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(54) **Title**: METHODS OF TREATING OF INFLAMMATORY BOWEL DISEASE AND PARASITE INFECTION

(57) **Abstract**: Described herein are methods for the induction of a TH2 immune response and for the treatment and/or prevention of diseases associated with pathological immune responses and parasitic infection.

METHODS OF TREATING OF INFLAMMATORY BOWEL DISEASE AND PARASITE INFECTION

RELATED APPLICATIONS

This application claims the benefit of priorirty to U.S. Provisional Patent Application serial number 62/290,734, filed February 3, 2016, the contents of which are hereby incorporated by reference in their entirety.

GOVERNMENT INTEREST

This invention was made with Government support under National Institutes of Health Grants F32DK098826, R01 CA154426 and R01 GM099531. The Government has certain rights in the invention.

BACKGROUND

Inflammatory bowel disease is group of inflammatory conditions of the colon and small intestine that cause over 50,000 deaths annually. The causes of inflammatory bowel disease are complex, and contributing factors may include diet, genetics, and the composition of an individual's gut microflora. Medical treatment is largely based on a factors specific to an individual.

Crohn's disease (CD) and ulcerative colitis (UC) are among the most common forms of inflammatory bowel disease. Both CD and UC are inflammatory diseases, but while UC is localized to the colon, Crohn's disease can affect any part of the gastrointestinal tract, from mouth to anus. Neither CD nor UC are currently medically curable, and current treatments range from surgical removal of parts of the intestine to administration of anti-inflammatory and/or immunosuppressive drugs. Unfortunately, current treatments for CD and UC are often ineffective and can result in significant side effects.

Parasitic diseases affect hundreds of millions of individuals, mostly in developing countries, where people are particularly susceptible to parasitic infection due to contaminated food and water and inadequate sanitation. The most common treatment for parasitic infection are antiparasitic drugs, such as albendazole and mebendazole. However, such treatments can be ineffective and repeated administration of such drugs can leave to drug resistance in the parasite populations.

Thus, there is a continuing need for new methods and compositions for the treatment of inflammatory bowel disease and parasitic diseases.

SUMMARY

In certain aspects, provided herein are methods and compositions for inducing a type 2 helper T cell (T_H2) immune response in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cells. In certain aspects, provided herein are methods and compositions for treating and/or preventing an inflammatory bowel disease and/or a parasitic infection in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cells. In some embodiments, provided herein are methods and compositions to protect, repair or regenerate the intestinal epithelium which has been damaged or depleted or has potential to be damaged or depleted as a result of inflammatory bowel disease (*e.g.*, ulcerative colitis, Crohn's disease). In some embodiments, the agent enhances the activity and/or expression of Trpm5, PLCB2 or gustducin. In some embodiments, the agent induces expression of IL-25 by the tuft cells and/or IL-13 by the subject.

In some aspects, provided herein are methods of inducing IL-25 expression by a tuft cell comprising contacting the tuft cell with an agent that enhances the taste-chemosensory signaling pathway in a tuft cells. In some embodiments, the tuft cell is contacted with the agent *in vitro*. In some embodiments, the tuft cell is administered to a subject after being contacted with the agent. In some embodiments, the tuft cell is isolated from the subject prior to being contacted with the agent. In some embodiments, the tuft cell is contacted with the agent *in vivo*.

In some embodiments of the methods provided herein, the agent is a small molecule agonist of Trpm5, PLCB2 or gustducin. In some embodiments, the agent is an antibody or antigen binding fragment thereof with binding specificity for Trpm5, PLCB2 or gustducin. In some embodiments, the agent comprises a nucleic acid (*e.g.*, an mRNA or an expression vector) that encodes Trpm5, PLCB2 or gustducin.

In some embodiments of the methods provided herein, the agent activates a taste receptor. In some embodiments, the agent is a taste receptor ligand. In some embodiments, the agent is an antibody or antigen binding fragment thereof with binding specificity for the taste receptor. In some embodiments, the agent is a small molecule agonist of the taste receptor.

In some embodiments of the methods provided herein, the subject has or is predisposed to a disease associated with a pathological immune response (*e.g.*, inflammatory

bowel disease) In some embodiments, the subject has or is predisposed to a protozoan infection and/or to a parasitic worm infection.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 includes eight panels (Panels A-H) showing that symbiotic protozoa or helminths increase intestinal tuft cell abundance. Panel A is a bar graph showing DCLK1$^+$ tuft cell frequency in small intestine (SI) from wild-type mice (WT) bred in-house (BIH) and at Jackson laboratories (JAX). Panel B shows H&E-stained SI sections from WT (BIH) and WT (JAX) scale bar, 50 μm (left) and higher magnification from WT (BIH), scale bar, 20μm (right). Panel C is an scanning electron micrograph of protozoa isolated from WT (BIH) mice, scale bar, 4 μm. Panel D is a bar graph showing *T. muris* abundance in stool DNA (*T. muris* 28S rRNA relative to Eubacteria 16S rRNA) by qPCR; not detectable (ND). Panel E is a representative SI images from uninfected and *T. muris* colonized mice and Panel F is a bar graph showing tuft cell frequency. Panel G is a representative SI images from uninfected and helminth colonized mice and Panel H shows tuft cell frequency. Scale bars 100μm in Panel E and Panel G. Each symbol represents an individual mouse and all data are representative of two (Panels D, F and H) or three (Panel A) independent experiments. *T. muris* infection was 17 days in (Panels E and F). In (Panles G and H) *Hp* infection was 21 days, *Ts* infection was 15 days, *Nb* infection was 8 days. Data plotted as mean with s.d. with ****P<0.0001, ***P=0.0001 calculated with one-way ANOVA or Mann-Whitney test.

Figure 2 includes 4 panels (Panels A-D) showing tuft cell frequency is equal when using the markers DCLK1 and Gfi1b. Panel A shows micrographs of small intestine from *Gfi1b*$^{EGFP/+}$ mice bred in-house (BIH). Scale bars 50μm. Panel B is a representative flow plot of the epithelium from the distal small intestine of *Gfi1b*$^{EGFP/+}$ (BIH) mice. Panel C is a bar graphing showing expression data when tuft cells and non-tuft cell epithelial cells from *Gfi1b*$^{EGFP/+}$ mice were sorted by FACS and *DCLK1* expression was determined by RT-qPCR. Data represent two independent experiments. Panel D is a bar graph showing the frequency of tuft cells (Gfi1b-GFP$^+$) in the total epithelium of *Gfi1b*$^{EGFP/+}$ (BIH) mice as determined by flow cytometry. Symbols represent data from individual mice and are reflective of 5 experiments.

Figure 3 shows feeding the cecal contents from WT (BIH) mice to WT (JAX) mice increases tuft cell abundance. Specifically, representative micrographs of the distal small intestine from WT (BIH) mice, WT (JAX) mice, and WT (JAX) mice 3 weeks after feeding

the cecal contents obtained from WT (BIH) mice. Scale bars 100μm. Data represent two independent experiments with 2-5 mice per group.

Figure 4 includes two panels showing metronidazole treatment of WT (BIH) mice reduces *Tritrichomonas muris* levels below the limit of detection in stool and concomitantly reduces tuft cell frequency in the epithelium. Panel A is a bar graph of quantitative PCR (qPCR) comparing *T. muris* 28S rRNA levels relative to eubacteria 16S rRNA from stool DNA isolated from WT (BIH) mice given 2.5g/L metronidazole in their drinking water or control mice; not detectable (ND). Panel B is a bar graph showing corresponding tuft cell frequency in either control or metronidazole-treated mice. $^{**}P$=0.0015, Mann-Whitney test. Data represent two independent experiments with 2-4 mice per group.

Figure 5 shows tritrichomonas colonizes germ-free mice and increases tuft cell abundance. Specifically, representative micrographs of the distal small intestine from germ-free C57BL/6 mice and germ-free C57BL/6 mice colonized with Tritrichomonas muris for 21 days. Scale bars 100μm. Data are representative of 5 mice per group.

Figure 6 is a simplified model of taste-chemosensation highlighting key taste-chemosensation effectors: gustducin, PLCβ2, and Trpm5.

Figure 7 includes 9 panels (Panels A-I) showing that tuft cells influence type 2 immunity via Trpm5. Panel A is three bar graphs showing *Gustducin*, *PLCβ2*, and *Trpm5* expression in sorted tuft cells compared to the non-tuft cell epithelium. Panel B shows representative images of *T. muris* (*Tm*) colonized WT and *Gustducin*$^{-/-}$ mice and tuft cell frequencies. Panel C shows representative image from Trpm5^{eGFP} mice. Panel D shows representative image of *T. muris* colonized *Trpm5-/-* mice and tuft cell frequencies. Scale bars 50μm (Panel B, C, and D). Panel E shows representative flow cytometry plots of IEC from uninfected (left) or *T. muris* colonized (right) WT (*Gfi1b*$^{EGFP/+}$) (top) and *Trpm5*$^{-/-}$ (*Gfi1b*$^{EGFP/+}$ *Trpm5*$^{-/-}$) (bottom) mice and Panel F is a bar graph showing tuft cell frequency. Panel G shows Goblet cells in SI sections stained with alcian blue/nuclear red in uninfected WT and *T. muris* colonized WT and *Trpm5*$^{-/-}$ mice and Panel H shows goblet cell frequency. Panel I is a bar graph showing eosinophil frequency in the distal SI lamina propria (LP) of uninfected and *T. muris* colonized WT and *Trpm5*$^{-/-}$ mice. Scale bars, 50 μm; each symbol represents an individual mouse and all data are representative of at least three independent experiments. Data plotted as mean with s.d.; $^{****}P$<0.0001; $^{***}P$=0.0001; $^{**}P$<0.01; not significant (ns) calculated with one-way ANOVA, Kruskal-Wallis, or Mann-Whitney tests.

Figure 8 includes 2 panels (Panels A and B) showing Trpm5-GFP^{+} cells in the distal

small intestine are restricted to the epithelium and colocalize with DCLK1. Panel A is a representative image of the distal small intestine of *Tritrichomonas muris* colonized Trpm5^{eGFP} mice. Scale bar 100μm. Panel is a plot showing the percentage of GFP^{+} cells in the distal small intestine from Trpm5^{eGFP} mice that are DCLK1^{+} EpCAM^{+} (tuft cells). Data represent 7 mice.

Figure 9 includes 3 panels (Panels A-C) showing data measuring *Tritrichomonas muris* and *Heligmosomoides polygyrus* colonization. Panel A is a plot showing data after *T. muris* was counted with a hemocytometer and correlated with Ct values obtained by qPCR. A semi-log regression curve was fit, R^2=0.998. Panel B is a bar graph showing the determination of *T. muris* abundance in the distal small intestine of WT, *Trpm5*$^{-/-}$, and *Gustducin*$^{-/-}$ 10-18 mice per group. Panel C is a bar graph showing *H. polygyrus* counts from the distal small intestine of WT and *Trpm5*$^{-/-}$ mice colonized for 36 days. ***P*=0.0087, Mann-Whitney test. Data represent 6 mice in each group and reflect 2 independent experiments.

Figure 10 includes 6 panels (Panels A-F) showing that tuft cells express IL-25 and elicit ILC2s, in a Trpm5 dependent manner, in response to symbiotic protozoa. Panel A is a bar graph showing *Il25* expression from sorted tuft cells. Panel B is plot showing WT (closed circles) and *Trpm5*$^{-/-}$ (open circles) mice were colonized with *T. muris* for 3, 7, 12, and 42 days. At each time point, epithelial cell *Il25* expression was measured (purple line) and *T. muris* colonization was quantified. Panel C is a bar graph showing the frequency of IL17RB^{+} (IL-25R) ILC2s in the distal SI LP of uninfected WT and WT and *Trpm5*$^{-/-}$ mice colonized with *T. muris* for 12 days. Panel D is a bar graph showing Eosinophil frequency in the distal SI lamina propria of uninfected WT or *T. muris* colonized *Trpm5*$^{-/-}$ mice i.p. injected with IL-25 or PBS control. Panel E is a bar graph showing tuft cell frequencies and Panel F shows flow plots of epithelial cells isolated from *Trpm5*$^{-/-}$ mice i.p. injected with IL-25 or PBS. Each symbol in C, D, and E represents an individual mouse and all data are representative of three independent experiments. Data plotted as mean with s.d.; ****P*<0.001; ***P*<0.01 calculated with Kruskal-Wallis or Mann-Whitney tests.

Figure 11 includes 2 panels (Panels A and B) showing Il-33, TSLP, Il17RB expression in tuft cells. Epithelial cells from *Gfi1b*$^{EGFP/+}$ mice were sorted into tuft cell and non-tuft cell fractions. Panel A is a bar graph showing expression of *Il-33* and *TSLP* determined by RT-qPCR. Panel B is a bar graph showing expression of *Il17RB* determined

by RT-qPCR. ***P=0.0006, **P=0.0043, Mann-Whitney test. Data represent three independent experiments.

Figure 12 includes 4 panels (Panels A-D) showing innate lymphoid cells and IL-13 increase tuft cells in organoids and the small intestine. Panel A shows differential interference contrast (DIC), fluorescent, and merged images of small intestinal organoids generated from *Gfi1b*$^{EGFP/+}$ mice, scale bars, 25μm. Panel B is a bar graph showing GFP^{+} tuft cell abundance by flow cytometry of WT and *Trpm5*$^{-/-}$ organoids treated with recombinant IL-13 or IL-25. Panel C shows Representative images of SI from WT, *Stat6*$^{-/-}$, *Rag2*$^{-/-}$, and *Rag2*$^{-/-}$*Il2rγ*$^{-/-}$ mice colonized with *T. muris* and Panel D shows tuft cell frequency. Scale bars, 100μm. Each symbol represents an individual mouse and all data are representative of (Panel D) two or (Panel B) three independent experiments. Data plotted as mean with s.d. with ****P<0.0001; not significant (ns) calculated with one-way ANOVA or Mann-Whitney tests.

Figure 13 includes 3 panels (Panels A-C) showing Il-13 increases tuft cell abundance in both WT and *Trpm5*$^{-/-}$organoids. Panel A shows representative flow cytometry plots of WT (*Gfi1b*$^{EGFP/+}$) and *Trpm5*$^{-/-}$ (*Gfi1b*$^{EGFP/+}$*Trpm5*$^{-/-}$) organoids treated with Il-13 and Il-25. Expression of *DCLK1* (Panel B) and *Trpm5* (Panel B) in organoids treated with Il-13 is shown. Data are plotted as mean with s.d. and are representative of three independent experiments.

Figure 14 is a bar graph showing tritrichomonas muris equivalently colonizes WT, Stat6-/-, Rag2-/-, and Rag2-/- IL2rγ-/- mice. Small intestinal contents were analyzed by qPCR for T. muris abundance. T. muris abundance was not significantly (ns) different between mice as calculated by Ordinary one-way ANOVA. Data are representative of two independent experiments, 5-10 mice per group.

Figure 15 depicts a model in accordance with the invention. (1) Tuft cells respond to lumenal parasites and utilize Trpm5 dependent upstream signaling pathways. (2) In response to parasite colonization, tuft cells produce IL-25 which expands and activates ILC2s (3) ILC2s then produce type 2 cytokines such as IL-13 that signal back to the epithelium to increase both goblet cells and tuft cells. (4) ILC2s control eosinophila through production of IL-5 and IL-13. Not wishing to be bound by any particular theory, it is proposed that IL-25 released by tuft cells may increase eosinophils through the accumulation and activation of ILC2s.

Figure 16 depicts a plot showing gating strategy for eosinophils. Cells were isolated from the distal small intestine lamina propria and gated on $CD45^+PI^-$ cells. Eosinophils were selected as $CD11b^+MHCII^-SiglecF^+$ and SSC^{hi}.

Figure 17 depicts a plot showing gating strategy for ILC2s. Cells from the distal small intestinal lamina propria were isolated and gated on viable $CD45^+$ cells. Il-25-responsive ILC2s were further selected as $Lin\text{-}IL7R\alpha^+KLRG1^+IL17RB^+$.

DETAILED DESCRIPTION

General

In certain aspects, provided herein are methods related to the administration of an agent to enhance the taste-chemosensory signaling pathway in tuft cells. As disclosed herein, the disruption of chemosensory signaling (*e.g.*, via loss of *Trpm5)* abrogates expansion of tuft cells, goblet cells, eosinophils, and type-2 innate lymphoid cells (ILC2s) during parasite colonization. Tuft cells are the primary source of the parasite-induced cytokine, IL-25, which indirectly induces tuft cells expansion by promoting IL-13 production by ILCs. As described herein, intestinal tuft cells are critical sentinels in the gut epithelium that promote type-2 immunity in response to intestinal parasites. As such, in some embodiments the methods disclosed herein are useful in treating or preventing diseases associated with a T_H1 and/or T_H17 immune response (*e.g.*, inflammatory bowel disease), and infections responsive to a T_H2 immune response (*e.g.*, parasitic infection) by inducing a type 2 helper T cell (T_H2) immune response in a subject. In some embodiments, provided herein are methods and compositions to protect, repair or regenerate the intestinal epithelium which has been damaged or depleted or has potential to be damaged or depleted as a result of inflammatory bowel disease (*e.g.*, ulcerative colitis, Crohn's disease). In certain embodiments, the instant invention relates to a method of inducing IL-25 expression by a tuft cell comprising contacting the tuft cell with an agent that enhances the taste-chemosensory signaling pathway in a tuft cells. Such cells can be, for example, induced to express IL-25 *ex vivo* and then transplanted into a subject to treat or prevent an inflammatory disease and/or a parasitic infection.

In certain embodiments, the agent administered in the methods disclosed herein is an agent that enhances the activity or expression of Trpm5, PLCB2, and/or gustducin, such as a small molecule, an antibody or a nucleic acid that enhances the activity or expression of Trpm5, PLCB2 and/or gustducin. In some embodiments the agent that administered according to the methods described herein activates a taste receptor on the tuft cells. In some

embodiments, the agent is a taste receptor ligand, an antibody or antigen binding fragment with binding specificity for the taste receptor, or a small molecule agonist of the taste receptor.

Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The articles "*a*" and "*an*" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article.

As used herein, the term "*administering*" means providing an agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering.

The terms "*agent*" are used herein to denote a chemical compound, a small molecule, a mixture of chemical compounds, a biological macromolecule (such as a nucleic acid, an antibody, a protein or portion thereof, *e.g.*, a peptide), or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. The activity of such agents may render them suitable as a "therapeutic agent" which is a biologically, physiologically, or pharmacologically active substance (or substances) that acts locally or systemically in a subject.

The term "*amino acid*" is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs having variant side chains; and all stereoisomers of any of any of the foregoing.

As used herein, the term "*antibody*" may refer to both an intact antibody and an antigen binding fragment thereof. Intact antibodies are glycoproteins that include at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain includes a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. Each light chain includes a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The V_H and V_L 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). Each V_H and V_L is 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. The variable

regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system. The term "antibody" includes, for example, monoclonal antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), single-chain antibodies and antigen-binding antibody fragments. An "*isolated antibody*," as used herein, refers to an antibody which is substantially free of other antibodies having different antigenic specificities. An isolated antibody may, however, have some cross-reactivity to other, related antigens.

The terms "*antigen binding fragment*" and "*antigen-binding portion*" of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to bind to an antigen. Examples of binding fragments encompassed within the term "antigen-binding fragment" of an antibody include Fab, Fab', $F(ab')_2$, Fv, scFv, disulfide linked Fv, Fd, diabodies, single-chain antibodies, NANOBODIES®, isolated CDRH3, and other antibody fragments that retain at least a portion of the variable region of an intact antibody. These antibody fragments can be obtained using conventional recombinant and/or enzymatic techniques and can be screened for antigen binding in the same manner as intact antibodies.

The terms "*CDR*", and its plural "*CDRs*", refer to a complementarity determining region (CDR) of an antibody or antibody fragment, which determine the binding character of an antibody or antibody fragment. In most instances, three CDRs are present in a light chain variable region (CDRL1, CDRL2 and CDRL3) and three CDRs are present in a heavy chain variable region (CDRH1, CDRH2 and CDRH3). CDRs contribute to the functional activity of an antibody molecule and are separated by amino acid sequences that comprise scaffolding or framework regions. Among the various CDRs, the CDR3 sequences, and particularly CDRH3, are the most diverse and therefore have the strongest contribution to antibody specificity. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (*i.e.*, Kabat *et al.*, Sequences of Proteins of Immunological Interest (National Institute of Health, Bethesda, Md. (1987), incorporated by reference in its entirety); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Chothia *et al.*, Nature, 342:877 (1989), incorporated by reference in its entirety).

As used herein, an "*effective amount*" is an amount effective in treating or preventing a disease associated with a pathological immune response, including, for example, inflammatory bowel disease.

As used herein, the term "*enhance*" refers to improve, increase, amplify, multiply, elevate, raise, and the like.

As used herein, the term "*humanized antibody*" refers to an antibody that has at least one CDR derived from a mammal other than a human, and a FR region and the constant region of a human antibody. A humanized antibody is useful as an effective component in a therapeutic agent according to the present invention since antigenicity of the humanized antibody in human body is lowered.

The term "*isolated polypeptide*" refers to a polypeptide, in certain embodiments prepared from recombinant DNA or RNA, or of synthetic origin, or some combination thereof, which (1) is not associated with proteins that it is normally found with in nature, (2) is isolated from the cell in which it normally occurs, (3) is isolated free of other proteins from the same cellular source, (4) is expressed by a cell from a different species, or (5) does not occur in nature.

The term "*isolated nucleic acid*" refers to a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which (1) is not associated with the cell in which the "isolated nucleic acid" is found in nature, or (2) is operably linked to a polynucleotide to which it is not linked in nature.

As used herein, the term "*monoclonal antibody*" refers to an antibody obtained from a population of substantially homogeneous antibodies that specifically bind to the same epitope, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The terms "*polynucleotide*", and "*nucleic acid*" are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer

RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified, such as by conjugation with a labeling component. The term "recombinant" polynucleotide means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a non-natural arrangement.

As used herein, the term "*subject*" means a human or non-human animal selected for treatment or therapy. In certain embodiments of the methods described herein, the subject is a human subject.

The term "*small molecule*" is art-recognized and refers to a composition which has a molecular weight of less than about 2000 amu, or less than about 1000 amu, and even less than about 500 amu. Small molecules may be, for example, nucleic acids, peptides, polypeptides, peptide nucleic acids, peptidomimetics, carbohydrates, lipids or other organic (carbon containing) or inorganic molecules. Many pharmaceutical companies have extensive libraries of chemical and/or biological mixtures, often fungal, bacterial, or algal extracts, which can be screened with any of the assays described herein. The term "small organic molecule" refers to a small molecule that is often identified as being an organic or medicinal compound, and does not include molecules that are exclusively nucleic acids, peptides or polypeptides.

"*Treating*" a disease in a subject or "*treating*" a subject having a disease refers to subjecting the subject to a pharmaceutical treatment, *e.g.*, the administration of a drug, such that at least one symptom of the disease is decreased or prevented from worsening.

Trpm5

In certain embodiments, the methods provided herein relate to agents that enhance the expression and/or activity or Trpm5. As used herein, the term "Trpm5" or "Trpm5 protein" refers to the transient receptor potential cation channel subfamily M member 5 protein, which is also known as the long transient receptor potential channel 5 protein. In humans, Trpm5 is encoded by the *TRPM5* gene. Exemplary human Trpm5 mRNA and

protein sequences are provided at NCBI accession numbers NM_014555.3 and NP_064673.2, respectively, each of which is hereby incorporated by reference.

PLCB2

In certain embodiments, the methods provided herein relate to agents that enhance the expression and/or activity or PLCB2. As used herein, the term "PLCB2" or "PLCB2 protein" refers to the 1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-2 protein. In humans, PLCB2 is encoded by the *PLCB2* gene. Exemplary human PLCB2 mRNA and protein sequences are provided at NCBI accession numbers NM_001284297.1 and NP_001271226.1, respectively, each of which is hereby incorporated by reference.

Gustducin

In certain embodiments, the methods provided herein relate to agents that enhance the expression and/or activity or Gustducin. Gustducin is a G protein associated with taste and the gustatory system found in certain taste receptor cells. Gustducin is a heterotrimeric protein composed of the protein products of the *GNAT3*, *GNB1* and *GNG13* genes. Exemplary human GNAT3 mRNA and protein sequences are provided at NCBI accession numbers NM_01102386.2 and NP_001095856.1, respectively, each of which is hereby incorporated by reference. Exemplary human GNB1 mRNA and protein sequences are provided at NCBI accession numbers NM_001282538.1 and NP_001269467.1, respectively, each of which is hereby incorporated by reference. Exemplary human GNG13 mRNA and protein sequences are provided at NCBI accession numbers NM_016541.2 and NP_057625.1, respectively, each of which is hereby incorporated by reference.

Taste-receptors

In certain embodiments, the methods provided herein relate to agents that enhance the activity and/or expression of a taste receptor. For example, in some embodiments, the agent is a small molecule agonist of a taste receptor, a taste receptor ligand, or an antibody or antigen binding fragment thereof with binding specificity for the taste receptor. In some embodiments, the taste receptor is a human taste receptor. In some embodiments, the taste receptor is expressed on a tuft cell.

In some embodiments, the agent enhances the activity or expression of any human taste receptor expressed on a tuft cell. In some embodiments, the agent enhances the activity and/or expression of a Type 1 taste receptor. In some embodiments, the agent enhances the activity and/or expression of a Type 2 taste receptor. In some embodiments, the agent enhances the activity or expression of TAS1R1. In some embodiments, the agent enhances

the activity or expression of TAS1R2. In some embodiments, the agent enhances the activity or expression of TAS1R3. In some embodiments, the agent enhances the activity or expression of TAS1R4. In some embodiments, the agent enhances the activity or expression of TAS2R1. In some embodiments, the agent enhances the activity or expression of TAS2R3. In some embodiments, the agent enhances the activity or expression of TAS2R4. In some embodiments, the agent enhances the activity or expression of TAS2R5. In some embodiments, the agent enhances the activity or expression of TAS2R7. In some embodiments, the agent enhances the activity or expression of TAS2R8. In some embodiments, the agent enhances the activity or expression of TAS2R9. In some embodiments, the agent enhances the activity or expression of TAS2R10. In some embodiments, the agent enhances the activity or expression of TAS2R12. In some embodiments, the agent enhances the activity or expression of TAS2R13. In some embodiments, the agent enhances the activity or expression of TAS2R14. In some embodiments, the agent enhances the activity or expression of TAS2R15. In some embodiments, the agent enhances the activity or expression of TAS2R16. In some embodiments, the agent enhances the activity or expression of TAS2R18. In some embodiments, the agent enhances the activity or expression of TAS2R19. In some embodiments, the agent enhances the activity or expression of TAS2R20. In some embodiments, the agent enhances the activity or expression of TAS2R22. In some embodiments, the agent enhances the activity or expression of TAS2R23. In some embodiments, the agent enhances the activity or expression of TAS2R30. In some embodiments, the agent enhances the activity or expression of TAS2R31. In some embodiments, the agent enhances the activity or expression of TAS2R33. In some embodiments, the agent enhances the activity or expression of TAS2R36. In some embodiments, the agent enhances the activity or expression of TAS2R37. In some embodiments, the agent enhances the activity or expression of TAS2R38. In some embodiments, the agent enhances the activity or expression of TAS2R39. In some embodiments, the agent enhances the activity or expression of TAS2R40. In some embodiments, the agent enhances the activity or expression of TAS2R41. In some embodiments, the agent enhances the activity or expression of TAS2R42. In some embodiments, the agent enhances the activity or expression of TAS2R43. In some embodiments, the agent enhances the activity or expression of TAS2R44. In some embodiments, the agent enhances the activity or expression of TAS2R45. In some

embodiments, the agent enhances the activity or expression of TAS2R46. In some embodiments, the agent enhances the activity or expression of TAS2R47. In some embodiments, the agent enhances the activity or expression of TAS2R48. In some embodiments, the agent enhances the activity or expression of TAS2R49. In some embodiments, the agent enhances the activity or expression of TAS2R50. In some embodiments, the agent enhances the activity or expression of TAS2R60.

Nucleic Acids

In some embodiments, the methods provided herein relate to the administration of a nucleic acid encoding one or more of the proteins described herein (*e.g.*, Trpm1, PLCB2, Gustducin and/or a taste receptor) to a subject and/or to a cell (*e.g.*, a tuft cell). In some embodiments, the nucleic acid is an mRNA molecule and/or a vector encoding an mRNA molecule. In some embodiments, the nucleic acid is linked to a promoter and/or other regulatory sequences. In some embodiments, the nucleic acid comprises a sequence that is at least about 80%, 85%, 90%, 95%, 98%, 99% or 100% identical to a nucleotide sequence provided herein.

In some embodiments, the nucleic acids described herein (*e.g.*, those encoding a protein of interest or functional homolog thereof, or a nucleic acid intended to enhance the production of a protein described herein) can be delivered to cells in culture, *ex vivo*, and *in vivo*. The delivery of nucleic acids can be delivered by any technique known in the art including, but not limited to, viral mediated gene transfer and liposome mediated gene transfer. Polynucleotides can be administered in any suitable formulations known in the art. These can be as virus particles, as naked DNA, in liposomes, in complexes with polymeric carriers, etc.

Nucleic acids can be delivered in any desired vector. These include viral or non-viral vectors, including adenovirus vectors, adeno-associated virus vectors, retrovirus vectors, lentivirus vectors, and plasmid vectors. Exemplary types of viruses include HSV (herpes simplex virus), AAV (adeno associated virus), HIV (human immunodeficiency virus), BIV (bovine immunodeficiency virus), and MLV (murine leukemia virus). Nucleic acids can be administered in any desired format that provides sufficiently efficient delivery levels, including in virus particles, in liposomes, in nanoparticles, and complexed to polymers.

A polynucleotide of interest can also be combined with a condensing agent to form a gene delivery vehicle. The condensing agent may be a polycation, such as polylysine,

polyarginine, polyornithine, protamine, spermine, spermidine, and putrescine. Many suitable methods for making such linkages are known in the art.

In an alternative embodiment, a polynucleotide of interest is associated with a liposome to form a gene delivery vehicle. Liposomes are small, lipid vesicles comprised of an aqueous compartment enclosed by a lipid bilayer, typically spherical or slightly elongated structures several hundred Angstroms in diameter. Under appropriate conditions, a liposome can fuse with the plasma membrane of a cell or with the membrane of an endocytic vesicle within a cell which has internalized the liposome, thereby releasing its contents into the cytoplasm. Prior to interaction with the surface of a cell, however, the liposome membrane acts as a relatively impermeable barrier which sequesters and protects its contents, for example, from degradative enzymes. Additionally, because a liposome is a synthetic structure, specially designed liposomes can be produced which incorporate desirable features. See Stryer, Biochemistry, pp. 236-240, 1975 (W.H. Freeman, San Francisco, CA); Szoka *et al.*, Biochim. Biophys. Acta 600:1, 1980; Bayer *et al.*, Biochim. Biophys. Acta. 550:464, 1979; Rivnay *et al.*, Meth. Enzymol. 149:119, 1987; Wang *et al.*, PROC. NATL. ACAD. SCI. U.S.A. 84: 7851, 1987, Plant *et al.*, Anal. Biochem. 176:420, 1989, and U.S. Patent 4,762,915. Liposomes can encapsulate a variety of nucleic acid molecules including DNA, RNA, plasmids, and expression constructs comprising growth factor polynucleotides such those disclosed in the present invention.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner *et al.*, Proc. Natl. Acad. Sci. USA 84:7413-7416, 1987), mRNA (Malone *et al.*, Proc. Natl. Acad. Sci. USA 86:6077-6081, 1989), and purified transcription factors (Debs *et al.*, J. Biol. Chem. 265:10189-10192, 1990), in functional form. Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. See also Felgner *et al.*, Proc. Natl. Acad. Sci. USA 91: 5148-5152.87, 1994. Other commercially available liposomes include Transfectace (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, *e.g.*, Szoka *et al.*, Proc. Natl. Acad. Sci. USA 75:4194-4198, 1978; and WO 90/11092 for descriptions of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

One or more polypeptide (*e.g.*, a Trpm5 protein, PLCB2 protein or gustducin protein) or nucleic acid of interest may be encoded by a single nucleic acid. Alternatively, separate nucleic acids may encode different protein or nucleic acids of interest. Different species of nucleic acids may be in different forms; they may use different promoters or different vectors or different delivery vehicles. Similarly, the same protein or nucleic acid of interest may be used in a combination of different forms.

In certain embodiments, the instant invention relates to vectors that contain the isolated nucleic acid molecules described herein. As used herein, the term "vector," refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby be replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

Antibodies

In certain embodiments, the methods provided herein relate to the delivery and/or use of antibodies and antigen binding fragments thereof that bind specifically to Trpm5, PLCB2, gustducin or a taste receptor described herein. Such antibodies can be polyclonal or monoclonal and can be, for example, murine, chimeric, humanized or fully human.

Polyclonal antibodies can be prepared by immunizing a suitable subject (*e.g.* a mouse) with a polypeptide immunogen (*e.g.*, a Trpm5, PLCB2, gustducin, or taste receptor

polypeptide). The polypeptide antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody directed against the antigen can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction.

At an appropriate time after immunization, *e.g.*, when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies using standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497) (see also Brown *et al.* (1981) *J. Immunol.* 127:539-46; Brown *et al.* (1980) *J. Biol. Chem.* 255:4980-83; Yeh *et al.* (1976) *Proc. Natl. Acad. Sci.* 76:2927-31; and Yeh *et al.* (1982) *Int. J. Cancer* 29:269-75), the more recent human B cell hybridoma technique (Kozbor *et al.* (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole *et al.* (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing monoclonal antibody hybridomas is well known (see generally Kenneth, R. H. in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); Lerner, E. A. (1981) *Yale J. Biol. Med.* 54:387-402; Gefter, M. L. *et al.* (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds to the polypeptide antigen, preferably specifically.

As an alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal specific for Trpm5, PLCB2, gustducin or a taste receptor can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library or an antibody yeast display library) with the appropriate polypeptide (*e.g.* a Trpm5, PLCB2, gustducin, or taste receptor polypeptide) to thereby isolate immunoglobulin library members that bind the polypeptide.

Additionally, recombinant antibodies specific for Trpm5, PLCB2, gustducin, or a taste receptor, such as chimeric or humanized monoclonal antibodies, can be made using standard recombinant DNA techniques. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in US Pat No. 4,816,567; US Pat. No. 5,565,332; Better *et al.*

(1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci.* 84:214-218; Nishimura *et al.* (1987) *Cancer Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *Biotechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

Human monoclonal antibodies specific for Trpm5, PLCB2, gustducin or a taste receptor can be generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than the mouse system. For example, "HuMAb mice" which contain a human immunoglobulin gene miniloci that encodes unrearranged human heavy (μ and γ) and κ light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, N. *et al.* (1994) Nature 368(6474): 856 859). Accordingly, the mice exhibit reduced expression of mouse IgM or κ, and in response to immunization, the introduced human heavy and light chain transgenes undergo class switching and somatic mutation to generate high affinity human IgGκ monoclonal antibodies (Lonberg, N. *et al.* (1994), supra; reviewed in Lonberg, N. (1994) Handbook of Experimental Pharmacology 113:49 101; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65 93, and Harding, F. and Lonberg, N. (1995) Ann. N. Y Acad. Sci 764:536 546). The preparation of HuMAb mice is described in Taylor, L. *et al.* (1992) Nucleic Acids Research 20:6287 6295; Chen, J. *et al.* (1993) International Immunology 5: 647 656; Tuaillon *et al.* (1993) Proc. Natl. Acad. Sci USA 90:3720 3724; Choi *et al.* (1993) Nature Genetics 4:117 123; Chen, J. *et al.* (1993) EMBO J. 12: 821 830; Tuaillon *et al.* (1994) J. Immunol. 152:2912 2920; Lonberg *et al.*, (1994) Nature 368(6474): 856 859; Lonberg, N. (1994) Handbook of Experimental Pharmacology 113:49 101; Taylor, L. *et al.* (1994) International Immunology 6: 579 591; Lonberg, N. and Huszar, D. (1995) Intern. Rev. Immunol. Vol. 13: 65 93; Harding, F. and Lonberg, N. (1995) Ann. N.Y. Acad. Sci 764:536 546; Fishwild, D. *et al.* (1996) Nature Biotechnology 14: 845 851. See further, U.S. Pat. Nos. 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,789,650; 5,877,397; 5,661,016; 5,814,318; 5,874,299; 5,770,429; and 5,545,807.

In certain embodiments, the antibodies described herein are able to bind to an epitope of Trpm5, PLCB2, gustducin or a taste receptor with a dissociation constant of no greater than 10^{-6}, 10^{-7}, 10^{-8} or 10^{-9} M. Standard assays to evaluate the binding ability of the

antibodies are known in the art, including for example, ELISAs, Western blots and RIAs. The binding kinetics (*e.g.*, binding affinity) of the antibodies also can be assessed by standard assays known in the art, such as by Biacore analysis.

Small molecules

In certain embodiments, the agent is a small molecule agonist of Trpm5, PLCB2, gustducin, or a taste receptor.

Agents (*e.g.*, small molecules) useful in the methods described herein may be obtained from any available source, including systematic libraries of natural and/or synthetic compounds. Agents may also be obtained by any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries (libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive; see, *e.g.*, Zuckermann *et al.*, 1994, *J. Med. Chem.* 37:2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of agents may be presented in solution (*e.g.*, Houghten, 1992, *Biotechniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria and/or spores, (Ladner, USP 5,223,409), plasmids (Cull *et al*, 1992, *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla *et al*, 1990, *Proc. Natl. Acad. Sci.* 87:6378-6382; Felici, 1991, *J. Mol. Biol.* 222:301-310; Ladner, *supra.*).

Formulations

As discussed herein, an agent described herein can be administered in any suitable formulation known in the art. Exemplary of routes of administration include oral administration, rectal administration, topical administration, inhalation (nasal) or injection. Administration by injection includes intravenous (IV), intramuscular (IM), intratumoral (IT) and subcutaneous (SC) administration. In certain preferred embodiments the agent is administered orally to the subject.

In some embodiments, the agent is delivered in a food product (*e.g.*, a food or beverage) such as a health food or beverage, a food or beverage for infants, a food or beverage for pregnant women, athletes, senior citizens or other specified group, a functional food, a beverage, a food or beverage for specified health use, a dietary supplement, a food or beverage for patients, or an animal feed. Specific examples of the foods and beverages include various beverages such as juices, refreshing beverages, tea beverages, drink preparations, jelly beverages, and functional beverages; alcoholic beverages such as beers; carbohydrate-containing foods such as rice food products, noodles, breads, and pastas; paste products such as fish hams, sausages, paste products of seafood; retort pouch products such as curries, food dressed with a thick starchy sauces, and Chinese soups; soups; dairy products such as milk, dairy beverages, ice creams, cheeses, and yogurts; fermented products such as fermented soybean pastes, yogurts, fermented beverages, and pickles; bean products; various confectionery products, including biscuits, cookies, and the like, candies, chewing gums, gummies, cold desserts including jellies, cream caramels, and frozen desserts; instant foods such as instant soups and instant soy-bean soups; microwavable foods; and the like. Further, the examples also include health foods and beverages prepared in the forms of powders, granules, tablets, capsules, liquids, pastes, and jellies.

In some embodiments the agent is delivered in a food product for animals, including humans. The animals, other than humans, are not particularly limited, and the composition can be used for various livestock, poultry, pets, experimental animals, and the like. Specific examples of the animals include pigs, cattle, horses, sheep, goats, chickens, wild ducks, ostriches, domestic ducks, dogs, cats, rabbits, hamsters, mice, rats, monkeys, and the like, but the animals are not limited thereto.

Exemplary methods of treatment and prevention of diseases

Provided herein are methods of treatment or prevention of conditions and diseases that can be improved by enhancing the taste-chemosensory signaling pathway in tuft cells.

The methods described herein can be used to treat any subject in need thereof. The terms "subject" or "patient" refers to any animal. A subject or a patient described as "in need thereof" refers to one in need of a treatment for a disease. Mammals (*i.e.*, mammalian animals) include humans, laboratory animals (*e.g.*, primates, rats, mice), livestock (*e.g.*, cows, sheep, goats, pigs), and household pets (*e.g.*, dogs, cats, rodents). In certain embodiments, the subject is human.

In certain aspects, provided herein are methods of inducing a type 2 helper T cell (T_H2) immune response in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell. In certain aspects, provided herein are methods of inhibiting a type 1 helper T cell (T_H1) immune response in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell. In certain aspects, provided herein are methods of inhibiting a type 17 helper T cell (T_H17) immune response in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell. In certain embodiments, the agent induces expression of IL-25 by the tuft cells. In certain embodiments, the agent induces expression of IL-13 by the subject.

In certain embodiments, the subject has a disease or disorder associated with a pathological immune response (*e.g.*, an inflammatory bowel disease), as well as any subject with an increased likelihood of acquiring a such a disease or disorder (*e.g.*, predisposed). . In some embodiments, the subject has a damaged or depleted intestinal epithelium e.g., as a result of a pathological immune response, such as an inflammatory bowel disease.

In some embodiments, the disease or disorder is an inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis). In some embodiments, provided herein are methods of treating an inflammatory bowel disease. Inflammatory bowel diseases include, for example, certain art-recognized forms of a group of related conditions. Several major forms of inflammatory bowel diseases are known, with Crohn's disease (regional bowel disease, *e.g.*, inactive and active forms) and ulcerative colitis (*e.g.*, inactive and active forms) the most common of these disorders. In addition, the inflammatory bowel disease encompasses irritable bowel syndrome, microscopic colitis, lymphocytic-plasmocytic enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis. Other less common forms of IBD include indeterminate colitis, pseudomembranous colitis (necrotizing colitis), ischemic inflammatory bowel disease, Behcet's disease, sarcoidosis, scleroderma,

IBD-associated dysplasia, dysplasia associated masses or lesions, and primary sclerosing cholangitis.

In certain embodiments, the subject has or is predisposed to a protozoan infection. Exemplary protozoan infections include but are not limited to leishmaniasis, trichomoniasis, trypanosomiasis (Chagas disease and sleeping sickness), toxoplasmosis, malaria, giardiasis, cryptosporidiosis, babesiosis, primary amoebic meningoencephalitis, amoebiasis, dientamoebiasis, rhinosporidosis, sarcocystosis, cyclosporiasis, isosporiasis, blastocystosis, balantidiasis, and granulomatous amoebic encephalitis.

In certain embodiments, the subject has or is predisposed to a parasitic worm infection. Exemplary parasitic worms include but are not limited to coenurosis, diphyllobothriasis, echinococcosis, hymenolepiasis, taeniasis, cysticercosis, bertiellíasis, sparganosis, schistosomiasis, clonorchiasis, fasciolosis, fasciolopsiasis, gnathostomiasis, metagonimiasis, paragonimiasis, opisthorchiasis, ascariasis, ancylostomiasis, angiostrongyliasis, baylisascariasis, filariasis, dracunculiasis, enterobiasis, halicephalobiasis, onchocerciasis, strongyloidiasis, thelaziasis, toxocariasis, trichinosis, trichuriasis, and elephantiasis.

In certain aspects, provided herein are methods of inducing IL-25 expression by a tuft cell comprising contacting the tuft cell with an agent that enhances the taste-chemosensory signaling pathway in a tuft cells. In some embodiments, the tuft cell is isolated from a subject (*e.g.*, a subject in need thereof) prior to induction of IL-25 expression. In certain embodiments, the tuft cell is contacted with the agent *in vitro* or *ex vivo*. In certain embodiments, the tuft cell is administered to the subject after being contacted with the agent in order to induce a T_H2 immune response in the subject.

In certain aspects, provided herein are methods of regenerating damaged intenstinal epithelium resulting from inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis) comprising contacting the epithelium cell (*e.g.*, tuft cell) with an agent that enhances the taste-chemosensory signaling pathway in the epithelium cell (*e.g.*, tuft cell).

In certain aspects, provided herein are methods of repairing damaged intenstinal epithelium resulting from inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis) comprising contacting the epithelium cell (*e.g.*, tuft cell) with an agent that enhances the taste-chemosensory signaling pathway in the epithelium cell (*e.g.*, tuft cell).

In certain aspects, provided herein are methods of repairing depleted intenstinal epithelium resulting from inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative

colitis) comprising contacting an epithelium cell (*e.g.*, tuft cell) with an agent that enhances the taste-chemosensory signaling pathway in the epithelium cell (*e.g.*, tuft cell).

EXEMPLIFICATION

The invention now being generally described will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention in any way.

Experimental procedures

Mice - Wild-type C57BL/6J mice designated as bred in-house (BIH) were bred and housed in microisolator cages in the specific-pathogen-free (SPF) barrier facility at the Harvard T.H. Chan School of Public Health. Wild-type C57BL/6J designated as (JAX), *Rag2*$^{-/-}$, *Gfi1b*$^{EGFP/+}$, and *Stat6*$^{-/-}$ mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. C57BL/6 *Rag2*$^{-/-}$*Il2rγ*$^{-/-}$ mice were obtained from Taconic Biosciences, Germantown, New York. C57BL/6J *Trpm5*$^{-/-}$, gustducin$^{-/-}$, and Trpm5^{eGFP} mice were generously provided by Dr. Robert Margolskee (Monell Chemical Senses Center). C57BL/6J *Gfi1b*$^{eGFP/+}$ *Trpm5*$^{-/-}$ mice were generated by breeding *Gfi1b*$^{eGFP/+}$ and *Trpm5*$^{-/-}$ mice. Germ-free WT C57BL/6 mice were bred and maintained in vinyl positive pressure isolators within the Germ-free and Gnotobiotic core facilities at the Harvard Digestive Diseases Center at Brigham and Women's Hospital. Animal studies and experiments were approved and carried out in accordance with Harvard Medical School's Standing Committee on Animals and the National Institutes of Health guidelines for animal use and care. Helminth infections were carried out at Weill Cornell Medical School, Tufts University School of Medicine or Harvard T.H. Chan School of Public Health according to Institutional Animal Care and Use Committees (IACUC), and all experiments were performed according to the guidelines of the relevant institution.

Antibiotic treatment - Mice were treated with metronidazole (2.5g/L) with 1% sucrose in the drinking water for 7 days. Control mice were given 1% sucrose water over the same time span. Fluid intake was monitored and metronidazole solution was changed 4 days after initiating antibiotic treatment.

Histology and Fluorescence microscopy - The small intestine was removed and divided into proximal and distal sections before fixation in 4% paraformaldehyde. The tissue was then embedded in paraffin and cut into 5μm thick sections. For both histology and immunofluorescence, sections were initially deparaffinized and rehydrated. Hematoxylin and

eosin (H&E) staining was performed using standard procedures. Goblet cells were identified by alcian blue/nuclear red staining and enumerated along the crypt-villus axis by quantitative microscopy. For immunofluorescence, heat-mediated antigen retrieval was performed in Tris-EDTA buffer 0.05% Tween-20 pH 9.0 for 20 minutes. Afterwards, the slides were washed in PBS and blocked in PBS containing 3% BSA, 3% donkey serum, 0.1% Triton X-100, 0.1% saponin for 1 hour at room temperature. Primary antibodies were incubated overnight at 4˚C and secondary antibodies were applied for 1.5 hours at room temperature. Primary antibodies included: rabbit anti-DCLK1 (1:250 dilution, ab37994, Abcam), mouse anti-E-Cadherin (1:400 dilution, 36/E-Cadherin, BD Biosciences) and DNA was labeled with DAPI (0.5 µg/ml). For colocalization of anti-DCLK1 and GFP, tissue was harvested from *Gfi1b*$^{EGFP/+}$ *or Trpm5*^{eGFP} mice and fixed as described above. The tissue was then incubated at 4˚C in PBS with 20% sucrose for 6 hours followed by PBS with 30% sucrose overnight prior to freezing in OCT compound. 8µm frozen sections were cut and labeled with the following primary antibodies for *Trpm5*^{eGFP}: anti-GFP (1:1500, ab13970), anti-DCLK1 (1:100 dilution, ab37994), DAPI (0.5 µg/ml), and either Phalloidin (1:400 dilution, Molecular probes) or APC-conjugated anti-EpCam (1:1200 dilution, clone G8.8, Biolegend). For localization of GFP and DCLK1 with *Gfi1b*$^{EGFP/+}$ the following antibody was used: anti-DCLK1 (1:100 dilution, ab37994) and DAPI (0.5 µg/ml). Images were captured with Nikon eclipse Ni-U microscope and processed with Nikon NIS-elements software.

Isolation and culture of Tritrichomonas muris -Isolation and culture of *Tritrichomonas muris* was performed using a modified protocol described by Saeki *et al.*, Nippon Juigaku Zasshi. 45, 151–156 (1982). Briefly, cecal contents were harvested from WT C57BL/6J (BIH) mice passed through a 40 µm filter and washed three times in PBS. Trichomonads were further purified at the interface of a 40%/80% percoll (GE healthcare) gradient after centrifugation at 1000 X g for 15 minutes without braking. The number and viability of the isolated *T. muris* was determined by counting with a hemocytometer. Approximately 5x10^5 *T. muris* trophozoites were inoculated per ml of growth media, which consisted of Trichosel™ broth (Becton Dickinson) suspended in cecal extract supplemented with 10% heat-inactivated horse serum, amphotericin B, gentamicin, penicillin, streptomycin, and vancomycin pH adjusted to 7.0. The culture of *T. muris* was then placed at 37˚C in an anaerobic cabinet.

Infection with protozoa and helminthes - Tritrichomonas muris was isolated and cultured as described above. The helminth maintenance and infection was performed as

previously described for *Heligmosmoides polygyrus* (Lin *et al.*, The Journal of Immunology. 185, 3184–3189 (2010)), *Trichinella spiralis* (Hotez *et al.*, Journal of Clinical Investigation. 118, 1311–1321 (2008)), and *Nippostrongylus brasiliensis* (H.-E. Liang *et al.*, Nat Immunol. 13, 58–66 (2011)). $5x10^6$ *T. muris,* 150 L3 *H. polygyrus*, or 500 *T. spiralis* muscle larvae were orally administered to mice. 500 L3 *N. brasiliensis* were subcutaneously injected into mice. Mice were infected and sacrificed according to the following schedule: *T. muris* were sacrificed 17 days post-infection, *H. polygyrus* 21 days post-infection, *T. spiralis* 15 days post-infection, and *N. brasiliensis* 8 days post-infection. For *H. polygyrus* counts, mice were inoculated with 150 L3 larvae and sacrificed 36 days later. The proximal small intestine was excised and worms were counted with a dissection microscope. For the *Tritrichomonas muris* time course, WT and *Trpm5*$^{-/-}$ mice were infected with $5x10^6$ *T. muris* and then sacrificed 3, 7, 12, and 42 days later. Germ-free mice were inoculated with approximately $5x10^6$ *T. muris* for 21 days before excising the distal small intestine and processing the tissue for frozen sections a described above.

Scanning electron microscopy - Tritrichomonas muris was isolated from the cecal contents of WT (BIH) mice as described above and suspended in PBS. Protozoa were adhered to poly-*L*-lysine coated coverslips and fixed in 2.5% glutaraldehyde in a 0.1 M cacodylate buffer, pH 7.2. Following 3 buffer rinses the protozoa were post-fixed for 30 min in 1% OsO4 in 0.1 M cacodylate buffer, dehydrated in a graded series of ethanol, and critical point dried with liquid CO_2. *T. muris* was then sputter-coated with 5 nm platinum and examined with a Hitachi S-4800 field emission scanning electron microscope.

Tritrichomonas muris enumeration - The distal 10 cm of small intestine was removed and flushed with ice-cold sterile PBS using a 19-gauge feeding needle. The intestinal contents were then pelleted by centrifugation and stored at -20°C. Genomic DNA was isolated from the stool with QIamp Fast DNA Stool mini kit (Qiagen) according to the manufacturer's directions. To detect and enumerate *Tritrichomonas muris,* quantitative PCR (qPCR) was performed using KAPPA SYBR fast Universal PCR (KAPPA Biosystems) with the following primers recognizing *T. muris* 28S rRNA gene: 5'-GCTTTTGCAAGCTAGGTCCC-3'and 5'- TTTCTGATGGGGCGTACCAC-3'. To convert qPCR values into parasite numbers, *T. muris* trophozoytes were isolated and counted using a hemocytomer before extracting genomic DNA and analyzing by qPCR. These results were plotted on a standard curve and a regression analysis was performed to convert Ct values to parasite numbers (Figure 9, Panel A). To determine *T. muris* abundance in stool as shown in Figure 1, Panel D and Figure 4,

Panel A, DNA was isolated as described above. The *T. muris* 28S rRNA gene and Eubacteria 16S rRNA gene were amplified by qPCR and *T. muris* 28S relative abundance was calculated as $2^{-\Delta Ct\,(28S\text{-}16S)}$.

Epithelial cell isolation and flow cytometry -The distal 10cm of small intestine was removed and flushed as described above. The intestine was opened longitudinally and gently agitated at 4˚C in PBS, 2% FBS, 5mM EDTA, 1mM DTT for 10 min. The tissue was then transferred into prewarmed PBS, 2% FBS, 5mM EDTA and rotated at 37˚C for 15 minutes followed by vigorous shaking to remove epithelial cells. This was repeated and epithelial cells from both fractions were combined and washed with PBS. The epithelium was then digested in DMEM containing 10% FBS, 0.5 units/ml Dispase II (StemCell Technologies), 50 µg/ml DNase (Roche) for 12 minutes at 37˚C. The resulting solution was passed through 40 µm filters and washed with PBS, 2% FBS, 1mM EDTA. The resulting single cell suspension was initially Fc blocked with anti-CD16/CD32 (clone 93, Biolegend) and then stained with the following antibodies: PacBlue-conjugated anti-CD45 (clone 30-F11, Biolegend), APC-conjugated anti-EpCam (clone G8.8, Biolegend). The cell viability was assessed by propidium iodide (PI) (Biolegend) staining.

Lamina propria cell isolation and flow cytometry - The distal 10 cm of small intestine was collected and epithelial cells were removed as detailed above. Afterwards the tissue was minced into approximately 1 mm^2 sections and digested in RPMI 10%FBS, 0.25mg/ml collagenase A (Roche), 0.1 units/ml Dispase II (StemCell Technologies), 50 µg/ml DNase (Roche) for 25 minutes followed by a second 40 minute digestion. The solution was passed through 40 µm filters and the resulting single-cell suspension was Fc blocked with anti-CD16/CD32 (clone 93, Biolegend) before staining with the following combination of antibodies (all antibodies are from Biolegend unless otherwise stated). For eosinophils: PacBlue-conjugated anti-CD45 (clone 30-F11), PE-Cy7-conjugated anti-I-A/I-E (clone M5/114.15.2), APC-conjugated anti-Siglec-F (clone E50-2440, BD biosciences), APC-Cy7-conjugated anti-CD11b (clone M1/70). PI (Biolegend) was used to exclude dead cells (gating strategy Figure 16). For innate lymphoid cells, the following antibodies were used: Alexa Fluor® 488-conjugated anti-CD45 (clone 30-F11), PE-conjugated anti-IL17RB (clone 12-7361, eBioscience), PerCP-Cy5.5 conjugated anti-KLRG1 (clone 2F1/KLRG1), APC-conjugated anti-IL7Ra (clone A7R34) and lineage markers PacBlue-conjugated anti-CD3 (clone 17A2), PacBlue-conjugated anti-GR-1 (clone RB6-8C5), PacBlue-conjugated anti-CD11b (clone M1/70), PacBlue-conjugated anti-B220 (clone RA3-6B2), PacBlue-

conjugated anti-Ter119 (clone Ter-119), PacBlue-conjugated anti-CD4 (clone RM4-5), PacBlue-conjugated anti-CD8a (clone 53-6.7), PacBlue-conjugated anti-NK1.1 (clone PK136). After antibody staining dead cells were excluded with Live/Dead fixable yellow dead cell kit (Invitrogen) (gating strategy Figure 17).

RNA isolation and RT-PCR for in vivo and vitro tuft cells - Epithelial cells from *Gfi1b*$^{EGFP/+}$ mice were isolated and stained for Fluorescence Activated Cell Sorting (FACS) as previously detailed. Tuft cells were sorted based on GFP^{+}EpCam^{+}CD45^{-}PI^{-} while the remaining epithelial cells were GFP^{-}EpCam^{+}CD45^{-}PI^{-}. RNA was then extracted from tuft cells and the remaining epithelium using RNeasy Micro Kit (Qiagen). Whole intestinal organoids were initially stored in RNA*later* solution (Ambion) before extracting RNA using Qiazol (Qiagen) according to the manufacturer's directions. cDNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad) and RT-qPCR was performed using the KAPA SYBR FAST Universal qPCR Kit (KAPA Biosystems). The following primers were used: DCLK1: 5'- CAGCCTGGACGAGCTGGTGG-3' and 5'-TGACCAGTTGGGGTTCACAT-3', Trpm5: 5'-CCTCCGTGCTTTTTGAACTCC-3' and 5'-CATAGCCAAAGGTCGTTCCTC-3', PLCβ2: 5'- AGATCTCGTCGATTTCTGGC-3' and 5'-GTGCTTGTCACCTTGCAAAA-3', Gustducin: 5'- GAGAGCAAGGAATCAGCCAG-3' and 5'-GTGCTTTTCCCAGATTCACC-3', IL-25: 5'-ACAGGGACTTGAATCGGGTC-3' and 5'- TGGTAAAGTGGGACGGAGTTG-3', IL-33: 5'-CCTCCCTGAGTACATACAATGACC-3' and 5'-GTAGTAGCACCTGGTCTTGCTCTT-3', TSLP: 5'-CGTGAATCTTGGCTGTAAACT-3' and 5'-GTCCGTGGCTCTCTTATTCT-3', IL-17RB: 5'-ACCGTCTGTCGCTTCACTG-3' and 5'-CCACTTTATCTGCCGCTTGC-3'.

Small intestine organoid culture and flow cytometry - Distal small intestinal organoids were prepared as previously described (Miyoshi *et al.*, Nat Protoc. 8, 2471–2482 (2013)). To assess cytokine effects on tuft cells abundance, IL-13 (10ng/ml, Biolegend, Endotoxin level <0.01ng/μg) or IL-25 (50ng/ml, Biolegend, Endotoxin level <0.01ng/μg) were added to organoid media for 48 hours. To perform flow cytometry, organoids were liberated from the matrigel matrix as described by Miyoshi *et al.* and digested in DMEM containing 10% FBS, 0.5 units/ml Dispase II (StemCell Technologies), 50 μg/ml DNase (Roche) for 8 minutes at 37°C. The resulting solution was filtered through 40 μm mesh and stained for flow cytometry with APC-conjugated anti-EpCam (clone G8.8, Biolegend) with cell viability assessed with PI (Biolegend).

In vivo IL-25 injections -*Gfi1b*$^{EGFP/+}$ mice without parasites and *Tritrichomonas muris* colonized *Gfi1b*$^{EGFP/+}$ *Trpm5*$^{-/-}$ mice were intraperitoneally (i.p.) injected daily with 0.5μg of recombinant IL-25 (R&D, Endotoxin level < 1.0 EU per 1 μg) or equivalent volume of sterile PBS for 7 days before harvesting the distal small intestine to examine the abundance of eosinophils and tuft cells as described above.

Statistical analyses - GraphPad Prism® Software was used for the calculation of statistical measures, including mean values, standard errors, Shapiro-Wilk normality test, Mann-Whitney test, Ordinary one-way ANOVA, and Kruskal-Wallis test.

Example 1: Evaluation DCLK1^{+} *tuft cells in the distal small intestine of wild type (WT) specific-pathogen-free mice*

The frequency of DCLK1^{+} tuft cells in the distal small intestine of wild type (WT) specific-pathogen-free mice that were bred in-house (BIH) was evaluated. Markedly more intestinal DCLK1^{+} tuft cells (7.2%) (Figure 1, Panel A) was found than previously known (0.4%) (*19*, *22*) and this discrepancy was confirmed with an alternative tuft cell marker, Gfi1b (*23*) (Figure 2). As inter-institutional microbiota differences can contribute to substantial variation of mucosal immune cell populations, tuft cell abundance in mice obtained from Jackson laboratories (JAX) was compared to BIH mice. Tuft cells constituted 1.0% of the total IEC population of JAX mice (Figure 1, Panel A). Feeding the cecal contents from BIH mice to JAX mice was sufficient to increase tuft cell populations to BIH levels (Figure 3), suggesting that transmissible components of the BIH microbiota may drive tuft cell expansion when introduced to JAX mice. In support of this idea, intestinal histology revealed numerous single-celled protozoa in BIH but not JAX mice (Figure 1, Panel B). To identify these protozoa, they were purified and imaged by scanning-electron microscopy (SEM) and identified as tritrichomonads (Figure 1, Panel C). Quantitative-PCR confirmed that they were *Tritrichomonas*.

To eradicate *T. muris* from BIH mice, metronidazole (2.5g/L) was added to their drinking water for 1 week. This eliminated *T. muris* and concomitantly reduced tuft cell abundance (Figure 4). Because this treatment does not exclude the possibility that other metronidazole-sensitive organisms may contribute to tuft cell expansion, *T. muris* was cultured and colonized unexposed mice. *T. muris* colonization significantly elevated tuft cell numbers in conventional (Figure 1, Panels E and F) and germ-free mice (Figure 5), suggesting that this symbiotic protozoa is sufficient to increase tuft cell frequency.

Example 2: Helminth infection on tuft cell abundance

To investigate the effect of helminth infection on tuft cell abundance, mice were infected with a diverse set of parasitic worms including: *Heligmosomoides polygyrus* (*Hp*), *Trichinella spiralis* (*Ts*), and *Nippostrongylus brasiliensis* (*Nb*). Similar to Example 1 with *T. muris*, infections with all three helminths increased tuft cell abundance, indicating that expansion of tuft cells is a broadly conserved feature of parasite colonization (Figure 1, Panels G and H).

Example 3 - Upstream pathways mediate tuft cell response

Because tuft cells are postulated to be chemosensory cells, whether perturbations to tuft chemosensory pathways may affect their expansion in response to parasites as well as the type 2 immune response typically initiated by parasites was considered. Multiple taste-GPCRs sense sweet, bitter, and umami compounds, yet engagement of these different receptors activates a common signal transduction pathway involving gustducin, PLCβ2, and Trpm5 (Figure 6). *Gfi1b*$^{+}$ tuft cells are the primary IEC subset expressing the canonical taste-associated components, *gustducin*, *PLCβ2*, and *Trpm5* (Figure 7, Panel A).

Tuft cell abundance in WT and *gustducin*$^{-/-}$ mice colonized with *T. muris* was compared and significantly fewer tuft cells in *gustducin*$^{-/-}$ animals (Figure 7, Panel B) was found. Using *Trpm5*^{eGFP} reporter mice, Trpm5 is restricted to the epithelium and expressed by DCLK1+ tuft cells in the distal small intestine was validated (Figure 7, Panel C and Figure 8). Given the multiplicity of taste-GPCRs, the established role of Trpm5 in taste-chemosensation, and predominant intestinal *Trpm5* expression by tuft cells, Trmp5-deficient mice were used to evaluate whether these pathways affect tuft cell parasite responses. Similar to *gustducin*$^{-/-}$ mice, tuft cells failed to expand in *Trpm5*$^{-/-}$ mice during *T. muris* colonization (Figure 7, Panels D, E, and F). To determine if the blunted response was due to reduced parasite colonization, *T. muris* in the distal small intestine (Figure 9) was measured. Slightly more parasites in both *gustducin*$^{-/-}$ and *Trpm5*$^{-/-}$ mice than WT (Figure 9, Panel B) was found, indicating the lack of tuft cell response was not due to decreased *T. muris* colonization. Because *T. muris* is a stable component of the microbiota, how loss of Trpm5 would affect clearance of a pathogenic helminth such as *H. polygyrus* was queried. After 36 days post-infection, *Trpm5*$^{-/-}$ mice had a significantly higher worm burden than WT (Figure 9, Panel C). Collectively, these data suggest that pathways initiated upstream of Trpm5 may mediate tuft cell response to intestinal parasites.

Consistent with helminth infections, *T. muris* colonization also induced goblet cell hyperplasia in WT ($P < 0.0001$), but not in *Trpm5*$^{-/-}$ mice (Figure 7, Panels G and H).

Similarly, eosinophilia in WT but not *Trpm5*$^{-/-}$ mice colonized with *T. muris* was observed (Figure 7, Panel I).

Example 4 - Determination of TSLP, IL33, and Il25 expression patterns

Because epithelial cells are a key source of the parasite-induced cytokines thymic stromal lymphopoietin (TSLP), interleukin-33 (IL-33) and interleukin-25 (IL-25), both tuft cells and the remaining epithelial fraction were isolated to determine *TSLP*, *Il33*, and *Il25* expression patterns. Tuft cells expressed less *TSLP* and *Il33* than other epithelial cells, and are the main source of epithelial *Il25* (Figure 10, Panel A and Figure 11, Panel A). To determine if Trpm5 affects parasite-induced *Il25* expression, WT and *Trpm5*$^{-/-}$ mice were infected with *T. muris* and both parasite colonization and the corresponding epithelial *Il25* expression were measured over time. *T. muris* rapidly colonized both WT and *Trpm5*$^{-/-}$ mice, but *Trpm5*$^{-/-}$ mice had significantly (P=0.0006) reduced *Il25* expression 12 days post-infection (Figure 10, Panel B).

IL-25 promotes proliferation and activation of type 2 innate lymphoid cells (ILC2s) via the receptor subunit, IL17RB. Accordingly, the frequency of intestinal lamina propria IL17RB^{+} ILC2s significantly increased in WT but not *Trpm5*$^{-/-}$ mice after 12 days of *T. muris* infection (Figure 10, Panel C). To determine if the parasite response in *Trpm5*$^{-/-}$ mice could be complemented by exogenous IL-25, IL-25 intraperitoneally (i.p.) was injected into *Trpm5*$^{-/-}$ mice and observed restoration of distal small intestinal eosinophilia and tuft cell abundance (Figure 10, Panels D-F), suggesting that tuft cells may influence their own abundance.

Epithelial cells are not only a crucial source of IL-25, but also signal in an autocrine manner via IL17RB. Therefore, tuft cell *Il17RB* expression was examined and found it was significantly higher (P=0.0043) than other epithelial cells (Figure 11, Panel B). This raised the question of whether IL-25 induces tuft cell expansion via autocrine signaling or indirectly through recruitment of ILC2s. To evaluate factors that affect tuft cell abundance independently of the microbiota or immune system, an *in vitro* primary intestinal organoid system was employed. Small intestinal organoids reconstitute all the epithelial subsets from IEC stem cells and by generating organoids from *Gfi1b*$^{EGFP/+}$ mice, and GFP^{+} tuft cells (Figure 12, Panel A and Figure 13, Panel A) was detected. Both WT and *Trpm5*$^{-/-}$ organoids contained approximately 0.3% tuft cells, but IL-25 did not increase tuft cell numbers (Figure 10, Panel and Figure 13, Panel A), suggesting that IL-25 does not act in an autocrine manner to expand tuft cell abundance. Since IL-25 promotes expansion of ILC2s, which are critical

sources of IL-13 (*11*, *36*, *40*); a cytokine previously shown to increase goblet cell numbers, whether IL-13 may also increase tuft cell abundance was considered. IL-13 significantly expanded tuft cells from 0.3% of total organoid cells to 11.9% and 10.9% (WT and *Trpm5*$^{-/-}$, respectively) (Figure 10, Panel B and Figure, 13, Panel A). In agreement with these results, expression of *Dclk1* and *Trpm5* also increased in IL-13 treated organoids (Figure 13, Panels B and C).

Example 5 - Tuft cells detect T. muris through Trpm5 taste-chemosensation

To determine if type 2 cytokine production by ILC2s may contribute to tuft cell expansion *in vivo*, WT, *Stat6*$^{-/-}$, *Rag2*$^{-/-}$, and *Rag2*$^{-/-}$*Il2rγ*$^{-/-}$ mice were colonized with *T. muris* (Figure 14). STAT6 is activated by the type 2 cytokines, IL-4 and IL-13, and is required for intestinal helminth expulsion. Consistent with the organoid data demonstrating that IL-13 potently induces of tuft cell expansion, tuft cells did not expand when *T. muris* colonized *Stat6*$^{-/-}$ mice (Figure 12, Panels C and D). While both T helper 2 (T_H2) and ILC2 cells can produce IL-13 in mucosal tissue, parasite-induced IL-25 potently activates *Il13* expression in ILCs. Tuft cell abundance was compared in *Rag2*$^{-/-}$ mice which lack T_H2 cells but contain ILC2s and *Rag2*$^{-/-}$*Il2rγ*$^{-/-}$ which lack both T_H2 and ILC2s cells. Infected *Rag2*$^{-/-}$ mice had elevated tuft cell abundance compared to uninfected WT mice, yet similar to both *Trpm5*$^{-/-}$ and *Stat6*$^{-/-}$ mice, *Rag2*$^{-/-}$*Il2rγ*$^{-/-}$ mice showed no tuft cell increase during *T. muris* infection (Figure 12, Panels C and D). Collectively, this suggests that tuft cells may detect *T. muris* through Trpm5 taste-chemosensation to elicit ILCs, which in turn produce IL-13 to expand tuft cell abundance (Figure 15).

Incorporation by Reference

All publications, patents, and patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A method of inducing a type 2 helper T cell (T_H2) immune response in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell.

2. The method of claim 1, wherein the agent enhances the activity or expression of Trpm5, PLCB2 or gustducin.

3. The method of claim 2, wherein the agent is a small molecule agonist of Trpm5, PLCB2 or gustducin.

4. The method of claim 2, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for Trpm5, PLCB2 or gustducin.

5. The method of claim 2, wherein the agent comprises a nucleic acid that encodes Trpm5, PLCB2 or gustducin.

6. The method of claim 5, wherein the nucleic acid is an mRNA.

7. The method of claim 5, wherein the nucleic acid is an expression vector.

8. The method of claim 1, wherein the agent activates a taste receptor.

9. The method of claim 8, wherein the taste receptor is selected from the group consisting of TAS1R1, TAS1R2, TAS1R3, TAS1R4, TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R12, TAS2R13, TAS2R14, TAS2R15, TAS2R16, TAS2R18, TAS2R19, TAS2R20, TAS2R22, TAS2R23, TAS2R30, TAS2R31, TAS2R33, TAS2R36, TAS2R37, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R42, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R60.

10. The method of claim 8 or 9, wherein the agent is a taste receptor ligand.

11. The method of claim 8 or 9, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for the taste receptor.

12. The method of claim 8 or 9, wherein the agent is a small molecule agonist of the taste receptor.

13. The method of any one of claims 1 to 12, wherein the agent induces expression of IL-25 by the tuft cells.

14. The method of any one of claims 1 to 13, wherein the agent induces expression of IL-13 by the subject.

15. The method of any one of claims 1 to 14, wherein the agent is delivered orally to the subject

16. The method of claim 15, wherein the agent is delivered in a food product.

17. The method of any one of claims 1 to 16, wherein the subject is human.

18. The method of any one of claims 1 to 17, wherein the subject has or is predisposed to a disease associated with a pathological immune response.

19. The method of claim 18, wherein the subject has or is predisposed to an inflammatory bowel disease.

20. The method of claim 19, wherein the inflammatory bowel disease is Crohn's disease, ulcerative colitis, irritable bowel syndrome, microscopic colitis, lymphocytic-plasmocytic enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis, indeterminate colitis, infectious colitis, pseudomembranous colitis, ischemic inflammatory bowel disease or Behcet's disease.

21. The method of any one of claims 1 to 17, wherein the subject has or is predisposed to a protozoan infection.

22. The method of claim 21, wherein the protozoan infection is selected from leishmaniasis, trichomoniasis, trypanosomiasis (Chagas disease and sleeping sickness), toxoplasmosis, malaria, giardiasis, cryptosporidiosis, babesiosis, primary amoebic meningoencephalitis, amoebiasis, dientamoebiasis, rhinosporidosis, sarcocystosis, cyclosporiasis, isosporiasis, blastocystosis, balantidiasis, and granulomatous amoebic encephalitis.

23. The method of any one of claims 1 to 17, wherein the subject has or is predisposed to a parasitic worm infection.

24. The method of claim 23, wherein the parasitic worm is selected from coenurosis, diphyllobothriasis, echinococcosis, hymenolepiasis, taeniasis, cysticercosis, bertielliasis, sparganosis, schistosomiasis, clonorchiasis, fasciolosis, fasciolopsiasis, gnathostomiasis, metagonimiasis, paragonimiasis, opisthorchiasis, ascariasis, ancylostomiasis, angiostrongyliasis, baylisascariasis, filariasis, dracunculiasis, enterobiasis, halicephalobiasis, onchocerciasis, strongyloidiasis, thelaziasis, toxocariasis, trichinosis, trichuriasis, and elephantiasis.

25. A method of treating or preventing an inflammatory bowel disease in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cells.

26. The method of claim 25, wherein the agent enhances the activity or expression of Trpm5, PLCB2 or gustducin.

27. The method of claim 26, wherein the agent is a small molecule agonist of Trpm5, PLCB2 or gustducin.

28. The method of claim 26, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for Trpm5, PLCB2 or gustducin.

29. The method of claim 26, wherein the agent comprises a nucleic acid that encodes Trpm5, PLCB2 or gustducin.

30. The method of claim 29, wherein the nucleic acid is an mRNA.

31. The method of claim 29, wherein the nucleic acid is an expression vector.

32. The method of claim 25, wherein the agent activates a taste receptor.

33. The method of claim 32, wherein the taste receptor is selected from the group consisting of TAS1R1, TAS1R2, TAS1R3, TAS1R4, TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R12, TAS2R13, TAS2R14, TAS2R15, TAS2R16, TAS2R18, TAS2R19, TAS2R20, TAS2R22, TAS2R23, TAS2R30, TAS2R31, TAS2R33, TAS2R36, TAS2R37, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R42, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R60.

34. The method of claim 32 or 33, wherein the agent is a taste receptor ligand.

35. The method of claim 32 or 33, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for the taste receptor.

36. The method of claim 32 or 33, wherein the agent is a small molecule agonist of the taste receptor.

37. The method of any one of claims 25 to 36, wherein the agent induces expression of IL-25 by the tuft cells.

38. The method of any one of claims 25 to 37, wherein the agent induces expression of IL-13 by the subject.

39. The method of any one of claims 25 to 38, wherein the agent is delivered orally to the subject

40. The method of claim 39, wherein the agent is delivered in a food product.

41. The method of any one of claims 25 to 40, wherein the subject is human.

42. The method of any one of claims 25 to 41, wherein the inflammatory bowel disease is Crohn's disease, ulcerative colitis, irritable bowel syndrome, microscopic colitis, lymphocytic-plasmocytic enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis, indeterminate colitis, infectious colitis, pseudomembranous colitis, ischemic inflammatory bowel disease or Behcet's disease.

43. A method of treating or preventing a parasitic infection in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cells.

44. The method of claim 43, wherein the agent enhances the activity or expression of Trpm5, PLCB2 or gustducin.

45. The method of claim 44, wherein the agent is a small molecule agonist of Trpm5, PLCB2 or gustducin.

46. The method of claim 44, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for Trpm5, PLCB2 or gustducin.

47. The method of claim 44, wherein the agent comprises a nucleic acid that encodes Trpm5, PLCB2 or gustducin.

48. The method of claim 47, wherein the nucleic acid is an mRNA.

49. The method of claim 47, wherein the nucleic acid is an expression vector.

50. The method of claim 43, wherein the agent activates a taste receptor.

51. The method of claim 50, wherein the taste receptor is selected from the group consisting of TAS1R1, TAS1R2, TAS1R3, TAS1R4, TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R12, TAS2R13, TAS2R14, TAS2R15, TAS2R16, TAS2R18, TAS2R19, TAS2R20, TAS2R22, TAS2R23, TAS2R30, TAS2R31, TAS2R33, TAS2R36, TAS2R37, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R42, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R60.

52. The method of claim 50 or 51, wherein the agent is a taste receptor ligand.

53. The method of claim 50 or 51, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for the taste receptor.

54. The method of claim 50 or 51, wherein the agent is a small molecule agonist of the taste receptor.

55. The method of any one of claims 43 to 54, wherein the agent induces expression of IL-25 by the tuft cells.

56. The method of any one of claims 43 to 55, wherein the agent induces expression of IL-13 by the subject.

57. The method of any one of claims 43 to 56, wherein the agent is delivered orally to the subject

58. The method of claim 57, wherein the agent is delivered in a food product.

59. The method of any one of claims 43 to 58, wherein the subject is human.

60. The method of any one of claims 43 to 59, wherein the parasitic infection is a protozoan infection.

61. The method of claim 60, wherein the protozoan infection is selected from leishmaniasis, trichomoniasis, trypanosomiasis (Chagas disease and sleeping sickness), toxoplasmosis, malaria, giardiasis, cryptosporidiosis, babesiosis, primary amoebic meningoencephalitis, amoebiasis, dientamoebiasis, rhinosporidosis, sarcocystosis, cyclosporiasis, isosporiasis, blastocystosis, balantidiasis, and granulomatous amoebic encephalitis.

62. The method of any one of claims 43 to 59, wherein the parasitic infection is a parasitic worm infection.

63. The method of claim 62, wherein the parasitic worm is selected from coenurosis, diphyllobothriasis, echinococcosis, hymenolepiasis, taeniasis, cysticercosis, bertielliasis, sparganosis, schistosomiasis, clonorchiasis, fasciolosis, fasciolopsiasis, gnathostomiasis, metagonimiasis, paragonimiasis, opisthorchiasis, ascariasis, ancylostomiasis, angiostrongyliasis, baylisascariasis, filariasis, dracunculiasis, enterobiasis, halicephalobiasis, onchocerciasis, strongyloidiasis, thelaziasis, toxocariasis, trichinosis, trichuriasis, and elephantiasis.

64. A method of inducing IL-25 expression by a tuft cell comprising contacting the tuft cell with an agent that enhances the taste-chemosensory signaling pathway in a tuft cells.

65. The method of claim 64, wherein the agent enhances the activity or expression of Trpm5, PLCB2 or gustducin.

66. The method of claim 65, wherein the agent is a small molecule agonist of Trpm5, PLCB2 or gustducin.

67. The method of claim 65, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for Trpm5, PLCB2 or gustducin.

68. The method of claim 65, wherein the agent comprises a nucleic acid that encodes Trpm5, PLCB2 or gustducin.

69. The method of claim 68, wherein the nucleic acid is an mRNA.

70. The method of claim 68, wherein the nucleic acid is an expression vector.

71. The method of claim 64, wherein the agent activates a taste receptor.

72. The method of claim 71, wherein the taste receptor is selected from the group consisting of TAS1R1, TAS1R2, TAS1R3, TAS1R4, TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R12, TAS2R13, TAS2R14, TAS2R15, TAS2R16, TAS2R18, TAS2R19, TAS2R20, TAS2R22, TAS2R23, TAS2R30, TAS2R31, TAS2R33, TAS2R36, TAS2R37, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R42, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R60.

73. The method of claim 72 or 73, wherein the agent is a taste receptor ligand.

74. The method of claim 72 or 73, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for the taste receptor.

75. The method of claim 72 or 73, wherein the agent is a small molecule agonist of the taste receptor.

76. The method of any one of claims 64 to 75, wherein the tuft cell is contacted with the agent *in vitro*.

77. The method of claim 76, wherein the tuft cell is administered to a subject after being contacted with the agent.

78. The method of claim 77, wherein the subject is human.

79. The method of claim 77 or 78, wherein the subject has or is predisposed to a disease associated with a pathological immune response.

80. The method of claim 79, wherein the subject has or is predisposed to an inflammatory bowel disease.

81. The method of claim 80, wherein the inflammatory bowel disease is Crohn's disease, ulcerative colitis, irritable bowel syndrome, microscopic colitis, lymphocytic-plasmocytic

enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis, indeterminate colitis, infectious colitis, pseudomembranous colitis, ischemic inflammatory bowel disease or Behcet's disease.

82. The method of claim 77 or 78, wherein the subject has or is predisposed to a protozoan infection.

83. The method of claim 82, wherein the protozoan infection is selected from leishmaniasis, trichomoniasis, trypanosomiasis (Chagas disease and sleeping sickness), toxoplasmosis, malaria, giardiasis, cryptosporidiosis, babesiosis, primary amoebic meningoencephalitis, amoebiasis, dientamoebiasis, rhinosporidosis, sarcocystosis, cyclosporiasis, isosporiasis, blastocystosis, balantidiasis, and granulomatous amoebic encephalitis.

84. The method of claim 77 or 78, wherein the subject has or is predisposed to a parasitic worm infection.

85. The method of claim 84, wherein the parasitic worm is selected from coenurosis, diphyllobothriasis, echinococcosis, hymenolepiasis, taeniasis, cysticercosis, bertielliasis, sparganosis, schistosomiasis, clonorchiasis, fasciolosis, fasciolopsiasis, gnathostomiasis, metagonimiasis, paragonimiasis, opisthorchiasis, ascariasis, ancylostomiasis, angiostrongyliasis, baylisascariasis, filariasis, dracunculiasis, enterobiasis, halicephalobiasis, onchocerciasis, strongyloidiasis, thelaziasis, toxocariasis, trichinosis, trichuriasis, and elephantiasis.

86. The method of any one of claim 77 to 85, wherein the tuft cell is isolated from the subject prior to being contacted with the agent.

87. The method of any one of claims 64 to 75, wherein the tuft cell is contacted with the agent *in vivo*.

88. A method of inducing the regeneration or repair of epithelium damaged by an inflammatory bowel disease in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cells.

89. The method of claim 88, wherein the agent enhances the activity or expression of Trpm5, PLCB2 or gustducin.

90. The method of claim 89, wherein the agent is a small molecule agonist of Trpm5, PLCB2 or gustducin.

91. The method of claim 89, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for Trpm5, PLCB2 or gustducin.

92. The method of claim 89, wherein the agent comprises a nucleic acid that encodes Trpm5, PLCB2 or gustducin.

93. The method of claim 92, wherein the nucleic acid is an mRNA.

94. The method of claim 92, wherein the nucleic acid is an expression vector.

95. The method of claim 88, wherein the agent activates a taste receptor.

96. The method of claim 95, wherein the taste receptor is selected from the group consisting of TAS1R1, TAS1R2, TAS1R3, TAS1R4, TAS2R1, TAS2R3, TAS2R4, TAS2R5, TAS2R7, TAS2R8, TAS2R9, TAS2R10, TAS2R12, TAS2R13, TAS2R14, TAS2R15, TAS2R16, TAS2R18, TAS2R19, TAS2R20, TAS2R22, TAS2R23, TAS2R30, TAS2R31, TAS2R33, TAS2R36, TAS2R37, TAS2R38, TAS2R39, TAS2R40, TAS2R41, TAS2R42, TAS2R43, TAS2R44, TAS2R45, TAS2R46, TAS2R47, TAS2R48, TAS2R49, TAS2R50, and TAS2R60.

97. The method of claim 95 or 96, wherein the agent is a taste receptor ligand.

98. The method of claim 95 or 96, wherein the agent is an antibody or antigen binding fragment thereof with binding specificity for the taste receptor.

99. The method of claim 95 or 96, wherein the agent is a small molecule agonist of the taste receptor.

100. The method of any one of claims 88 to 99, wherein the agent induces expression of IL-25 by the tuft cells.

101. The method of any one of claims 88 to 100, wherein the agent induces expression of IL-13 by the subject.

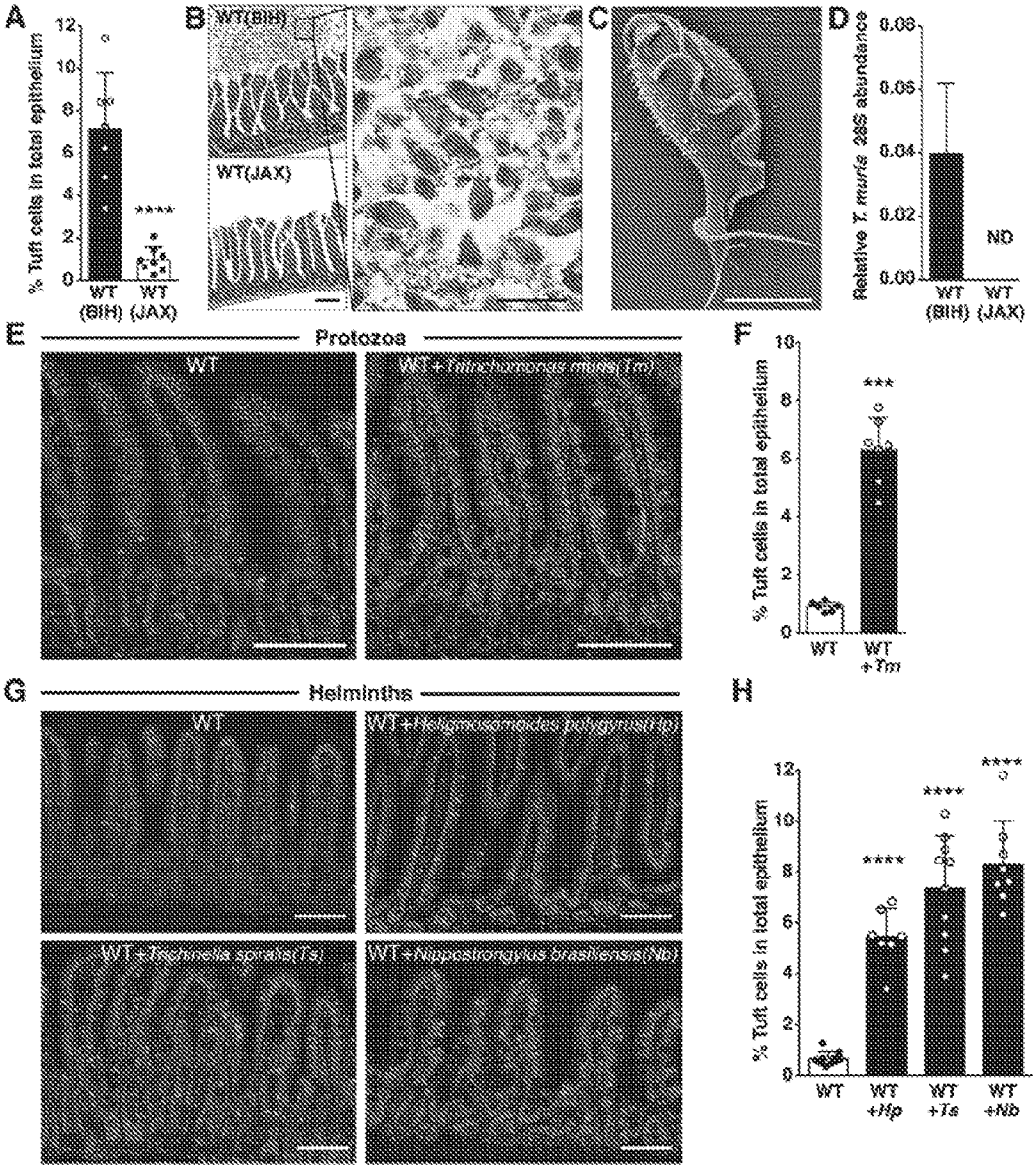
102. The method of any one of claims 88 to 101, wherein the agent is delivered orally to the subject

103. The method of claim 102, wherein the agent is delivered in a food product.

104. The method of any one of claims 88 to 103, wherein the subject is human.

105. The method of any one of claims 88 to 104, wherein the inflammatory bowel disease is Crohn's disease, ulcerative colitis, irritable bowel syndrome, microscopic colitis, lymphocytic-plasmocytic enteritis, coeliac disease, collagenous colitis, lymphocytic colitis and eosinophilic enterocolitis, indeterminate colitis, infectious colitis, pseudomembranous colitis, ischemic inflammatory bowel disease or Behcet's disease.

Figure 1


A
% Tuft cells in total epithelium
WT (BIH)
WT (JAX)
****
B
WT(BIH)
WT(JAX)
C
D
Relative T. muris 28S abundance
ND
WT (BIH)
WT (JAX)
E
Protozoa
WT
WT + Tritrichomonas muris (Tm)
F
% Tuft cells in total epithelium
***
WT
WT +Tm
G
Helminths
WT
WT + Heligmosomoides polygyrus (Hp)
WT + Trichinella spiralis (Ts)
WT + Nippostrongylus brasiliensis (Nb)
H
% Tuft cells in total epithelium
****
WT
WT +Hp
WT +Ts
WT +Nb

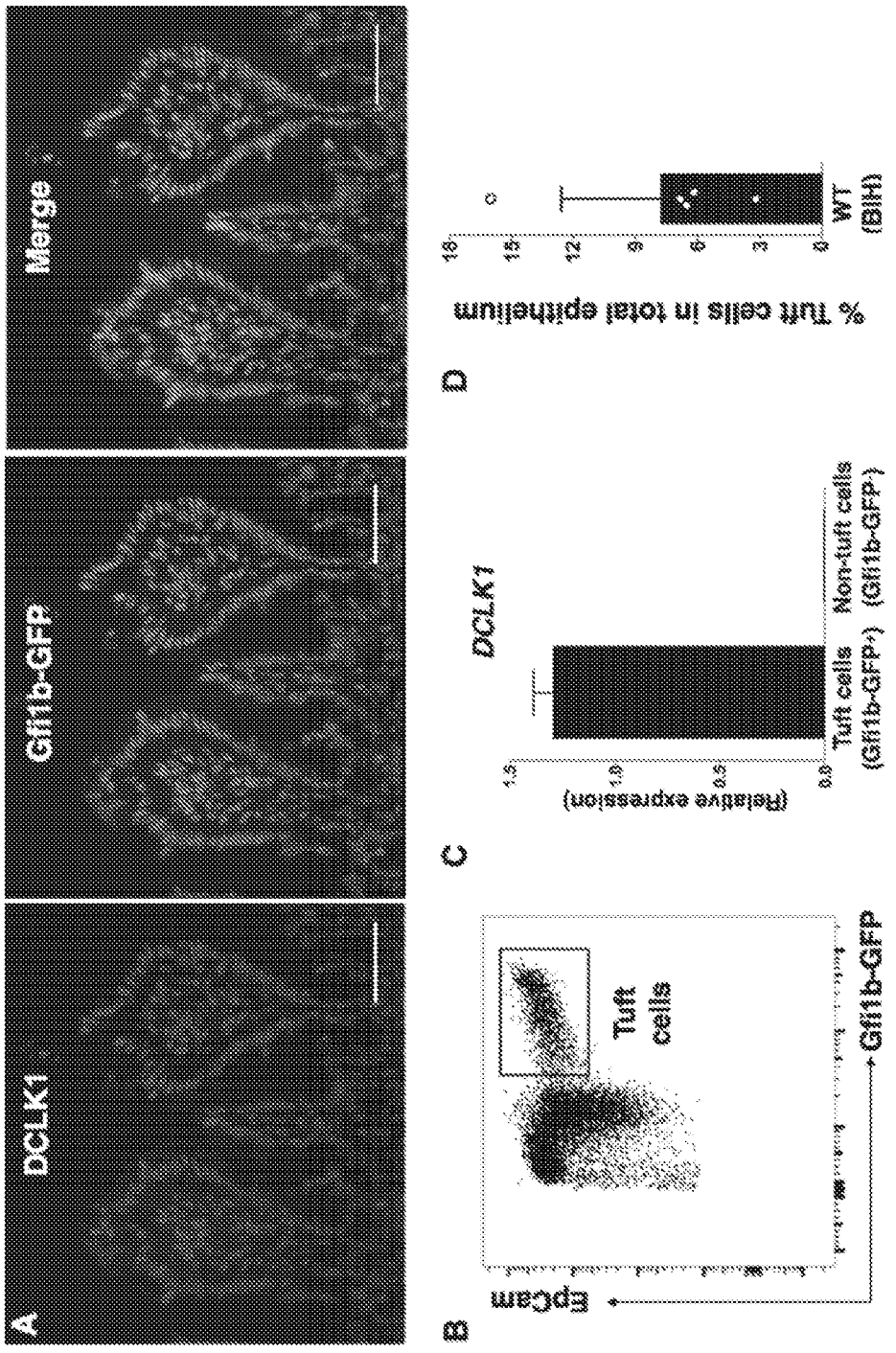

A
DCLK1
Gfi1b-GFP
Merge
B
EpCam
Gfi1b-GFP
Tuft cells
C
DCLK1
(Relative expression)
1.5
1.0
0.5
0.0
Tuft cells (Gfi1b-GFP+)
Non-tuft cells (Gfi1b-GFP-)
D
% Tuft cells in total epithelium
18
15
12
9
6
3
0
WT (BIH)

Figure 2

Figure 3

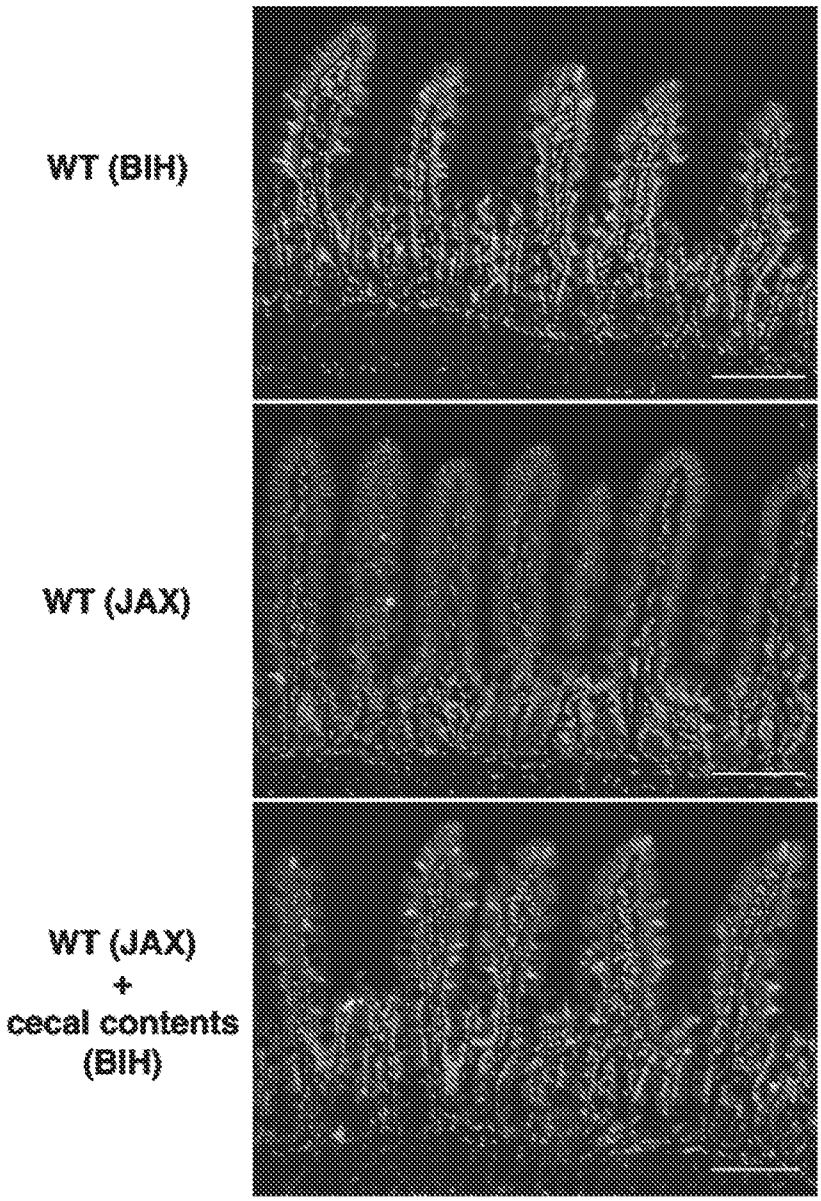
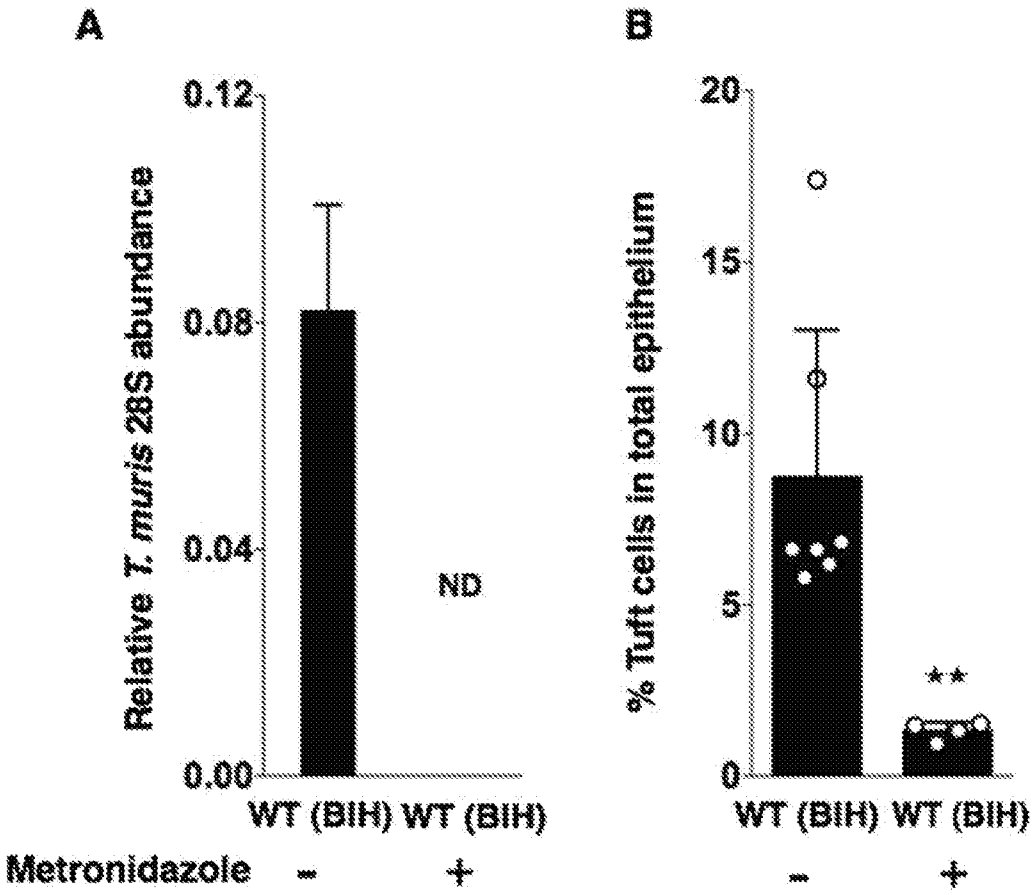


Figure 4


A
Relative T. muris 28S abundance
0.12
0.08
0.04
0.00
ND
WT (BIH) WT (BIH)
Metronidazole - +
B
% Tuft cells in total epithelium
20
15
10
5
0
**
WT (BIH) WT (BIH)
- +

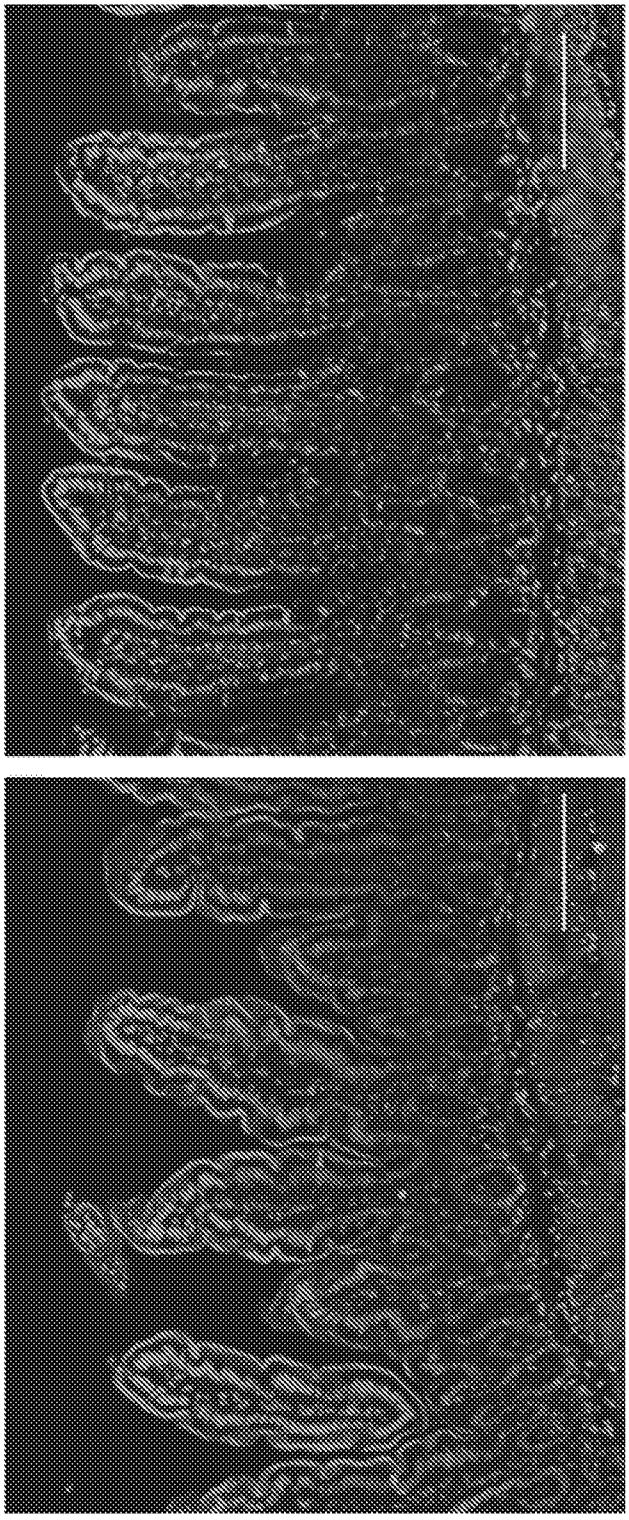


Figure 5

Figure 6

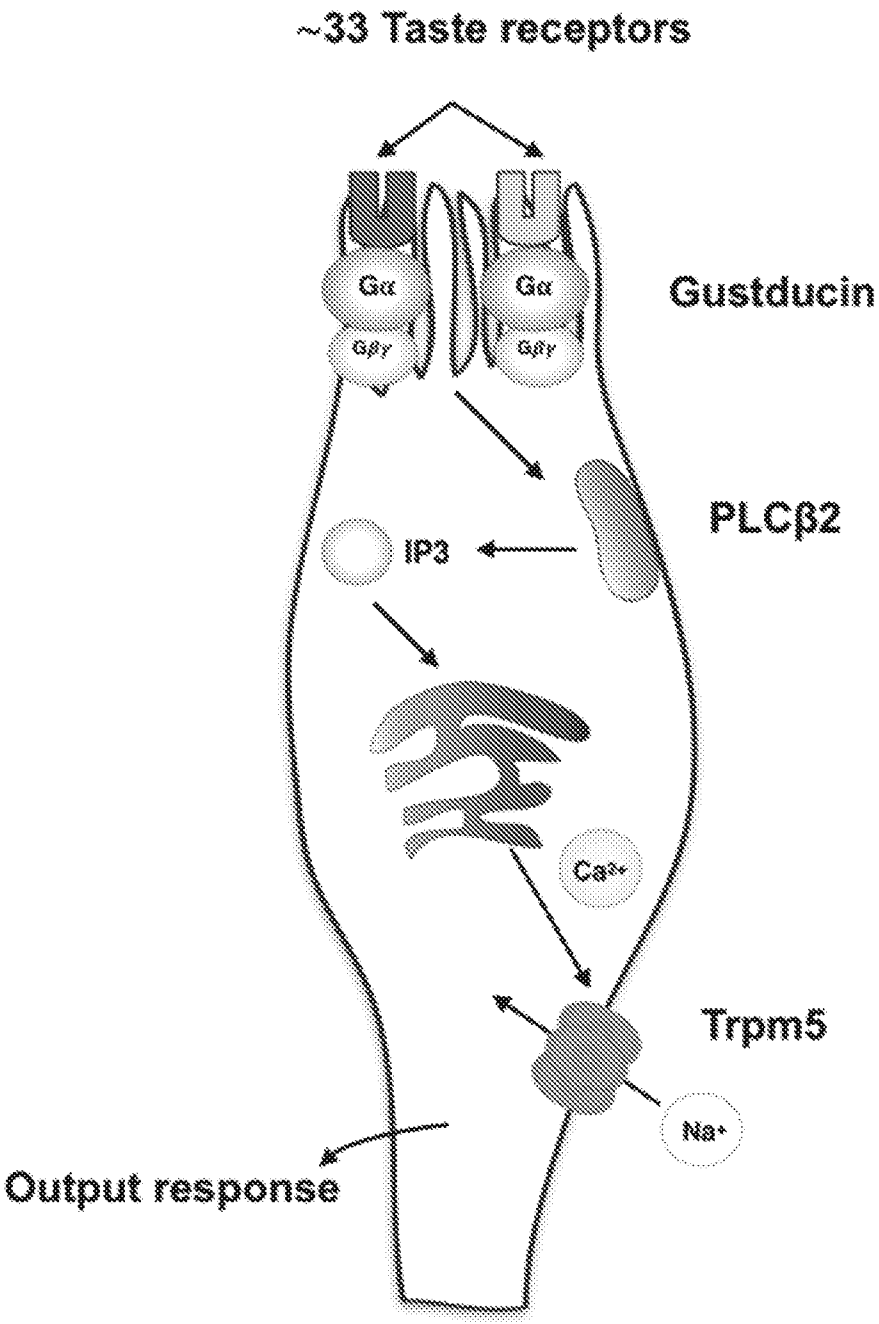
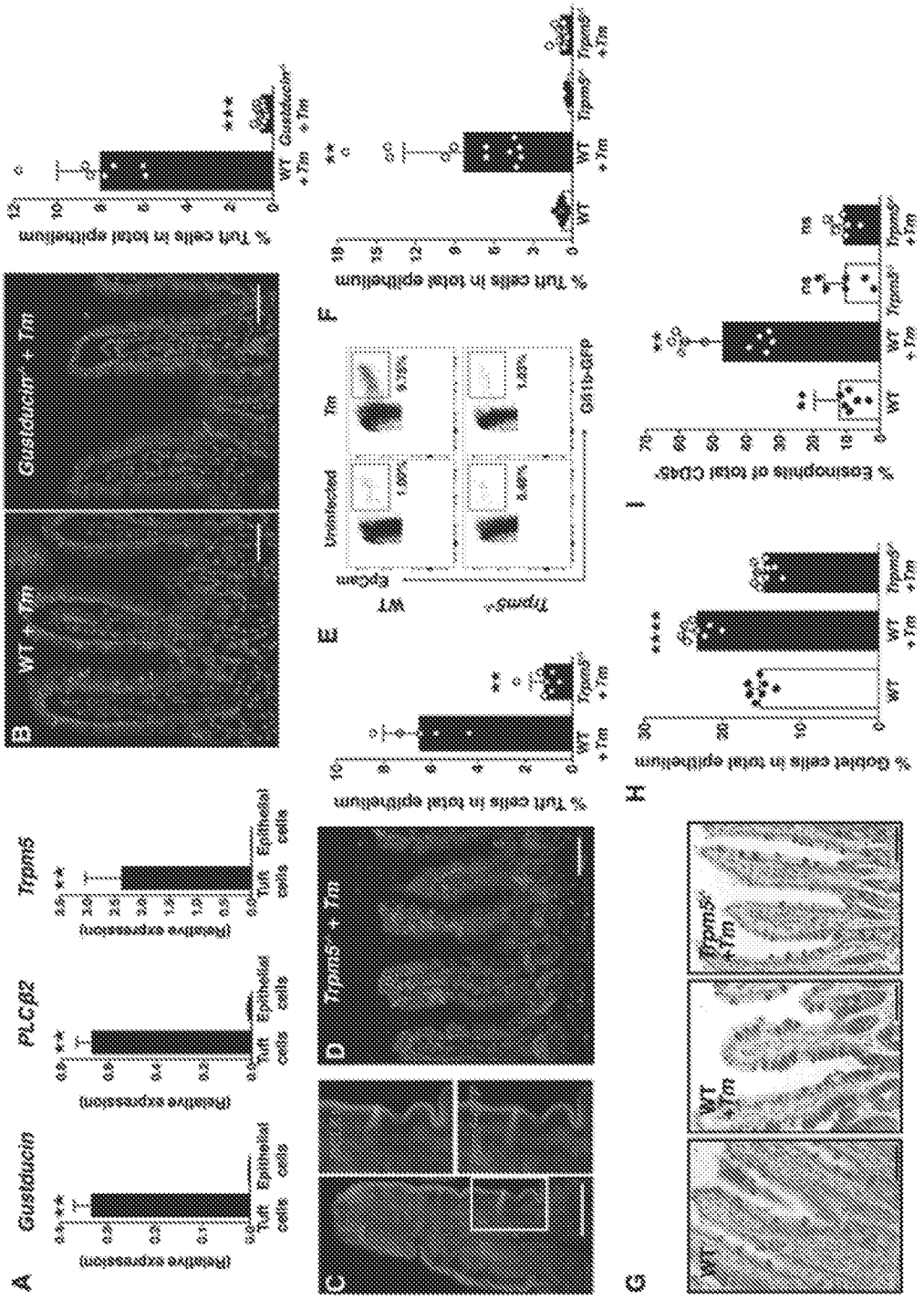
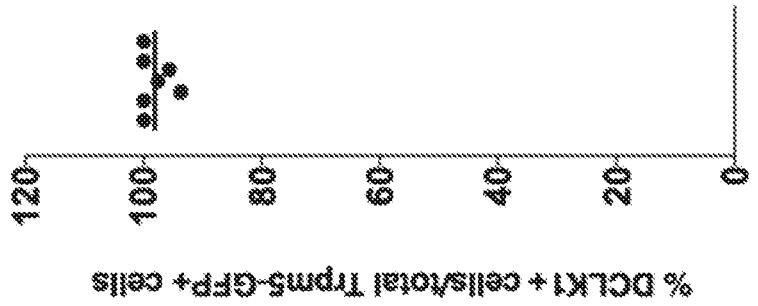

~33 Taste receptors
Gα
Gβγ
Gα
Gβγ
Gustducin
PLCβ2
IP3
Ca2+
Trpm5
Na+
Output response

Figure 7


A
Gustducin
PLCβ2
Trpm5
Tuft cells
Epithelial cells
Relative expression
B
WT + Tm
Gustducin⁻ + Tm
% Tuft cells in total epithelium
WT
Gustducin⁻
+Tm
C
D
Trpm5⁻ + Tm
E
Untreated
Tm
WT
Trpm5⁻
EYFP
G8I1a-GFP
1.66%
4.78%
1.85%
3.46%
F
Trpm5⁻
G
H
% Goblet cells in total epithelium
I
% Eosinophils of total CD45⁺
ns


120
100
80
60
40
20
0
% DCLK1+ cells/total Trpm5-GFP+ cells

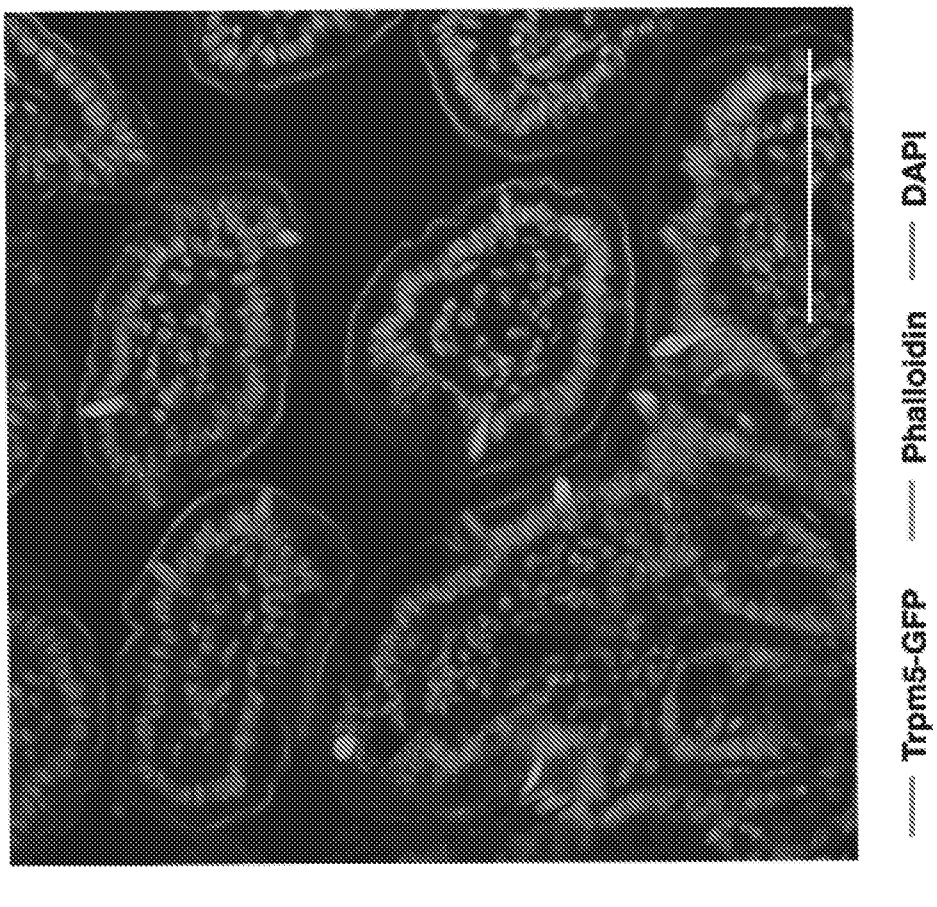

B
A
Trpm5-GFP
Phalloidin
DAPI

Figure 8

Figure 9

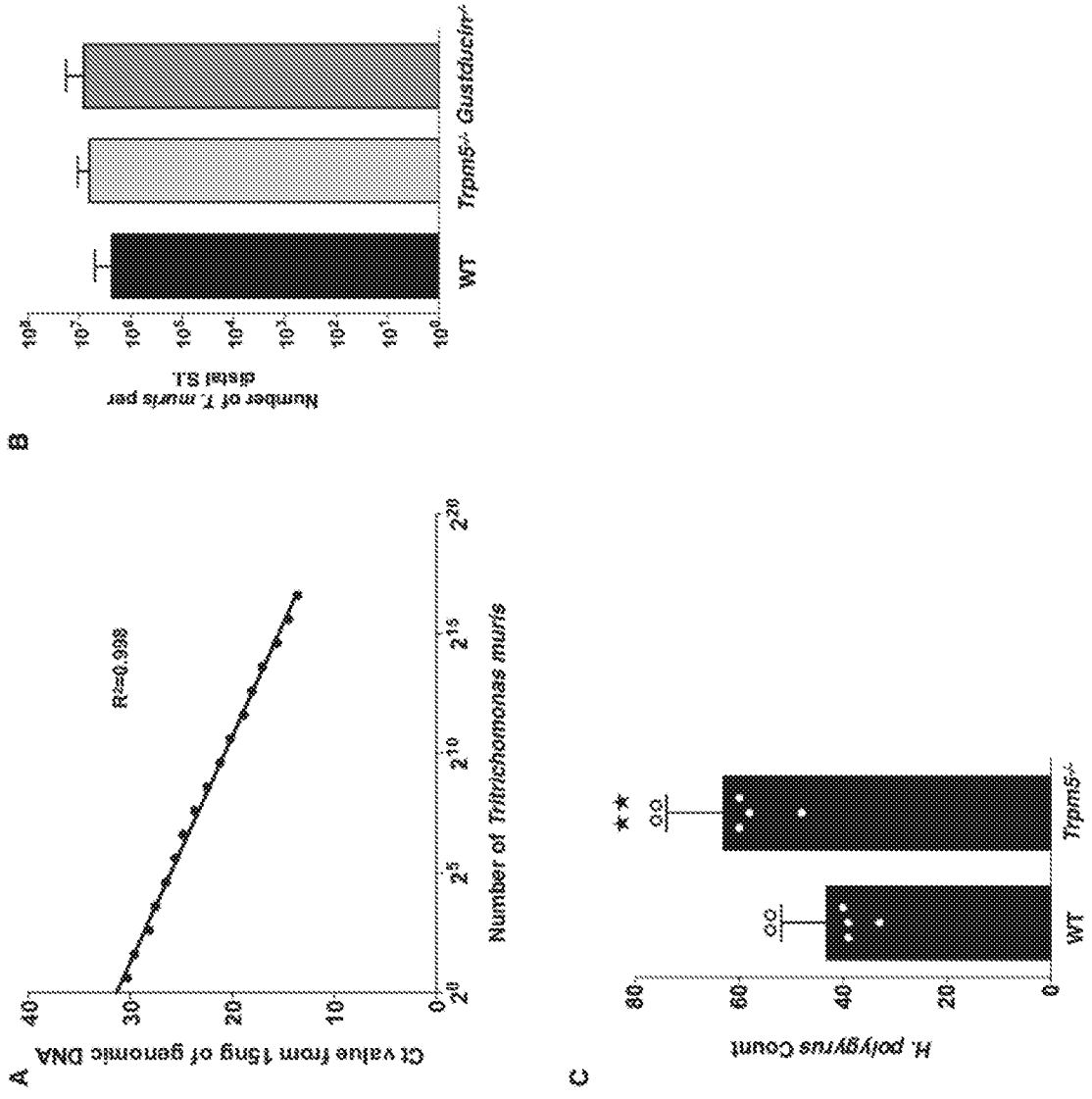
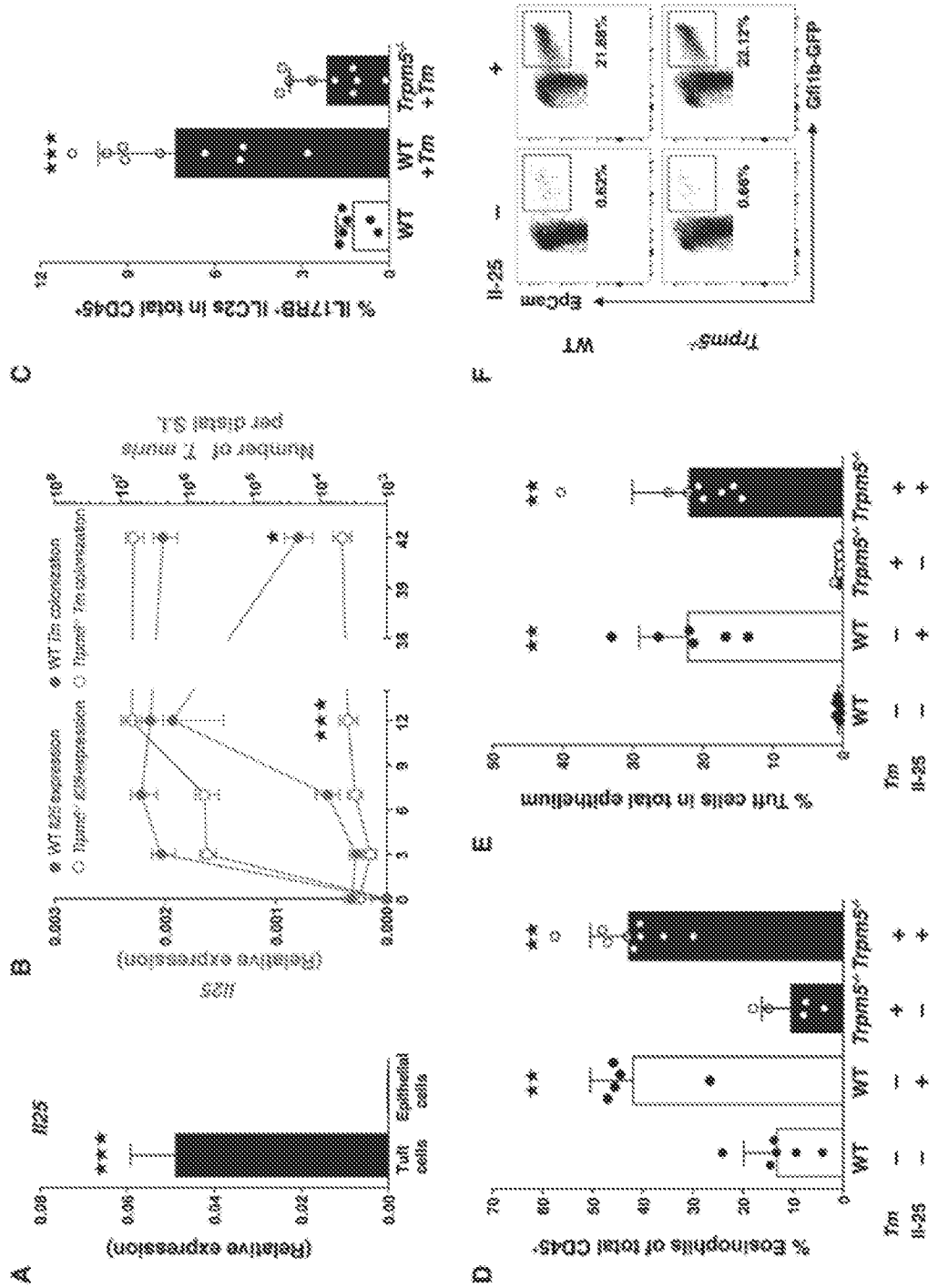

A
Ct value from 15ng of genomic DNA
R²=0.998
Number of Trichomonas muris
B
Number of T. muris per distal SI.
WT
Trpm5-/- Gustducin-/-
C
H. polygyrus Count
**
WT
Trpm5-/-

Figure 10


A
Il25
Tuft cells
Epithelial cells
(Relative expression)
B
Il25
(Relative expression)
Il25 expression
Trpm5-/- Il25 expression
WT Tm colonization
Trpm5-/- Tm colonization
Number of T. muris per distal SI
C
% Il17RB+ ILC2s in total CD45+
WT
WT +Tm
Trpm5-/- +Tm
D
% Eosinophils of total CD45+
Tm
Il-25
WT
Trpm5-/-
E
% Tuft cells in total epithelium
F
WT
Trpm5-/-
Il-25
epCam
Gfi1b-GFP
0.82%
0.66%
21.86%
23.12%

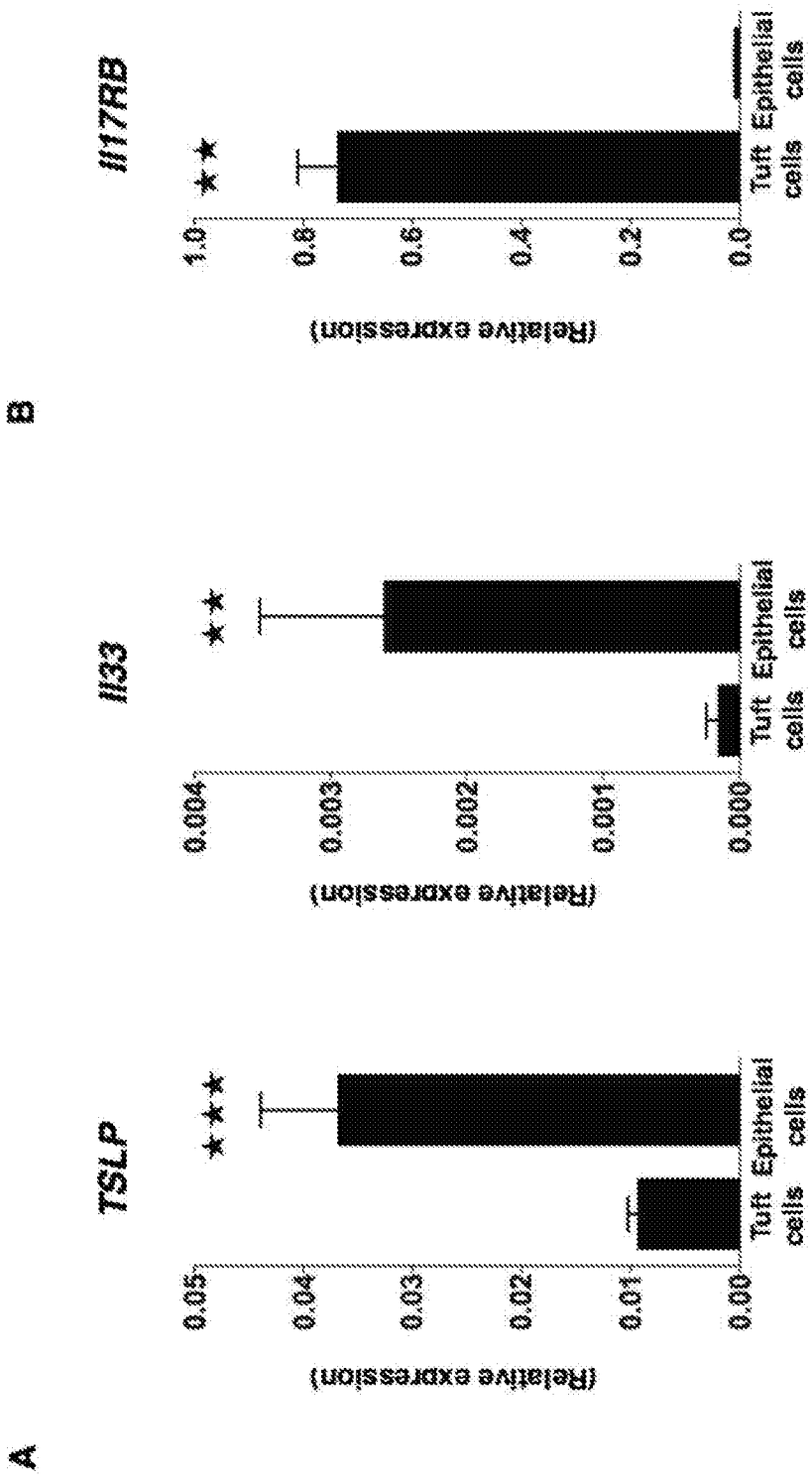

A
TSLP
★★★
0.05
0.04
0.03
0.02
0.01
0.00
(Relative expression)
Tuft cells
Epithelial cells
B
Il33
★★
0.004
0.003
0.002
0.001
0.000
(Relative expression)
Tuft cells
Epithelial cells
Il17RB
★★
1.0
0.8
0.6
0.4
0.2
0.0
(Relative expression)
Tuft cells
Epithelial cells

Figure 11

Figure 12

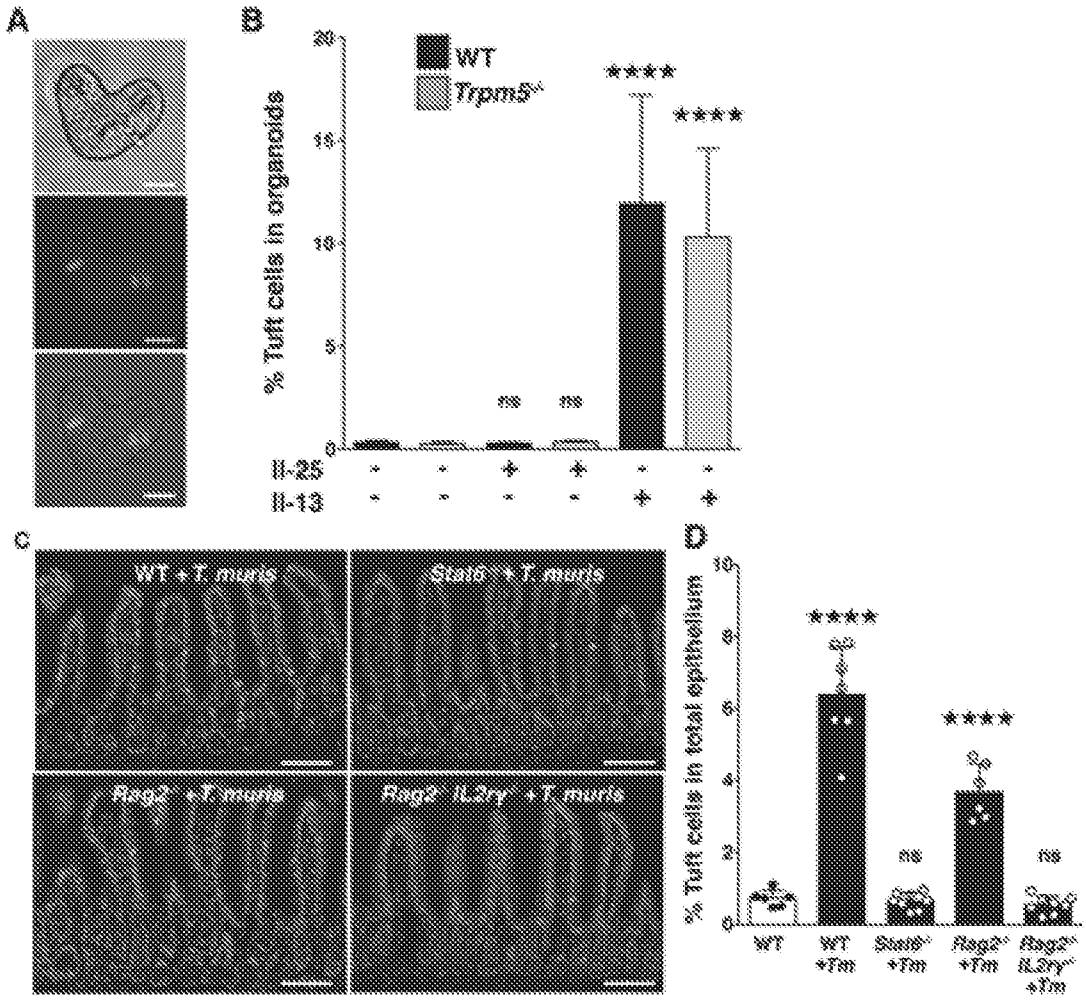

A
B
WT
Trpm5-/-
% Tuft cells in organoids
****
****
ns
ns
Il-25
Il-13
C
WT + T. muris
Stat6-/- + T. muris
Rag2-/- + T. muris
Rag2-/- Il2rg-/- + T. muris
D
% Tuft cells in total epithelium
****
****
ns
ns
WT
WT +Tm
Stat6-/- +Tm
Rag2-/- +Tm
Rag2-/- Il2rg-/- +Tm

Figure 13

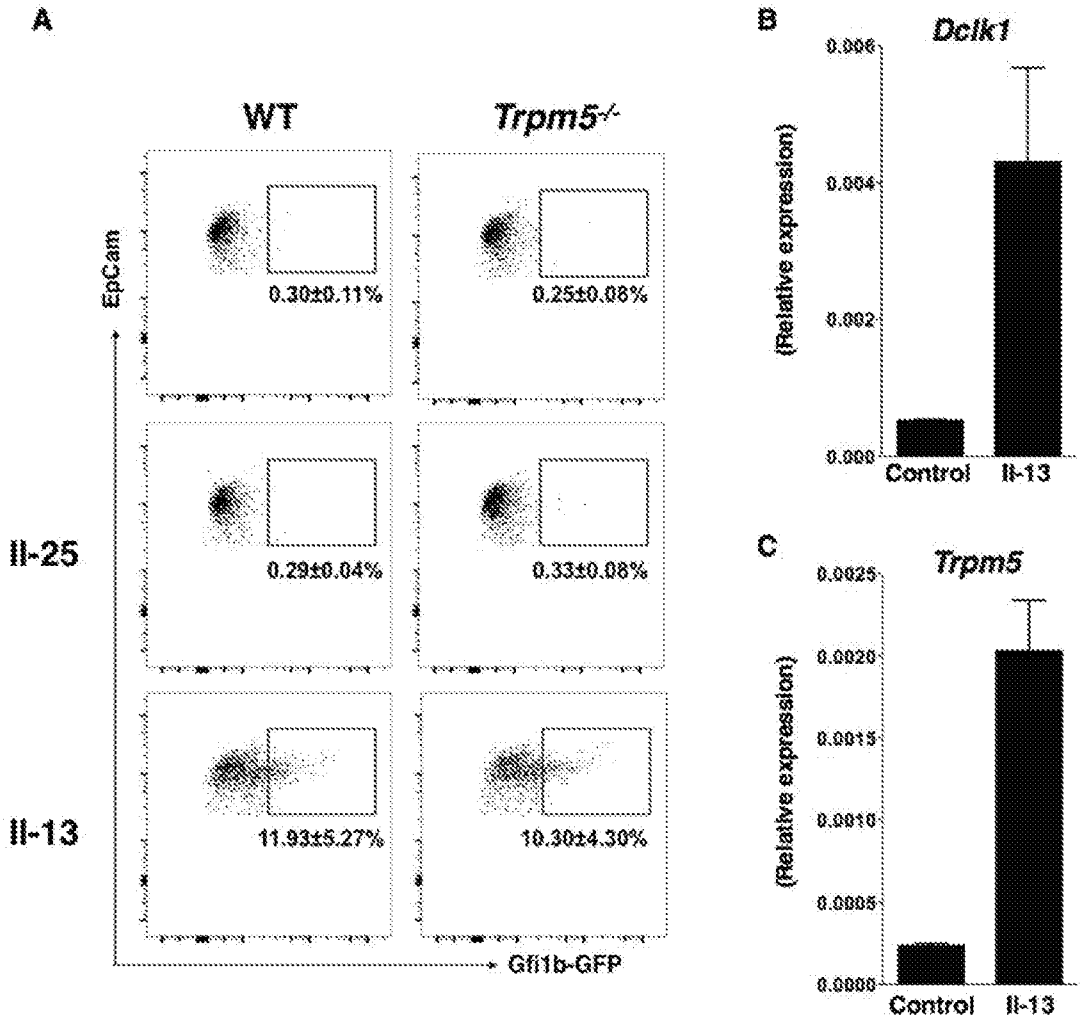
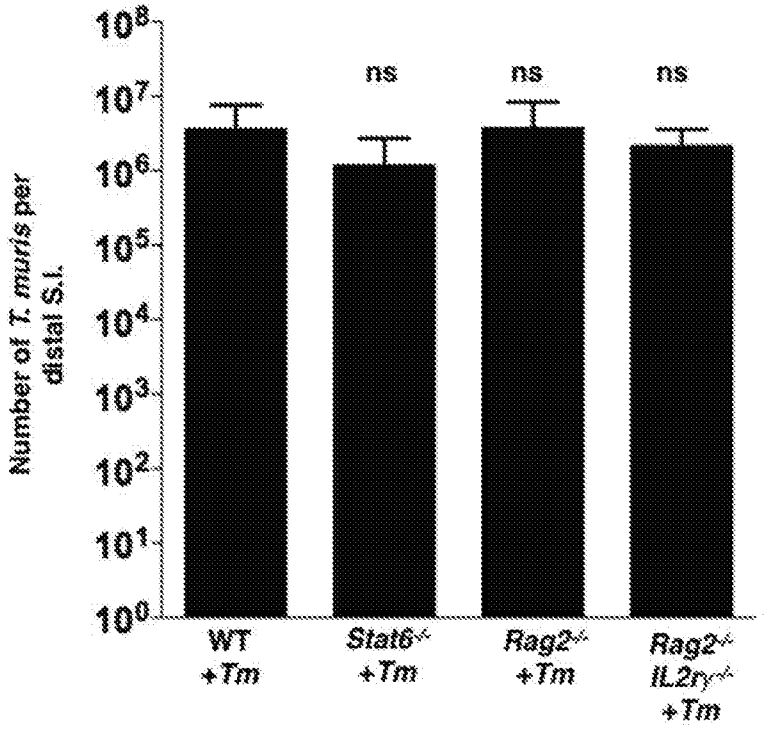

A
WT
Trpm5-/-
EpCam
0.30±0.11%
0.25±0.08%
Il-25
0.29±0.04%
0.33±0.08%
Il-13
11.93±5.27%
10.30±4.30%
Gfi1b-GFP
B
Dclk1
(Relative expression)
0.006
0.004
0.002
0.000
Control
Il-13
C
Trpm5
(Relative expression)
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
Control
Il-13

Figure 14


Number of T. muris per distal S.I.
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
ns
ns
ns
WT +Tm
Stat6-/- +Tm
Rag2-/- +Tm
Rag2-/- IL2rγ-/- +Tm

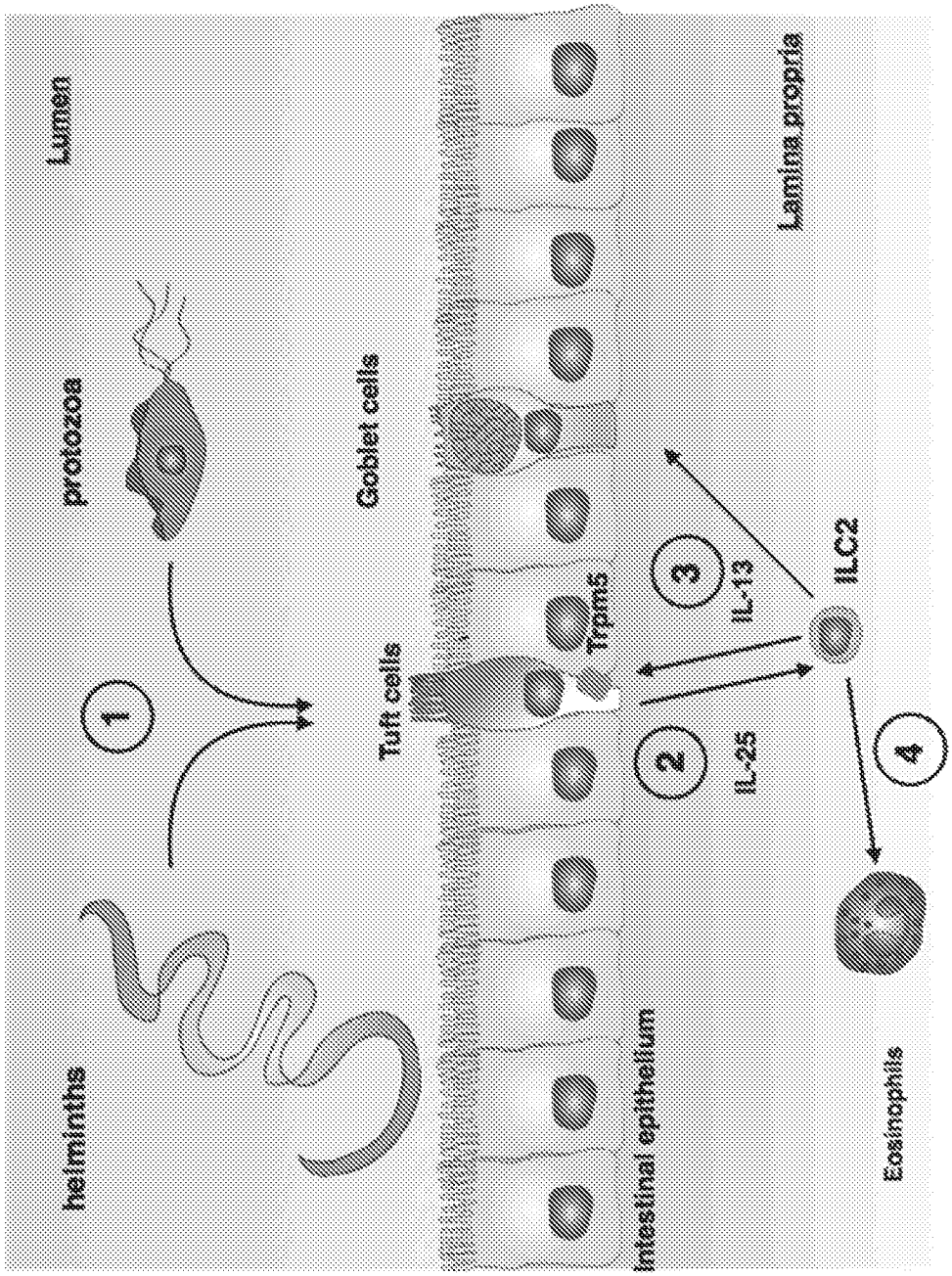

Lumen
protozoa
helminths
1
Goblet cells
Tuft cells
Trpm5
3
IL-13
ILC2
2
IL-25
4
Intestinal epithelium
Lamina propria
Eosinophils

Figure 15

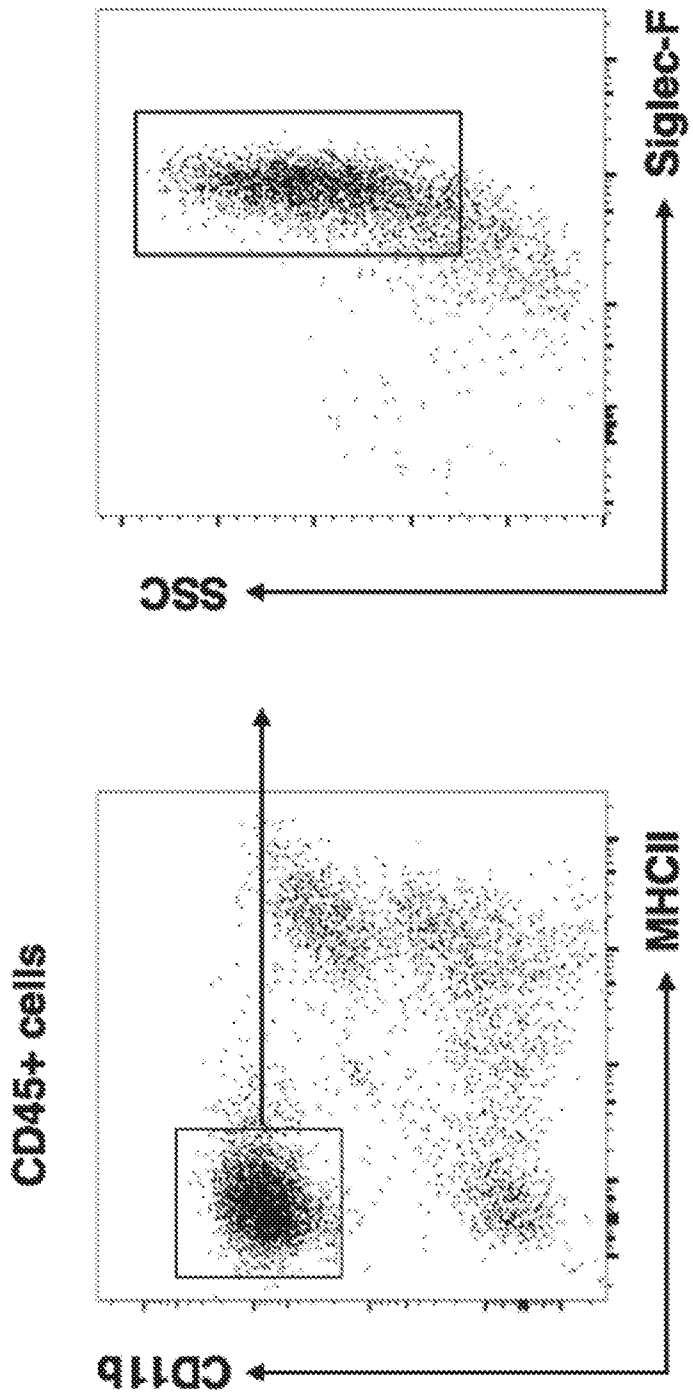

Eosinophil gating strategy
CD45+ cells
CD11b
MHCII
SSC
Siglec-F

Figure 16

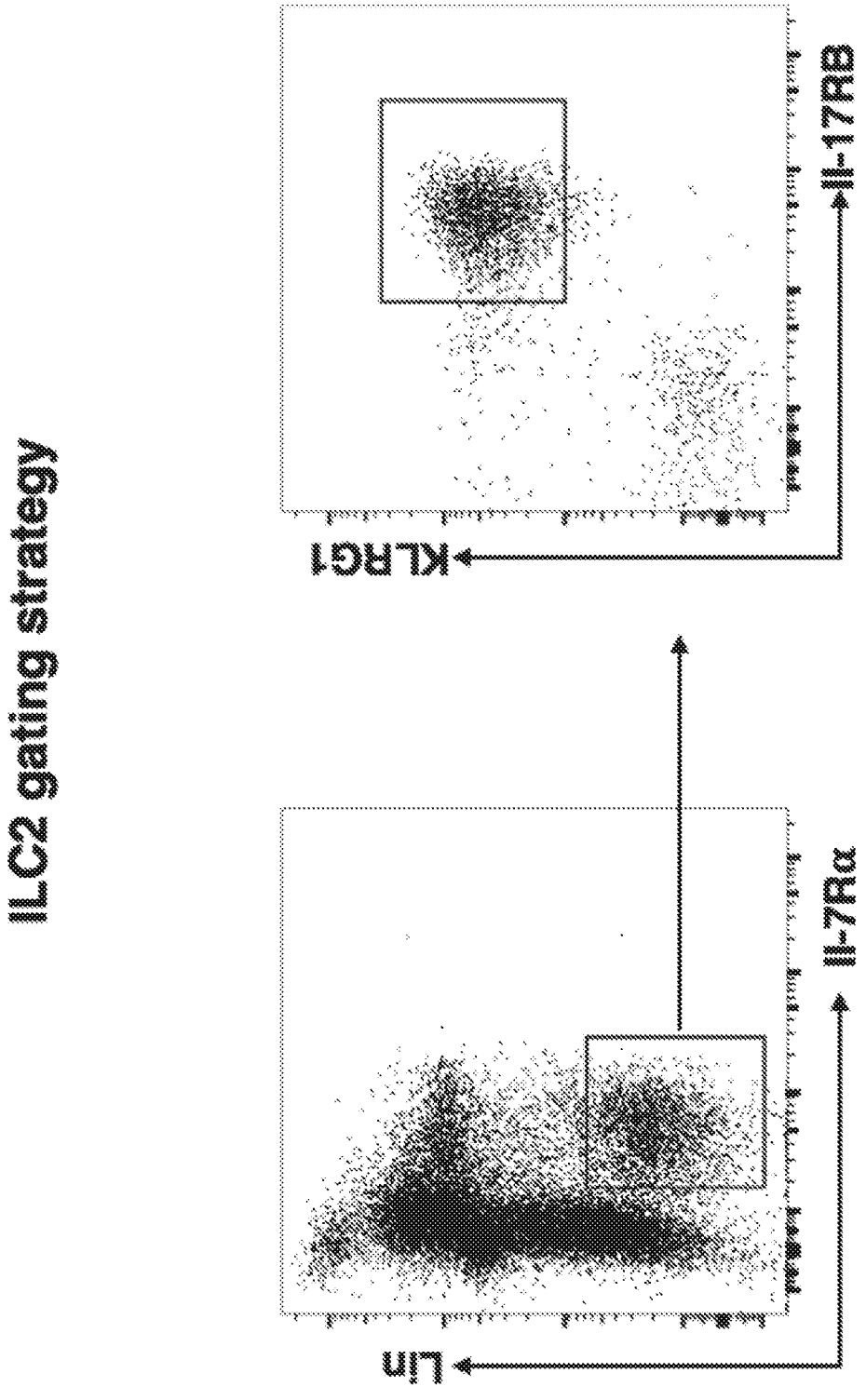


Figure 17

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/016447

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/02 A61K39/39
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	M. R. HOWITT ET AL: "Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut", SCIENCE, vol. 351, no. 6279, 4 February 2016 (2016-02-04), pages 1329-1333, XP055371373, ISSN: 0036-8075, DOI: 10.1126/science.aaf1648 the whole document ----- -/--	1-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2017	19/07/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheffzyk, Irmgard

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/016447

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JAKOB VON MOLTKE ET AL: "Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit", NATURE, vol. 529, no. 7585, 14 December 2015 (2015-12-14), pages 221-225, XP055371288, United Kingdom ISSN: 0028-0836, DOI: 10.1038/nature16161 abstract ----- FRANÇOIS GERBE ET AL: "Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites", NATURE, vol. 529, no. 7585, 13 January 2016 (2016-01-13), pages 226-230, XP055371369, United Kingdom ISSN: 0028-0836, DOI: 10.1038/nature16527 abstract ----- CATHERINE M. D. MILLER ET AL: "Immunological Interactions between 2 Common Pathogens, Th1-Inducing Protozoan Toxoplasma gondii and the Th2-Inducing Helminth Fasciola hepatica", PLOS ONE, vol. 4, no. 5, 25 May 2009 (2009-05-25), page e5692, XP055113901, DOI: 10.1371/journal.pone.0005692 abstract -----	1-24 1-24 1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/016447

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-24

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-24

   Method of inducing a type 2 helper T cell immune response in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell

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2. claims: 25-42

   Method of treating or preventing an inflammatory bowel disease in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell

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3. claims: 43-63

   Method of treating or preventing a parasitic infection in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell

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4. claims: 64-87

   Method of inducing IL-25 expression by a tuft cell comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cell

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5. claims: 88-105

   Method of inducing the regeneration or repair of epithelium damaged by an inflammatory bowel disease in a subject comprising administering to the subject an agent that enhances the taste-chemosensory signaling pathway in a tuft cells

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