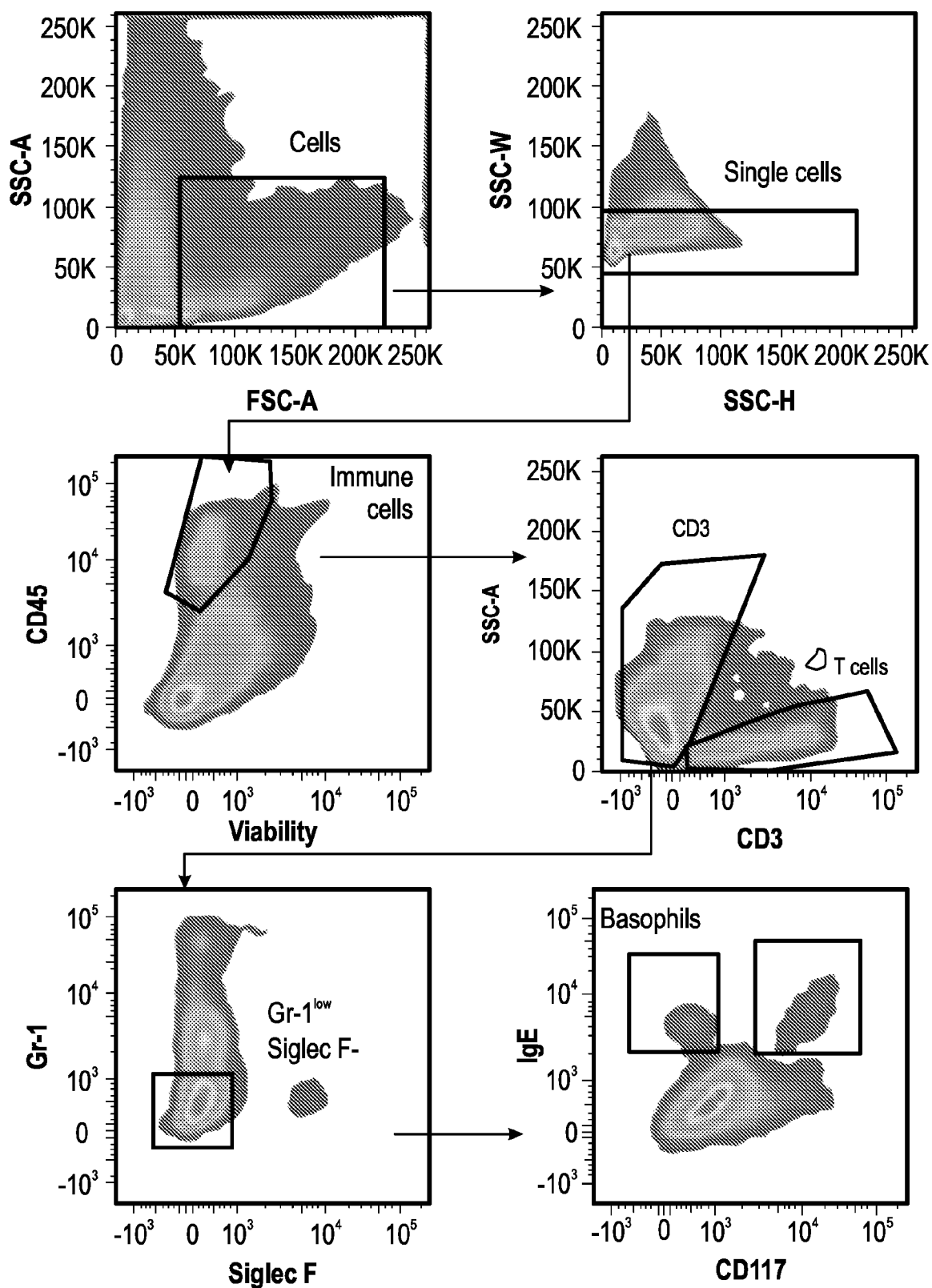
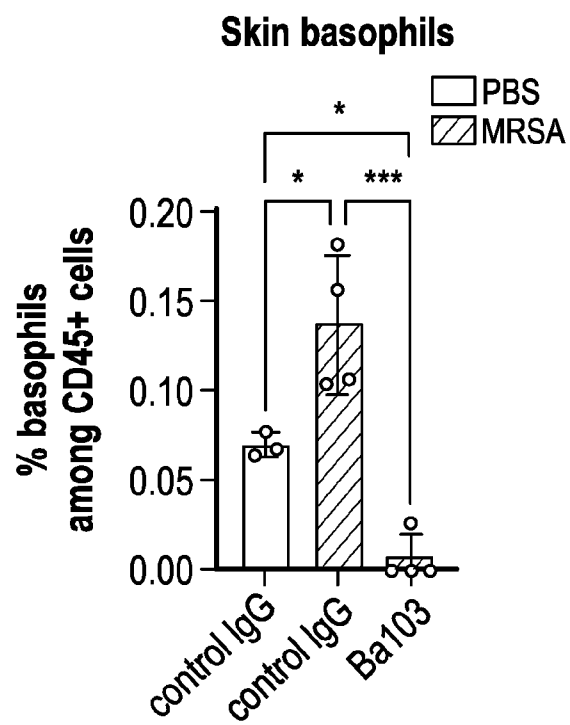


FIG.9K

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**FIG.9L**

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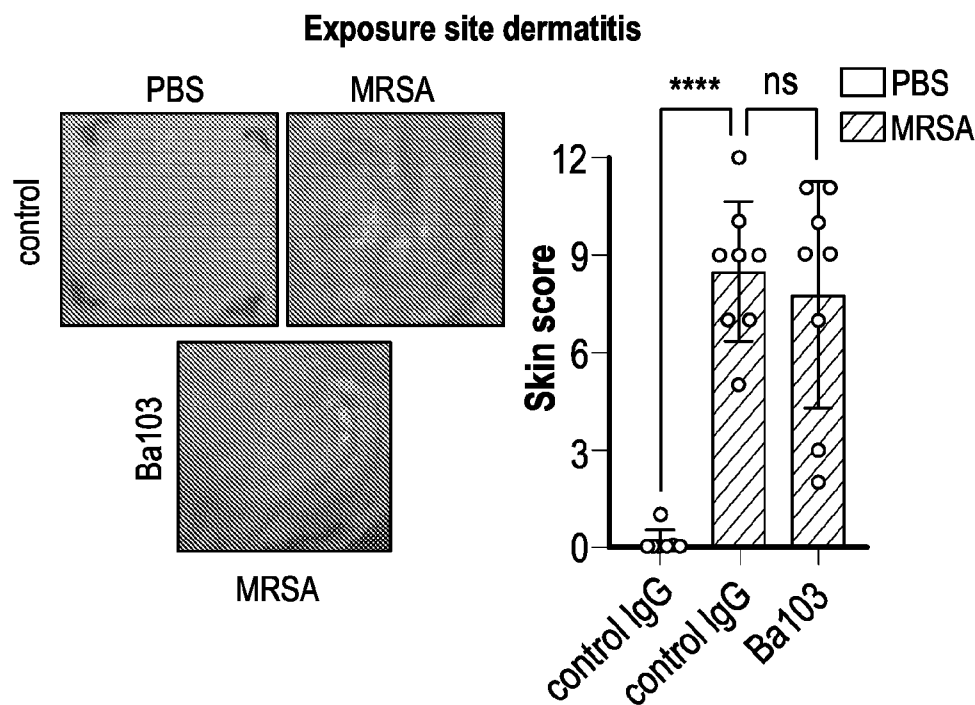


FIG.9M

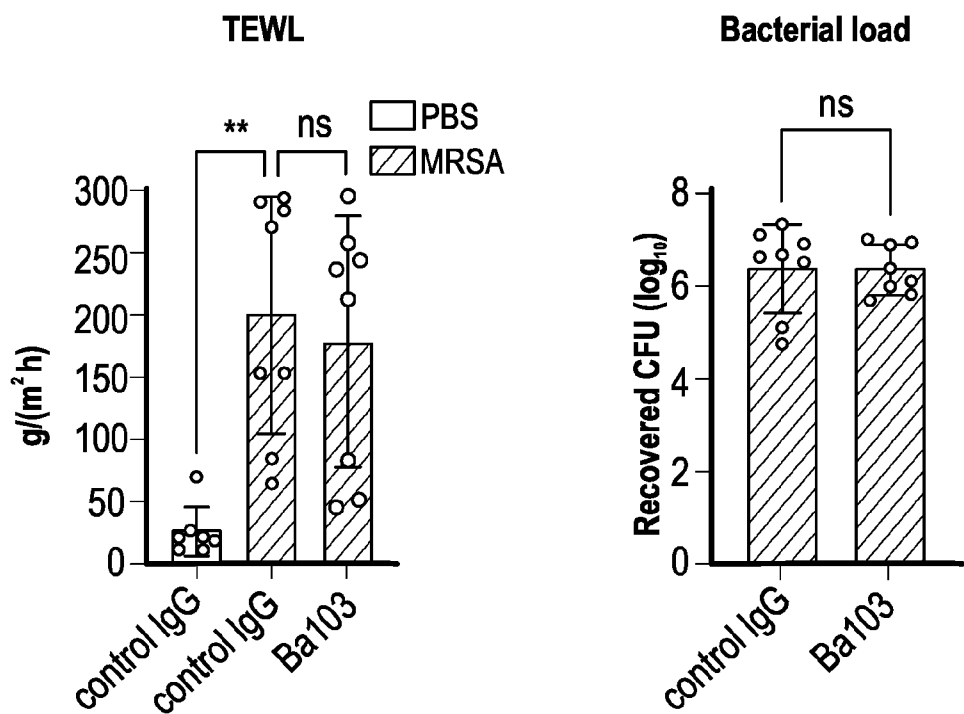


FIG.9N

FIG.9O

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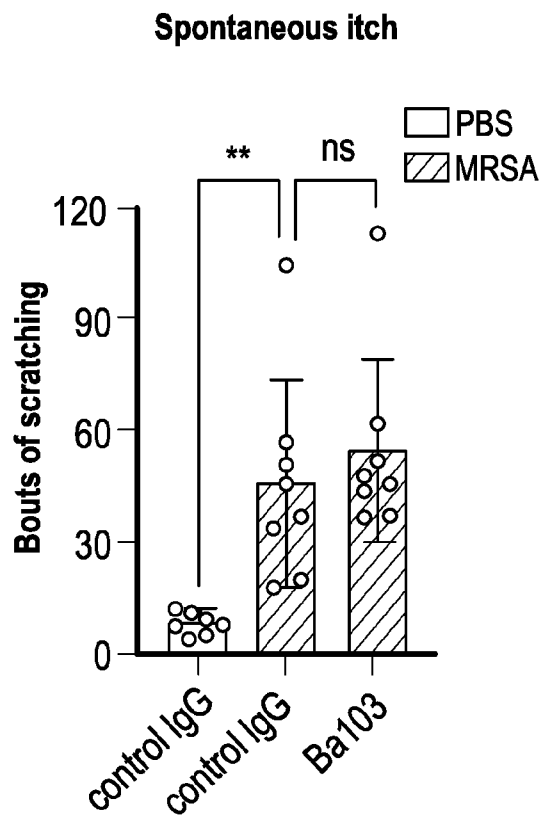


FIG.9P

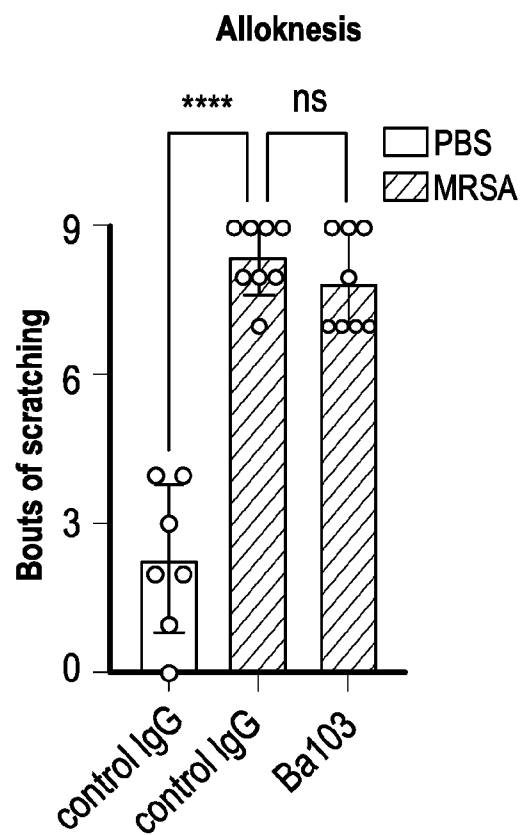
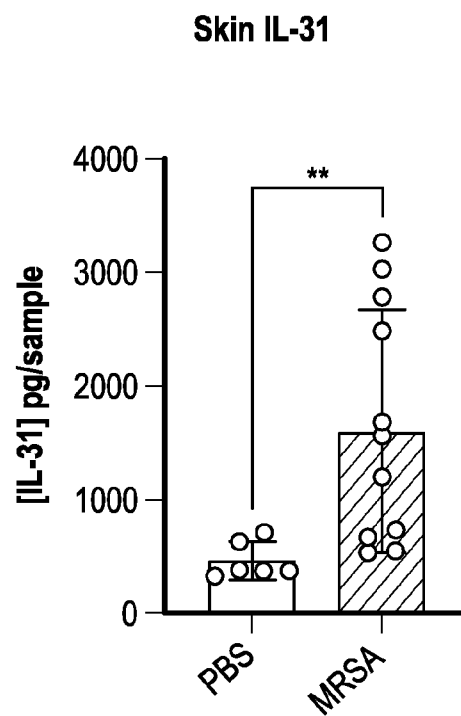


FIG.9Q

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**FIG. 10A**

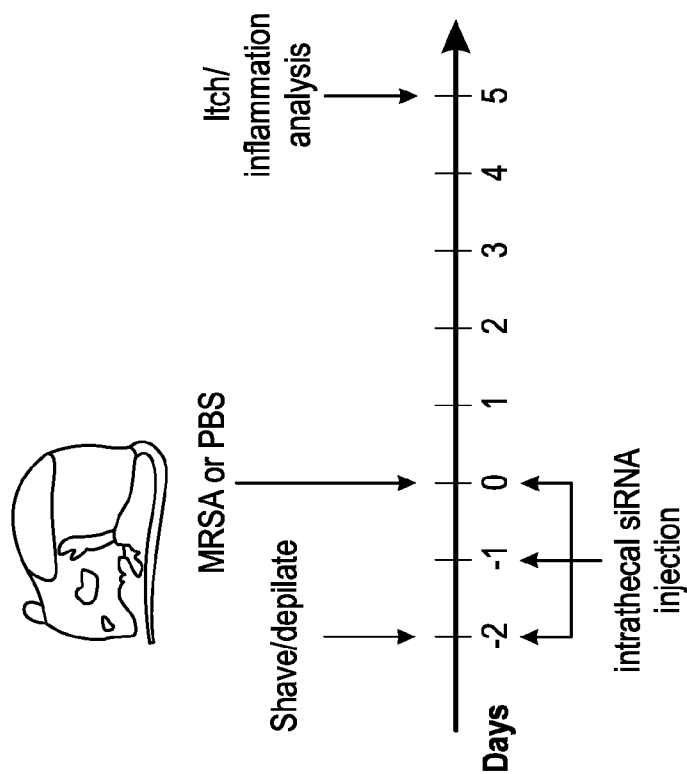
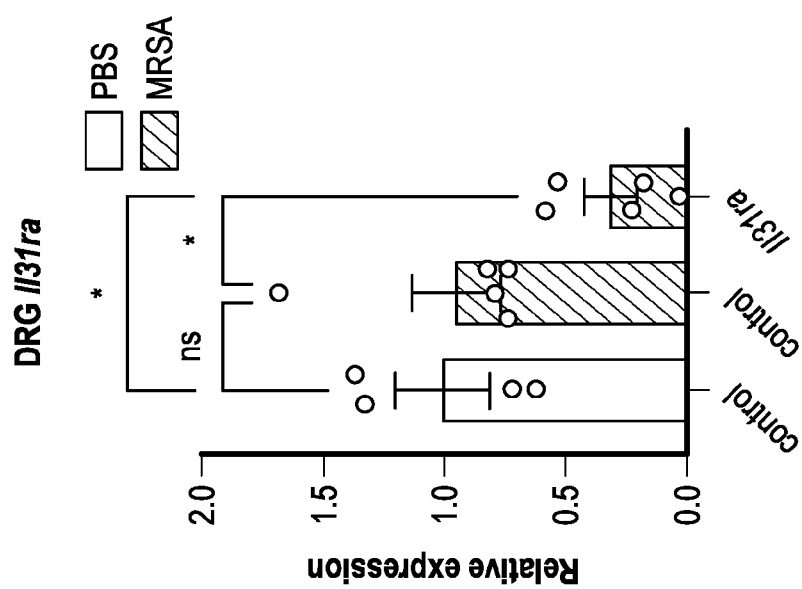


FIG. 10B

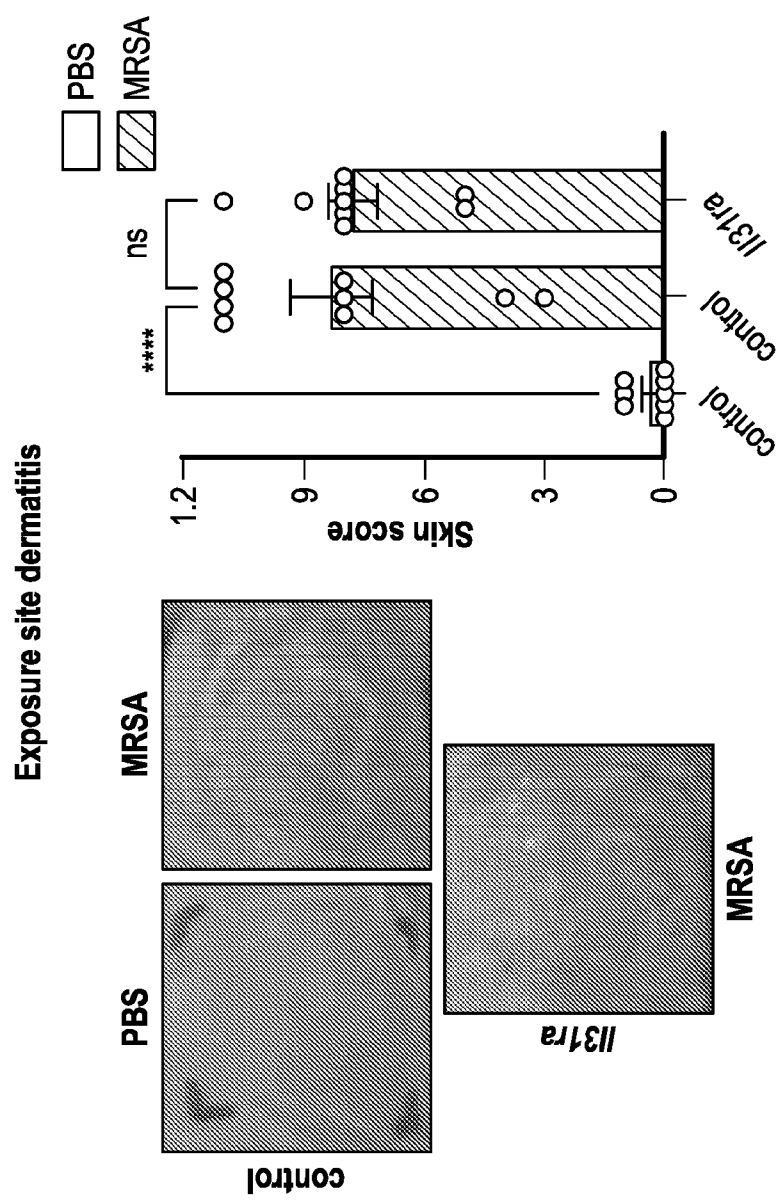


FIG. 10C

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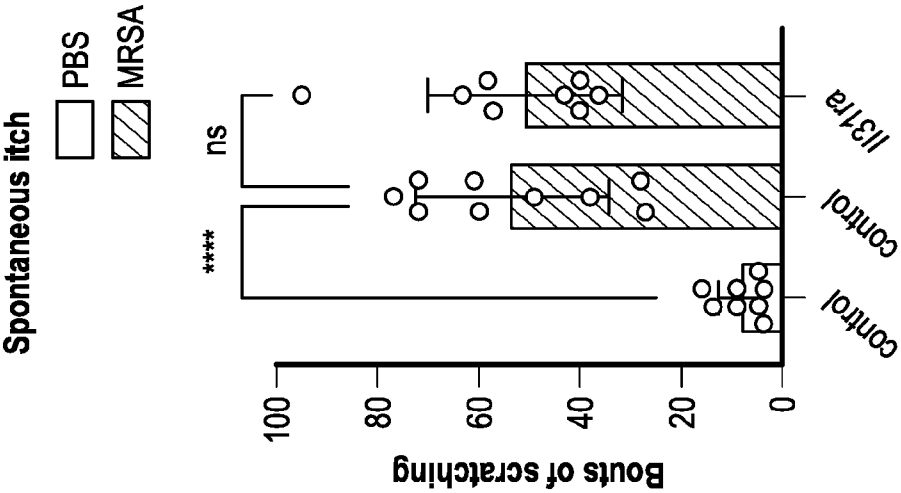


FIG. 10F

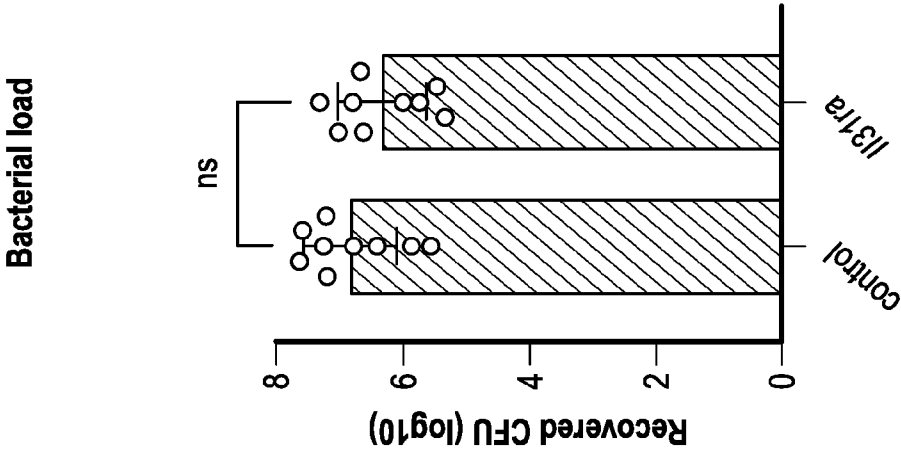


FIG. 10E

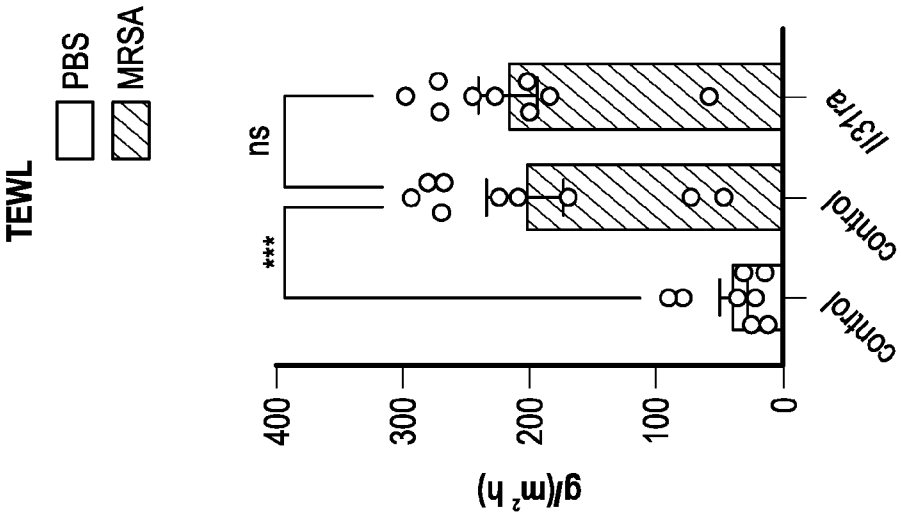
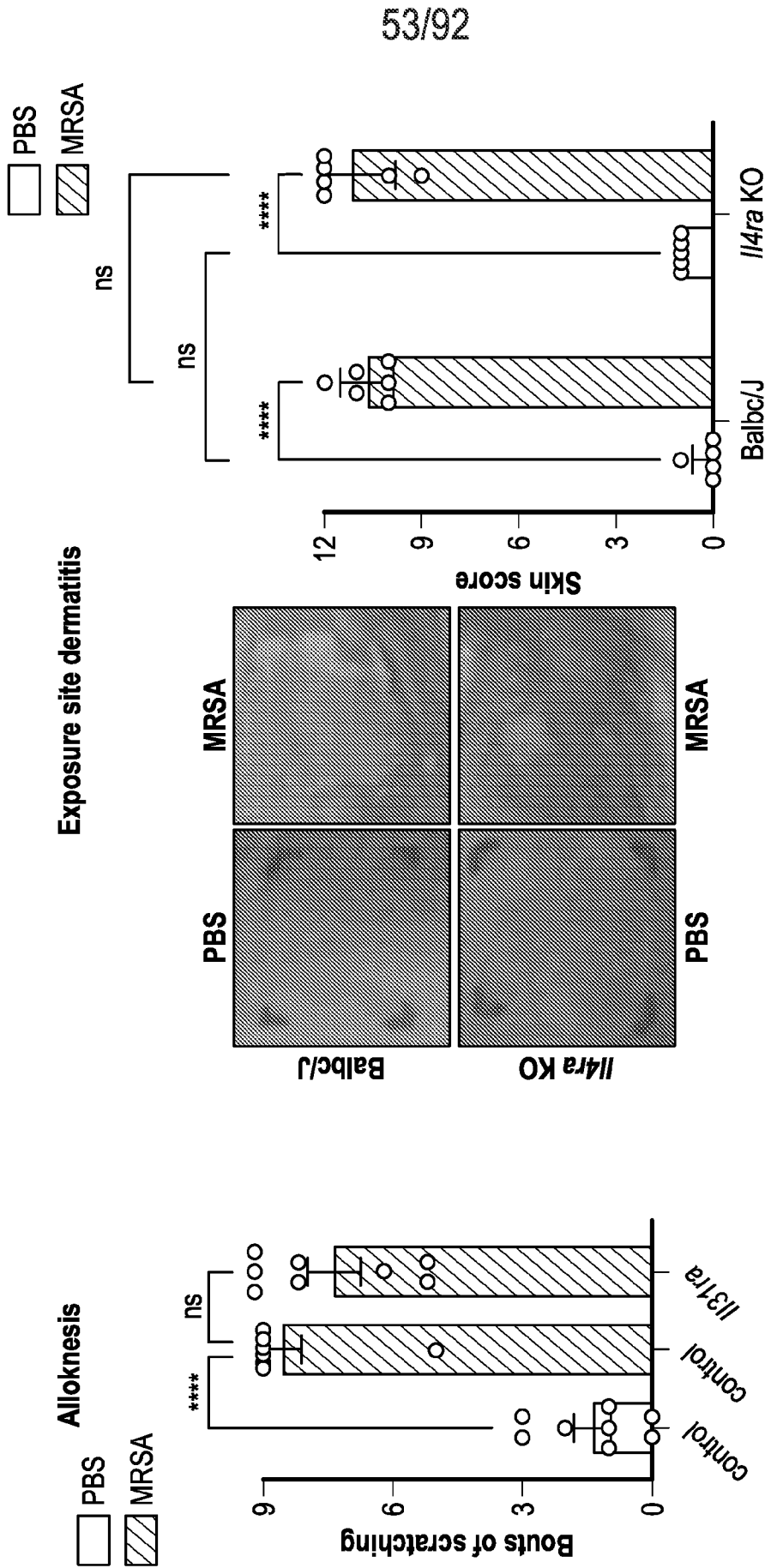


FIG. 10D



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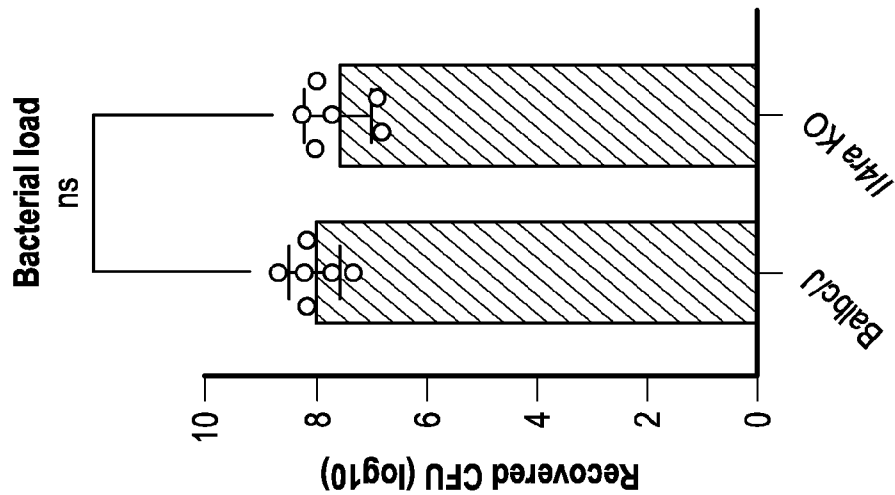


FIG. 10J

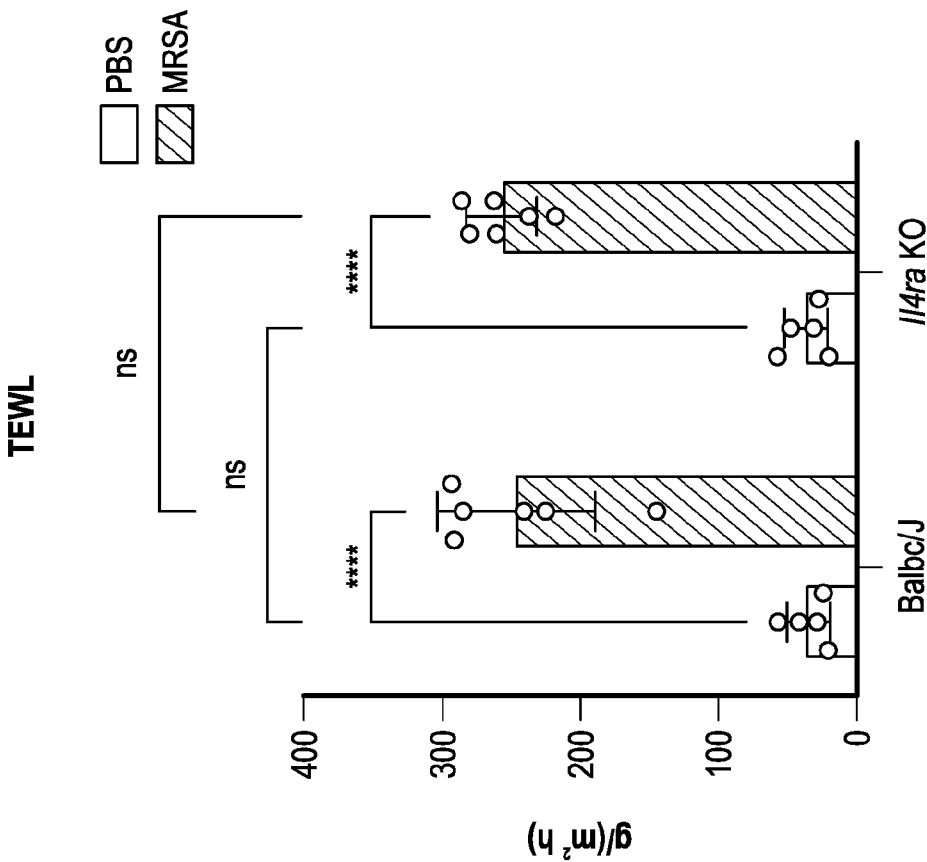


FIG. 10I

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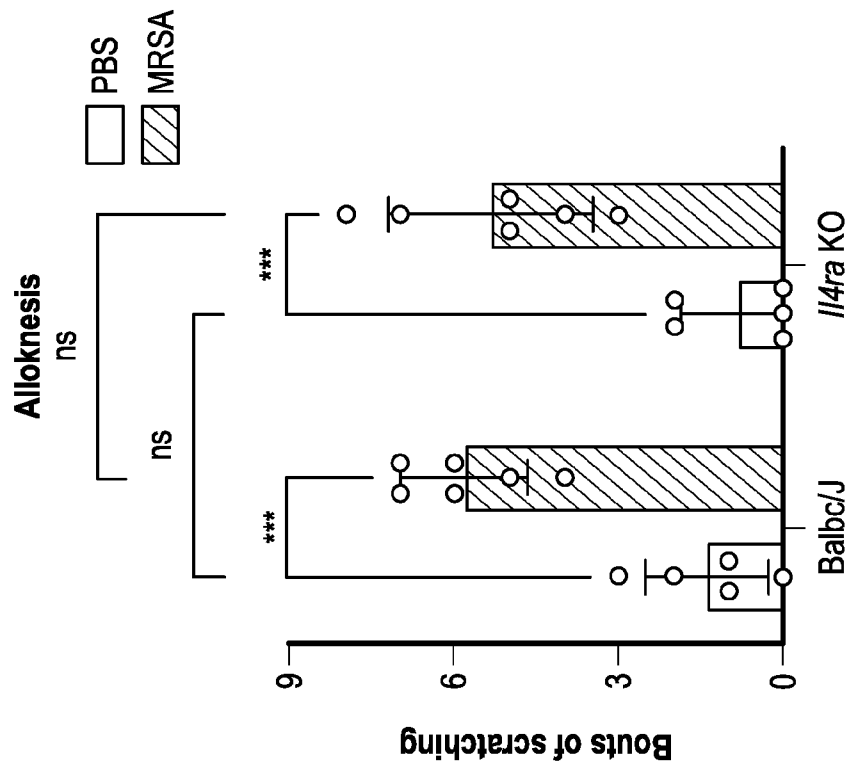


FIG. 10L

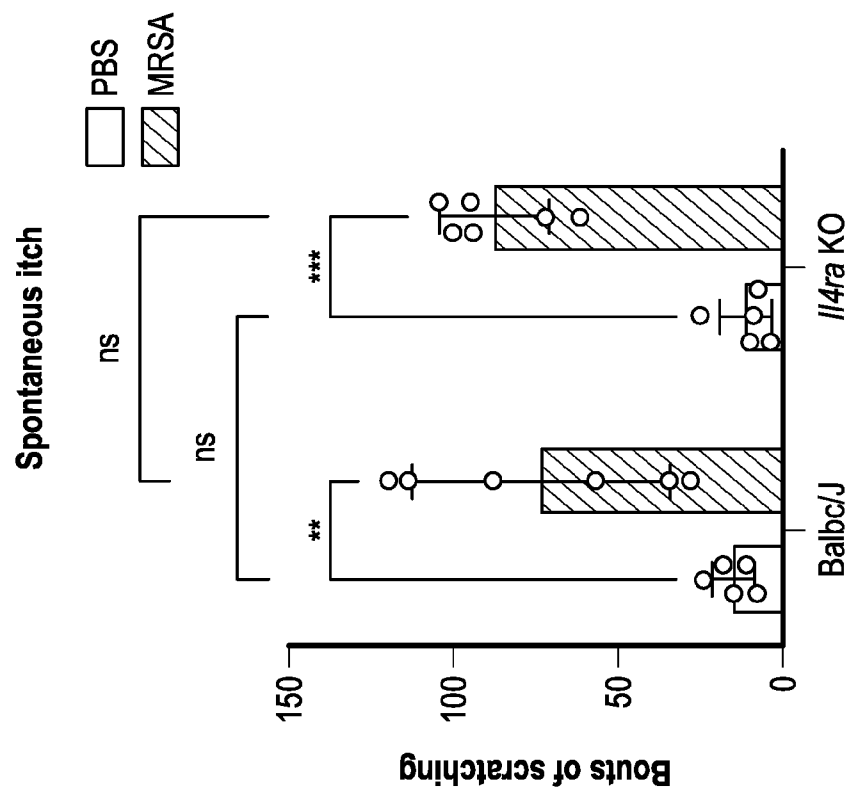


FIG. 10K

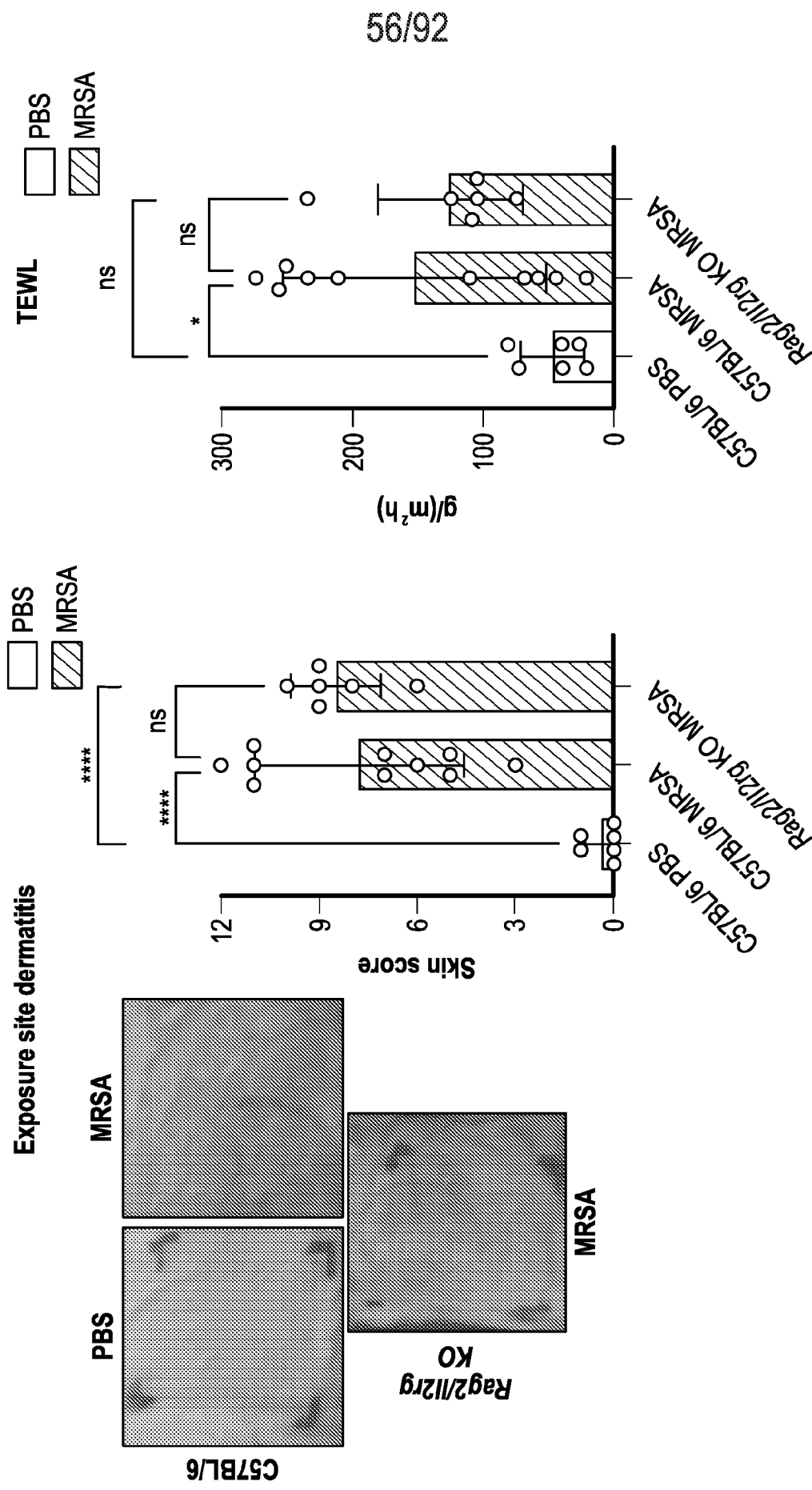


FIG. 10N

FIG. 10M

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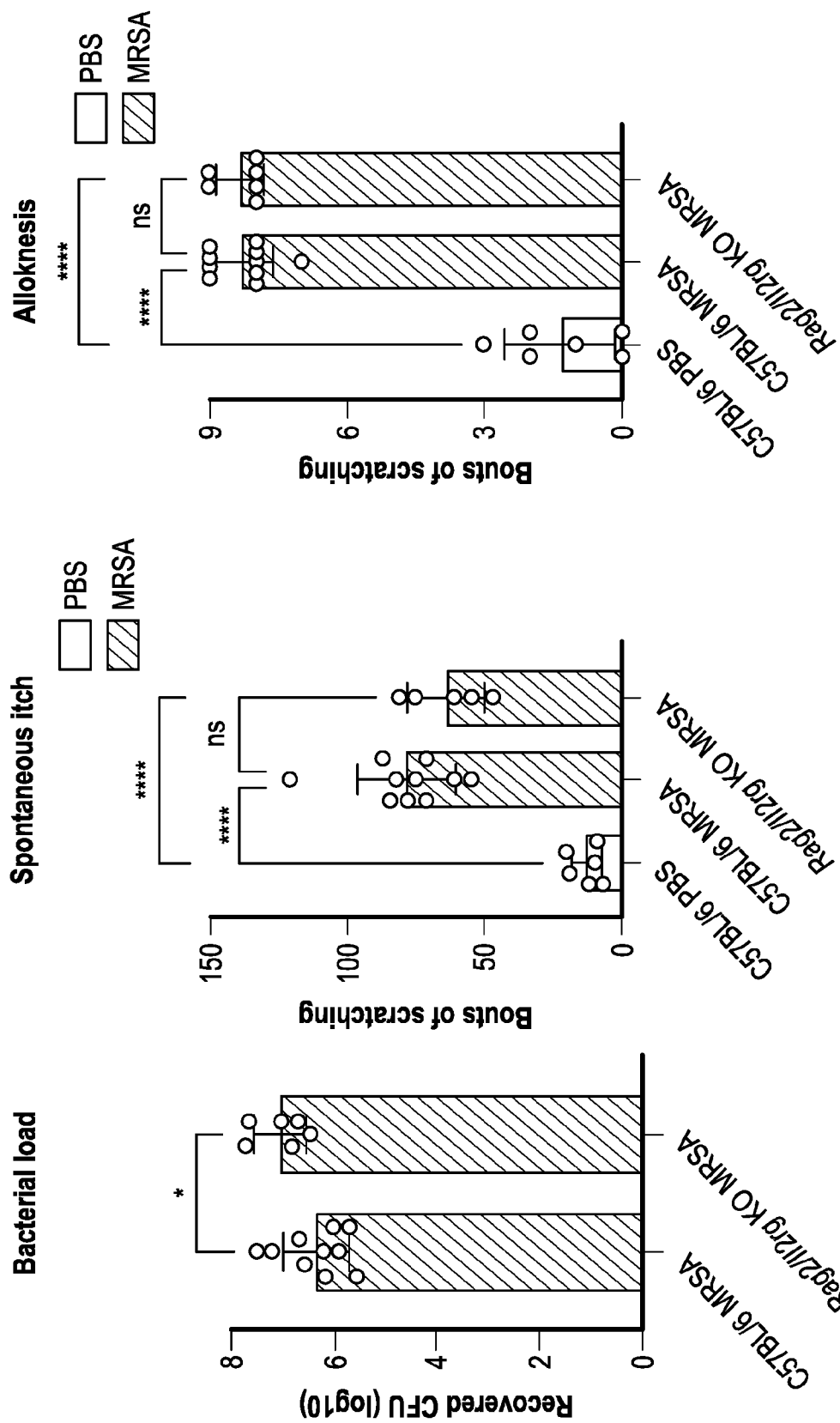


FIG. 10Q

FIG. 10P

FIG. 10O

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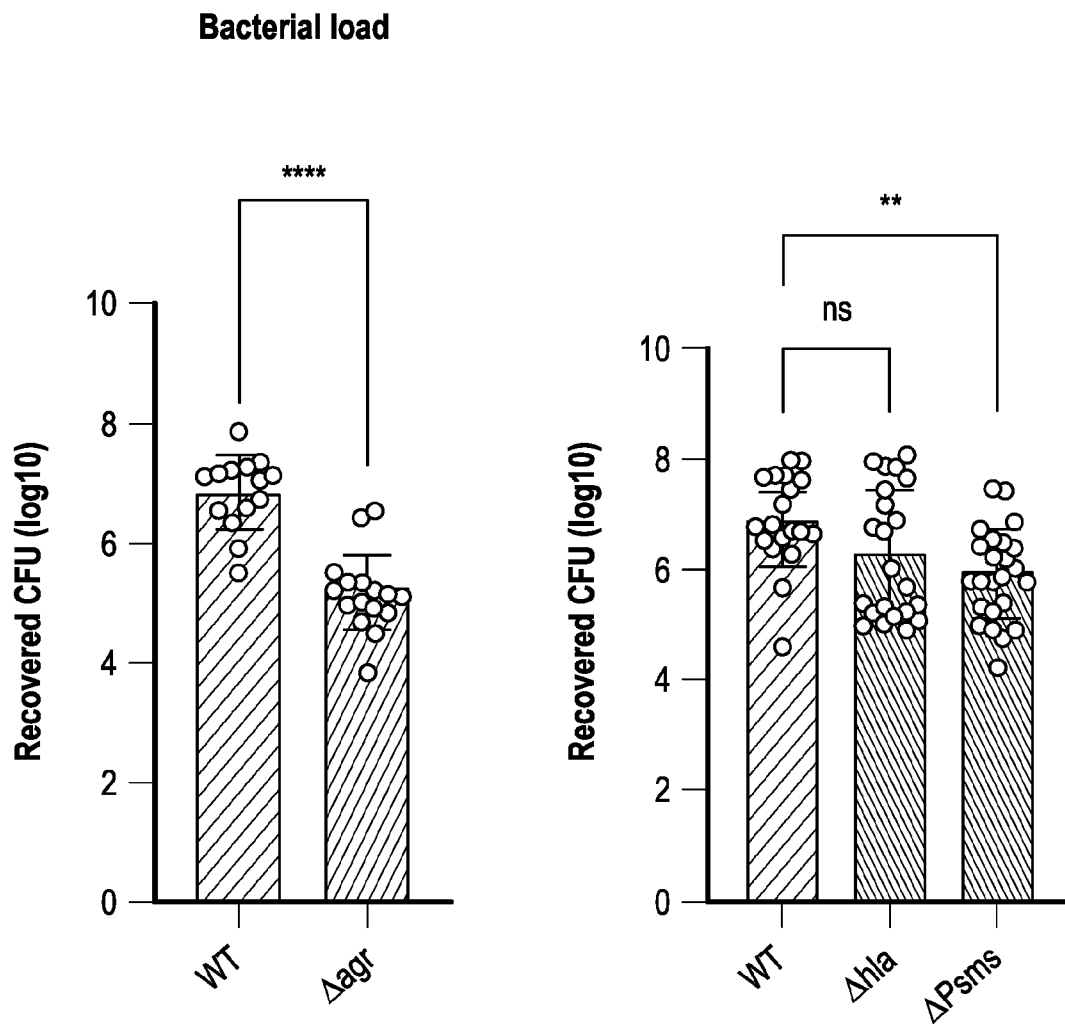


FIG. 11A (Cont...)

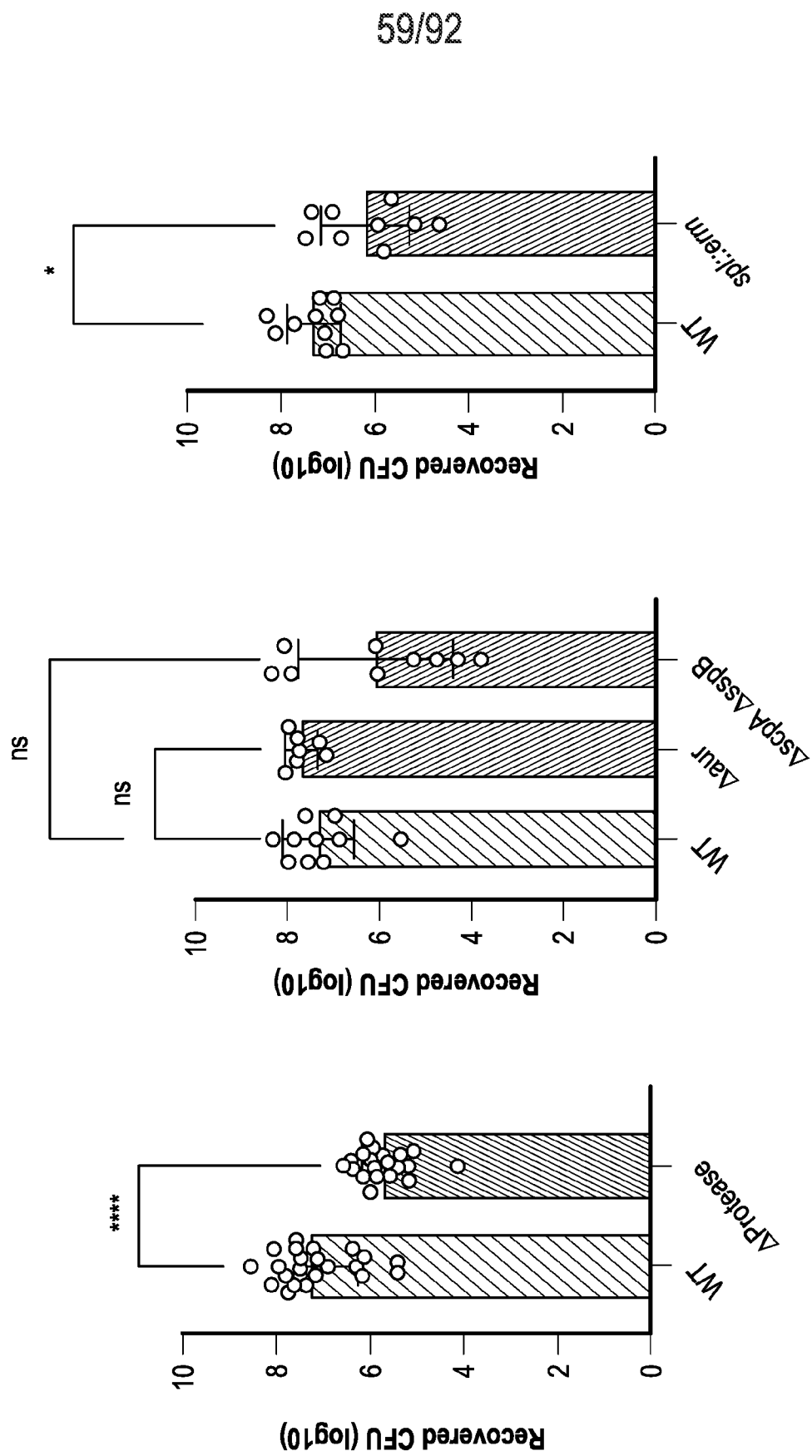


FIG. 11A

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Exposure site dermatitis

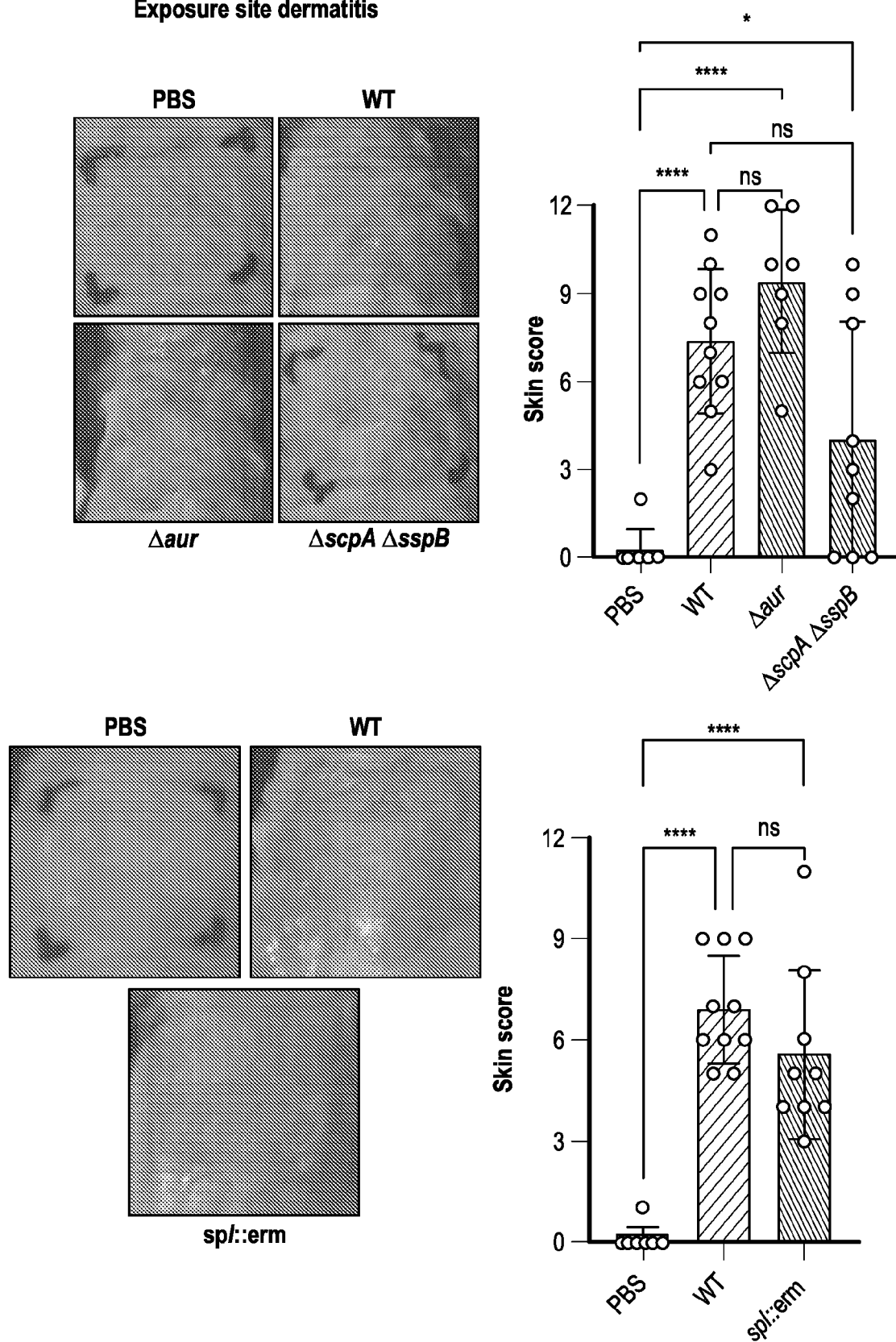
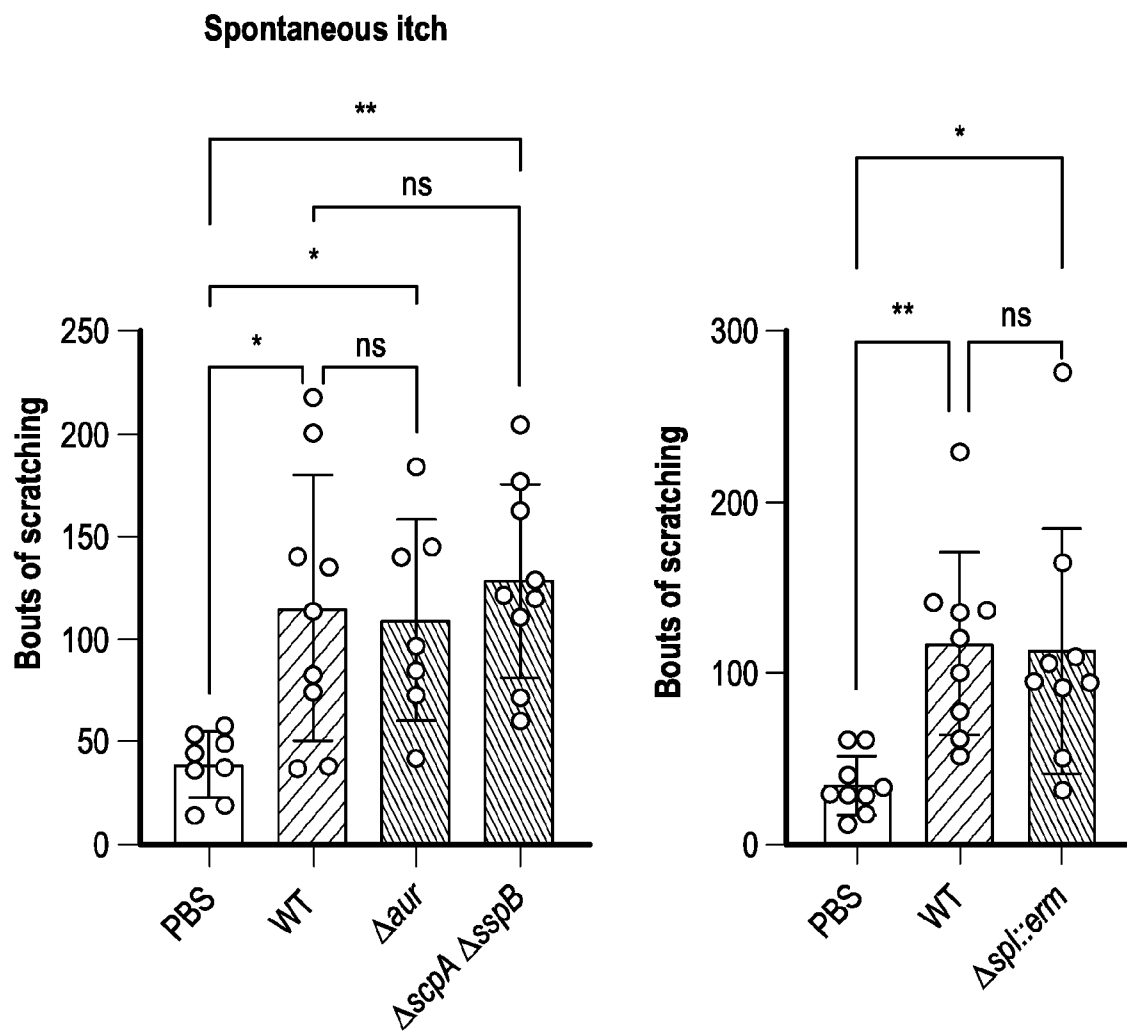
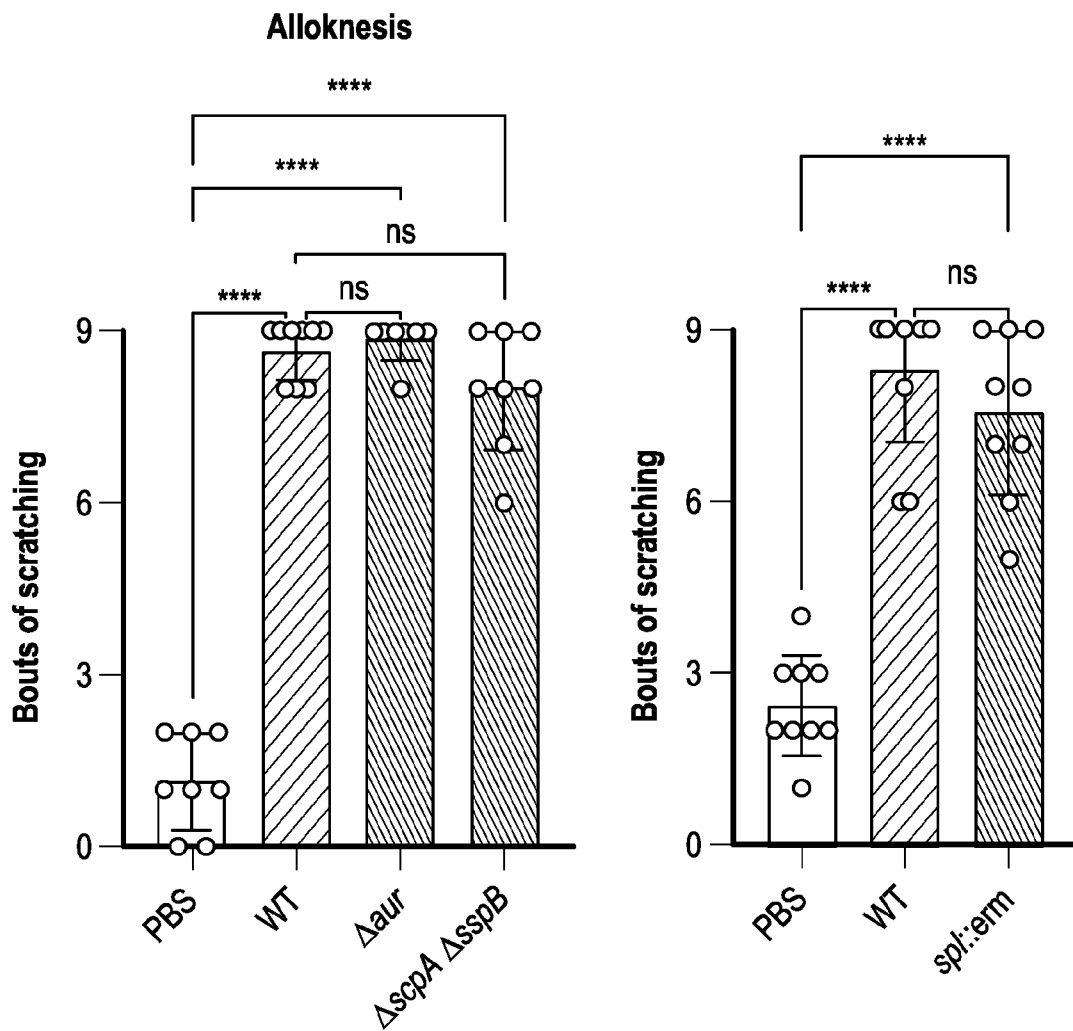


FIG. 11B

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**FIG. 11C**

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**FIG. 11D**

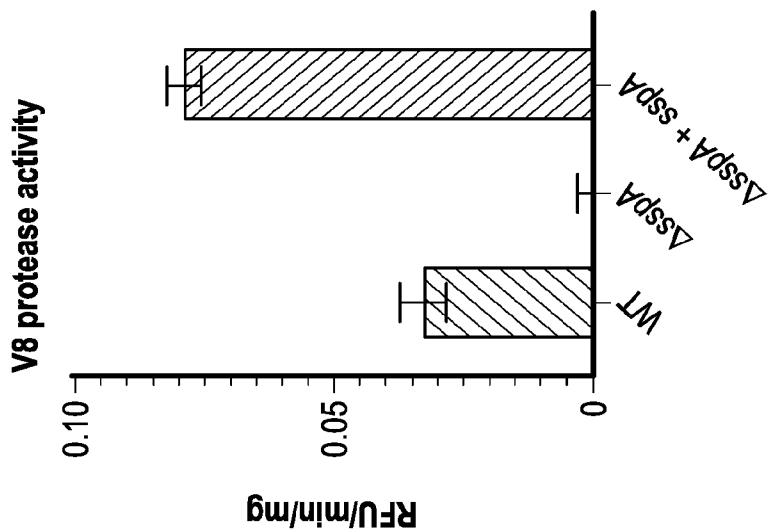
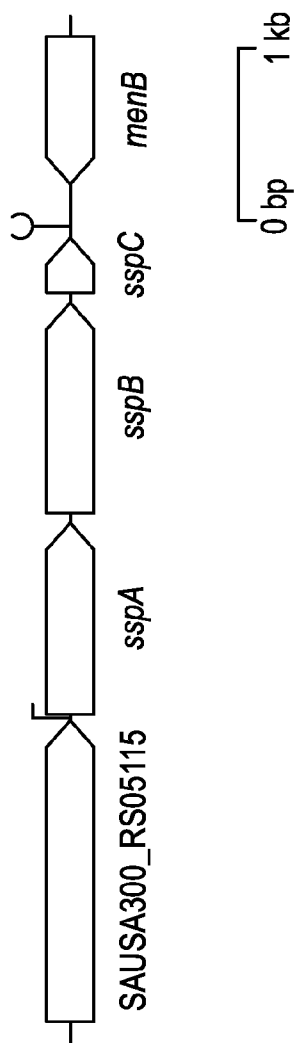


FIG. 12A

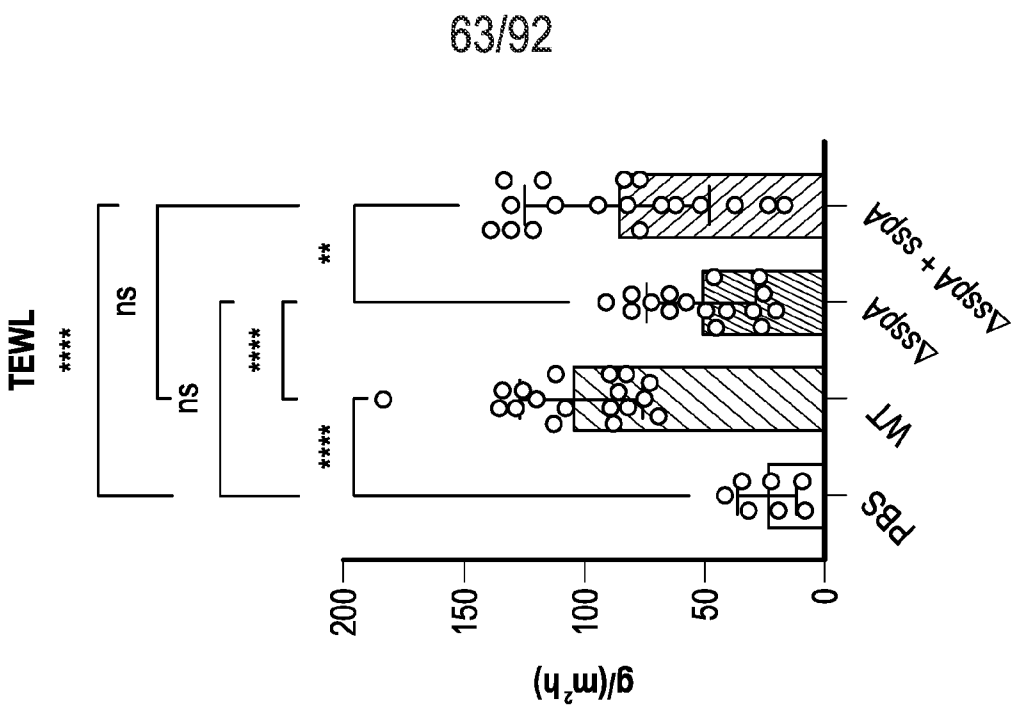
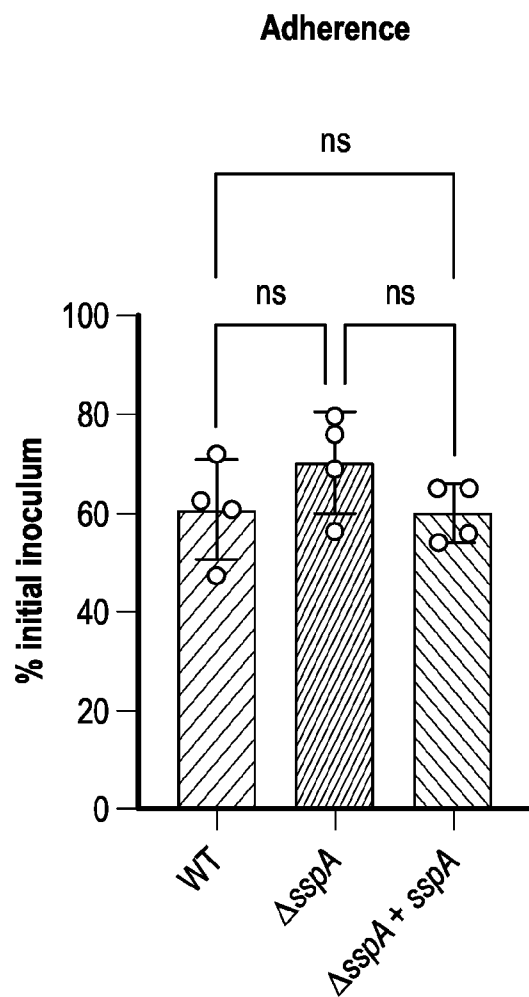
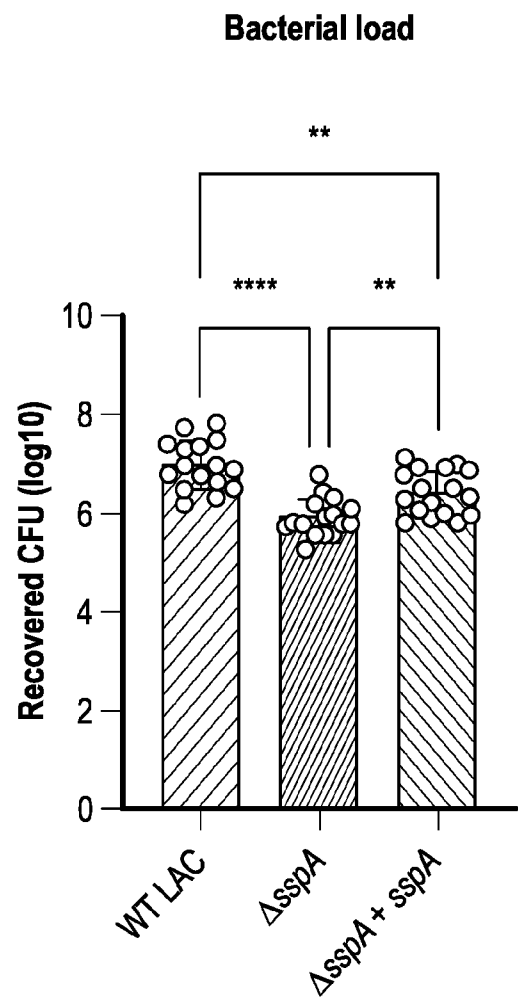


FIG. 12B

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**FIG. 12C****FIG. 12D**

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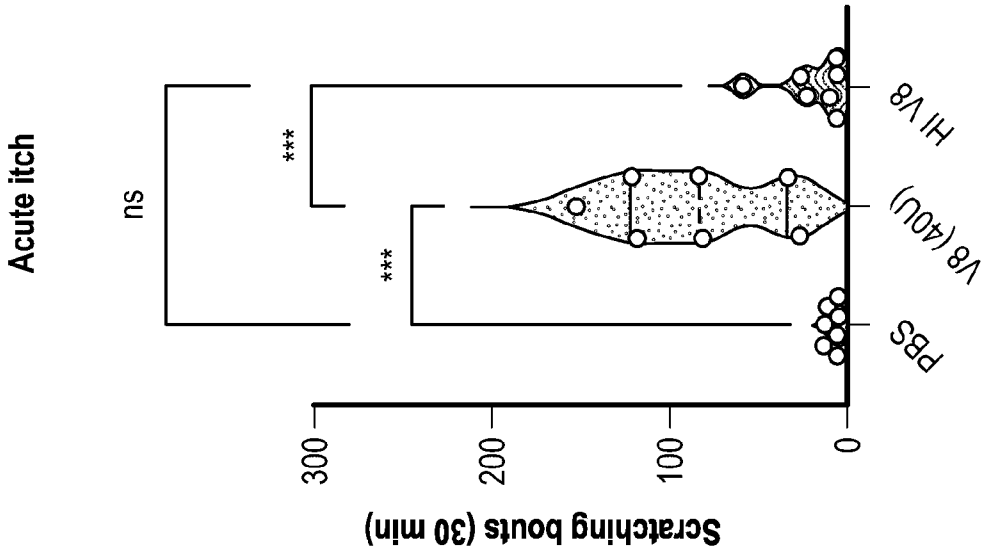


FIG. 12F

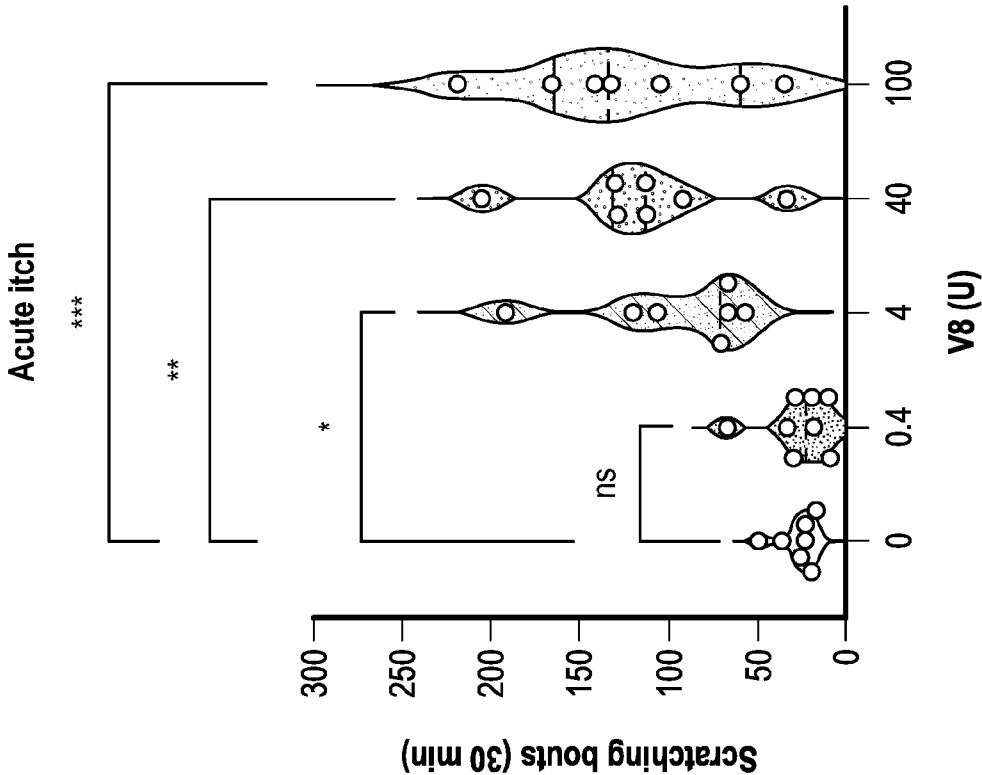


FIG. 12E

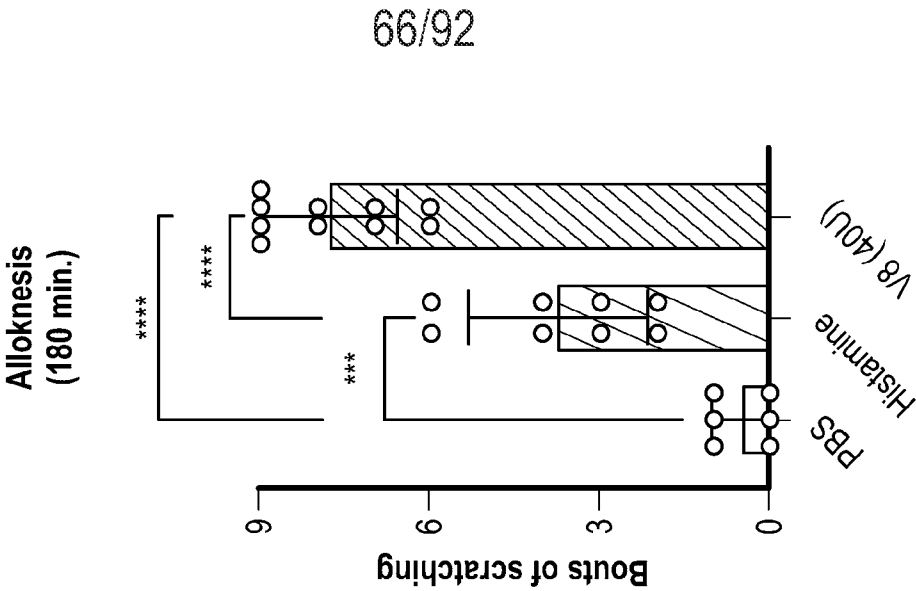


FIG. 12I

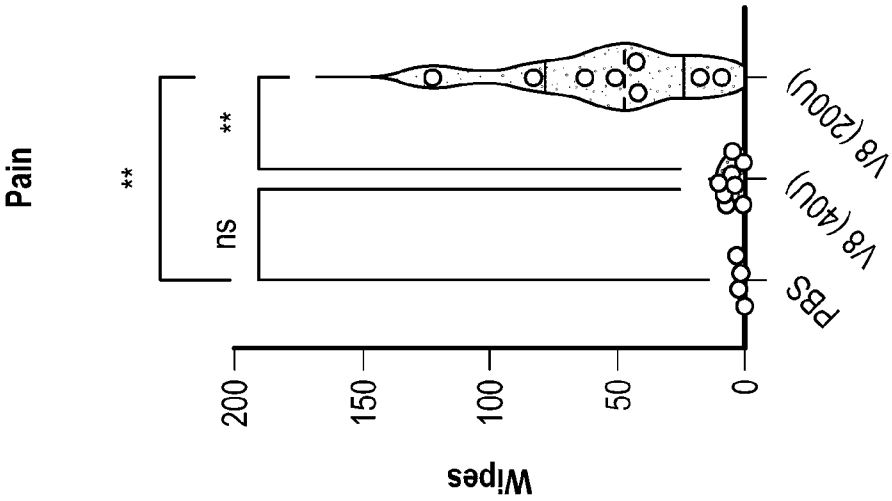


FIG. 12H

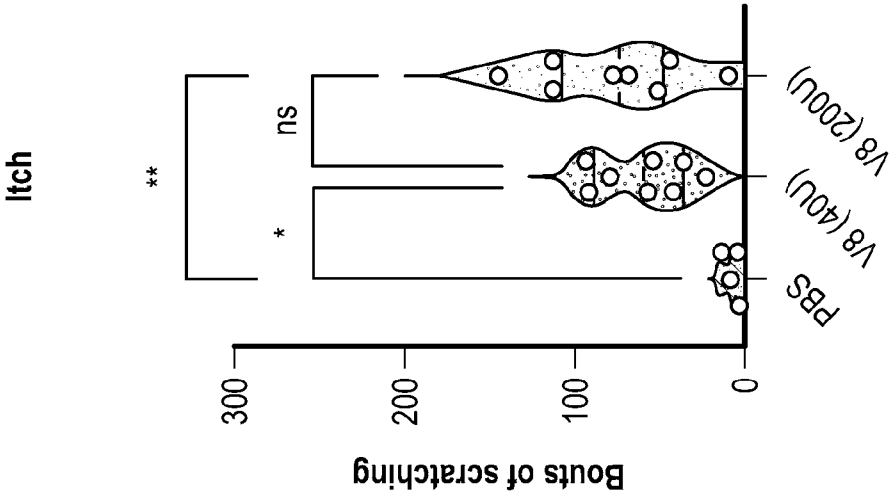


FIG. 12G

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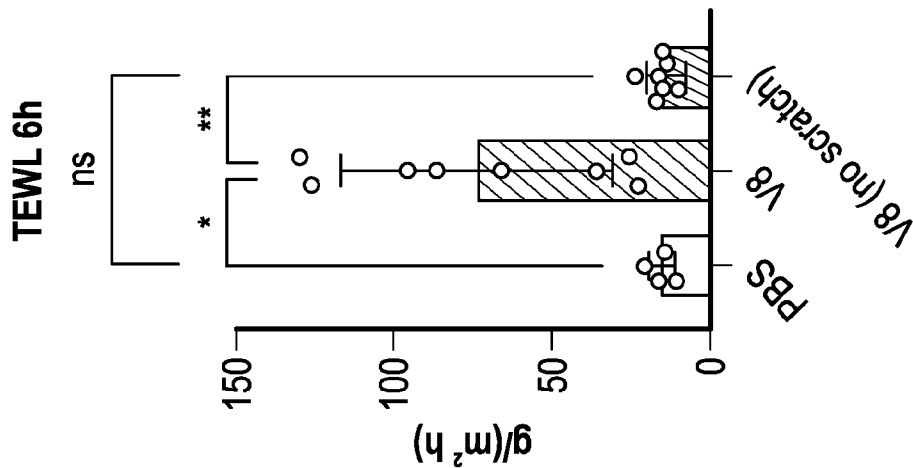


FIG. 12L

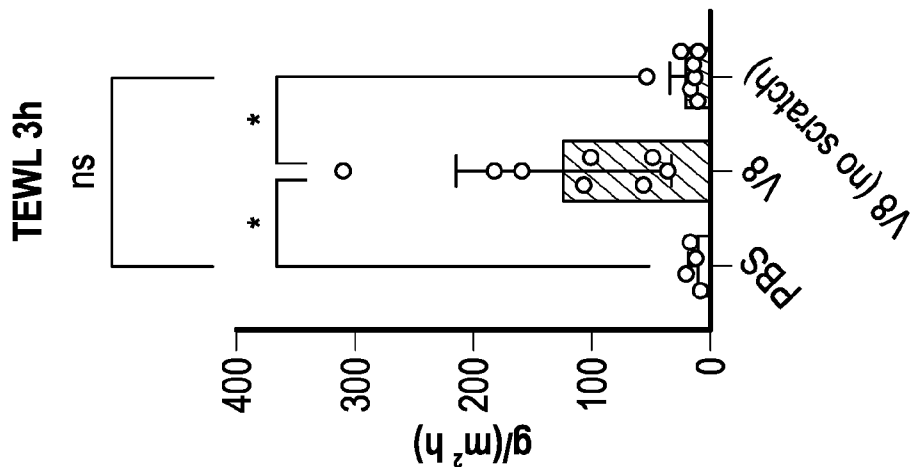


FIG. 12K

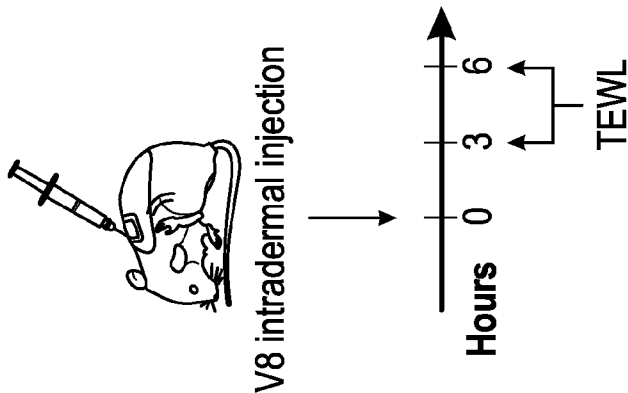
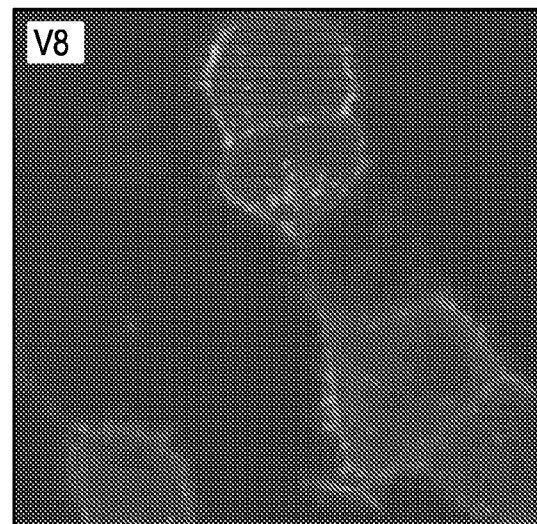
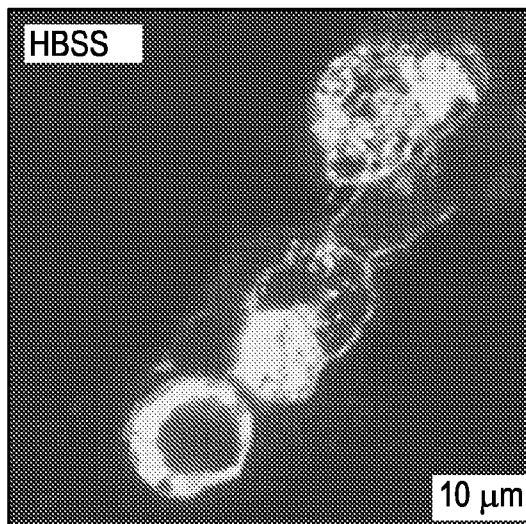
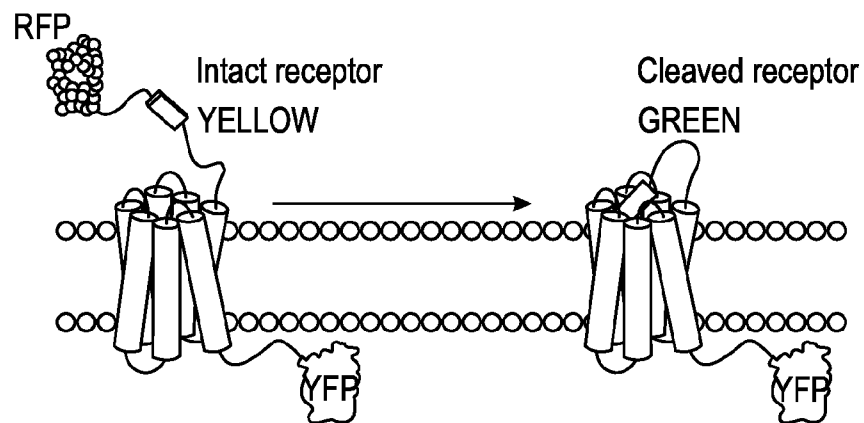


FIG. 12J

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Double brilliant PAR1 cleavage**FIG. 13A**

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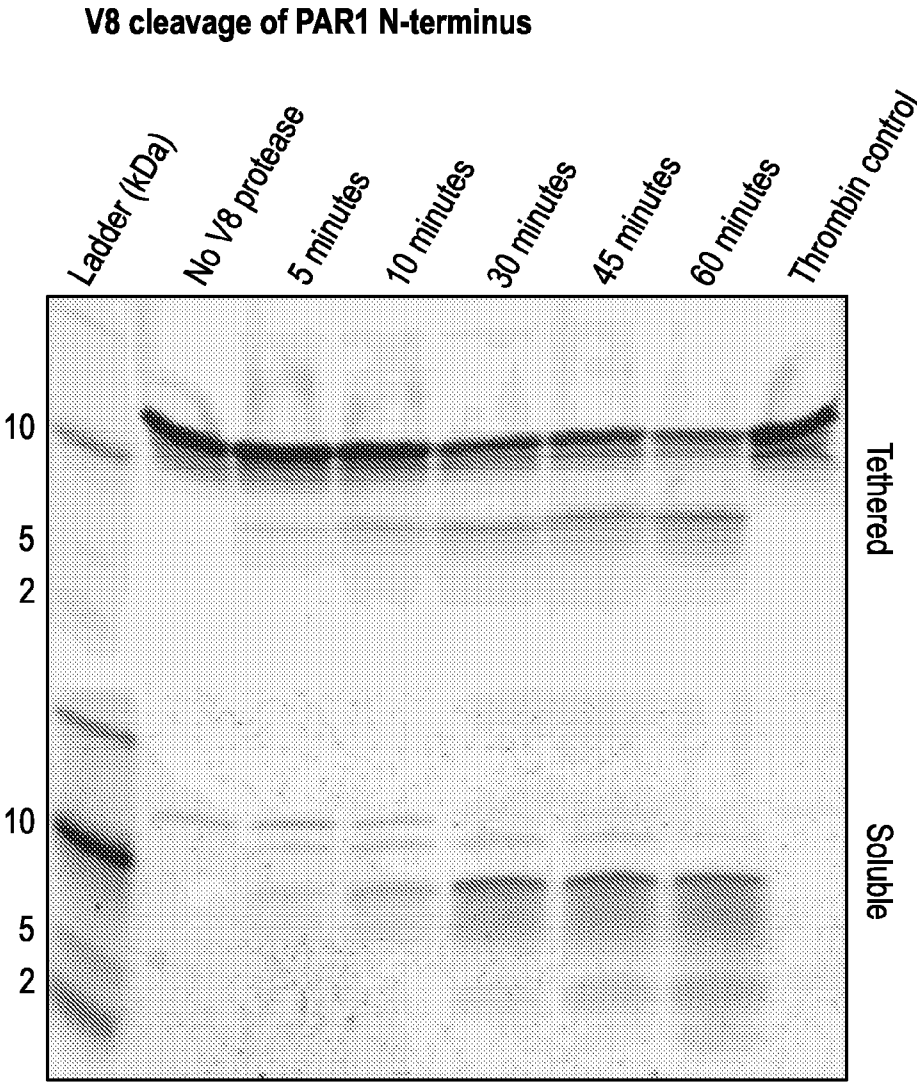


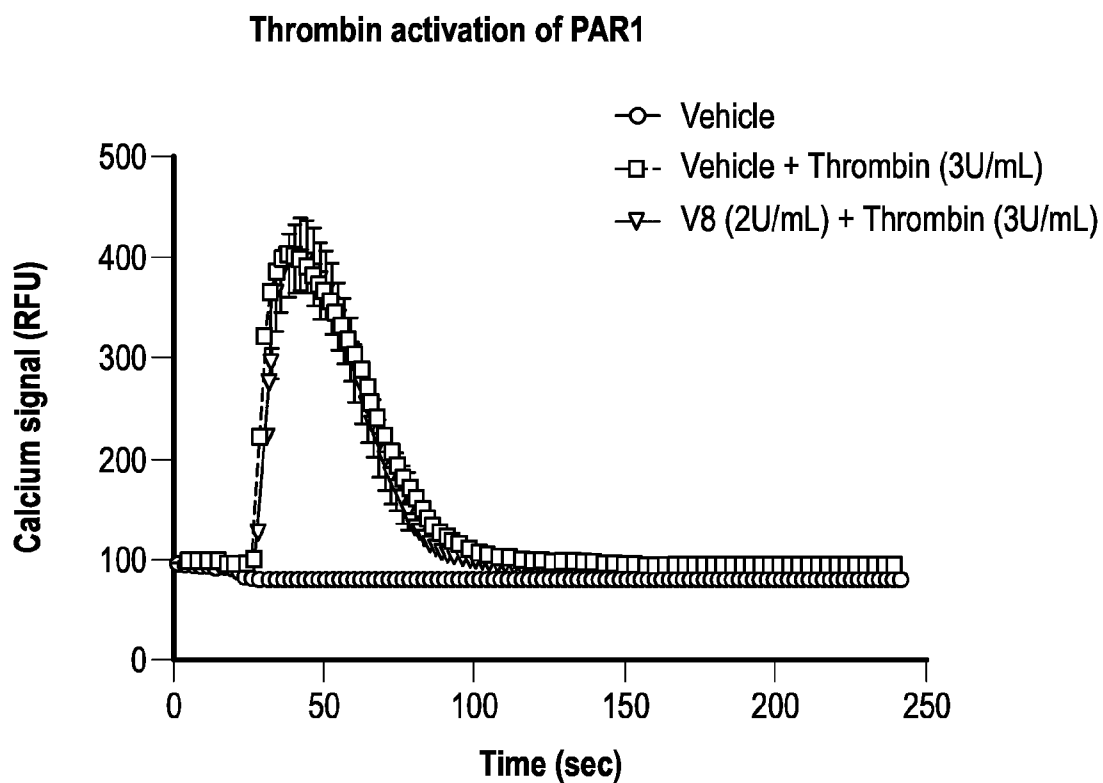
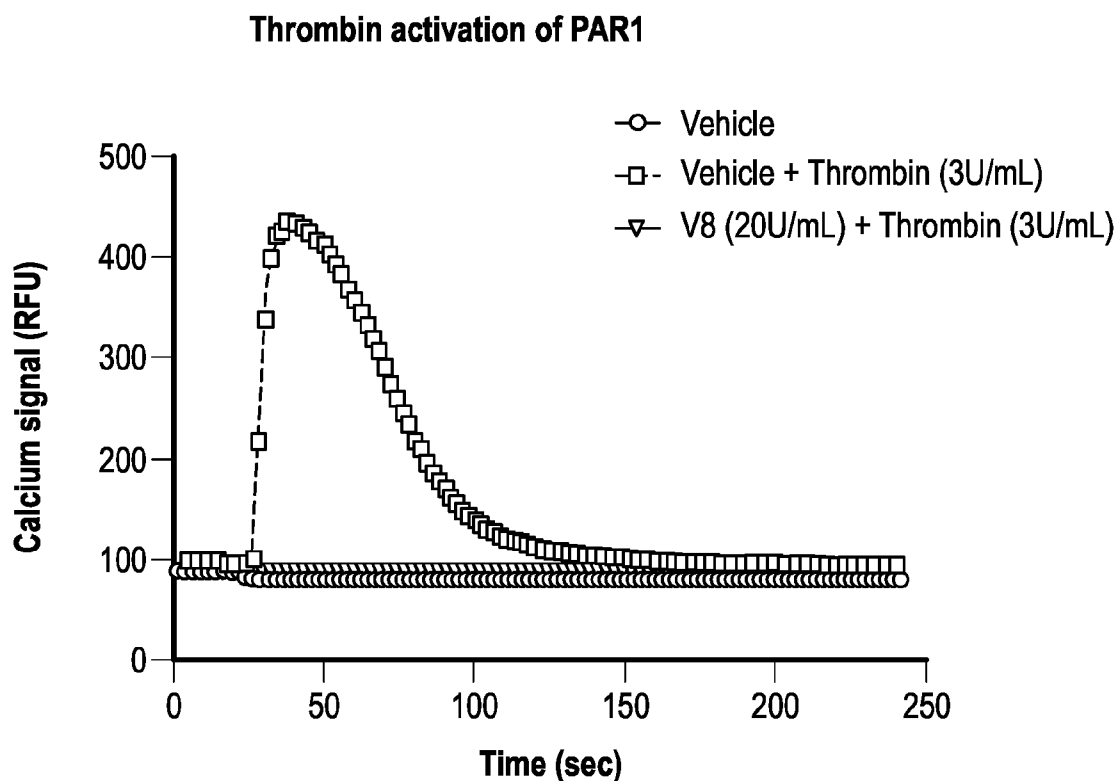
FIG. 13B

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Peptide (30 minutes)	Total Spectral Count	
	Soluble Fraction	Tothered Fraction
ASGYLTSSWLTE	4	2
ASGYLTSSWLTENLYFQGHHHHH		4
DASGYLTSSWLTE	60	50
DASGYLTSSWLTENLYFQGHHHHH	1	25
DEEKNESGLTE	8	2
EKKNESGLTE	1	
EKNESGLTE	3	1
KNESGLTE	4	4
KNESGLTEYRLVSINKSSPLQKQLPAFISEDASGYLTSSWLTENLYFQGHHHHH		1
KYEPFWE	1	
KYEPFWEDEEKNE	2	2
KYEPFWEDEEKNESGLTE	2	
NLYFQGHHHHH	2	227
SGLTEYRLVSINKSSPLQKQLPAFISE		1
SGLTEYRLVSINKSSPLQKQLPAFISEDASGYLTSSWLTE	1	7
SGLTEYRLVSINKSSPLQKQLPAFISEDASGYLTSSWLTENLYFQGHHHHH		10
SKATNATLDPRSFLRNPN	1	
SKATNATLDPRSFLRNPNNDKYEPFWE	8	4
SKATNATLDPRSFLRNPNNDKYEPFWEDE	2	
SKATNATLDPRSFLRNPNNDKYEPFWEDEE	1	
SKATNATLDPRSFLRNPNNDKYEPFWEDEEKNE	11	2
YRLVSINKSSPLQKQLPAFISE	40	10
YRLVSINKSSPLQKQLPAFISED	2	1
YRLVSINKSSPLQKQLPAFISEDASGYLTSSWLTE	105	92
YRLVSINKSSPLQKQLPAFISEDAGYLTSSWLTENLYFQGHHHHH	5	151

FIG. 13B(Cont...)

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**FIG. 13C****FIG. 13D**

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TFLLR-NH2 activation of PAR1

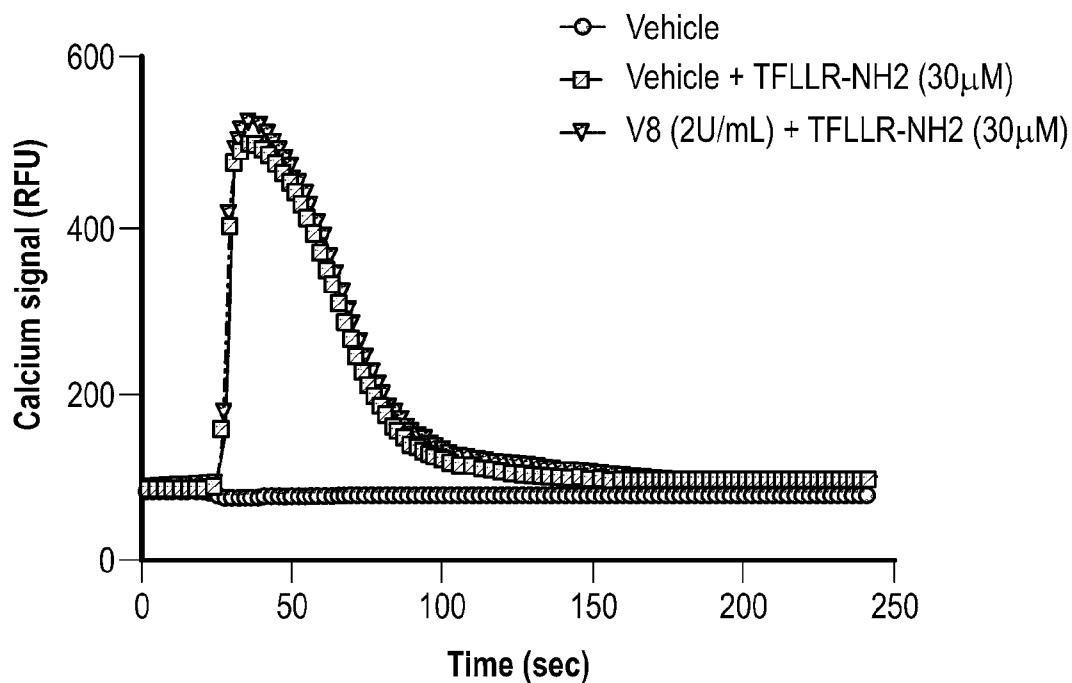


FIG. 13E

TFLLR-NH2 activation of PAR1

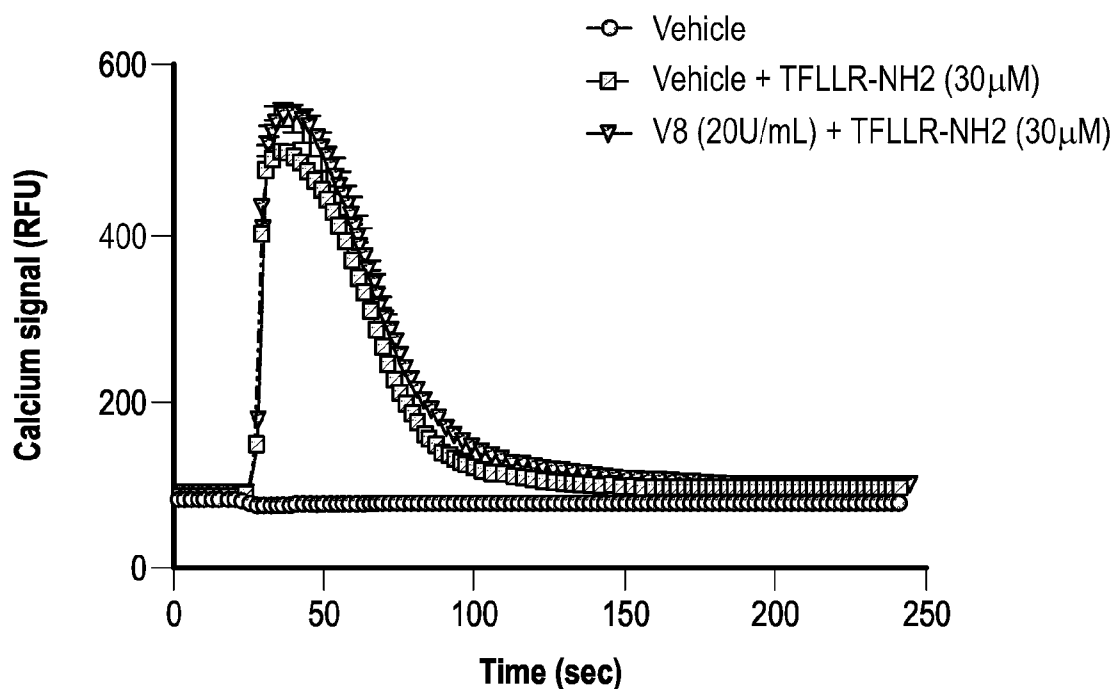


FIG. 13F

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Mouse DRG neurons

	Peptidergic								Neurofilament			Non Peptidergic						
	TrpM8	TrpM8	TrpM8	PEP1.2	PEP1.3	PEP1.1	PEP1.4	PEP2	NF2/3	NF4/5	NF1	TH	NP1.1	NP1.2	NP2.1	NP2.2	NP3	
Scn 10a	0.00	0.08	0.00	0.20	1.56	1.16	2.51	3.28	0.61	0.04	0.03	1.08	3.71	5.28	5.47	4.99	4.00	
Trpv1	2.21	0.67	0.31	3.07	2.68	1.02	3.26	0.56	0.00	0.05	0.00	0.01	0.06	0.05	0.04	0.73	1.95	
Trpa1	0.00	0.00	0.00	0.00	0.20	0.00	0.12	0.00	0.00	0.00	0.00	0.05	0.36	0.31	0.07	0.00	0.13	
Mrgprd	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.05	0.00	0.01	10.60	9.00	1.20	1.43	0.48	
Mrgpra3	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.10	0.00	0.00	0.00	0.01	8.40	3.30	0.06	
Mrgprx1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	
Nppb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.04	0.00	9.17	
Hrh1	0.00	0.00	0.00	0.00	0.12	0.00	0.02	0.01	0.10	0.00	0.03	0.01	0.00	0.01	0.25	0.63	0.47	
S1pr3	0.07	0.05	0.11	0.57	3.54	1.29	2.05	2.89	2.31	0.26	0.97	0.07	0.06	0.08	0.00	0.10	0.51	
F2r11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.02	0.18	0.03	0.97	
F2r13	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.02	0.00	0.02	0.00	0.00	0.01	0.01	0.04	0.00	0.01	
F2r	0.04	0.06	0.02	0.03	0.81	0.00	0.01	0.54	0.55	0.11	0.26	0.05	0.03	0.28	0.56	0.15	0.05	

FIG. 14A

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Human DRG neurons

	C-LTMRs	Nociceptors						Mechanoreceptors			Prop.
		Pruri- ceptor	Silent	TRPA1+	PENK+	A δ HTMRs	Cold	A β noc.	A δ LTMRs	A β RA LTMRs	
SNAP25	3.09	3.98	4.68	6.02	8.35	9.96	8.51	9.22	10.19	10.52	15.11
TRPV1	2.18	3.29	3.07	2.06	0.76	1.36	0.54	0.70	0.37	0.35	0.28
SCN10A	0.74	1.20	0.95	0.84	0.52	1.18	0.95	0.74	0.09	0.12	0.07
SCN11A	3.11	6.69	2.64	0.92	0.58	0.92	2.08	0.82	0.16	0.24	0.20
GFRA2	1.58	2.71	0.12	0.11	0.11	0.11	0.10	0.22	0.25	0.27	0.08
IL31RA	1.23	2.13	4.92	0.36	0.10	0.09	0.14	0.13	0.14	0.10	0.10
NPPB	0.27	0.57	0.60	0.04	0.01	0.02	0.01	0.05	0.01	0.03	0.02
F2R	0.02	0.09	0.02	0.03	0.02	0.02	0.03	0.02	0.02	0.02	0.02

FIG. 14B

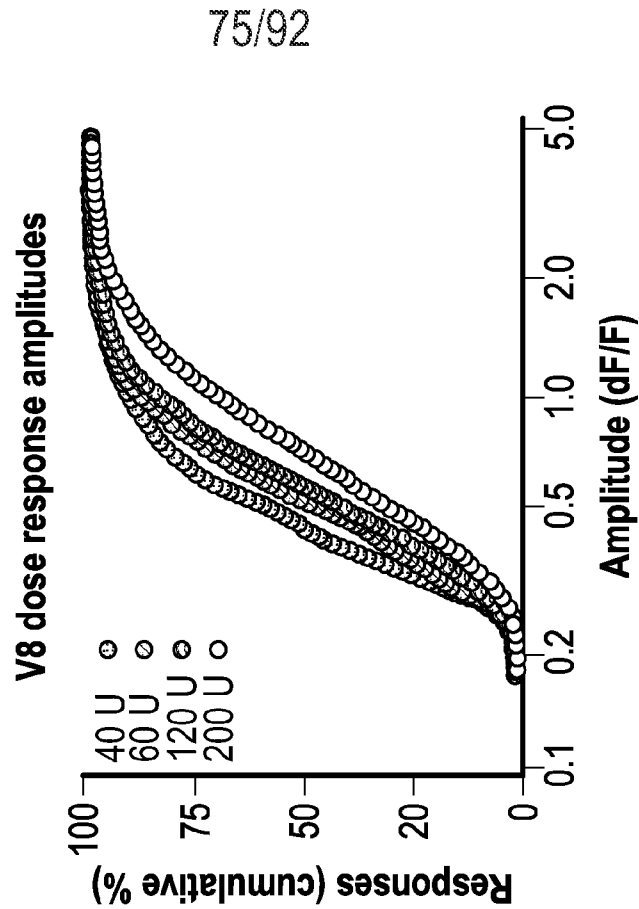


FIG. 14D

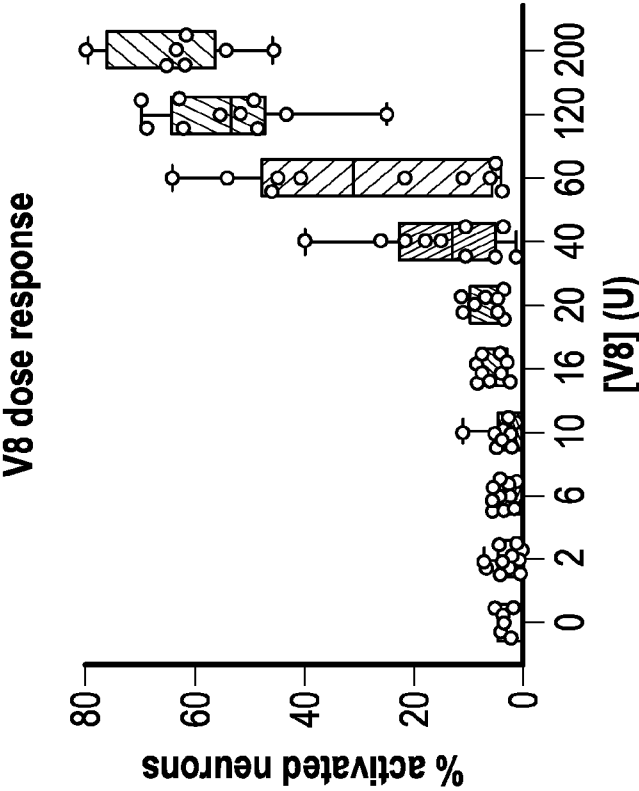
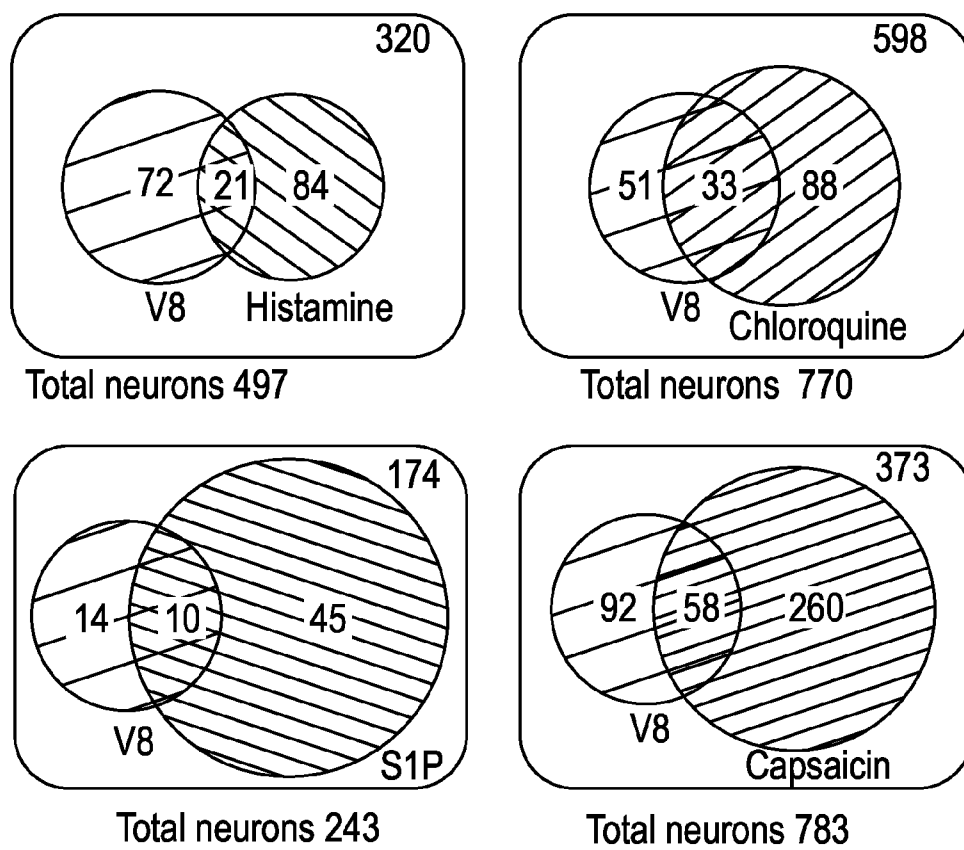


FIG. 14C

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**FIG. 14E**

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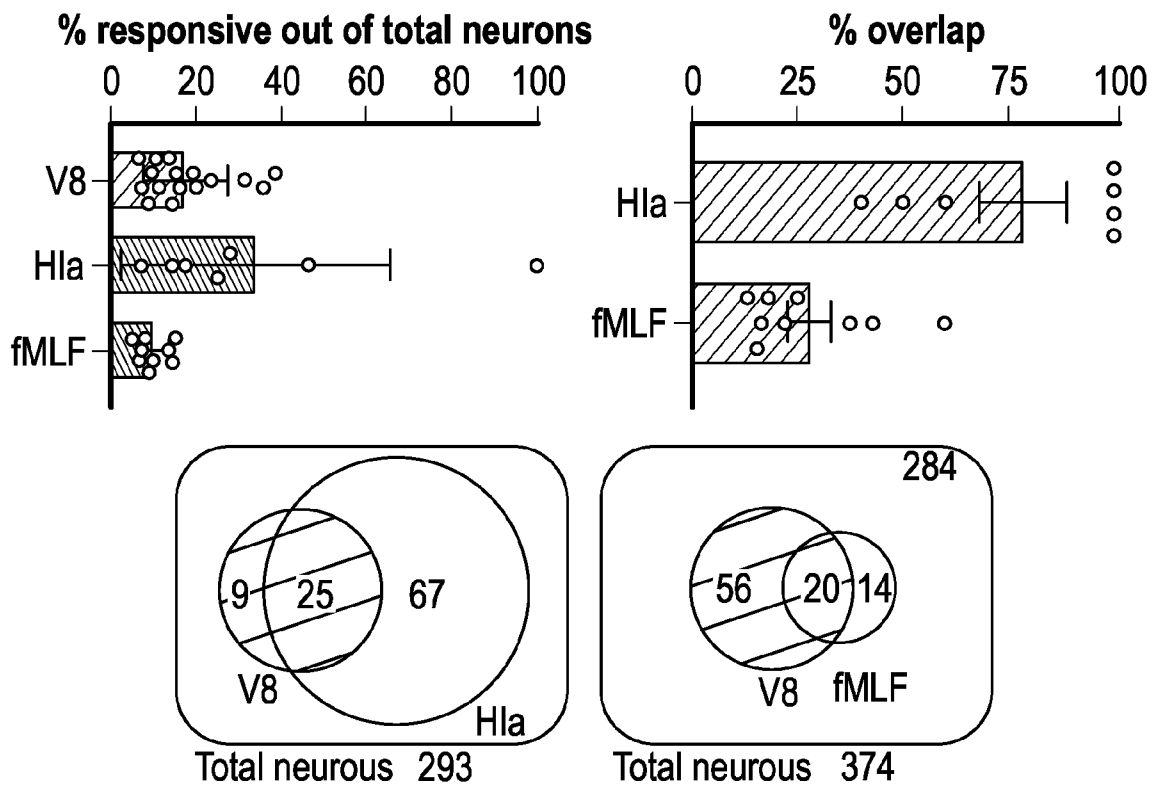


FIG. 14F

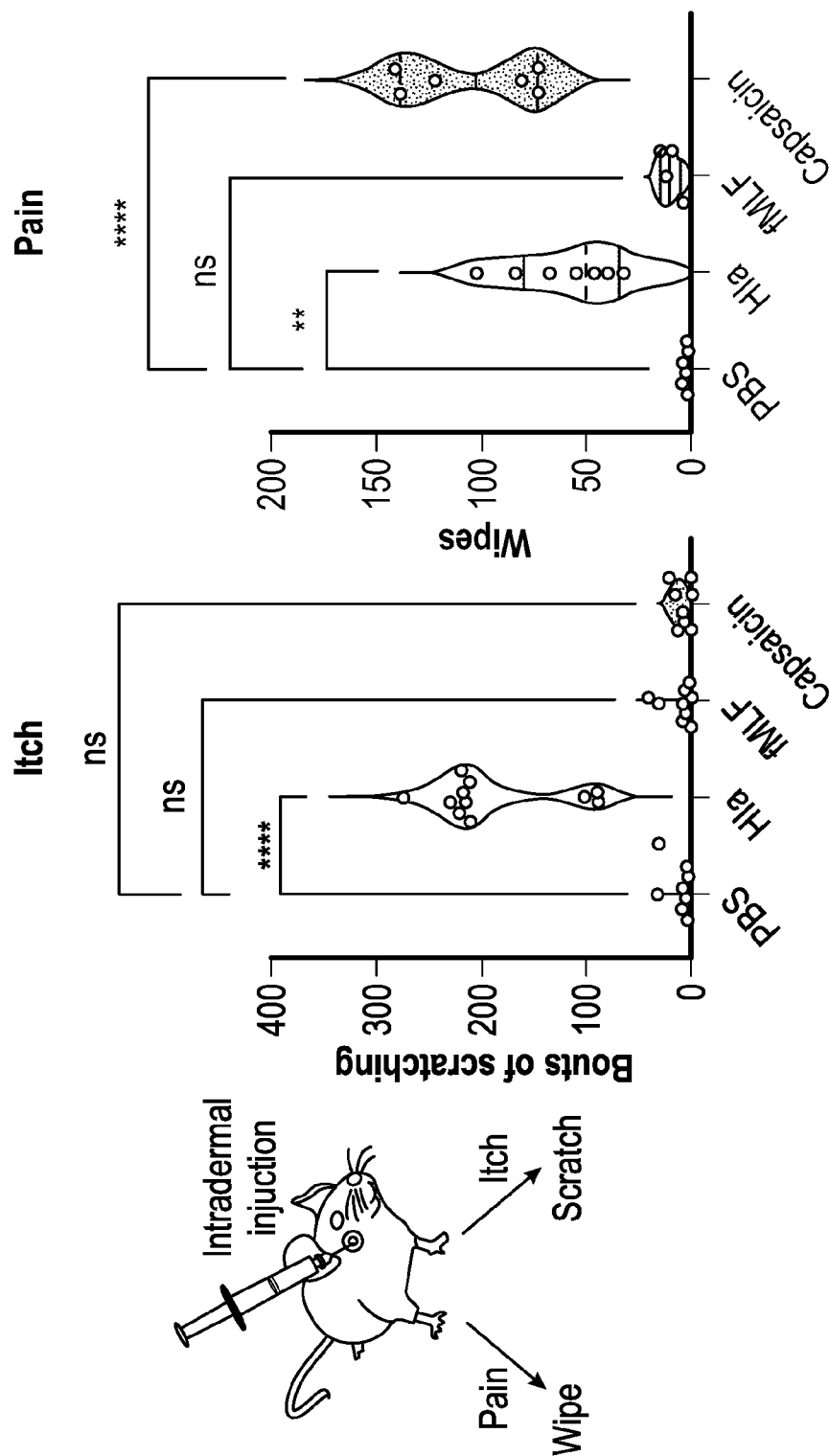


FIG. 14G

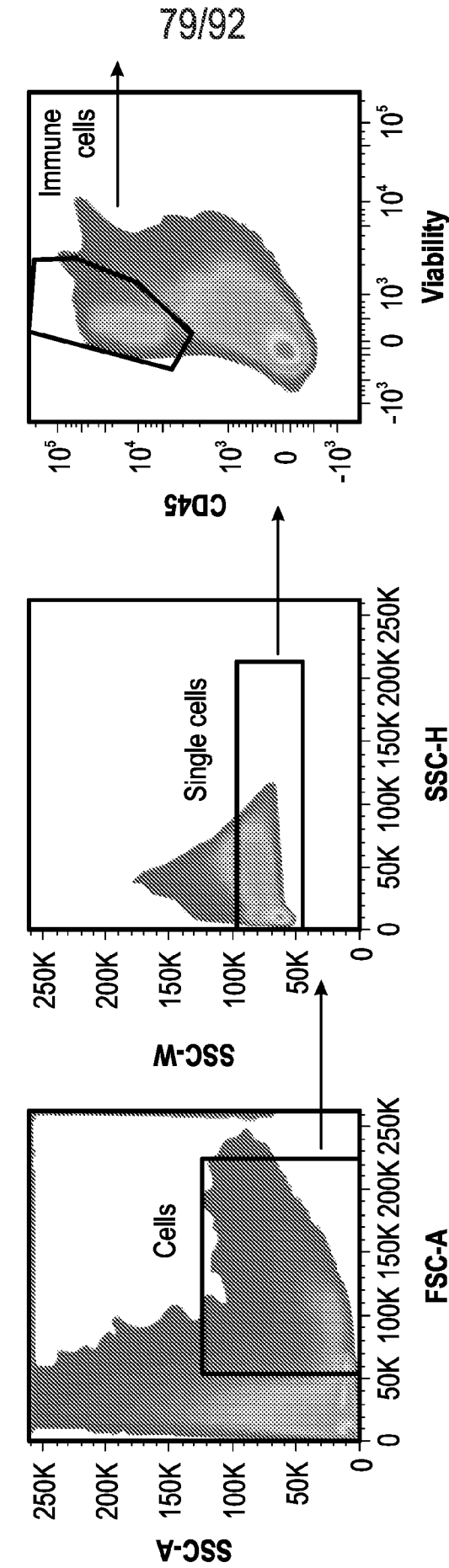


FIG. 15A(Cont...)

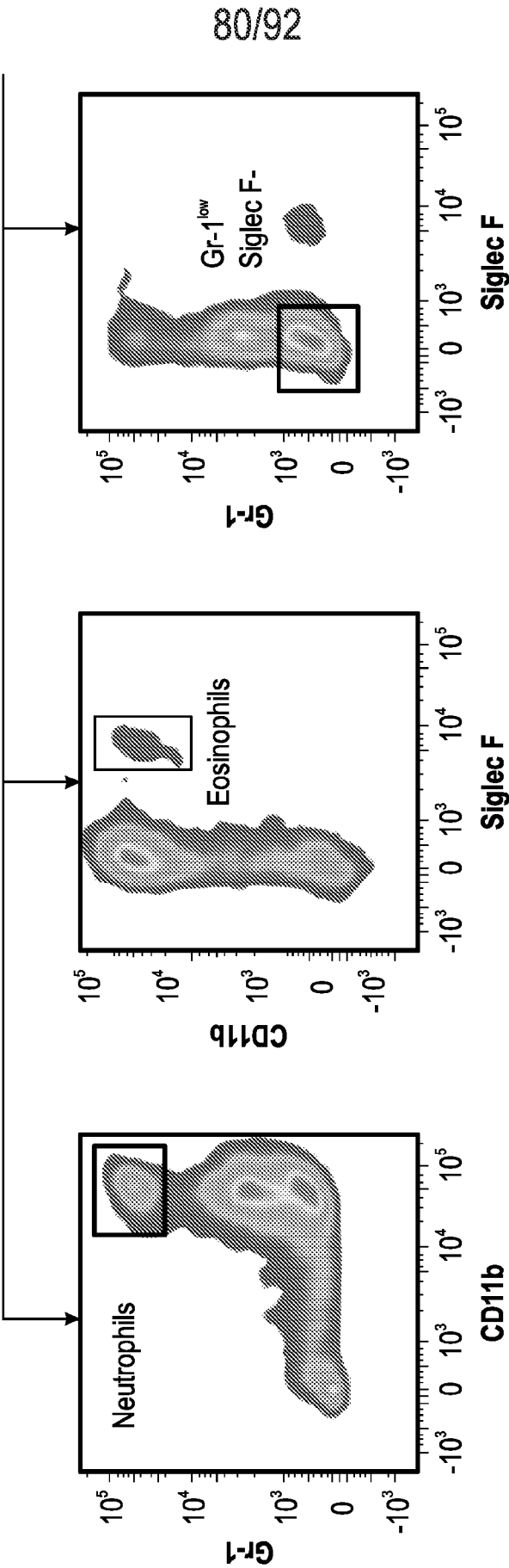


FIG. 15A (cont...)

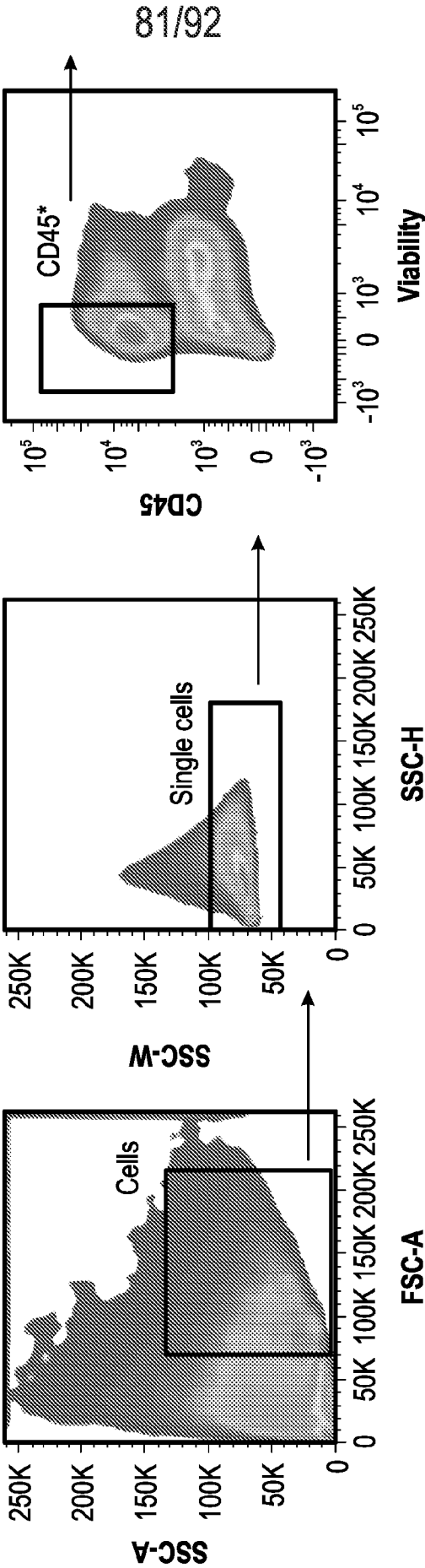


FIG. 15A (cont...)

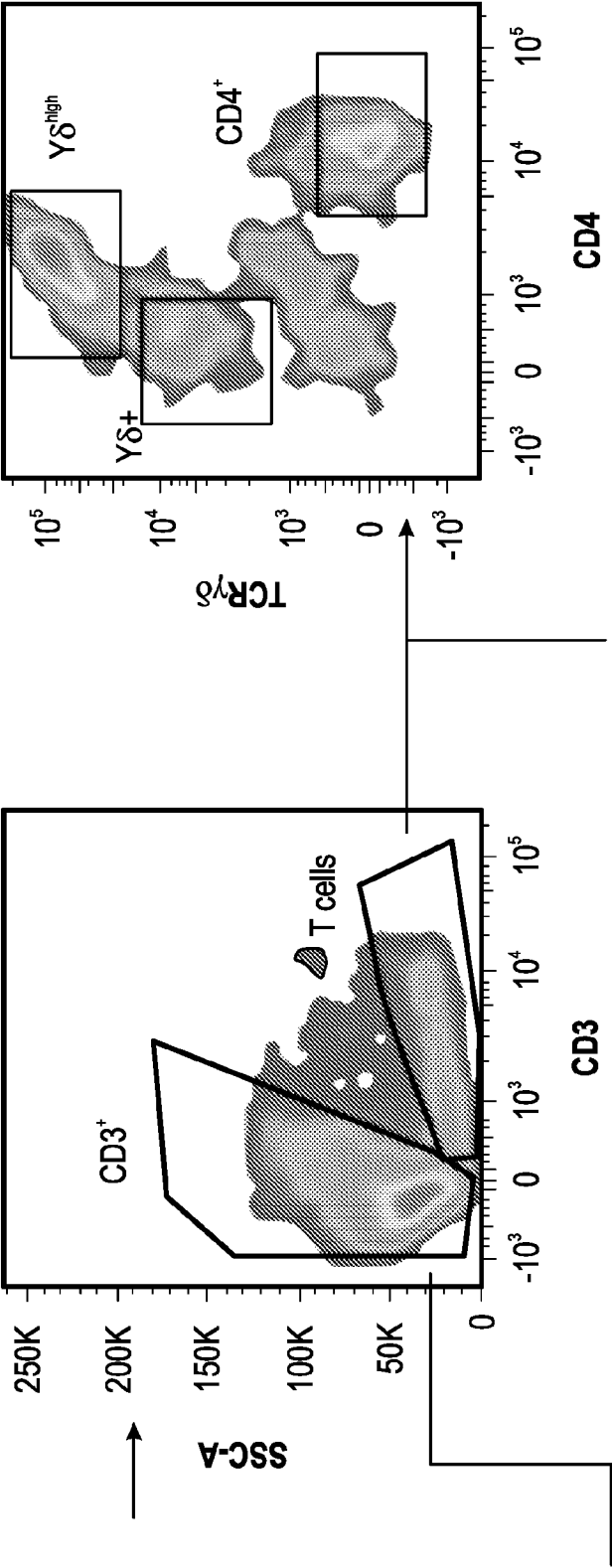


FIG. 15A (cont...)

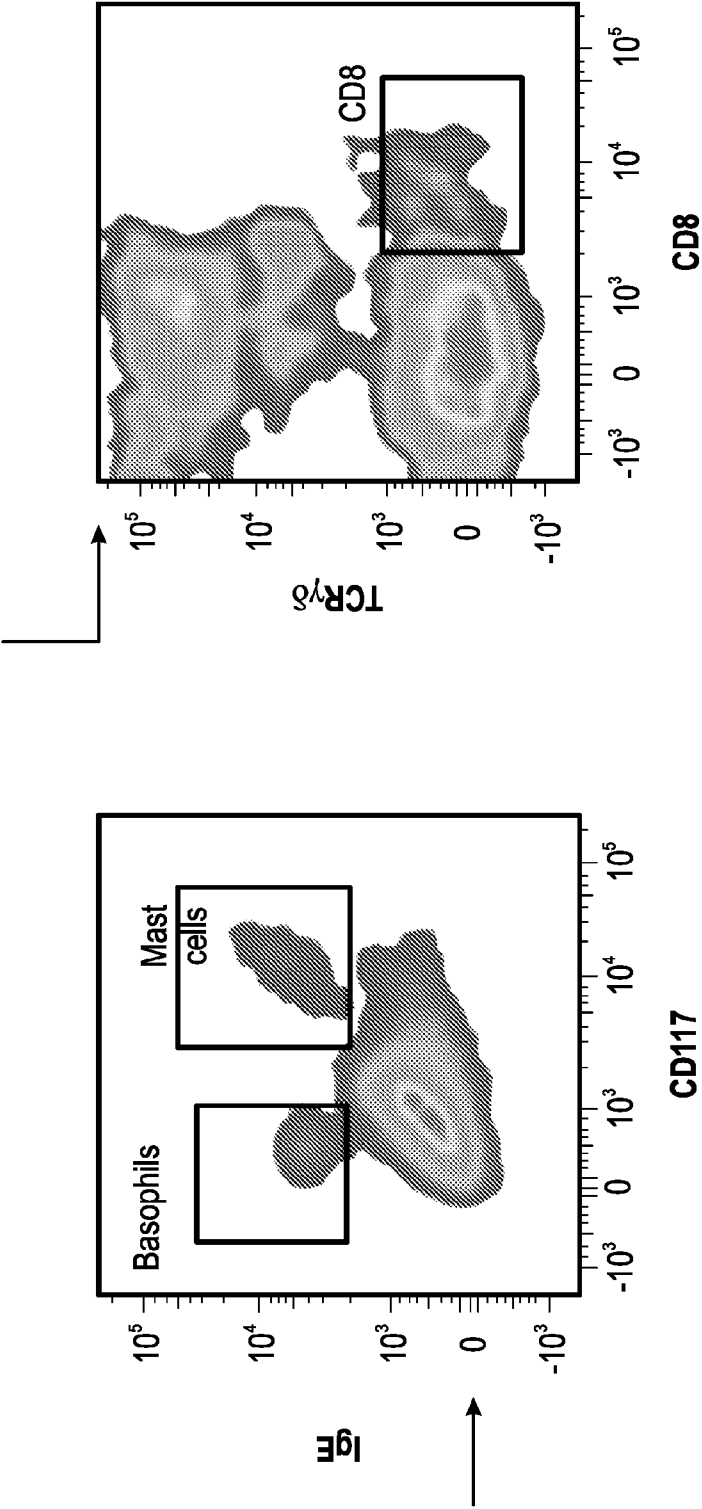


FIG. 15A (cont...)

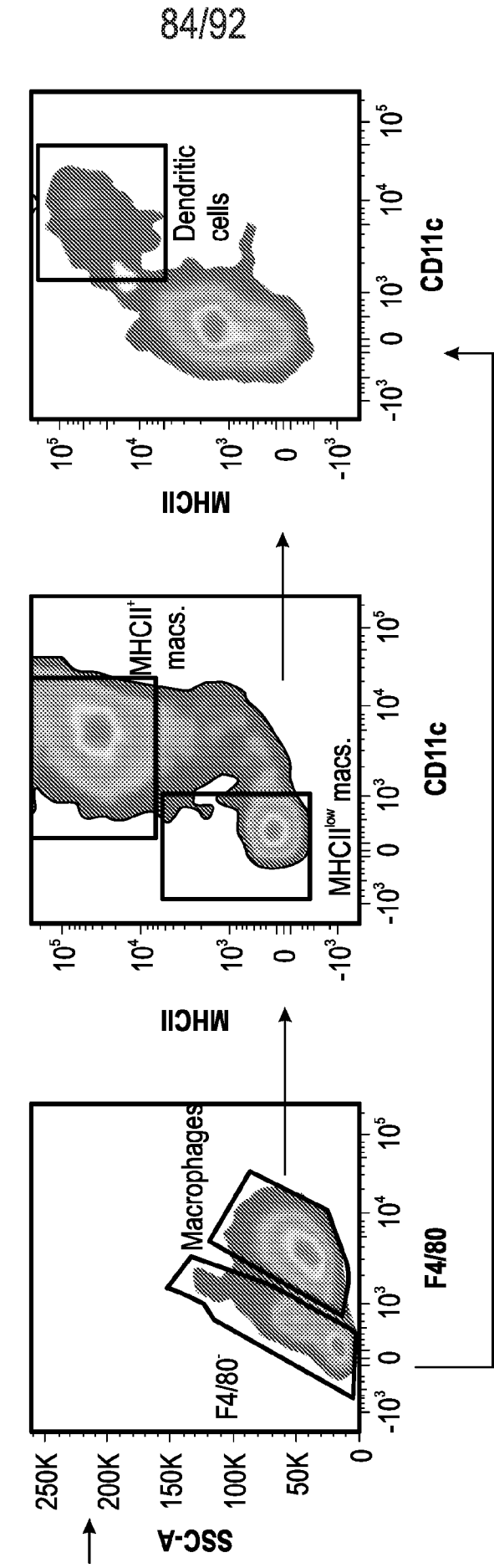
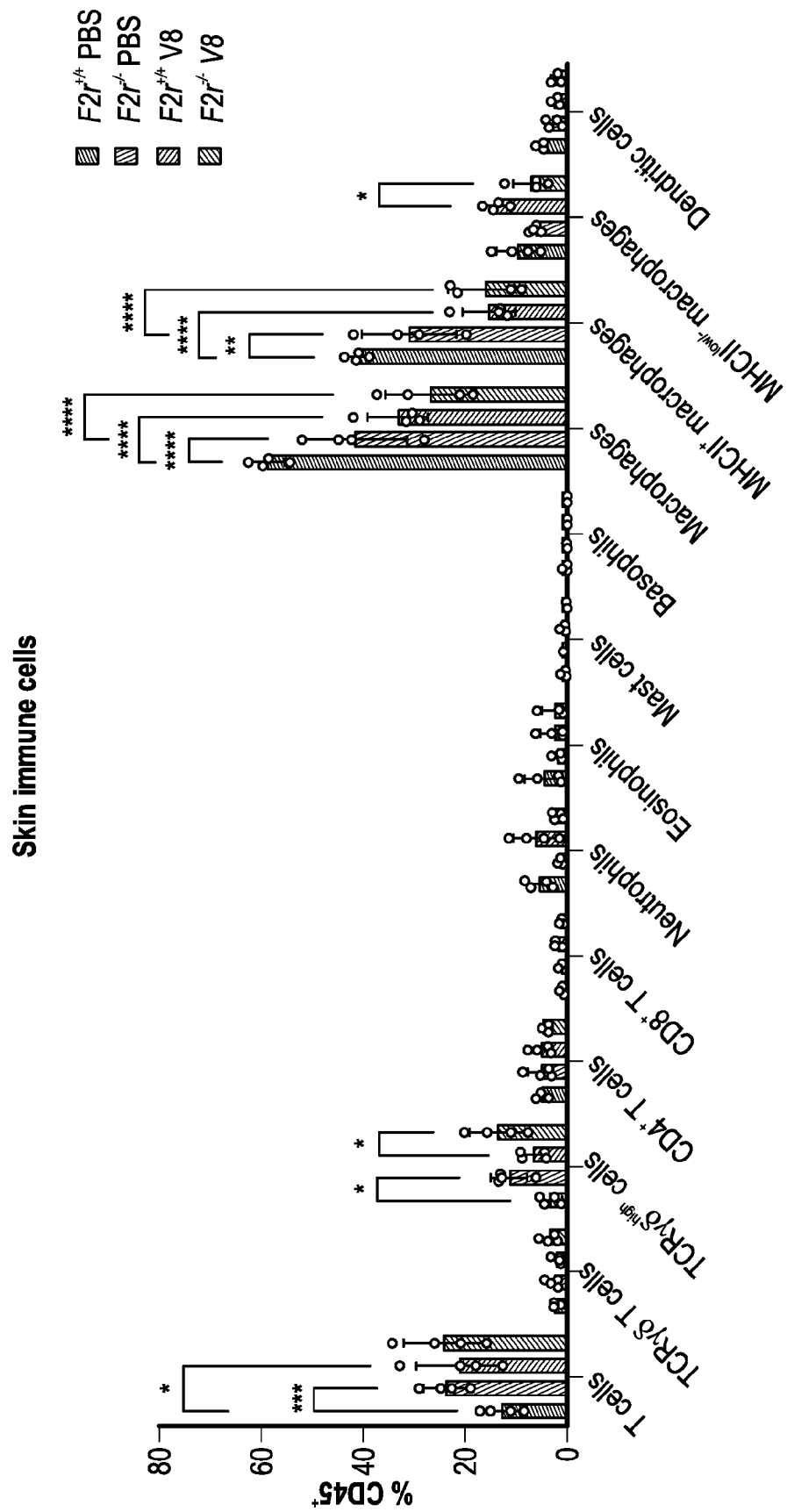


FIG. 15A



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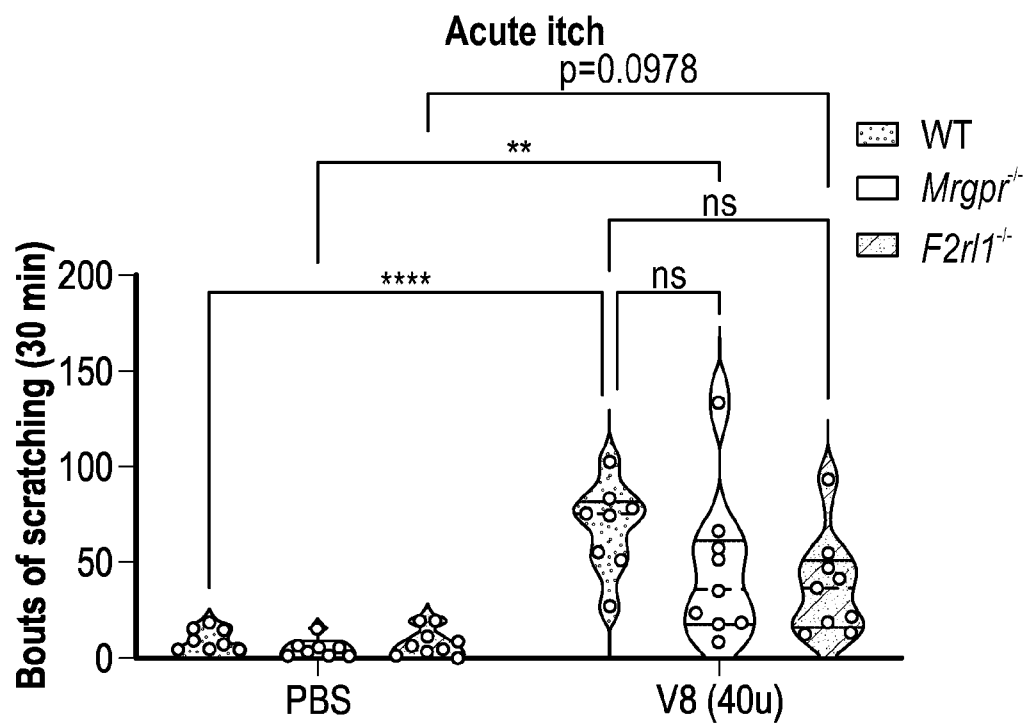


FIG. 16A

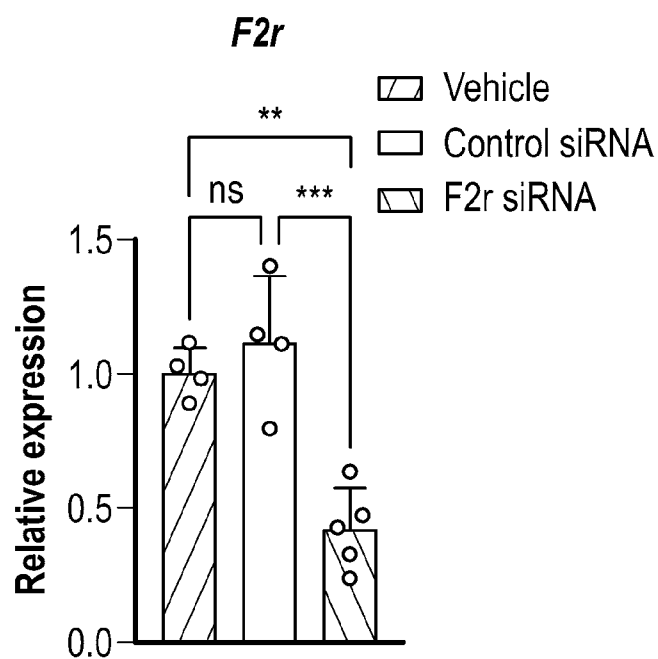


FIG. 16B

Exposure site dermatitis

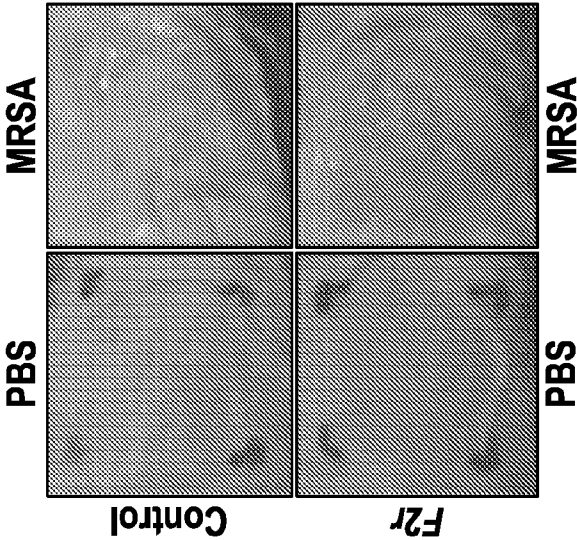
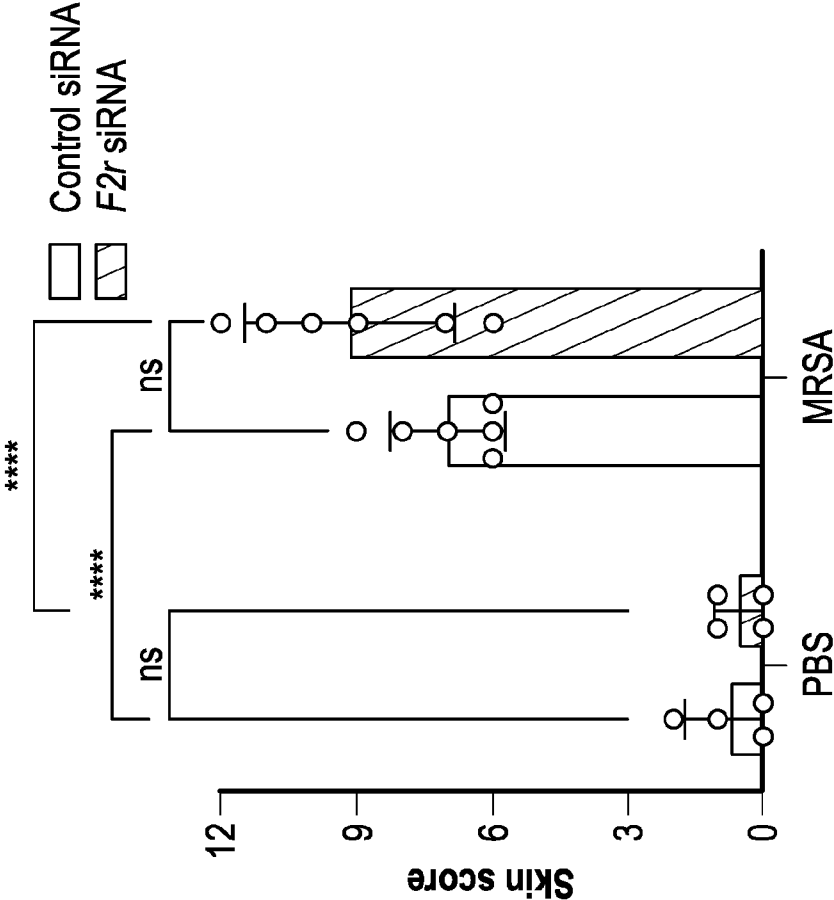


FIG. 16C

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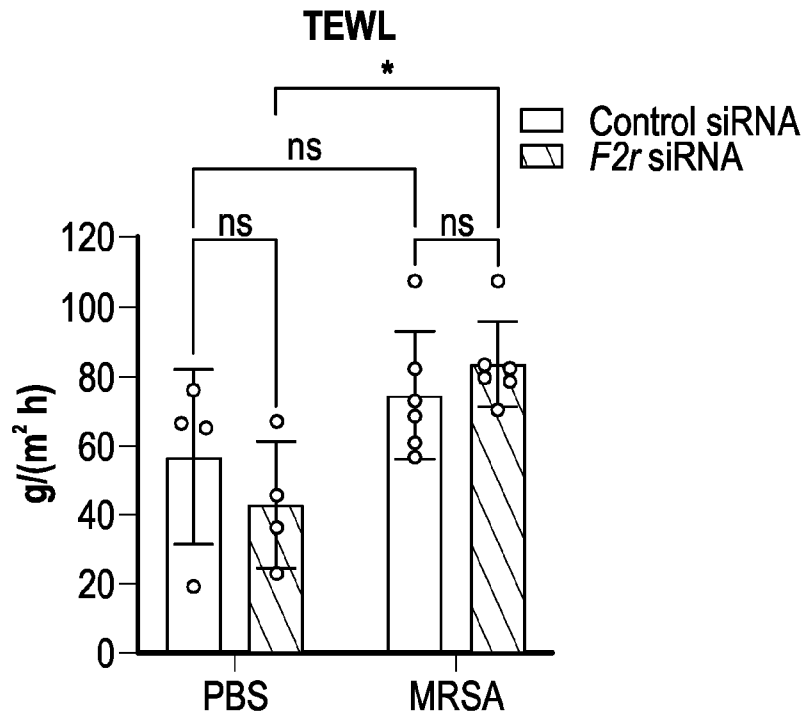


FIG. 16D

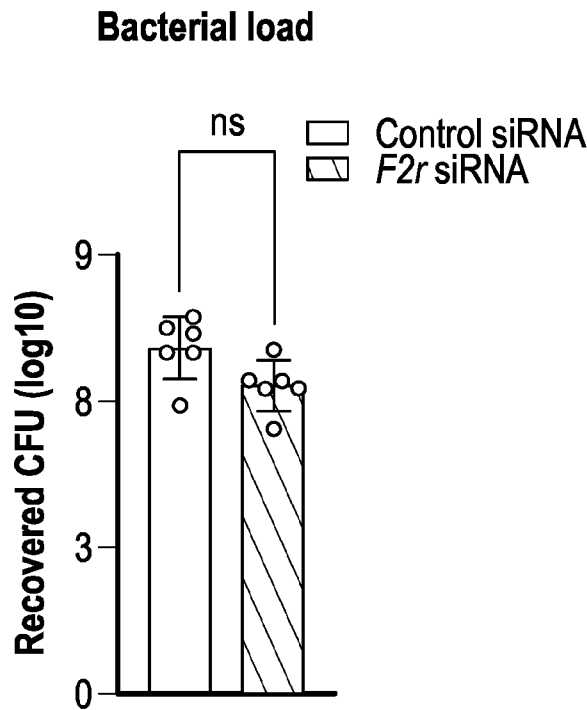
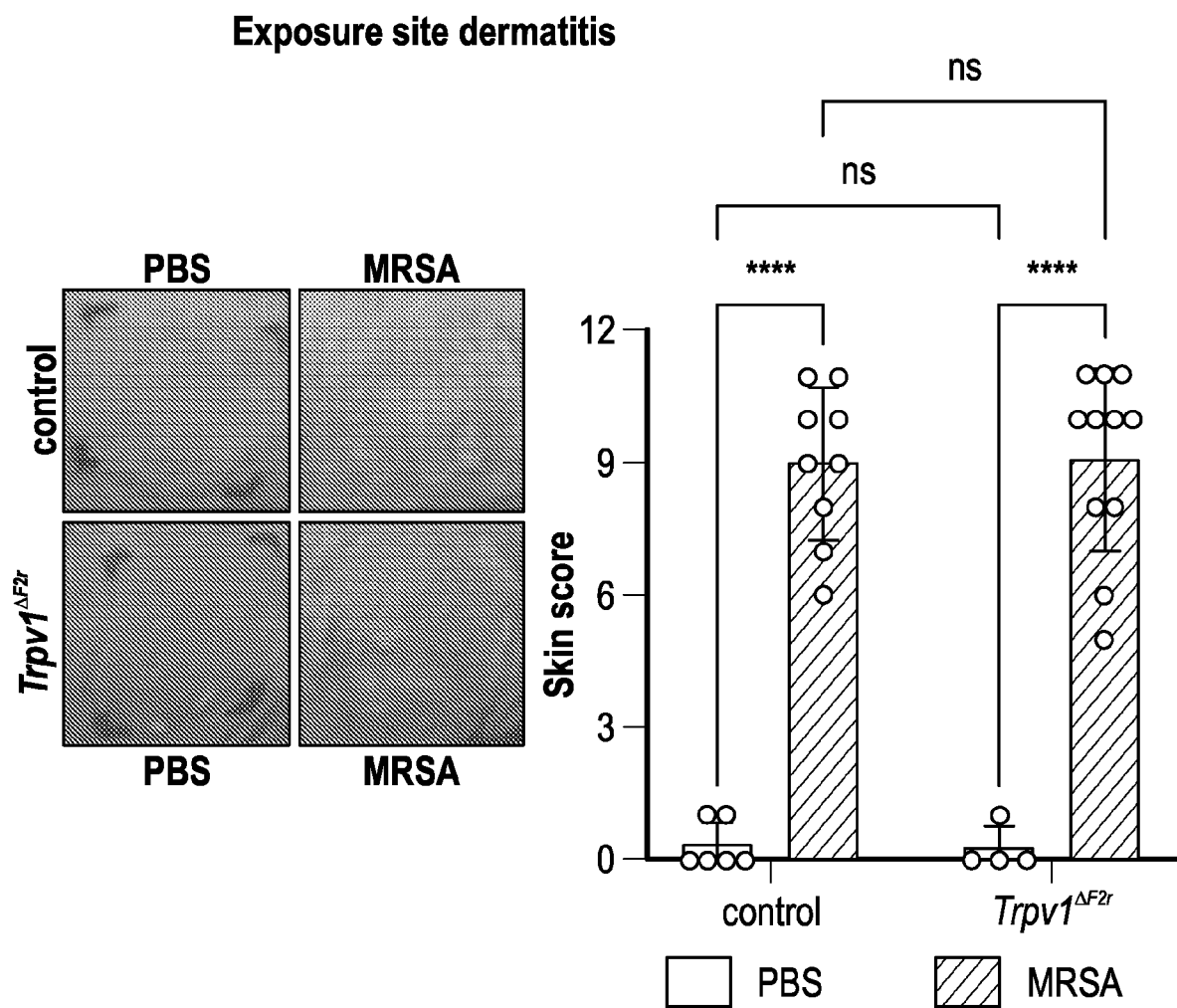
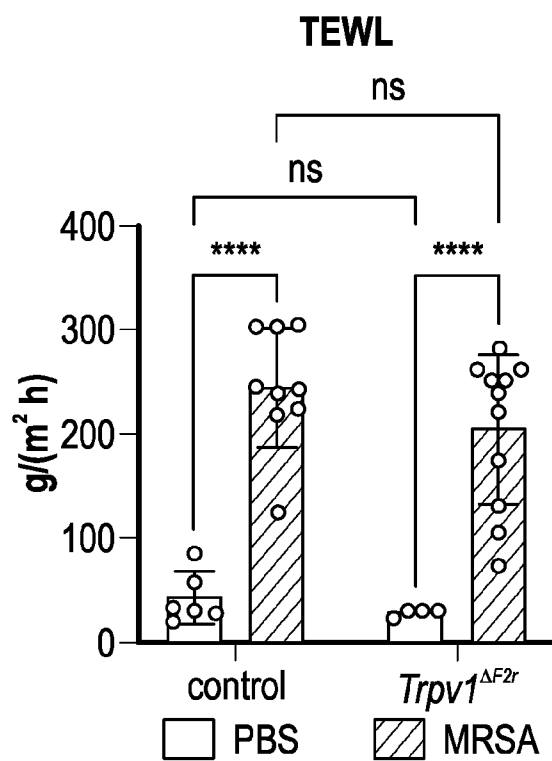
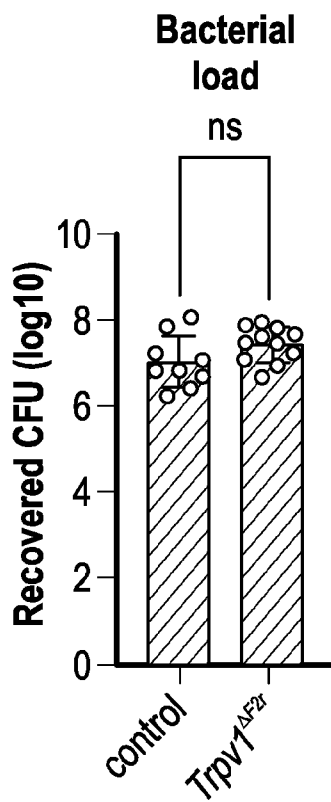


FIG. 16E

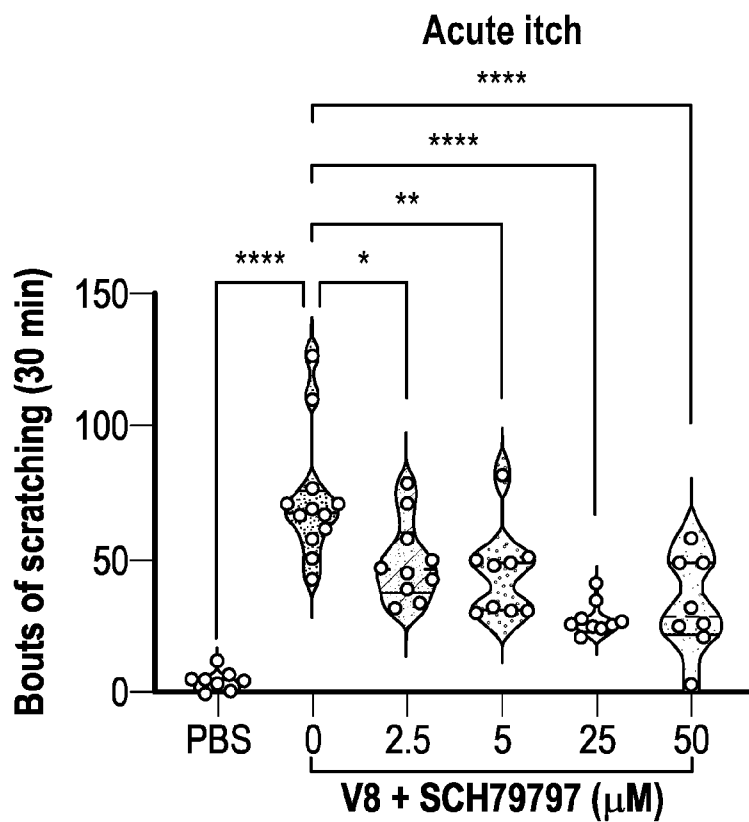
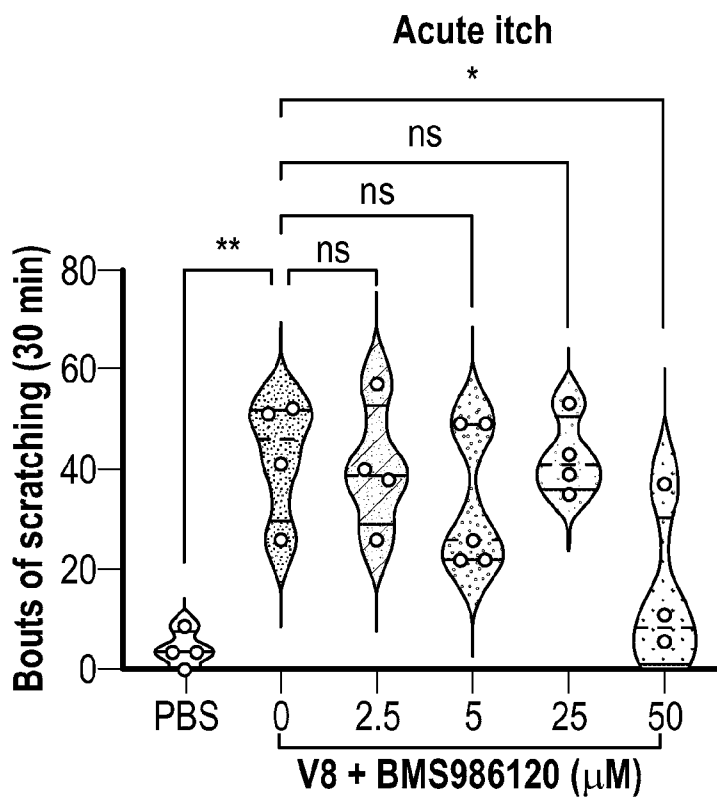
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**FIG. 16F**

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**FIG. 16G****FIG. 16H**

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**FIG. 16I****FIG. 16J**

