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A family of novel feline bitter taste receptors, referred to as feline TAS2R (fTAS2R), are disclosed herein. Isolated polynucleotides encoding the novel feline bitter taste receptors and chimeric polypeptides are also disclosed, as are expression vectors and host cells for expression of the novel feline bitter taste receptors. Methods of identifying compounds that bind to the novel feline bitter taste receptors and modulate their activity are disclosed.

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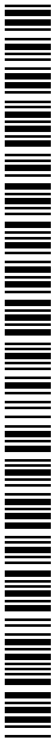

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(54) Title: FELINE BITTER TASTE RECEPTORS AND METHODS

(57) Abstract: A family of novel feline bitter taste receptors, referred to as feline TAS2R (fTAS2R), are disclosed herein. Isolated polynucleotides encoding the novel feline bitter taste receptors and chimeric polypeptides are also disclosed, as are expression vectors and host cells for expression of the novel feline bitter taste receptors. Methods of identifying compounds that bind to the novel feline bitter taste receptors and modulate their activity are disclosed.

FELINE BITTER TASTE RECEPTORS AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of US Provisional Application No. 61/788,528, filed March 15, 2013, and US Provisional Application No. 61/945,500, filed February 27, 2014.

BACKGROUND

[0001] The taste system provides sensory information about the chemical composition of the external world. Mammals are believed to have at least five basic taste modalities: sweet, bitter, sour, salty, and umami. Each taste modality is thought to be mediated by a distinct protein receptor or receptors that are expressed in taste receptor cells found on the surface of the tongue. The taste receptors that recognize bitter, sweet, and umami taste stimuli belong to the G-protein-coupled receptor (GPCR) superfamily. Subtle differences in a receptor may alter which ligands bind and what signal is generated once the receptor is stimulated.

[0002] Various members of the GPCR superfamily mediate many other physiological functions, such as endocrine function, exocrine function, heart rate, lipolysis, and carbohydrate metabolism. The biochemical analysis and molecular cloning of a number of such receptors has revealed many basic principles regarding the domain structure and function of these receptors.

[0003] The ability of mammals to taste the five primary modalities is thought to be largely similar, however due to diet and environmental differences, taste receptors have evolved to be somewhat different across mammalian species. For example, the gene encoding the Taste Receptor, Type 1 protein, member 2, TAS1R2, a component of the receptor for sweet compounds, has mutated to a nonfunctional pseudogene in felines and several other obligate carnivores, while aquatic mammals such as dolphin have lost most functional taste receptors.

[0004] The bitter taste modality is usually described as disagreeable. Many natural and synthetic toxins have been characterized as bitter tastants. As a result, it is hypothesized that bitter taste perception has evolved as a means to discourage the consumption of toxic compounds often found in plants. Estimates for the number of bitter tasting compounds are in the tens of thousands. Compounds that block bitter taste perception have also been identified, for example p-(dipropylsulfamoyl)benzoic acid (probenecid) which acts on a

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subset of Taste Receptor, Type 2 ("TAS2R") proteins, a family of monomeric G protein-coupled receptors, embedded in the surface of taste cells.

[0005] Research has shown that molecular diversity in the TAS2Rs of humans and other primates leads to functional differences in individuals' bitter taste perception (Imai *et al.*, 2012, Biol Lett. 8(4): 652–656; Li *et al.*, 2011, Human Biology 83: 363-377). The exposure to the specific flora of a geographic region is thought to be a major driving force of selection on TAS2Rs.

[0006] Humans encode about 26 functional TAS2Rs, allowing for the detection of an enormous number of compounds. About 550 compounds have been identified thus far as bitter tastants for humans. A subset of human TAS2Rs (hTAS2Rs) are currently believed to be promiscuous, i.e., activated by multiple ligands belonging to several chemical classes, while other hTAS2Rs bind ligands of only particular chemical classes. Additionally, several hTAS2Rs are orphan receptors, with no compounds identified as yet that stimulate them.

[0007] Signal transduction of bitter stimuli is accomplished via the α-subunit of gustducin. This G protein subunit activates a taste phosphodiesterase and decreases cyclic nucleotide levels. Further steps in the transduction pathway are still unknown. The βγ-subunit of gustducin also mediates taste by activating IP3 (inositol triphosphate) and DAG (diglyceride). These second messengers may open gated ion channels or may cause release of internal calcium. Though all TAS2Rs are located in gustducin-containing cells, knockout of gustducin does not completely abolish sensitivity to bitter compounds, suggesting a redundant mechanism for bitter tasting.

[0008] hTAS2R38 is the most extensively studied bitter taste receptor. Early in the twentieth century a dichotomy in the perception of phenylthiocarbamide (PTC), a bitter tasting compound, was observed in a sample of people. Most people could taste PTC, but about 25% could not. Researchers noticed the taster/non-taster phenotype had a degree of heritability. Later it was determined that the difference in phenotype between the two groups could be ascribed to a difference in genotype, more specifically single nucleotide polymorphisms (SNPs) at three positions within the hTAS2R38 DNA.

[0009] Other species display a TAS2R repertoire much different from that of humans. For example, the mouse has 34 full-length TAS2Rs encoded in its genome, while the chicken has only 3 (Go *et al.,* Genetics. 2005 May; 170(1):313-26). Although some compounds can be detected by multiple TAS2Rs, it is almost certain that differences in TAS2R repertoire across species result in differences in bitter taste perception.

[0010] Bitter taste perception is mediated by G protein-coupled receptors (GPCRs) of

the taste receptor 2 family (TAS2R). The TAS2R genes encode a family of related seven transmembrane G-protein coupled receptors involved in taste transduction, which interact with a G-protein to mediate taste signal transduction. In particular, TAS2Rs interact in a ligand-specific manner with the G protein Gustducin.

[0011] To date, much work has been done to characterize human TAS2Rs (hTAS2Rs). The human genome encodes about 26 functional TAS2Rs that are glycoproteins. All hTAS2Rs share a conserved site for Asn-linked glycosylation within the center of the second extracellular loop. The hTAS2Rs also have the ability to form homo- and hetero-oligomers with other GPCR when expressed *in vitro*, however at present no evidence exists that TAS2R receptor oligomerization has functional implications.

[0012] Bitter taste receptor cells represent a distinct subpopulation of chemosensory cells characterized by the expression of TAS2R genes and completely segregated from those receptor cells devoted to the detection of other taste stimuli. Each bitter taste receptor cell expresses multiple bitter taste receptors, although the extent of co-expression is still a matter of debate.

[0013] In addition to their expression in the gustatory system, TAS2Rs are found in non-gustatory tissues. Among these extra-oral sites are the respiratory epithelia, gastrointestinal tissues, reproductive organs, and brain. Bitter taste receptors are implicated in differentiation or maturation of sperm in mice. The non-gustatory expression of TAS2Rs is known to be used to regulate digestion and respiration.

[0014] Activation of TAS2R receptors in an enteroendocrine cell line (STC-1 cells) results in release of the peptide hormone cholecystokinin (CCK), which can reduce gut motility. Consequently, intake of a potential toxin that activates the TAS2R pathway may decrease the rate at which food passes through the stomach and lower the drive for continued eating. The release of CCK also excites sensory nerve processes of the vagus nerve to carry the signal to the brain, suggesting that regulation of food intake involves both peripheral and central controls. Activation of the TAS2R signaling network may also or alternatively indirectly increase elimination of absorbed toxins from gut epithelium before the toxins can enter circulation since some data suggest that the CCK-secreting enteroendocrine cells are involved in a paracrine signaling system that reduces transfer of toxic substances from the gut into the circulation. Lower in the gut, activation of TAS2R receptors has a different effect. When some bitter-tasting ligands are applied to the colonic epithelium, they induce the secretion of anions, which leads to fluid secretion by the epithelium which may flush out any noxious irritant from the colon.

[0015] Solitary chemosensory cells (SCCs) are also present throughout the upper respiratory system and express the entire suite of taste-related signaling molecules, including TAS2R receptors, PLCβ2, gustducin, and the transduction channel TrpM5. The SCCs synapse onto polymodal pain fibers of the trigeminal nerve. Inhalation of a toxin that activates TAS2R receptors of the SCCs will be irritating and evoke trigeminally-mediated reflex changes in respiration. Additionally, the activated trigeminal nerve fibers release peptide modulators that result in local neurogenic inflammation of the respiratory epithelium, activating the immune system in response to the presence of the toxins.

[0016] The human bitter taste receptors, hTAS2R2, hTAS2R41, hTAS2R42, hTAS2R45, hTAS2R48, and hTAS2R60 are still considered orphan GPCRs since ligands have not yet been identified for these receptors.

[0017] Until recently, hTAS2R2 was annotated as a pseudogene due to a two base deletion at codon 160 found in sequences collected from 10 human populations (Karitiana, Surui, Waorani Indians from South America, Russians from Eastern Europe, Druze from the Middle East, Atayal, Chinese, Japanese from Eastern Asia, and Khmers and Melanesians from Southeast Asia) and from GenBank resources. hTAS2R2 has been found to be polymorphic with respect to that deletion, with the intact gene found in the Adygei (Eastern European), Mbuti (African Pygmies), and Biaka (African Pygmies) (Go Y *et al.*, Genetics May 1, 2005, 170 (1): 313-326).

[0018] The feline genome has been sequenced with minimal coverage (Mullikin *et al.* BMC Genomics 2010 11: 406; Pontius *et al.*, Genome Research 2007 17: 1675-1689). As a result, major gaps exist in the feline genome sequence and only slightly over 2000 feline genes have been annotated to date. As a comparison, the human genome has about 25,000 genes annotated. The sequences prior to a gap in the genomic assembly are of poor quality, so in addition to information that is missing, a large portion of the data present is of poor quality. Consequently, there is much to be discovered within feline genomics and in determining the molecular basis of feline taste perception. No feline TAS2R (fTAS2R) has been annotated in the feline genome or investigated biochemically to date. Additionally, with many feline breeds originating in a particular geographic region and therefore being exposed to unique flora, breed specific TAS2R differences may exist.

[0019] The identification and characterization of the feline TAS2R bitter receptors is useful to gain understanding of the taste profile of felines and its modulation.

SUMMARY

[0020] Disclosed herein are novel feline TAS2R receptors.

[0021] In an embodiment, an isolated feline TAS2R (fTAS2R) receptor polypeptide comprises an extracellular domain of a feline TAS2R receptor; a transmembrane region of a feline TAS2R receptor, or an intracellular domain of a feline TAS2R receptor, wherein the fTAS2R receptor comprises a sequence selected from SEQ ID NO:18, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26, wherein the isolated fTAS2R receptor polypeptide does not consist of the amino acid sequence of SEQ ID NOs: 2, 4, 6, or 10.

[0022] In an embodiment, the isolated fTAS2R receptor polypeptide comprises an extracellular domain of a feline TAS2R receptor polypeptide comprising amino acids 1, 68-84; 146-179; or 249-257 of SEQ ID NO:2; amino acids 1-10, 73-88; 151-186; or 256-264 of SEQ ID NO:4; amino acids 1-8; 72-88; 150-186; or 256-265 of SEQ ID NO:6; amino acids 1-2; 69-87; 151-183; or 253-261 of SEQ ID NO:8; amino acids 1-8; 72-88; 150-187; or 257-265 of SEQ ID NO:10; amino acids 1-6; 72-88; 150-183; or 253-262 of SEQ ID NO:12; amino acids 1; 69-87; 150-181; or 251-260 of SEQ ID NO:14; amino acids 1-8; 69-88; 150-185; or 252-261 of SEQ ID NO:16; amino acids 1-17: 83-98; 161-198; or 268-277 of SEQ ID NO:18; amino acids 1; 69-88; 150-185; or 255-264 of SEQ ID NO:20; amino acids 1-2; 69-87; 149-181; or 251-260 of SEQ ID NO:22; amino acids 1-2; 69-87; 149-181; or 251-259 of SEQ ID NO:24; or amino acids 1-8; 72-88; 150-185; or 254-263of SEQ ID NO:26; a transmembrane region of the feline TAS2R receptor polypeptide comprising amino acids 2-22, 47-67, 85-105, 125-145, 180-200, 228-248, or 258-278 of SEQ ID NO:2; amino acids 11-31, 52-72, 89-109, 130-150, 187-207, 235-255, or 265-285 of SEQ ID NO:4; amino acids 9-29, 51-71, 89-109, 129-149, 187-207, 235-255, or 266-286 of SEQ ID NO:6; amino acids 3-23, 48-68, 88-108, 130-150, 184-204, 232-252, or 262-282 of SEQ ID NO:8; amino acids 9-29, 51-71, 89-109, 129-149, 188-208, 236-256, or 266-286 of SEQ ID NO:10; amino acids 7-27, 51-71, 89-109, 129-149, 184-204, 232-252, or 263-283 of SEQ ID NO:12; amino acids 2-22, 2. 48-68, 88-108, 129-149, 182-202, 230-250, or 261-281 of SEQ ID NO:14; amino acids 9-29, 48-68, 89-109, 129-149, 186-206, 231-251, or 262-282 of SEQ ID NO:16; amino acids 18-38, 62-82, 99-119, 140-160, 199-219, 247-267, or 278-298 of SEQ ID NO:18; amino acids 2-22, 48-68, 89-109, 129-149, 186-206, 234-254, or 265-285 of SEQ ID NO:20; amino acids 3-23, 48-68, 88-108, 128-148, 182-202, 230-250, or 261-281 of SEQ ID NO:22;

amino acids 3-23, 48-68, 88-108, 128-148, 182-202, 230-250, or 260-280 of SEQ ID NO:24; or amino acids 9-29, 51-71, 89-109, 129-149, 186-206, 233-253, or 264-284 of SEQ ID NO:26, or an intracellular domain comprising: amino acids 23-46; 106-124; 201-227; or 279-298 of SEQ ID NO:2; amino acids 32-51; 110-129; 208-234; or 286-304 of SEQ ID NO:4; amino acids 30-50; 110-128; 208-234; or 287-316 of SEQ ID NO:6; amino acids 24-47; 109-129; 205-231; or 283-306 of SEQ ID NO:8; amino acids 30-50; 110-128; 209-235; or 287-311 of SEQ ID NO:10; amino acids 28-50; 110-128; 205-231; or 284-337 of SEQ ID NO:12; amino acids 23-48; 109-128; 203-229; or 282-300 of SEQ ID NO:14; amino acids 30-47; 110-128; 207-230; or 283-309 of SEQ ID NO:16; amino acids 39-61; 120-139; 220-246; or 299-334 of SEQ ID NO:18; amino acids 23-47; 110-128; 207-233; or 286-322 of SEQ ID NO:20; amino acids 24-47; 109-127; 203-229; or 282-299 of SEQ ID NO:22; amino acids 24-47; 109-127; 203-229; or 281-308 of SEQ ID NO:24; or amino acids 30-50; 110-128; 207-232; or 285-312 of SEQ ID NO:26.

[0023] A polynucleotide encoding the novel feline TAS2R receptor, fragment thereof, is also disclosed.

[0024] In an embodiment, the polynucleotide comprises a nucleotide sequence selected from: the nucleotide sequence of SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25; a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; a nucleotide sequence that hybridizes to the complement of the polynucleotide having SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25 under high stringency conditions; and the complement of the foregoing nucleotide sequences.

[0025] Expression vectors and host cells comprising the polynucleotides, as well as oligonucleotides are also disclosed.

[0026] Antibodies and kits for detecting the fTAS2R receptor are also disclosed.

[0027] Also disclosed herein are methods for identifying compounds that interact with or modulate activity of a fTAS2R receptor polypeptide.

[0028] In an embodiment, the method comprises contacting a TAS2R receptor polypeptide herein with a test compound, and detecting interaction between the receptor polypeptide and the test compound.

[0029] In an embodiment, the method comprises contacting a TAS2R receptor

polypeptide disclosed herein with a receptor ligand in the presence or absence of a test compound, and determining whether the test compound modulates binding of the ligand to the receptor or activation of the receptor by the ligand.

[0030] Additional methods are also disclosed.

[0031] In an embodiment, a method of preparing edible compositions comprises contacting an edible composition or a component thereof with a feline TAS2R receptor polypeptide for a time sufficient to reduce the amount of a bitter compound from the edible composition or component thereof.

[0032] In an embodiment, a method of preparing edible compositions for controlling palatability to an animal comprises adding a compound to an edible composition to decrease the palatability of the edible composition to an animal, wherein the compound is an agonist of or a positive modulator of a feline TAS2R receptor polypeptide.

[0033] In an embodiment, a method of formulating an edible composition with enhanced palatability comprises determining the presence of a compound which is an agonist, antagonist, or modulator of a feline TAS2R receptor polypeptide in an edible composition; and enhancing palatability of the edible composition by: if the compound is an agonist or a positive modulator, increasing the amount of an antagonist for the receptor in the edible composition or reducing the amount of the compound in the edible composition, or if the compound is an antagonist or a negative modulator, increasing the amount of the compound in the edible composition.

[0034] In an embodiment, a method of administering a bitter compound to an animal in need thereof comprises administering an edible composition to an animal, wherein the edible composition comprises a bitter compound and a compound that is an antagonist, or modulator of a feline TAS2R receptor polypeptide that alters acceptance of the edible composition by the animal compared to acceptance of the edible composition without the compound. The bitter compound can comprise a pharmaceutical, an oral care material, a nutritional supplement, or a repellant.

[0035] Also disclosed are flavor compositions for coating or incorporating into an edible composition to be administered to an animal and methods of manufacture thereof.

[0036] In an embodiment, the flavor composition comprises an agonist or an antagonist of a feline TAS2R receptor polypeptide, wherein the agonist is denatonium, aloin, or PTC and the antagonist is probenecid; optionally, a palatability enhancer; optionally, a compound to help adhere the flavor composition to the edible composition; and optionally, a compound for providing color or aroma; wherein the flavor composition is a liquid, solid, powder, paste,

gel, spreadable formulation, granule, or sprayable formulation.

[0037] In an embodiment, the method of making the flavor composition comprises mixing an agonist or an antagonist of a feline TAS2R receptor polypeptide, wherein the agonist is denatonium, aloin, or PTC and the antagonist is probenecid; optionally, a palatability enhancer; optionally, a compound to help adhere the flavor composition to the edible composition; and optionally, a compound for providing color or aroma with an ingredient selected from the group consisting of meat products, meat by-products, fish products, fish by-products, dairy products, dairy by-products, sources of microbial proteins, vegetable proteins, carbohydrates and amino acids to obtain a flavor composition, wherein the flavor composition is a liquid, solid, powder, paste, gel, spreadable formulation, granule, or sprayable formulation.

[0037a] Also provided is an isolated feline TAS2R (fTAS2R) receptor polypeptide comprising a sequence selected from SEQ ID NO:18, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26.

[0037b] Also provided is an isolated polynucleotide comprising a nucleotide sequence selected from: the nucleotide sequence of SEQ ID NO: 17, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25; a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 18, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; and the complement of the foregoing nucleotide sequences

[0038] These and other advantages, as well as additional inventive features, will be apparent from the following Drawings, Detailed Description, Examples, and Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] Fig. 1 is a sequence alignment displaying the 3rd through the 7th transmembrane (TM) region (transmembrane regions in grey) of several human and feline bitter receptors: human TAS2R16 (SEQ ID NO:30), TAS2R4 (SEQ ID NO:27), TAS2R9 (SEQ ID NO:28), TAS2R10 (SEQ ID NO:29) AND TAS2R38 (SEQ ID NO:31); and feline bitter receptors, TAS2R4 (SEQ ID NO:8), 9 (SEQ ID NO:12), 10 (SEQ ID NO:14), 12 (SEQ ID NO:16), and 38 (SEQ ID NO:18).

[0040] Fig. 2 shows a sequence alignment for human TAS2R38 polypeptide (SEQ ID NO:31) and feline TAS2R38 polypeptide (SEQ ID NO:18) determined from sequencing of

genomic DNA of five individual cats.

DETAILED DESCRIPTION

[0041] A family of novel feline bitter taste receptors, feline TAS2R (fTAS2R), are disclosed herein. These G-protein coupled receptors (GPCRs) are components of the feline taste transduction pathway, specifically, part of the bitter taste transduction pathway, and are involved in feline taste detection of bitter substances such as 6-n-propylthiouracil, sucrose octaacetate, raffinose undecaacetate, cycloheximide, denatonium, copper glycinate, and quinine. Polynucleotides encoding the novel feline bitter taste receptors are also disclosed, as are expression vectors and host cells for expression of the novel feline bitter taste receptors. Methods of expressing and isolating the nucleic acids and encoded polypeptides are also

disclosed.

[0042] The nucleic acids provide probes for identification of cells in which the nucleic acids are expressed, e.g., taste cells. For example, probes for expression of TAS2R polypeptides can be used to identity taste cells present in foliate, circumvallate, and fungiform papillae. In particular, the TAS2R probes are useful to identify bitter sensing cells and can serve as tools for the generation of anatomical maps that elucidate the relationship between the bitter sensing cells and their projections into the central nervous system. Methods of identifying compounds that bind to the novel feline bitter taste receptors and modulate their activity are disclosed. In the methods, members of the fTAS2R family act as direct or indirect reporter molecules to identify modulators of taste receptor expressing cellular activity. Such compounds are useful for modulation of feline bitter taste receptor activity. Modulating the activity of feline bitter receptors receptor can be achieved by agonists, antagonists, inhibitors, and/or enhancers. These modulatory compounds can be used in the food and pharmaceutical industries to customize taste of foods or drugs, for example, to decrease the bitter taste of foods or drugs. Thus, the methods disclosed herein are useful for designing or formulating food, food palatants, treats, and medications in which aversive compounds are avoided or blocked, particularly for felines.

[0043] An “agonist”, or “receptor agonist” as used herein, refers to a molecule that has an affinity for and stimulates functional activity of a cell receptor. The level of stimulation of the functional activity at the receptor can be, e.g., at least 5%, at least 10%, at least 30%, at least 50%, at least 80%, at least 100%, at least 200%, at least 300%, at least 500%, at least 1,000%, at least 10,000% over baseline.

[0044] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics means chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring

amino acid. Amino acids may be referred to herein by either their commonly known three-letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0045] A receptor “antagonist” as used herein refers to a type of receptor ligand that binds to the receptor at the same site as an agonist, but does not activate the functional response initiated by the active form of the receptor. Once bound, an antagonist will block agonist binding thereby inhibiting the functional response produced by agonist binding. Since agonists and antagonists "compete" for the same binding site on the receptor, the level of activity of the receptor will be determined by the relative affinity of each molecule for the site and their relative concentrations. The inhibition of the functional response elicited by an agonist by an antagonist applied prior, concomitantly or after the application of the agonist can be e.g., at least by 10%, at least 15%; at least 20%; at least 30%; at least 40%; at least 50%; at least 60%; at least 70%; at least 80%; at least 90%; at least 95%; at least 98%; at least 99%; at least 99.5%; or at least 100%. In certain embodiments, the antagonist and agonist are applied at the same molar concentration.

[0046] "Antibody" refers to a polypeptide that specifically binds and recognizes an antigen. The term “antibody” or “immunoglobulin,” as used interchangeably herein, includes whole antibodies and any antigen binding fragment (antigen-binding portion) or single chain cognates thereof. Antibodies may be polyclonal or monoclonal. The term “monoclonal antibody” means an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. In some embodiments, the term “monoclonal antibody” refers to an antibody derived from a single cell clone.

[0047] An “antibody” comprises at least one heavy (H) chain and one light (L) chain. In naturally occurring IgGs, for example, these heavy and light chains are inter-connected by disulfide bonds and there are two paired heavy and light chains; these two are also inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2, and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more

conserved, termed framework regions (FR) or Joining (J) regions (JH or JL in heavy and light chains respectively). Each VH and VL is composed of three CDRs three FRs and a J domain, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, J. The variable regions of the heavy and light chains bind 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) or humoral factors such as the first component (Clq) of the classical complement system.

[0048] The term “antigen-binding portion” or “antigen-binding fragment” of an antibody, as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that certain fragments of a full-length antibody can perform the antigen-binding function of an antibody. Examples of binding fragments denoted as an antigen-binding portion or fragment of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb including VH and VL domains; (vi) a dAb fragment (Ward et al. (1989) Nature 341, 544-546), which consists of a VH domain; (vii) a dAb which consists of a VH or a VL domain; and (viii) an isolated complementarity determining region (CDR) or (ix) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions are paired to form monovalent molecules (such a single chain cognate of an immunoglobulin fragment is known as a single chain Fv (scFv). Such single chain antibodies are also encompassed within the term “antibody fragment.” Antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same general manner as are intact antibodies. Antigen-binding fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

[0049] An "anti-TAS2R" or a “TAS2R” antibody is an antibody or antibody fragment that specifically binds a polypeptide encoded by a TAS2R gene, cDNA, or a subsequence thereof.

[0050] The term “chimeric polypeptide” refers to a molecule, which does not occur in nature, in which all or a portion of an fTAS2R polypeptide sequence is part of the linear chimeric polypeptide sequence. The portion of an fTAS2R polypeptide sequence can be the amino acid sequence of one or more domains of the complete fTAS2R polypeptide. For example, the portion can be an extracellular domain of a fTAS2R polypeptide. The chimeric polypeptide can be made by any method known in the art. For example, the chimeric polypeptide can be made by a recombinant expression system or can be synthesized.

[0051] "Codon optimization" describes a method applied to nucleotide sequences encoding a polypeptide to modify the nucleotide sequence for enhanced expression of the polypeptide in the cells of a non-feline organism of interest, e.g. *Drosophila melanogaster* or *Saccharomyces cerevisae*, by replacing at least one, more than one, or all, codons of the native feline sequence with codons that are more frequently or most frequently used in the genes of the expression organism without changing the amino acids of the expressed polypeptide. In preferred embodiments, all codons of the nucleic acid encoding a polypeptide sequence, or fragment thereof, are codon-optimized. Many organisms display a bias for use of particular codons to code for insertion of a particular amino acid in a growing peptide chain. Differences in codon usage, sometimes referred to as codon bias or preference, between organisms is afforded by degeneracy of the genetic code, and is well documented among many organisms. Codon bias often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, inter alia, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization. Methods of codon-optimization are known in the art, for example the free, internet-accessible program JCat (Grote A, et al. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Res. 2005 Jul 1;33(Web Server issue):W526-31.) or methodology disclosed in US20130017217 or WO2004058166.

[0052] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large

number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence. One of skill will further recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0053] "C terminal domain" refers to the region that spans the end of the last transmembrane domain and the C-terminus of the protein, and which is normally located within the cytoplasm.

[0054] "Cytoplasmic domains" or “intracellular domains” refers to the domains of TAS2R proteins that face the inside of the cell, e. g., the "C terminal domain" and the intracellular loops of the transmembrane domain, e. g., the intracellular loops between transmembrane regions 1 and 2, the intracellular loops between transmembrane regions 3 and 4, and the intracellular loops between transmembrane regions 5 and 6.

[0055] The term "extracellular domains" refers to the domains of TAS2R polypeptides that protrude from the cellular membrane and are exposed to the extracellular face of the cell. Such domains include the "N terminal domain" that is exposed to the extracellular face of the cell, as well as the extracellular loops of the transmembrane domain that are exposed to the extracellular face of the cell, i.e., the loops between transmembrane regions 2 and 3, and between transmembrane regions 4 and 5. The "N terminal domain" region starts at the N-terminus and extends to a region close to the start of the transmembrane domain. These extracellular domains are useful for *in vitro* ligand binding assays, both soluble and solid

phase.

[0056] The term “feline” refers herein to any member of the *Felidae* family, including domestic cats and nondomestic cats. In some embodiments felines can include both wild or captive cats, including wild and exotic cats, such as cougars, cheetah, lynxes, ocelots, lions, tigers, jaguars, panthers, and leopards.

[0057] As used herein, “heterologous” means that the sequence or cell originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention, or that the sequence is designed de novo without reference to any natural sequence. For example, a promoter operably linked to a heterologous polynucleotide is from a species different from the species from which the polynucleotide was derived, or, if from the same or an analogous species, one or both are substantially modified from their original form and/or genomic locus, or the promoter is not the native promoter for the operably linked polynucleotide. "Heterologous sequences" are those that are not operatively linked or are not contiguous to each other in nature. A “heterologous polypeptide” as used herein refers to a polypeptide which is not naturally included in the polypeptide sequence of the fTAS2R receptor polypeptide. A “heterologous cell” for expression of a polypeptide or nucleic acid refers to a cell that does not normally express that polypeptide or nucleic acid.

[0058] "Homology" refers to the percent identity between polynucleotide or polypeptide molecules. Two DNA, or two polypeptide sequences are "substantially homologous" to each other when the sequences exhibit at least about 50% , specifically at least about 75%, more specifically at least about 80%-85%, at least about 90%, and most specifically at least about 95%-98% sequence identity over a defined length of the molecules. As used herein, substantially homologous also refers to sequences showing complete identity to the specified DNA or polypeptide sequence.

[0059] In general, "identity" refers to an exact nucleotide-to-nucleotide or amino acid-to- amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

[0060] The term "immunoassay" is an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

[0061] As used herein “inhibition” or “blocking” of activity of a TAS2R receptor, or a ligand-binding fragment thereof, means that the functional response of a TAS2R receptor, or fragment, to an agonist is reduced or prevented when in the presence of the inhibitor, for

example the TAS2R receptor interacts with an intracellular signaling pathway to produce a smaller functional response, e.g. the TAS2R receptor interacts with a G-protein to promote signal transduction that produces a smaller increase in intracellular Ca2+ than is elicited by the agonist in the absence of inhibition.

[0062] “Interaction” of a compound with a TAS2R receptor can mean binding of the compound to the receptor or modulation of a functional response of the receptor by the compound.

[0063] The terms "isolated" or “purified”, used interchangeably herein, refers to a nucleic acid, a polypeptide, or other biological moiety that is removed from components with which it is naturally associated. The term "isolated" can refer to a polypeptide that is separate and discrete from the whole organism with which the molecule is found in nature or is present in the substantial absence of other biological macro-molecules of the same type. The term "isolated" with respect to a polynucleotide can refer to a nucleic acid molecule devoid, in whole or part, of sequences normally associated with it in nature; or a sequence, as it exists in nature, but having heterologous sequences in association therewith; or a molecule disassociated from the chromosome. Purity and homogeneity are typically determined using analytical chemistry techniques, for example polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. In particular, an isolated TAS2R nucleic acid is separated from open reading frames that flank the TAS2R gene and encode proteins other than a TAS2R. In some embodiments, the term "purified" means that the nucleic acid or protein is at least 85% pure, specifically at least 90% pure, more specifically at least 95% pure, or yet more specifically at least 99% pure.

[0064] A “ligand” as used herein refers to a molecule that binds to a macromolecule, such as a TAS2R receptor. The ligand can be a small molecule, or a biological moiety, such as a protein, a sugar, nucleic acid or lipid. The ligand can be a molecule that modulates TAS2R receptor activity. A molecule that modulates activity of a receptor can be an agonist, an antagonist, or a modulator as defined herein.

[0065] Ligands for various TAS2R receptors are known in the art. For example, ligands of a mammalian TAS2R1 can include adhumulone, adlupulone, amarogentin, arborescin, cascarillin, chloramphenicol, cis-isocohumulone, cis-isoloadhumulone, cohumulone, colupulone, dextromethorphan, diphenidol (diphenylthiourea, sulfocarbanilide, sym-diphenylthiourea, or thiocarbanilide), humulon (humulone), isoxanthohumol, lupulon, lupulone, parthenolide, picrotoxinin, sodium cyclamate, sodium thiocyanate, thiamine,

trans-isoadhumulone, trans-isocohumulone, trans-isohumulone, xanthohumol, and yohimbine. The mammalian TAS2R1 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R1 is a feline TAS2R1.

[0066] Ligands of a mammalian TAS2R3 can include chloroquine. The mammalian TAS2R3 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R3 is a feline TAS2R3.

[0067] Ligands of a mammalian TAS2R4 can include amarogentin, arborescin, artemorin, azathioprine, brucine, campher, chlorpheniramine, colchicine, dapsone, denatonium benzoate, diphenidol, parthenolide, quassin, quinine, and yohimbine. The mammalian TAS2R4 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R4 is a feline TAS2R4.

[0068] Ligands of a mammalian TAS2R7 can include caffeine, chlorpheniramine, cromolyn, diphenidol, papaverine, and quinine. The mammalian TAS2R7 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R7 is a feline TAS2R7.

[0069] Ligands of a mammalian TAS2R9 can include ofloxacin, pirenzapin, and procainamid. The mammalian TAS2R9 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R9 is a feline TAS2R9.

[0070] Ligands of a mammalian TAS2R10 can include (-)-alpha thujone, absinthin, arborescin, arglabin, artemorin, azathioprine, benzoin , caffeine, campher, cascarillin, chloramphenicol, chloroquine, chlorpheniramine, coumarin, cucurbitacin b, cucurbitacin e, cucurbitacins, cycloheximid, cycloheximide, dapsone, denatonium benzoate, dextromethorphan, diphenidol, erythromycin, famotidine, haloperidol, papaverine, parthenolide, picrotoxinin, quassin, quinine, strychnine, and yohimbine. The mammalian TAS2R10 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R10 is a feline TAS2R10.

[0071] Ligands of a mammalian TAS2R38 can include 6-methyl-2-thiouracil, acetylthiourea, allyl isothiocyanate, caprolactam, chlorpheniramine, dimethylthioformamide, diphenidol, (diphenylthiourea, sulfocarbanilide, sym-diphenylthiourea, thiocarbanilide), ethylene thiourea, n,n-ethylene thiourea, ethylpyrazine, limonin, methimazole, n-ethylthiourea, n-methylthiourea, phenethyl isothiocyanate, phenylthiocarbamide (ptc), probenecid, propylthiouracil, sinigrin, sodium cyclamate, sodium_thiocyanate, and yohimbine. The mammalian TAS2R38 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R38 is a feline TAS2R38.

[0072] Ligands of a mammalian TAS2R43 can include acesulfame K, aloin, amarogentin, arborescin, arglabin, aristolochic acid, caffeine, chloramphenicol, cromolyn, denatonium benzoate, diphenidol, falcarindiol, grosheimin (grossheimin), helicin, probenecid, quinine, and saccharin. The mammalian TAS2R43 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R43 is a feline TAS2R43.

[0073] Ligands of a mammalian TAS2R44 can include acesulfame K, aloin, aristolochic acid, diphenidol, famotidine, parthenolide, quinine, and saccharin. The mammalian TAS2R44 can be from a human, a rodent, a canine, or a feline. In an embodiment, the mammalian TAS2R44 is a feline TAS2R44.

[0074] The term "ligand-binding fragment" of a TAS2R receptor, as used herein, refers to one or more fragments of the TAS2R receptor retaining the ability to specifically bind to a ligand of the TAS2R receptor.

[0075] A "modulator" is a molecule that modulates the functional response of a receptor by binding to a binding site that is distinct from the agonist binding site. A positive modulator or "enhancer" enhances the functional response of a receptor, while a negative modulator or "inhibitor" inhibits the functional response of a receptor. An "allosteric modulator" induces a conformational change in the receptor, which alters the affinity of the receptor for ligands, particularly at the agonist binding site. Positive allosteric modulators increase the affinity for ligands at the agonist binding site and/or enhance functional activity of a receptor, while negative allosteric modulators decrease the affinity for ligands at the agonist binding site and/or inhibit functional activity of a receptor. Modulators can include non-peptide molecules such as non-peptide mimetics, non-peptide allosteric effectors, and peptides.

[0076] The "modulating" or "altering" activity of a TAS2R receptor herein can refer to any change in TAS2R receptor activity occurring in response to binding of an agonist, antagonist, or modulator to the TAS2R receptor or a ligand binding fragment thereof, that is the alteration can be stimulating, antagonizing, or modulating the functional response of the receptor.

[0077] "Non-naturally occurring" in reference to a polynucleotide means that the polynucleotide sequence does not occur in nature in genomic DNA of an organism.

[0078] The term "nucleic acid", "polynucleotide", or "oligonucleotide" includes DNA molecules and RNA molecules. A polynucleotide may be single-stranded or double-stranded. Polynucleotides can contain known nucleotide analogs or modified backbone

residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs). A polynucleotide can be obtained by a suitable method known in the art, including isolation from natural sources, chemical synthesis, or enzymatic synthesis. Nucleotides may be referred to by their commonly accepted single-letter codes.

[0079] The term “operably linked” refers to a nucleic acid sequence placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. A nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence. With respect to transcription regulatory sequences, operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

[0080] A “palatability enhancer” or “palatant” for animal edible composition, e.g., a food, is an additive that provides an aroma, taste, aftertaste, mouth feel, texture, and/or organoleptic sensation that is appealing to the target animal.

[0081] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a molecule formed from the linking, in a defined order, of at least two amino acids. The link between one amino acid residue and the next is an amide bond and is sometimes referred to as a peptide bond. A polypeptide can be obtained by a suitable method known in the art, including isolation from natural sources, expression in a recombinant expression system, chemical synthesis, or enzymatic synthesis. The terms also apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid

polymers and non-naturally occurring amino acid polymer.

[0082] Macromolecular structures of polypeptides can be described in terms of various levels of organization. "Primary structure" refers to the amino acid sequence of a particular peptide. "Secondary structure" refers to locally ordered, three dimensional structures within a polypeptide. These structures are commonly known as domains. Domains are portions of a polypeptide that form a compact unit of the polypeptide and are typically 50 to 350 amino acids long. Typical domains are made up of sections of lesser organization such as stretches of beta-sheet and alpha-helices. "Tertiary structure" refers to the complete three-dimensional structure of a polypeptide monomer. "Quaternary structure" refers to the three-dimensional structure formed by the noncovalent association of independent tertiary units.

[0083] The term "primer" refers to an isolated single-stranded oligonucleotide of between about 10 to 50 nucleotides in length, preferably between about 15 to 50, more preferably 15 to 30 nucleotides in length and most preferably between about 18 and 28 nucleotides in length, that forms a duplex with a single stranded nucleic acid sequence of interest, and which is capable of acting as a point of initiation of nucleic acid synthesis to allow for polymerization of a complementary strand using a polymerase under appropriate conditions (i.e., in the presence of nucleotides and an inducing agent such as DNA polymerase and at a suitable temperature and pH). The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer and the use of the method. Preferably, the primer is an oligodeoxyribonucleotide. In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their 5' ends. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. A “primer pair” is a pair of primer sequences chosen to amplify a particular DNA target sequence by PCR. One primer of the pair is complementary to the 3' end of the “sense” strand of the DNA target, e.g. a cDNA, and the other is complementary to the 3' end of the “anti-sense” strand of the DNA target.

[0084] As used herein, the term "probe" refers to an oligonucleotide which is capable of hybridizing to another nucleic acid of interest. A probe may be single-stranded or double-stranded. A probe herein is an oligonucleotide of between about 10 to 100 nucleotides in length, preferably between about 15 to 80, more preferably 20 to 50 nucleotides in length. Probes are useful in the detection, identification and isolation of particular nucleic acid

sequences, for example via Southern hybridization or other methods known in the art. It is contemplated that any probe used in the present invention will be labeled with any "reporter molecule," so that it is detectable in any detection system, including, but not limited to enzyme (e.g., ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

[0085] The term "recombinant" can be used to describe a nucleic acid molecule and refers to a polynucleotide of genomic, RNA, DNA, cDNA, viral, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation is not associated with all or a portion of the polynucleotide with which it is associated in nature. The term "recombinant" as used with respect to a protein or polypeptide can refer to a polypeptide produced by expression of a recombinant polynucleotide. In general, the gene of interest is cloned and then expressed in transformed organisms, by a method known in the art. The host organism expresses the foreign gene to produce the protein under expression conditions.

[0086] The term “solid support” refers to a material or group of materials having a rigid or semi-rigid surface or surfaces. Examples of materials include plastics (e.g., polycarbonate), complex carbohydrates (e.g., agarose and sepharose), acrylic resins (e.g., polyacrylamide and latex beads), nitrocellulose, glass, silicon wafers, and positively charged nylon. In some aspects, at least one surface of the solid support can be substantially flat, although in some aspects it may be desirable to physically separate regions for different molecules with, for example, wells, raised regions, pins, etched trenches, or the like. In certain aspects, the solid support(s) will take the form of beads, resins, gels, microspheres, or other geometric configurations.

[0087] The phrase "specifically (or selectively) binds" to an antibody or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and do not substantially bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies raised to a fTAS2R can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with the fTAS2R protein or an immunogenic portion thereof and not with other proteins, except for orthologs or polymorphic variants and alleles of the TAS2R

protein. This selection may be achieved by subtracting out antibodies that cross-react with TAS2R molecules from other species or other TAS2R molecules. Antibodies can also be selected that recognize only fTAS2R GPCR family members but not other GPCRs. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, e.g., Harlow & Lane, Antibodies, A Laboratory Manual (1988), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

[0088] With respect to receptors, the terms “specific binding,” “specifically binds,” “selective binding,” and “selectively binds” mean that a receptor, such as a TAS2R receptor, exhibits appreciable affinity for a particular ligand. "Appreciable" binding affinity includes binding with an affinity of at least 10^4 M^{-1}, at least 10^5 M^{-1}, specifically at least 10^6 M^{-1}, more specifically at least 10^7 M^{-1}, yet more specifically at least 10^8 M^{-1}, or even yet more specifically at least 10^9 M^{-1}. A binding affinity can also be indicated as a range of affinities, for example, 10^4 M^{-1} to 10^{10} M^{-1}, specifically 10^5 M^{-1} to 10^{10} M^{-1}, more specifically 10^6 M^{-1} to 10^{10} M^{-1}. Specific binding can be determined according to any art-recognized means for determining such binding. In some embodiments, specific binding is determined according to Scatchard analysis and/or competitive binding assays.

[0089] As used herein, “stimulation” or “activation” of a TAS2R receptor, or a ligand-binding fragment thereof, means that the TAS2R receptor, or fragment, is placed in a state in which it produces a functional response, for example the TAS2R receptor interacts with an intracellular signaling pathway to produce the functional response, e.g. the TAS2R receptor interacts with a G-protein to promote signal transduction that produces increased intracellular Ca2+.

[0090] "Substantially the same" biological activity refers to a polypeptide fragment, derivative, homolog, analog, or variant retaining at least about 50%, 55%, 60%, 65%, 70%, preferably at least about 75%, 80%, 85%, 90%, more preferably at least about 91%, 92%, 93%, 94%, 95%, and most preferably at least about 96%, 97%, 98%, 99% or greater biological activity of the parent polypeptide. The extent to which a polypeptide fragment, derivative, homolog, analog, or variant retains the biological activity of the parent polypeptide may be assessed by any means available in the art, including, but not limited to, the assays listed or described herein.

[0091] A"TAS2R binding partner" is a compound that directly or indirectly binds a TAS2R polypeptide disclosed herein.

[0092] A "TAS2R receptor polypeptide" (or TAS2R receptor or TAS2R) for use in assays described herein to measure ligand binding or receptor activity can comprise a TAS2R receptor; a domain of a TAS2R receptor, such as an extracellular domain, transmembrane region, transmembrane domain, cytoplasmic domain, a ligand-binding fragment, subunit association domain, active site, and the like; or a chimeric protein in which either a TAS2R receptor or a domain thereof is covalently linked to a heterologous protein.

[0093] Herein a "tastant" means a ligand that can bind to a specific TAS2R receptor or set of TAS2R receptors.

[0094] The term "taste perception" as used herein refers to a response (e.g., biochemical, behavioral) or sensitivity of a TAS2R receptor to a taste stimulus. Modification of taste perception includes an alteration of (enhancement of, reduction of, or change to) a biochemical response, an ingestive response, a taste preference, a metabolic response, or a general behavior of a mammal in response to a tastant. "Taste perception" does not require, though it can include, transmission of a neural signal resulting in the *in vivo* sensation of taste by a mammal.

[0095] The "transmembrane domain," which comprises the seven transmembrane regions, refers to the domain of TAS2R polypeptides that lies within the plasma membrane, and may also include the corresponding cytoplasmic (intracellular) and extracellular loops, also referred to as transmembrane domain "regions." Transmembrane regions can also bind ligand either in combination with the extracellular domain or alone, and are therefore also useful for *in vitro* ligand binding assays.

[0096] The term "transmembrane region" as used herein denotes a three-dimensional protein structure which is thermodynamically stable in a membrane, e.g., a single transmembrane alpha helix or a transmembrane beta barrel.

[0097] The term "vector" means a nucleic acid sequence to express a target gene in a host cell. Examples include a plasmid vector, a cosmid vector, a bacteriophage vector, and a viral vector. Examples of viral vectors include a bacteriophage vector, an adenovirus vector, a retrovirus vector, and an adeno-associated virus vector. For example, the vector may be an expression vector including a membrane targeting or secretion signaling sequence or a leader sequence, in addition to an expression control element such as promoter, operator, initiation codon, termination codon, polyadenylation signal, and enhancer. The vector may be manufactured in various ways known in the art depending on the purpose. An expression

vector may include a selection marker for selecting a host cell containing the vector. Further, a replicable expression vector may include an origin of replication. The term "recombinant vector" or “expression vector” means a vector operably linked to a heterologous nucleotide sequence for the purpose of expression, production, and isolation of the heterologous nucleotide sequence. The heterologous nucleotide sequence can be a nucleotide sequence encoding all or part of a fTAS2R receptor or a chimeric polypeptide disclosed herein.

[0098] Human TAS2R (hTAS2R) gene and pseudogene nucleotide sequences were used as references to identify, via a bioinformatics approach, previously unknown feline TAS2R (fTAS2R) genes. Subsequently, isolated feline genomic DNA was used to clone the fTAS2R genes. The nucleotide sequence of the cloned fTAS2R genes of several felines was then determined by sequencing, e.g., Sanger sequencing, and used to establish a consensus nucleotide sequence for the gene, and to identify any variant sites in the sequence.

[0099] Polynucleotides encoding a fTAS2R receptor are disclosed. In an embodiment, the polynucleotides are isolated. The polynucleotide can comprise a nucleotide sequence selected from the nucleotide sequence of SEQ ID NO:1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25; a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO:10, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; a nucleotide sequence encoding a fTAS2R having an amino acid sequence having at least 70% homology to the amino acid sequence of SEQ ID NO:2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO:10, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; a nucleotide sequence encoding a fTAS2R and having at least 70% homology to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25; a nucleotide sequence that hybridizes to the complement of the polynucleotide having SEQ ID NO:1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25 under high stringency conditions; a nucleotide sequence comprising at least 15 contiguous nucleotides of the nucleotide sequence of any one of the foregoing nucleotide sequences; and the complement of any one of the foregoing nucleotide sequences. In an embodiment, the percent homology is at least 90%. In

an embodiment, the percent homology is at least 95%, preferably at least 98%, more preferably at least 99%. In an embodiment, the polynucleotide comprises a nucleotide sequence selected from: the nucleotide sequence of SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25; a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; a nucleotide sequence that hybridizes to the complement of the polynucleotide having SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25 under high stringency conditions; and the complement of the foregoing nucleotide sequences. In an embodiment, the polynucleotide comprises a nucleotide sequence selected from: the nucleotide sequence of SEQ ID NO: 17; a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 18; a nucleotide sequence that hybridizes to the complement of the polynucleotide having SEQ ID NO: 17 under high stringency conditions; and the complement of the foregoing nucleotide sequences. In an embodiment, the nucleotide sequence is codon-optimized for expression in a non-feline cell. In an embodiment, the non-feline cell is *Escherichia coli*, a *Saccharomyces cerevisae* cell, a *Drosophila melanogaster* cell, a *Caenorhabditis elegans* cell, or a mammalian cell. In an embodiment, the mammalian cell is a human or murine cell. Examples of codon-optimized sequences for expression of the novel fTAS2R receptor polypeptides in *Escherichia coli*, *Saccharomyces cerevisae* cell, *Drosophila melanogaster*, *Caenorhabditis elegans*, human, or murine cells are disclosed in SEQ ID NOs: 58-135.

[00100] Further disclosed are polynucleotides comprising a sequence having at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% homology with SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or the complement of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, or 25.

[00101] Also disclosed are compositions comprising at least two polynucleotides disclosed herein. In an embodiment each polynucleotide encodes a portion of a different fTAS2R receptor. In an embodiment, the composition comprises at least 3, 4, or 5 of the polynucleotides disclosed herein.

[00102] In an embodiment, the composition comprises at least 6, 7, 8, 9, 10, 11, 12, or 13 of the polynucleotides disclosed herein. In an embodiment each polynucleotide of the composition encodes a different fTAS2R receptor, or fragment thereof. In an embodiment, the composition comprises a polynucleotide comprising SEQ ID NO: 17 and/or SEQ ID NO:

21. In an embodiment, the composition comprises a primer pair for amplifying a portion of a nucleic acid encoding a feline TAS2R polypeptide. In an embodiment, the primer pairs are selected from the primer pairs of Table 5. The primer pairs disclosed herein are useful for determination of the nucleotide sequence of a particular TAS2R polynucleotide, or fragment thereof, using PCR. The pairs of single-stranded DNA primers can be annealed to sequences within or surrounding the fTAS2R gene in order to prime amplifying DNA synthesis of the fTAS2R gene itself. Allele-specific primers can also be used. Such primers anneal only to particular fTAS2R mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

[00103] A single nucleotide polymorphism in the nucleic acid sequence encoding fTAS2R38 was identified at nucleotide 220 of the cDNA sequence (SEQ ID NO:17) from sequencing amplified feline genomic DNA from multiple subjects. The two alleles observed at the nucleotide 220 were G and A. The G220A nucleic acid variation corresponds to an amino acid variation D74N in the fTAS2R38 protein sequence (SEQ ID NO:18). In an embodiment, a disclosed polynucleotide comprises a nucleotide sequence of at least 15 contiguous nucleotides of SEQ ID NO:17 containing nucleotide 220, wherein an A is present at nucleotide 220; or the complement of the nucleotide sequence. In an embodiment, the polynucleotide comprises at least 20 contiguous nucleotides of SEQ ID NO:17 containing nucleotide 220, wherein an A is present at nucleotide 220; or the complement of the nucleotide sequence. In an embodiment, a disclosed fTAS2R38 polypeptide comprises SEQ ID NO:18 with N present at residue 74 of the sequence, or a fragment thereof comprising the N74 residue.

[00104] In another aspect, isolated fTAS2R receptor polypeptides are disclosed.

[00105] In an embodiment, the isolated fTAS2R polypeptide is encoded by a polynucleotide disclosed herein.

[00106] In an embodiment, the isolated fTAS2R polypeptide can comprise the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26; or an amino acid sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% homology with the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26. In an embodiment, the isolated fTAS2R polypeptide comprises the amino acid sequence of SEQ ID

NO:8, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26; or an amino acid sequence having at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% homology with one of the foregoing amino acid sequences. In an embodiment, the isolated fTAS2R polypeptide comprises the amino acid sequence of SEQ ID NO:18 or SEQ ID NO:22.

[00107] Sensory GPCRs, such as the TAS2R bitter taste receptors, have a domain structure including an N-terminal domain; extracellular domains; a transmembrane domain comprising seven transmembrane regions, cytoplasmic, and extracellular loops; cytoplasmic domains; and a C-terminal domain. These domains can be structurally identified using methods known in the art, such as sequence analysis programs that identify hydrophobic and hydrophilic domains. Such domains are useful for making chimeric proteins and for *in vitro* assays disclosed herein, e.g., ligand binding assays.

[00108] The seven transmembrane regions and extracellular and cytoplasmic loops can be identified using standard methods known in the art. For example, transmembrane regions of the fTAS2R proteins can be identified using software, TOPCONS, available on the internet from the Stockholm Bioinformatics Center, Stockholm University (Andreas Bernsel, et al. (2009) Nucleic Acids Research 37(Webserver issue), W465-8). The seven transmembrane regions and extracellular and cytoplasmic loops of the fTAS2R identified by TOPCONS are shown in the following table:

Table 1. TOPCONS prediction of seven transmembrane regions, extracellular loops, and intracellular loops

fTAS2R1 (SEQ ID NO:2)
Transmembrane domains: 1: 2-22, 2: 47-67, 3: 85-105, 4: 125-145, 5: 180-200, 6: 228-248, 7. 258-278
Extracellular domain: 1, 68-84; 146-179; 249-257.
Intracellular domain: 23-46; 106-124; 201-227; 279-298
fTAS2R2 (SEQ ID NO:4)
Transmembrane domains: 1: 11-31, 2: 52-72, 3: 89-109, 4: 130-150, 5: 187-207, 6: 235-255, 7: 265-285
Extracellular domain: 1-10, 73-88; 151-186; 256-264
Intracellular domain: 32-51; 110-129; 208-234; 286-304
fTAS2R3 (SEQ ID NO:6)
Transmembrane domains: 1: 9-29, 2: 51-71, 3: 89-109, 4: 129-149, 5: 187-207, 6: 235-255, 7: 266-

286
Extracellular domain: 1-8; 72-88; 150-186; 256-265
Intracellular domain: 30-50; 110-128; 208-234; 287-316
fTAS2R4 (SEQ ID NO:8)
Transmembrane domains: 1: 3-23, 2: 48-68, 3: 88-108, 4: 130-150, 5: 184-204, 6: 232-252, 7: 262-282
Extracellular domain: 1-2; 69-87; 151-183; 253-261
Intracellular domain: 24-47; 109-129; 205-231; 283-306
fTAS2R7 (SEQ ID NO:10)
Transmembrane domains: 1: 9-29, 2: 51-71, 3: 89-109, 4: 129-149, 5: 188-208, 6: 236-256, 7: 266-286
Extracellular domain: 1-8; 72-88; 150-187; 257-265
Intracellular domain: 30-50; 110-128; 209-235; 287-311
fTAS2R9(SEQ ID NO:12)
Transmembrane domains: 1: 7-27, 2: 51-71, 3: 89-109, 4: 129-149, 5: 184-204, 6: 232-252, 7: 263-283
Extracellular domain: 1-6; 72-88; 150-183; 253-262
Intracellular domain: 28-50; 110-128; 205-231; 284-337
fTAS2R10 (SEQ ID NO:14)
Transmembrane domains: 1: 2-22, 2. 48-68, 3: 88-108, 4: 129-149, 5: 182-202, 6: 230-250, 7: 261-281
Extracellular domain: 1; 69-87; 150-181; 251-260
Intracellular domain: 23-48; 109-128; 203-229; 282-300
fTAS2R12 (SEQ ID NO:16)
Transmembrane domains: 1: 9-29, 2: 48-68, 3: 89-109, 4: 129-149, 5: 186-206, 6: 231-251, 7: 262-282
Extracellular domain: 1-8; 69-88; 150-185; 252-261
Intracellular domain: 30-47; 110-128; 207-230; 283-309
fTAS2R38 (SEQ ID NO:18)
Transmembrane domains: 1: 18-38, 2: 62-82, 3: 99-119, 4: 140-160, 5: 199-219, 6: 247-267, 7: 278-298
Extracellular domain: 1-17: 83-98; 161-198; 268-277
Intracellular domain: 39-61; 120-139; 220-246; 299-334
fTAS2R42 (SEQ ID NO:20)
Transmembrane domains: 1: 2-22, 2: 48-68, 3: 89-109, 4: 129-149, 5: 186-206, 6: 234-254, 7: 265-285
Extracellular domain: 1; 69-88; 150-185; 255-264
Intracellular domain: 23-47; 110-128; 207-233; 286-322

fTAS2R43 (SEQ ID NO:22)
Transmembrane domains: 1: 3-23, 2: 48-68, 3: 88-108, 4: 128-148, 5: 182-202, 6: 230-250, 7: 261-281
Extracellular domain: 1-2; 69-87; 149-181; 251-260
Intracellular domain: 24-47; 109-127; 203-229; 282-299
fTAS2R44 (SEQ ID NO:24)
Transmembrane domains: 1: 3-23, 2: 48-68, 3: 88-108, 4: 128-148, 5: 182-202, 6: 230-250, 7: 260-280
Extracellular domain: 1-2; 69-87; 149-181; 251-259
Intracellular domain: 24-47; 109-127; 203-229; 281-308
fTAS2R67 (SEQ ID NO:26)
Transmembrane domains: 1: 9-29, 2: 51-71, 3: 89-109, 4: 129-149, 5: 186-206, 6: 233-253, 7: 264-284
Extracellular domain: 1-8; 72-88; 150-185; 254-263
Intracellular domain: 30-50; 110-128; 207-232; 285-312

[00109] Alternative predictions of the transmembrane regions and extracellular and cytoplasmic loops of the fTAS2R proteins can be generated using different software also available on the internet from the Stockholm Bioinformatics Center, including SCAMPI (Andreas Bernsel, et al. (2008) Proc. Natl. Acad. Sci. USA. 105, 7177-7181.); PRODIV (Håkan Viklund and Arne Elofsson (2004) Protein Science 13, 1908-1917), and OCTAPUS (Håkan Viklund and Arne Elofsson (2008) Bioinformatics. 24, 1662-1668.) Additional methods known in the art to predict the structural regions include hydropathy prediction methods of Goldman-Engleman-Steitz, or Kyte-Doolittle (J. Mol. Biol. 157: 105-132 (1982), or Hopp-Woods. Secondary structure prediction methods include Garnier-Robson, or Deléage & Roux or Chou-Fasman. As known in the art, the various available algorithms may predict slightly different boundaries for transmembrane regions based on the amino acid sequence.

[00110] In an embodiment, the isolated TAS2R receptor polypeptide can comprise at least one extracellular domain of a feline TAS2R receptor; at least one transmembrane domain of a feline TAS2R receptor; or at least one intracellular domain of a feline TAS2R receptor, wherein the feline TAS2R receptor comprises the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26; or an amino acid sequence having at least 70%, at least 80%, at least 90%, at least 95% homology, specifically at least 97% homology, more specifically at least 99%

homology with the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26, wherein the isolated fTAS2R receptor polypeptide does not consist of the amino acid sequence of SEQ ID NOs: 2, 4, 6, or 10..

[00111] In an embodiment, the extracellular domain of the fTAS2R polypeptide can comprise amino acids 1, 68-84; 146-179; or 249-257 of SEQ ID NO:2; amino acids 1-10, 73-88; 151-186; or 256-264 of SEQ ID NO:4; amino acids 1-8; 72-88; 150-186; or 256-265 of SEQ ID NO:6; amino acids 1-2; 69-87; 151-183; or 253-261 of SEQ ID NO:8; amino acids 1-8; 72-88; 150-187; or 257-265 of SEQ ID NO:10; amino acids 1-6; 72-88; 150-183; or 253-262 of SEQ ID NO:12; amino acids 1; 69-87; 150-181; or 251-260 of SEQ ID NO:14; amino acids 1-8; 69-88; 150-185; or 252-261 of SEQ ID NO:16; amino acids 1-17: 83-98; 161-198; or 268-277 of SEQ ID NO:18; amino acids 1; 69-88; 150-185; or 255-264 of SEQ ID NO:20; amino acids 1-2; 69-87; 149-181; or 251-260 of SEQ ID NO:22; amino acids 1-2; 69-87; 149-181; or 251-259 of SEQ ID NO:24; or amino acids 1-8; 72-88; 150-185; or 254-263of SEQ ID NO:26.

[00112] In an embodiment, the transmembrane domain of the fTAS2R polypeptide can comprise amino acids 2-22, 47-67, 85-105, 125-145, 180-200, 228-248, or 258-278 of SEQ ID NO:2; amino acids 11-31, 52-72, 89-109, 130-150, 187-207, 235-255, or 265-285 of SEQ ID NO:4; amino acids 9-29, 51-71, 89-109, 129-149, 187-207, 235-255, or 266-286 of SEQ ID NO:6; amino acids 3-23, 48-68, 88-108, 130-150, 184-204, 232-252, or 262-282 of SEQ ID NO:8; amino acids 9-29, 51-71, 89-109, 129-149, 188-208, 236-256, or 266-286 of SEQ ID NO:10; amino acids 7-27, 51-71, 89-109, 129-149, 184-204, 232-252, or 263-283 of SEQ ID NO:12; amino acids 2-22, 2. 48-68, 88-108, 129-149, 182-202, 230-250, or 261-281 of SEQ ID NO:14; amino acids 9-29, 48-68, 89-109, 129-149, 186-206, 231-251, or 262-282 of SEQ ID NO:16; amino acids 18-38, 62-82, 99-119, 140-160, 199-219, 247-267, or 278-298of SEQ ID NO:18; amino acids 2-22, 48-68, 89-109, 129-149, 186-206, 234-254, or 265-285 of SEQ ID NO:20; amino acids 3-23, 48-68, 88-108, 128-148, 182-202, 230-250, or 261-281of SEQ ID NO:22; amino acids 3-23, 48-68, 88-108, 128-148, 182-202, 230-250, or 260-280 of SEQ ID NO:24; or amino acids 9-29, 51-71, 89-109, 129-149, 186-206, 233-253, or 264-284of SEQ ID NO:26.

[00113] In an embodiment, the intracellular domain of the fTAS2R polypeptide can comprise amino acids 23-46; 106-124; 201-227; or 279-298 of SEQ ID NO:2; amino acids 32-51; 110-129; 208-234; or 286-304 of SEQ ID NO:4; amino acids 30-50; 110-128; 208-

234; or 287-316 of SEQ ID NO:6; amino acids 24-47; 109-129; 205-231; or 283-306 of SEQ ID NO:8; amino acids 30-50; 110-128; 209-235; or 287-311 of SEQ ID NO:10; amino acids 28-50; 110-128; 205-231; or 284-337 of SEQ ID NO:12; amino acids 23-48; 109-128; 203-229; or 282-300 of SEQ ID NO:14; amino acids 30-47; 110-128; 207-230; or 283-309 of SEQ ID NO:16; amino acids 39-61; 120-139; 220-246; or 299-334 of SEQ ID NO:18; amino acids 23-47; 110-128; 207-233; or 286-322 of SEQ ID NO:20; amino acids 24-47; 109-127; 203-229; or 282-299 of SEQ ID NO:22; amino acids 24-47; 109-127; 203-229; or 281-308 of SEQ ID NO:24; or amino acids 30-50; 110-128; 207-232; or 285-312 of SEQ ID NO:26.

[00114] In an embodiment, the fTAS2R receptor polypeptide comprises a transmembrane region 2, a transmembrane region 3, a transmembrane region 4, a transmembrane region 5, a transmembrane region 6, and a transmembrane region 7, wherein each transmembrane region comprises at least 20 consecutive amino acids of the corresponding transmembrane region sequence independently selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26; or a transmembrane region 3, a transmembrane region 6, and a transmembrane region 7, wherein each transmembrane region comprises at least 20 consecutive amino acids of the corresponding transmembrane region sequence independently selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26; an extracellular domain 3 comprising at least 15 consecutive amino acids selected from amino acids 146-179 of SEQ ID NO:2; amino acids 151-186 of SEQ ID NO:4; amino acids 150-186 of SEQ ID NO:6; amino acids 151-183 of SEQ ID NO:8; amino acids 150-187 of SEQ ID NO:10; amino acids 150-183 of SEQ ID NO:12; amino acids 150-181 of SEQ ID NO:14; amino acids 150-185 of SEQ ID NO:16; amino acids 161-198 of SEQ ID NO:18; amino acids 150-185 of SEQ ID NO:20; amino acids 149-181 of SEQ ID NO:22; amino acids 149-181 of SEQ ID NO:24; and amino acids 150-185 of SEQ ID NO:26; and an extracellular domain 4 comprising at least 8 consecutive amino acids selected from amino acids 249-257of SEQ ID NO:2; amino acids 256-264 of SEQ ID NO:4; amino acids 256-265 of SEQ ID NO:6; amino acids 253-261 of SEQ ID NO:8; amino acids 257-265 of SEQ ID NO:10; amino acids 253-262 of SEQ ID NO:12; amino acids 251-260 of SEQ ID NO:14; amino acids 252-261 of SEQ ID NO:16; amino acids 268-277 of SEQ ID NO:18; amino acids 255-264 of SEQ ID NO:20; amino acids 251-260 of SEQ ID NO:22; amino acids 251-259 of SEQ ID NO:24; and amino acids 254-263 of SEQ ID NO:26.

[00115] Also disclosed are polynucleotides encoding the polypeptide comprising at least one extracellular domain of a feline TAS2R receptor; at least one transmembrane domain of a feline TAS2R receptor; or at least one intracellular domain of a feline TAS2R receptor.

[00116] In another aspect, chimeric polypeptides comprising an extracellular domain, an intracellular domain, or a transmembrane region of a feline TAS2R receptor polypeptide, and further comprising a heterologous polypeptide are disclosed. The intracellular domain, extracellular domain, or the transmembrane region of the feline TAS2R receptor polypeptide can be any of those disclosed herein.

[00117] The heterologous polypeptide can be any suitable polypeptide known in the art, or a portion of such polypeptide as may be useful herein. The heterologous polypeptide can be, for example, a sequence to determine cellular localization and expression, to permit proper folding of the chimeric polypeptide in an expression system, and/or to facilitate isolation of the chimeric polypeptide. The heterologous polypeptide can be linked to any portion of the chimeric polypeptide, for example to the amino terminal end or the carboxy terminal end of the fTAS2R sequence. For example, the heterologous polypeptide can be the first 45 amino acids of rat somatostatin, the FLAG® tag, a 6x histidine (his) tag, MYC, a fluorescent protein tag, V5, and/or glutathione S-transferase (GST). When the heterologous polypeptide is the first 45 amino acids of rat somatostatin, it is typically placed at the amino terminal end of the chimeric polypeptide to permit membrane targetting. When the heterologous polypeptide is a tag to permit easier isolation of the chimeric polypeptide, e.g., a 6x histidine tag, it can be placed at the amino terminus of the chimeric polypeptide. Determination of a suitable location for the heterologous polypeptide in the chimeric polypeptide relative to the amino end or the carboxy end of the fTAS2R sequence to obtain a particular functional aspect of the heterologous polypeptide on the chimeric polypeptide can be made by one of skill in the art.

[00118] Also disclosed are polynucleotides encoding the chimeric polypeptides.

[00119] Also disclosed is a composition comprising at least two fTAS2R polypeptides disclosed herein. In an embodiment, the composition comprises at least 3, 4, or 5 polypeptides disclosed herein. In an embodiment, the composition comprises at least 6, 7, 8, 9, 10, 11, 12, or 13 polypeptides disclosed herein. In an embodiment each polypeptide in the composition is a different fTAS2R receptor. In an embodiment, the composition comprises a polypeptide comprising SEQ ID NO:18 and a polypeptide comprising SEQ ID NO:22.

[00120] Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof. A conservative amino acid

substitution in a polypeptide sequence includes the substitution of an amino acid in one class by an amino acid of the same class, where a class is defined by common physicochemical amino acid side chain properties and high substitution frequencies in homologous proteins found in nature, as determined, for example, by a standard Dayhoff frequency exchange matrix or BLOSUM matrix. Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution. One of skill in the art can readily determine regions of the molecule of interest that can tolerate change by reference to Hopp/Woods and Kyte- Doolittle plots.

[00121] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

[00122] Percent identity (homology) can be determined by a direct comparison of the sequence information between two molecules by aligning the sequences, counting the exact number of matches between the two aligned sequences, dividing by the length of the shorter sequence, and multiplying the result by 100. Readily available computer programs can be used to aid in the analysis, such as ALIGN (Dayhoff, M.O. in Atlas of Protein Sequence and Structure M.O. Dayhoff ed., 5 Suppl. 3:353-358, National Biomedical Research Foundation, Washington, DC), which adapts the local homology algorithm of Smith and Waterman 1981 Advances in Appl Math 2:482-489, for peptide analysis. Programs for determining nucleotide sequence identity are available in the Wisconsin Sequence Analysis Package, Version 8 (available from Genetics Computer Group, Madison, WI) for example, the BESTFIT, FASTA and GAP programs, which also rely on the Smith and Waterman algorithm. These programs are readily utilized with the default parameters recommended by the manufacturer and described in the Wisconsin Sequence Analysis Package referred to above. For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions.

[00123] Alternatively, nucleotide homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s), and size determination of the digested fragments. DNA sequences that are substantially homologous can be identified in a Southern hybridization experiment under, for example, stringent conditions, as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y. (1989) or Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1-6.3.6, 1991. In an embodiment, high stringency conditions are 6X SSC (1X SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7) at 45° C., followed by a wash in 0.2X SSC, 0.1% SDS at 65° C or an equivalent thereto. Moderate hybridization conditions are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30° C., followed by a wash in 1X SSC, 0.1% SDS at 50° C. Highly stringent conditions are known in the art, and for purposes herein, include conditions equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45° C., followed by a wash in 0.2X SSC, 0.1% SDS at 65° C.

[00124] Disclosed herein is an expression vector comprising a polynucleotide encoding a feline TAS2R polypeptidedisclosed herein, or a fragment thereof. In an embodiment, the recombinant vector comprises a polynucleotide consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, or 25; a polynucleotide consisting of the complement of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, or 25; or a polynucleotide consisting of a sequence having at least 90%, at least 95%, at least 97%, at least 98%, at least 99% homology with SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or the complement of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, or 25. In an embodiment, the recombinant vector comprises a polynucleotide comprising a nucleotide sequence selected from: the nucleotide sequence of SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25; a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; a nucleotide sequence that hybridizes to the complement of the polynucleotide having SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25 under high stringency conditions; and the complement of the foregoing nucleotide sequences. In an embodiment, the vector comprises a polynucleotide sequence of SEQ ID

NO:17 or SEQ ID NO:21. Also disclosed is an expression vector comprising a polynucleotide encoding a chimeric polypeptide disclosed herein.

[00125] The recombinant vector may be constructed for use in prokaryotic or eukaryotic host cells. For example, when a prokaryotic cell is used as a host cell, the expression vector used generally includes a strong promoter capable of initiating transcription (for example, pLλ promoter, *trp* promoter, *lac* promoter, *tac* promoter, T7 promoter), a ribosome binding site for initiating translation, and a transcription/translation termination sequence. When a eukaryotic cell is used as a host cell, the vector used generally includes the origin of replication acting in the eukaryotic cell, for example f1 origin of replication, SV40 origin of replication, pMB1 origin of replication, adeno origin of replication, AAV origin of replication, or BBV origin of replication, but is not limited thereto. A promoter in an expression vector for a eukaryotic host cell may be a promoter derived from the genomes of mammalian cells (for example, a metallothionein promoter or an EF-1alpha promoter) or a promoter derived from mammalian viruses (for example, an adenovirus late promoter, a *Vaccinia* virus 7.5K promoter, a Sindbis promoter, a SV40 promoter, a cytomegalovirus promoter, and a *tk* promoter of HSV). A transcription termination sequence in an expression vector for a eukaryotic host cell may be, in general, a polyadenylation sequence.

[00126] Further disclosed is a host cell comprising an expression vector or a polynucleotide disclosed herein. A suitable host cell can be transformed with at least one of the recombinant vectors or at least one polynucleotide disclosed herein, for example a polynucleotide consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, or 25.

[00127] The host cell of the vector may be any cell that can be practically utilized by the expression vector. For example, the host cell may be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell. Further, the host cell may be a prokaryotic cell, such as a bacterial cell. A prokaryotic host cell may be a *Bacillus* genus bacterium, such as *E. coli* JM109, *E. coli* BL21, *E. coli* RR1, *E. coli* LE392, *E. coli* B, *E. coli* X 1776, *E. coli* W3110, *Bacillus subtilis*, and *Bacillus thuringiensis*; or an intestinal bacterium, such as *Salmonella typhimurium*, *Serratia marcescens*, and various *Pseudomonas* species. A eukaryotic host cell may be a yeast (e.g., *Saccharomyces cerevisiae*), an insect cell, a plant cell, or an animal cell, for example, mouse Sp2/0, CHO (Chinese hamster ovary) K1, CHO DG44, PER.C6, W138, BHK, COS-7, 293, HepG2, Huh7, 3T3, RIN, HeLa, HEK-293, or a MDCK cell line. In some embodiments, fish cells are useful herein.

[00128] The polynucleotide or recombinant vector including the polynucleotide may be transferred into the host cell using a method known in the art. For example, when a

prokaryotic cell is used as the host cell, the transfer may be performed using a $CaCl_2$ method or an electroporation method, and when a eukaryotic cell is used as the host cell, the transfer may be performed by microinjection, calcium phosphate precipitation, electroporation, liposome-mediated transfection, LIPOFECTAMINE® (Life Technologies Corporation) transfection, or gene bombardment, but is not limited thereto.

[00129] After the expression vector is introduced into the cells, the transfected cells can be cultured under conditions favoring expression of the fTAS2R. The fTAS2R can be recovered from the culture using standard techniques known in the art.

[00130] The expression vectors disclosed herein are particularly useful for assays to identify and characterize tastants. Means to introduce/express the nucleic acids and vectors, either individually or as libraries, are well known in the art. A variety of individual cell, organ, or whole animal parameters can be measured by a variety of means. The disclosed fTAS2R sequences can be expressed, for example, in animal taste tissues by delivery with a transmissible agent, e.g., adenovirus expression vector.

[00131] Nucleic acid assays for the presence of DNA and RNA for a TAS2R family member in a sample include numerous techniques known to those skilled in the art, such as Southern analysis, Northern analysis, dot blots, RNase protection, S1 analysis, amplification techniques such as polymerase chain reaction (PCR) and ligase chain reaction (LCR), and in situ hybridization. In addition, a TAS2R protein can be detected with the various immunoassay techniques known in the art. The test sample is typically compared to both a positive control (e.g., a sample expressing a recombinant TAS2R protein) and a negative control.

[00132] The nucleic acid and amino acid sequence information disclosed herein also makes possible identification of binding partner compounds with which a TAS2R polypeptide or polynucleotide will interact. Methods to identify binding partner compounds include solution assays, *in vitro* assays wherein TAS2R polypeptides are immobilized, and cell-based assays.

[00133] Specific binding molecules, including natural ligands and synthetic compounds, can be identified or developed using isolated or recombinant TAS2R products, TAS2R variants, or cells expressing such products. Binding partners are useful for purifying TAS2R products and detection or quantification of TAS2R products in fluid and tissue samples using known immunological procedures. Binding molecules are also useful in modulating (i.e., blocking, inhibiting or stimulating) biological activities of TAS2R, especially those activities involved in signal transduction. Binding molecules also are useful in methods for predicting

the taste perception of an organism such as a mammal by detecting a TAS2R polypeptide in a biological sample of a feline.

[00134] Methods to identify compounds that bind and/or modulate fTAS2R receptors are disclosed.

[00135] In an embodiment, the method comprises contacting TAS2R receptor with a test compound suspected of binding TAS2R receptor; and detecting binding between the compound and the TAS2R receptor. Binding can be determined by any binding assay known to the skilled artisan, including gel-shift assays, Western blots, radiolabeled competition assay, phage-based expression cloning, co-fractionation by chromatography, co-precipitation, cross-linking, interaction trap/two-hybrid analysis, southwestern analysis, and ELISA. The methods may also use ligands that are attached to a label, such as a radiolabel (e. g., ^{125}I, ^{35}S, ^{32}P, ^{33}P, ^{3}H), a fluorescence label, a chemiluminescent label, an enzymic label, and an immunogenic label. In one variation, a composition comprising a cell expressing TAS2R receptor on its surface is used in the method. In another variation, isolated TAS2R receptor or cell membranes comprising TAS2R receptor are employed. The binding may be measured directly, e. g., by using a labeled compound, or may be measured indirectly. Compounds identified as binding a TAS2R receptor may be further tested in other assays including TAS2R activity assays and/or *in vivo* models, in order to confirm or quantitate their activity.

[00136] Ligand binding to a TAS2R protein, a domain, or chimeric protein can be tested in solution, in a bilayer membrane, attached to a solid phase, in a lipid monolayer, or in vesicles. Ligand binding to a TAS2R receptor can be tested using, e.g., changes in spectroscopic characteristics (e.g., fluorescence, absorbance, refractive index), or in hydrodynamic (e.g., shape), chromatographic, or solubility properties.

[00137] The TAS2R polypeptide or polynucleotide employed in such a test may either be free in solution, attached to a solid support, borne on a cell surface, located intracellularly, or associated with a portion of a cell. One skilled in the art can, for example, measure the formation of complexes between a TAS2R receptor or polynucleotide and the compound being tested. Alternatively, one skilled in the art can examine the diminution in complex formation between a TAS2R receptor or polynucleotide and its substrate caused by the compound being tested. In some embodiments, the recognition sites of the TAS2R receptor or polynucleotide are coupled with a monitoring system, either electrical or optical. An appropriate chemical stimulus can bind to the receptor's ligand binding domain, changing the receptor conformation to a degree that the coupled electronics or optical changes can be observed on a read-out. In an embodiment, the solid support is formulated into a feline-

specific electronic tongue or biosensor.

[00138] In an embodiment of a solution assay, the methods can comprise the steps of contacting a TAS2R receptor with one or more test compound and identifying the compounds that bind to the TAS2R receptor. Identification of the compounds that bind the TAS2R receptor can be achieved by isolating the TAS2R polypeptide/binding partner complex, and separating the binding partner compound from the TAS2R polypeptide. In one aspect, the TAS2R polypeptide/binding partner complex is isolated using an antibody immunospecific for either the TAS2R receptor or the test compound. In still other embodiments, either the TAS2R receptor or the test compound comprises a label or tag that facilitates its isolation, and methods to identify binding partner compounds include a step of isolating the TAS2R polypeptide/binding partner complex through interaction with the label or tag.

[00139] In one variation of an *in vitro* assay, the method comprises the steps of contacting an immobilized TAS2R receptor with a test compound and detecting binding of the test compound to the TAS2R receptor. In an alternative embodiment, the test compound is immobilized and binding of TAS2R receptor is detected. Immobilization is accomplished using any of the methods well known in the art, including covalent bonding to a support, a bead, or a chromatographic resin, as well as non-covalent, high affinity interactions such as antibody binding, or use of streptavidin/biotin binding wherein the immobilized compound includes a biotin moiety. The support may, for example, be formulated into a feline-specific electronic tongue or biosensor.

[00140] In another embodiment, cell-based assays are used to identify binding partner compounds of a TAS2R receptor. In one embodiment, the method comprises the steps of contacting a TAS2R receptor expressed on the surface of a cell with a test compound and detecting binding of the test compound to the TAS2R receptor. In some embodiments, the detection comprises detecting a physiological event in the cell caused by the binding of the molecule.

[00141] In another embodiment, high throughput screening (HTS) for compounds having suitable binding affinity to TAS2R receptor is employed. Briefly, large numbers of different test compounds are synthesized on a solid substrate. The test compounds are contacted with TAS2R receptor and washed. Bound TAS2R receptor is then detected by methods well known in the art. Purified polypeptides of the invention can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the protein and immobilize it on the solid support.

[00142] Generally, an expressed TAS2R receptor can be used for HTS binding assays in

conjunction with a ligand, such as an amino acid or carbohydrate. The identified ligand is labeled with a suitable radioisotope, including, ^{121}I, ^{3}H, ^{35}S or ^{32}P, by methods that are well known to those skilled in the art. Alternatively, the ligands may be labeled by well-known methods with a suitable fluorescent derivative (Baindur et al., Drug Dev. Res., 1994, 33, 373-398; Rogers, Drug Discovery Today, 1997,2, 156-160). Radioactive ligand specifically bound to the receptor in membrane preparations made from the cell line expressing the recombinant protein can be detected in HTS assays in one of several standard ways, including filtration of the receptor-ligand complex to separate bound ligand from unbound ligand. Alternative methods include a scintillation proximity assay (SPA) or a FlashPlate format in which such separation is unnecessary. Binding of fluorescent ligands can be detected in various ways, including fluorescence energy transfer (FRET), direct spectrophotofluorometric analysis of bound ligand, or fluorescence polarization.

[00143] In still other embodiments, either the TAS2R receptor or the test compound comprises a label or tag that facilitates its isolation, and methods to identify test compounds include a step of isolating the TAS2R polypeptide/test compound complex through interaction with the label or tag. An exemplary tag of this type is a poly-histidine sequence, generally around six histidine residues, that permits isolation of a compound so labeled using nickel chelation. Other labels and tags, such as the FLAG tag (Eastman Kodak, Rochester, NY), are well known and routinely used in the art.

[00144] Detection of binding can be accomplished using a radioactive label on the compound that is not immobilized, using a fluorescent label on the non-immobilized compound, using an antibody immunospecific for the non-immobilized compound, using a label on the non- immobilized compound that excites a fluorescent support to which the immobilized compound is attached, as well as other techniques well known and routinely practiced in the art.

[00145] Other assays may be used to identify specific ligands of a TAS2R receptor, including assays that identify ligands of the target protein through measuring direct binding of test ligands to the target, as well as assays that identify ligands of target proteins through affinity ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Alternatively, such binding interactions are evaluated indirectly using the yeast two- hybrid system, a genetic assay for detecting interactions between two proteins or polypeptides.

[00146] In any of the methods disclosed herein, to be considered a ligand of the TAS2R receptor polypeptide, the test compound must alter the measured interaction by an amount

sufficient to achieve a statistically significant difference between the responses in the presence vs. the absence of the test compound. In an embodiment, to be considered a ligand, the test compound must alter the measured interaction by an amount sufficient to achieve a statistically significant difference between the responses in the presence vs. the absence of the test compound. Statistical significance can be determined by any appropriate statistical test known in the art, such as a t-test. For example, to be of statistical significance, the p-value is at least 0.05, at least 0.01, or at least 0.001.

[00147] Also disclosed are methods of identifying compounds that modulate (i.e., increase or decrease) activity of TAS2R receptor comprising contacting a TAS2R receptor with a compound, and determining whether the compound modifies activity of TAS2R receptor. In another embodiment, the method comprises contacting a TAS2R receptor with a known TAS2R receptor ligand in the presence or absence of a test compound. The activity in the presence of the test compound is compared to the activity in the absence of the test compound. Where the activity of the sample containing the test compound is higher than the activity in the sample lacking the test compound, the compound is an agonist. Similarly, where the activity of the sample containing the test compound is lower than the activity in the sample lacking the test compound, the compound is an antagonist.

[00148] In an embodiment, TAS2R protein activity is measured by expressing a TAS2R gene in a heterologous cell with a promiscuous G-protein that links the receptor to a phospholipase C signal transduction pathway (see Offermanns & Simon, J. Biol. Chem. 270:15175-15180 (1995)). Optionally the cell line is a eukaryotic cell line which does not naturally express TAS2R genes (e.g., Life Technologies Cat# R700-07) and the promiscuous G-protein is Gα15 (Offermanns & Simon, supra).

[00149] In an embodiment, a TAS2R polypeptide is expressed in a eukaryotic cell as a chimeric receptor with a heterologous, chaperone sequence that facilitates its maturation, targeting through the secretory pathway or membrane localization. In a preferred embodiment, the heterologous sequence is a rhodopsin sequence, such as an N-terminal fragment of a rhodopsin. Such chimeric TAS2R receptors can be expressed in any eukaryotic cell, such as Life Technologies Cat# R700-07 cells. Preferably, the cells comprise a functional G protein, e.g., Gα15, that is capable of coupling the receptor to an intracellular signaling pathway or to a signaling protein such as phospholipase Cβ. Activation of such expressed receptors in such cells can be detected using any standard method, such as by detecting changes in intracellular calcium by detecting FURA-2 dependent fluorescence in the cell.

[00150] In another embodiment, transcription levels can be measured to assess the effects of a test compound on signal transduction. A host cell containing a TAS2R protein of interest is contacted with a test compound for a sufficient time to effect any interactions, and then the level of gene expression is measured. The amount of time to effect such interactions may be empirically determined, such as by running a time course and measuring the level of transcription as a function of time. The amount of transcription may be measured by using any method known to those of skill in the art to be suitable. For example, mRNA expression of the protein of interest may be detected using Northern blots or their polypeptide products may be identified using immunoassays. Alternatively, transcription based assays using a reporter gene may be used as described in U.S. Pat. 5,436,128. The reporter genes can be, e.g., chloramphenicol acetyltransferase, luciferase, [beta]-galactosidase and alkaline phosphatase. Furthermore, the protein of interest can be used as an indirect reporter via attachment to a second reporter such as green fluorescent protein (see, e.g., Mistili & Spector, Nature Biotechnology 15:961-964 (1997)). The amount of transcription is then compared to the amount of transcription in either the same cell in the absence of the test compound, or it may be compared with the amount of transcription in a substantially identical cell that lacks the protein of interest. A substantially identical cell may be derived from the same cells from which the recombinant cell was prepared but which had not been modified by introduction of heterologous DNA. Any difference in the amount of transcription indicates that the test compound has in some manner altered the activity of the protein of interest.

[00151] In an embodiment, a method for identifying an agonist of a feline TAS2R receptor comprises contacting a feline Tas2R receptor polypeptide disclosed herein with a test compound; and detecting an increase in biological activity of the receptor in the presence of the compound relative to biological activity of the polypeptide in the absence of the compound.

[00152] In an embodiment, a method for identifying an antagonist of a feline Tas2R receptor comprises contacting a feline Tas2R receptor polypeptide disclosed herein with a test compound; and detecting a decrease in biological activity of the receptor in the presence of the compound relative to biological activity of the polypeptide in the absence of the compound.

[00153] Receptor-G-protein interactions can also be examined. For example, binding of the G-protein to the receptor or its release from the receptor can be examined. For example, in the absence of GTP, an agonist will lead to the formation of a tight complex of a G protein

(all three subunits) with the receptor. This complex can be detected in a variety of ways, as noted above. Such an assay can be modified to search for antagonists, e.g., by adding an agonist to the receptor and G protein in the absence of GTP, which form a tight complex, and then screen for antagonists by looking at dissociation of the receptor-G protein complex. In the presence of GTP, release of the alpha subunit of the G protein from the other two G protein subunits serves as a criterion of activation.

[00154] In some embodiments, TAS2R-Gustducin interactions are monitored as a function of TAS2R receptor activation. Ligand dependent coupling of TAS2R receptors with Gustducin can be used as a marker to identify modifiers of any member of the TAS2R family.

[00155] An activated or inhibited G-protein will in turn alter the properties of target enzymes, channels, and other effector proteins. The classic examples are the activation of cGMP phosphodiesterase by transducin in the visual system, adenylate cyclase by the stimulatory G-protein, phospholipase C by Gq and other cognate G proteins, and modulation of diverse channels by Gi and other G proteins. Downstream consequences can also be examined such as generation of diacyl glycerol and IP3 by phospholipase C, and in turn, for calcium mobilization by IP3.

[00156] Receptor activation typically initiates subsequent intracellular events, e.g., increases in second messengers such as IP3, which releases intracellular stores of calcium ions. Activation of some G-protein coupled receptors stimulates the formation of inositol triphosphate (IP3) through phospholipase C-mediated hydrolysis of phosphatidylinositol (Berridge & Irvine, Nature 312:315-21 (1984)). IP3 in turn stimulates the release of intracellular calcium ion stores. Thus, a change in cytoplasmic calcium ion levels, or a change in second messenger levels such as IP3 can be used to assess G-protein coupled receptor function. Cells expressing such G-protein coupled receptors may exhibit increased cytoplasmic calcium levels as a result of contribution from both intracellular stores and via activation of ion channels, in which case it may be desirable although not necessary to conduct such assays in calcium-free buffer, optionally supplemented with a chelating agent such as EGTA, to distinguish fluorescence response resulting from calcium release from internal stores. The generation of IP3 can be measured using various commercially available kits. Some exemplary kits to detect the generation of IP3 use antibodies specific for IP3 which can detect IP3 in a cell lysate in a western blot or an ELISA; alternatively the antibodies are fluorescently labeled and detected using a plate reader.

[00157] Modulation of receptor activity (taste transduction) can be assayed by measuring

changes in intracellular Ca2+ levels, which change in response to modulation of the TAS2R signal transduction pathway via administration of a molecule that associates with a TAS2R protein. Changes in Ca2+ levels are optionally measured using fluorescent Ca2+ indicator dyes and fluorometric imaging.

[00158] In an embodiment, assays for G-protein coupled receptors include cells that are loaded with ion or voltage sensitive dyes to report receptor activity. Assays for determining activity of such receptors can also use known agonists and antagonists for other G-protein coupled receptors as positive or negative controls to assess activity of tested compounds. In assays for identifying modulatory compounds (e.g., agonists, antagonists, modulators), changes in the level of ions in the cytoplasm or membrane voltage will be monitored using an ion sensitive or membrane voltage fluorescent indicator, respectively. Ion-sensitive indicators and voltage probes that may be employed are commercially available from a variety of sources. For G-protein coupled receptors, promiscuous G-proteins such as Gα15 and Gα16 can be used in the assay of choice. Such promiscuous G-proteins allow coupling of a wide range of receptors.

[00159] Activated GPCR proteins become substrates for kinases that phosphorylate the C-terminal tail of the receptor (and possibly other sites as well). Thus, agonists will promote the transfer of ^{32}P from gamma-labeled GTP to the receptor, which can be assayed with a scintillation counter. The phosphorylation of the C-terminal tail will promote the binding of arrestin-like proteins and will interfere with the binding of G-proteins. The kinase/arrestin pathway plays a key role in the desensitization of many GPCR proteins. For example, compounds that modulate the duration a taste receptor stays active would be useful as a means of prolonging a desired taste or cutting off an unpleasant one.

[00160] Changes in ion flux may be assessed by determining changes in polarization (i.e., electrical potential) of the cell or membrane expressing a TAS2R protein. One means to determine changes in cellular polarization is by measuring changes in current (thereby measuring changes in polarization) with voltage-clamp and patch-clamp techniques, e.g., the "cell-attached" mode, the "inside-out" mode, and the "whole cell" mode. Whole cell currents are conveniently determined using standard methodology known in the art. Other known assays include: radiolabeled ion flux assays and fluorescence assays using voltage-sensitive dyes. Generally, the compounds to be tested are present in the range from 1 pM to 100 mM.

[00161] Other assays can involve determining the activity of receptors which, when activated, result in a change in the level of intracellular cyclic nucleotides, e.g., cAMP or cGMP, by activating or inhibiting enzymes such as adenylate cyclase. There are cyclic

nucleotide-gated ion channels, e.g., rod photoreceptor cell channels and olfactory neuron channels that are permeable to cations upon activation by binding of cAMP or cGMP (see, e.g., Altenhofen et al., Proc. Natl. Acad. Sci. U.S.A. 88:9868-9872 (1991) and Dhallan et al., Nature 347:184-187 (1990)). In cases where activation of the receptor results in a decrease in cyclic nucleotide levels, it may be preferable to expose the cells to agents that increase intracellular cyclic nucleotide levels, e.g., forskolin, prior to adding a receptor-activating compound to the cells in the assay. Cells for this type of assay can be made by co-transfection of a host cell with DNA encoding a cyclic nucleotide-crated ion channel, GPCR phosphatase and DNA encoding a receptor (e.g., certain glutamate receptors, muscarinic acetylcholine receptors, dopamine receptors, serotonin receptors, and the like), which, when activated, causes a change in cyclic nucleotide levels in the cytoplasm.

[00162] In one embodiment, the changes in intracellular cAMP or cGMP can be measured using immunoassays. The method described in Offermanns & Simon, J. Biol. Chem. 270:15175-15180 (1995) may be used to determine the level of cAMP. Also, the method described in Felley-Bosco et al., Am. J. Resp. Cell and Mol. Biol. 11:159-164 (1994) may be used to determine the level of cGMP. Further, an assay kit for measuring cAMP and/or cGMP is described in U.S. Pat. No. 4,115,538.

[00163] In another embodiment, phosphatidyl inositol (PI) hydrolysis can be analyzed according to U.S. Pat. No. 5,436,128. Briefly, the assay involves labeling of cells with 3H-myoinositol for 48 or more hrs. The labeled cells are treated with a test compound for one hour. The treated cells are lysed and extracted in chloroform-methanol-water after which the inositol phosphates were separated by ion exchange chromatography and quantified by scintillation counting. Fold stimulation is determined by calculating the ratio of counts per minute (cpm) in the presence of agonist to cpm in the presence of buffer control. Likewise, fold inhibition is determined by calculating the ratio of cpm in the presence of antagonist to cpm in the presence of buffer control (which may or may not contain an agonist).

[00164] The effects of the test compounds upon the function of the polypeptides can be measured by examining any of the parameters described above. Any suitable physiological change that affects GPCR activity can be used to assess the influence of a test compound on the polypeptides disclosed herein. When the functional consequences are determined using intact cells, animals or animal behavior, one can also measure a variety of effects such as neurotransmitter release, hormone release, transcriptional changes to both known and uncharacterized genetic markers (e.g., Northern blots), changes in cell metabolism such as

cell growth or pH changes, and changes in intracellular second messengers such as Ca^{2+}, IP3, cGMP, or cAMP.

[00165] Samples or assays that are treated with a test compound that is a potential TAS2R agonist are compared to control samples without the test compound, to examine the extent of modulation. Activation of a TAS2R protein is achieved when the TAS2R activity value relative to the control is 110%, optionally 150%, 200-500%, or 1000-2000%.

[00166] Samples or assays that are treated with a known agonist and a test compound that is a potential TAS2R antagonist are compared to control samples treated with the known agonist without the test compound, to examine the extent of modulation. The control samples are assigned a relative value of 100%. Inhibition of a TAS2R protein is achieved when the TAS2R activity value relative to the control is about 90%, optionally 50%, optionally 25-0%.

[00167] Agents that modulate TAS2R receptor activity or expression also may be identified, for example, by incubating a putative modulator with a cell containing a TAS2R polypeptide or polynucleotide and determining the effect of the putative modulator on TAS2R receptor activity or expression. In an embodiment, to be considered a modulator, the putative modulator must alter the measured interaction by an amount sufficient to achieve a statistically significant difference between the responses in the presence vs. the absence of the putative modulator. Statistical significance can be determined by any appropriate statistical test known in the art, such as a t-test. For example, to be of statistical significance, the p-value is at least 0.05, at least 0.01, or at least 0.001. The selectivity of a compound that modulates the activity of TAS2R receptor can be evaluated by comparing its effects on TAS2R receptor to its effect on other TAS2R receptors. Selective modulators may include, for example, antibodies and other proteins, peptides, or organic molecules that specifically bind to a TAS2R polypeptide or a TAS2R receptor-encoding nucleic acid. Compounds identified as modulating TAS2R receptor activity may be further tested in other assays including *in vivo* models, in order to confirm or quantitate their activity.

[00168] TAS2R polynucleotides and polypeptides, and their homologs, are useful tools for identifying taste receptor expressing cells, for taste perception, and for examining taste transduction. TAS2R family member-specific reagents that specifically hybridize to TAS2R nucleic acids, such as TAS2R probes and primers, and TAS2R specific reagents that specifically bind to a TAS2R protein, e.g., TAS2R antibodies are used to examine taste cell expression and taste transduction regulation. For example, a TAS2R antibody can be used to identify and/or isolate feline taste cells expressing the particular TAS2R from a mixed feline cell population. For example, polynucleotide probes disclosed herein may be used in tissue

distribution studies and diagnostic assays.

[00169] Also provided are kits for screening for modulators of TAS2R family members. Such kits can be prepared from readily available materials and reagents. For example, such kits can comprise any one or more of the following materials: TAS2R nucleic acids or proteins, reaction tubes, and instructions for testing TAS2R activity. Optionally, the kit contains a biologically active TAS2R receptor. A wide variety of kits and components can be prepared, depending upon the intended user of the kit and the particular needs of the user.

[00170] Antibodies to the fTAS2R receptors and the chimeric polypeptides are also disclosed.

[00171] For preparation of monoclonal or polyclonal anti-fTAS2R antibodies, any technique known in the art can be used. Techniques for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce antibodies to polypeptides disclosed herein. Also, transgenic mice, or other organisms such as other mammals, may be used to express humanized antibodies. Alternatively, phage display technology can be used to identify antibodies and heteromeric Fab fragments that specifically bind to selected antigens. In one embodiment isolated DNA sequences encoding a monoclonal antibody or a binding fragment thereof are obtained by screening a DNA library from human B cells according to the general protocol outlined by Huse et al., Science 246:1275-1281 (1989).

[00172] Monoclonal antibodies and polyclonal sera can be collected and titered against the protein immunogen in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Typically, polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against non-TAS2R proteins, or even other TAS2R family members or other related proteins from other organisms, using a competitive binding immunoassay. Specific polyclonal antisera and monoclonal antibodies will usually bind with a K_d of at least about 0.1 mM, more usually at least about 1 μM, specifically at least about 0.1 μM or better, and more specifically 0.01 μM or better.

[00173] Immunoassays can be used to detect, qualitatively or quantitatively, a fTAS2R, e.g., to identify taste receptor cells, especially bitter taste receptor cells, and variants of TAS2R family members.

[00174] The anti-fTAS2R antibodies can also be used to isolate feline taste cells from a mixed population of cells obtained from a feline. In an embodiment, isolation of the feline taste cells bound to the anti-fTAS2R antibody can be achieved by flow cytometry. Other methods known in the art can also be used.

[00175] As known in the art, taste behavior can be determined in a short term assay

which directly measures taste preferences by counting licking responses of an animal, e.g., a mouse, using a multi-channel gustometer (e.g., the Davis MS160-Mouse gustometer, DiLog instruments, Tallahassee, FL). The mean rate that a mouse will lick a tastant relative to their sampling of an appropriate control (ratio defined as lick rate relative to control) indicates whether the stimulus is appetitive, neutral or aversive. In addition, the change in intake of a palatable stimulus can be evaluated in the presence of the test stimulus to assess enhancement or suppression of the palatable stimulus.

[00176] In a further embodiment, animals can be trained to discriminate qualitatively distinct stimuli using operant testing methods known in the art. These animals can then be used to determine qualitative similarity between two stimuli, regardless of palatability or preference.

[00177] To determine if the fTAS2R receptors are activating brain areas reported to be involved in appetitive or aversive taste responses, electrodes may be attached to these brain areas and animals tested in an awake or anesthetized state.

[00178] Alternatively, other noninvasive methods to monitor neural activity such as positron emission tomography (PET) or electroencephalography may be used to monitor neural activity associated with appetitive or aversive taste responses. Such methods may also be used to evaluate the impact of various factors such as age, experience or nutritional state on neural activity elicited by stimuli identified in cell-based experiments to modify receptor function.

[00179] Also provided are kits comprising at least one composition, polypeptide, or nucleic acid disclosed herein, optionally contained in a single package. The kits may optionally include, e.g., instructions for use of the kit components in detecting a fTAS2R receptor or a polynucleotide encoding a fTAS2R receptor, or compounds altering the activity of a TAS2R receptor.

[00180] In an embodiment, the kit comprises at least one anti-TAS2R antibody disclosed herein and reagents for detecting a complex between the antibody and the TAS2R antigen. For example, the kit can include a buffer that enables binding reaction between the antibody and the TAS2R antigen in a biological sample, or components for producing the buffer.

[00181] The activity of TAS2R polypeptides can be assessed using a variety of *in vitro* and *in vivo* assays to determine functional, chemical, and physical effects, e.g., measuring ligand binding (e.g., radioactive ligand binding), second messengers (e.g., cAMP, cGMP, IP3, DAG, or Ca^{2+}), ion flux, phosphorylation levels, transcription levels, neurotransmitter levels, and the like. Furthermore, such assays can be used to test for inhibitors and activators

of TAS2R family members. Such modulators of taste transduction activity are useful for customizing taste perception, for example to modify the detection of bitter tastes.

[00182] The TAS2R protein of the assay will typically be selected from a polypeptide having a sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26; a conservatively modified variant of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26; or a sequence that is at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26. In an embodiment, the polypeptide has a sequence of SEQ ID NO:18 or SEQ ID NO:22.

[00183] In some embodiments, the polypeptide of the assays will comprise a domain of a TAS2R protein, such as an extracellular domain, transmembrane region, transmembrane domain, cytoplasmic domain, ligand binding domain, subunit association domain, active site, and the like. Either the TAS2R protein or a domain thereof can be covalently linked to a heterologous protein to create a chimeric protein used in the assays described herein. In an embodiment, the polypeptide has a domain from SEQ ID NO:18 or SEQ ID NO:22.

[00184] Modulators of TAS2R receptor activity are tested using TAS2R polypeptides as described above, either recombinant or naturally occurring. The protein can be isolated, expressed in a cell, expressed in a membrane derived from a cell, expressed in tissue or in an animal, either recombinant or naturally occurring. For example, sections or dissociated cells from a TAS2R-expressing tissue, transformed cells, or membranes can be used. Assays may also be prepared using TAS2R polypeptides in artificial / synthetic membrane systems. Modulation is tested using any of the *in vitro* or *in vivo* assays described herein. Taste transduction can also be examined *in vitro* with soluble or solid state reactions, using a full-length TAS2R-GPCR or a chimeric molecule such as an extracellular domain or transmembrane region, or combination thereof, of a TAS2R receptor covalently linked to a heterologous signal transduction domain, or a heterologous extracellular domain and/or transmembrane region covalently linked to the transmembrane and/or cytoplasmic domain of a TAS2R receptor. Furthermore, ligand-binding domains of the protein of interest can be used *in vitro* in soluble or solid state reactions to assay for ligand binding. In numerous

embodiments, a chimeric receptor will be made that comprises all or part of a TAS2R polypeptide as well an additional sequence that facilitates the localization of the TAS2R to the membrane, such as a rhodopsin, e.g., an N-terminal fragment of a rhodopsin protein.

[00185] The compounds tested as modulators or ligands of a TAS2R family member can be any compound, including small molecules, or more complex molecules such as biological molecules, for example a protein, sugar, nucleic acid or lipid. Alternatively, modulators can be genetically altered versions of a TAS2R gene. Essentially any chemical compound can be used as a potential modulator or ligand in the assays and methods disclosed herein. In certain useful embodiments the compounds can be dissolved in aqueous or organic solutions (for example, DMSO solutions). The assays are designed to screen libraries of chemicals, including large libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays).

[00186] Knowledge of the structure of two or more agonists for a single receptor allows the skilled person to rationally design further libraries of compounds to screen for interaction with the receptor. Computer modeling of such compounds is also facilitated. Screening the compound libraries enables the development of compositions to suppress or eliminate bitter tasting components of food in particular animal foods, nutrients and dietary supplements and pharmaceutical or homeopathic preparations containing such phytochemicals. Alternatively the screen allows for the identification of structurally related agonists to enhance a bitter response in the production of appetite suppressants, animal repellents, and the like.

[00187] Flavor compositions, edible compositions, and methods of manufacturing the edible compositions and flavor compositions are disclosed herein

[00188] A flavor composition is a composition which can be added to an edible composition for an animal to improve acceptance of the edible composition for consumption by the animal. Examples of edible compositions include foods, treats, nutritional supplements, pharmaceuticals, oral care materials such as dental products, chewable products, drinkable products, and the like. The edible composition can be in the form of a tablet, capsule, caplet, edible film, wet food, liquid food, treat or kibble.

[00189] In one aspect, a flavor composition comprises a compound that is an agonist, antagonist, or modulator of a feline TAS2R receptor. In an embodiment; the flavor composition further comprises a palatability enhancer; optionally, an adhesive compound to help adhere the flavor composition to the edible composition; and optionally, a compound for providing color or aroma for a human, wherein the flavor composition is a solid, liquid,

powder, paste, gel, sprayable formulation or spreadable formulation. In an embodiment, the flavor composition is a coating composition and further comprises the adhesive compound. Alteration or masking of perceived bitterness of an edible composition can be tested using any of the behavioral assays for palatability disclosed herein, such as a standard two bowl comparison.

[00190] "Basal food composition," as used herein, refers to an animal food combinable with the flavor composition. In one embodiment, the animal food is formulated for felines, and includes dry food, canned food, semi-dry food, edible treats, and the like, and combinations comprising one or more of the foregoing foods. Various sizes and shapes of the basal food composition may be employed as long as the food is acceptably consumable by a recipient (such as an animal, particularly a feline) in an amount so that the animal receives a normal daily ration providing the known essential nutrients. A basal food composition may be uncoated, or may be coated, for example, with a coating comprising lipids. If desired, feeding may be carried out by feeding the animal one or more times per day.

[00191] In an embodiment, the flavor composition is combined with an edible composition, for example a basal food composition (e.g. for a feline), in an amount effective to impart increased palatability of the edible composition to the animal. Effective amounts of such flavor compositions are readily determined by one of ordinary skill in the art without undue experimentation, particularly in view of the general guidance provided below.

[00192] In an embodiment, the flavor composition may be combined with a basal food composition in a manner such that the flavor composition is incorporated into the basal food composition. By incorporated it is meant that the flavor composition is intimately associated with the edible composition and does not become substantially dissociated, for example, during normal storage conditions. In one embodiment, the flavor composition is substantially uniformly dispersed throughout the edible composition. In other embodiments, the distribution of the flavor composition may intentionally not be uniform. In such embodiments the flavor composition may provide bits or pieces that are intermixed with the basal food. In various embodiments, the flavor composition may be deposited in the edible composition in an amount effective to provide about 0.5 wt% to about to about 3 wt%, specifically about 0.8 wt% to about 2.5 wt%, and more specifically about 1 wt% to about 2 wt% of the dry weight of the edible composition.

[00193] In another embodiment, the flavor composition is deposited on the surface of the edible composition, for example in the form of a coating. Coating the edible composition

includes the topical deposition of the flavor composition onto the surface of the edible composition, such as by spraying, dusting, and the like. The coating comprising the flavor composition may comprise one or more fats to help adhere the flavor composition to the surface. It may further or alternatively comprise other components useful to facilitate adhesion of the flavor composition to the surface of the edible composition. It is possible, although not required, that the flavor composition be coated onto the edible composition uniformly or that uniform distribution of the flavor composition be achieved, for example, by repeatedly tumbling the coated food. One or more coats may be applied. The flavor composition may be deposited onto the surface of the edible composition in an amount effective to provide about 0.5 wt% to about to about 3 wt%, specifically about 0.8 wt% to about 2.5 wt%, and more specifically about 1 wt% to about 2 wt% of the dry weight of the basal animal food composition.

[00194] The flavor composition may be both dispersed in and coated onto the edible composition, such as a dry animal food composition. In one embodiment, the finished animal food product is packaged for sale and ultimately fed to the animal. In other embodiments, the flavor composition may be packaged for combination with a food prior to serving. In some embodiments, the animal is a feline.

[00195] In an embodiment, the flavor composition may further comprise an additional palatability enhancer such as a flavoring. Suitable flavorings include, for example, a vegetable flavoring, a meat flavoring, (e.g., liver flavoring), a cheese flavoring, yeast, sodium pyrophosphate, a fat, an acid phosphate, a phosphate salt, and/or other food or flavor ingredients utilized by the flavor industry in order to improve palatability. Suitable meat flavorings include, for example, meat-derived flavorings (e.g., beef, pork, bacon, lamb, ham, fish, chicken, turkey, and/or other poultry flavoring).

[00196] Palatability or acceptance of a food refers to the overall willingness of an animal, such as a feline, to eat a certain food. Developing preferred flavorants and palatability enhancers for animals such as pets is subjective. Flavorants which work for humans do not always work with felines. Similarly, a flavorant which is effective with one animal species may not work as well with a different animal species. The skilled artisan will appreciate that palatability testing is routinely used to determine preferences for animals with respect to food and flavorants. For purposes herein, such palatability testing will be effective and straight forward to implement for testing preferences for flavorants for any animal, including felines. Traditional methods of developing flavor compositions for increasing palatability employ a variety of candidate flavorants selected empirically, based on knowledge of how these

ingredients are perceived by humans, and a "trial and error" approach is used to empirically test each candidate relative to a known target product or to identify more preferable palatants. The disclosed feline TAS2R receptor polypeptides permit intentional design of palatability enhancers based on the taste receptors for the target species and will substantively improve and shorten the process for palatant development.

[00197] In one embodiment, the flavor composition is a palatant for a feline food and the flavor composition exhibits improved palatability for the feline compared to the feline food without the flavor composition, as measured by improved consumption of the feline food comprising the palatant compared to the animal food in the absence of the palatant.

[00198] The flavor composition may be used as a liquid flavor in either unconcentrated or concentrated form. If the flavor composition is to be a dry flavor composition, the flavor composition may be dried in a suitable dryer such as, for example, a spray dryer, or an oven. The flavor composition may comprise a variety of other useful components, for example, maltodextran, gum, or a combination which may be useful for providing the composition with one or more preferred functionalities such as the ability to bind to a food or to retain a desired texture, viscosity, flowability, color, aroma or the like. Such components and their uses will be readily understood by the skilled food scientist.

[00199] Palatability testing can be performed by a standard two bowl comparison. In this test, each animal is presented with two bowls of food, each containing a measured amount of either a control ration or a test ration. The control and test rations contain the same basal compositions. The animal is allowed to select the food it prefers. The amount of food eaten from each bowl is measured. A direct comparison of the amount eaten from the two rations gives a reliable indication of relative palatability.

[00200] For example, a feline may be given two bowls with equal amounts of food, one containing the flavor composition to be tested and the other not containing the flavor composition. The amount of food in the two bowls is weighed prior to giving them to the feline. During the test, steps should be taken to ensure that the feline does not finish one bowl and continue to the other because it is still hungry. This can be accomplished, for example, by limiting the time of the feline with the two bowls, or by providing enough food in each bowl to fully satisfy the feline.

[00201] At the end of the test, the two bowls are weighed again to determine the amount of food eaten from each bowl. If more food is eaten from the bowl with the test flavor composition (bowl A), the ingestion ratio is recorded as a positive value to indicate that the flavor composition had a positive effect on the animal preference. If more food was eaten

from the bowl with the control food (bowl B), the ratio is recorded as a negative value to indicate that the flavor composition did not perform as well as the control food.

[00202] For example, the flavor compositions are applied to a dry basal feline food composition and multiple felines, e.g., ten, are fed for a period of time (e.g. two days). The bowl position is changed daily to eliminate bias due to the animals showing a preference for right or left placement of the bowls. The preference of each animal for each bowl can be calculated as an intake ratio (IR) for that particular animal, for example the IR for animal 1 = (grams consumed from bowl A)/(total grams consumed from bowl A + bowl B). The average preference is calculated as the average value of each day for the duration of the test period. Thus an IR value close to 0.5 indicates equal preference. IR values greater than 0.5 and typically above 0.55 indicate preference. The degree of preference estimation based on IR scores can be determined by number of animals used and statistical analysis of the data.

[00203] A method for making the flavor composition for coating or incorporating into an edible composition to be administered to an animal is disclosed.

[00204] In an embodiment, the method comprises mixing an agonist, an antagonist, or a modulator of a feline TAS2R receptor polypeptide; optionally, a palatability enhancer; optionally, a compound to help adhere the flavor composition to the edible composition; and optionally, a compound for providing color or aroma with an ingredient selected from the group consisting of meat products, meat by-products, fish products, fish by-products, dairy products, dairy by-products, sources of microbial proteins, vegetable proteins, carbohydrates and amino acids carrier to obtain a flavor composition, wherein the flavor composition is a liquid, solid, powder, paste, gel, spreadable formulation, granule, or sprayable formulation. In an embodiment, an agonist or an antagonist of the feline TAS2R receptor polypeptide is mixed into the composition. In an embodiment, the agonist is denatonium, aloin, or PTC and the antagonist is probenecid.

[00205] To make a liquid flavor composition, for example, commercially available liquid ingredients are combined in a mixer with an agonist, an antagonist, or a modulator of a feline TAS2R receptor polypeptide. Wet ingredients are ground or emulsified to a slurry and the liquid ingredients are combined therewith. A commercially available protease may be added to the slurry to hydrolyze proteins, and later inactivated with heat, acid or another method. Preservatives such as sorbic acid can also be added. Water is added to adjust the viscosity and the solids content of the slurry to facilitate spray application.

[00206] A dry formulation of the flavor composition can be prepared by combining commercially available dry ingredients, including amino acids, inorganic salts and organic

materials with an agonist, an antagonist, or a modulator of a feline TAS2R receptor polypeptide in the desired proportions in a batch mixer and blending to homogeneity prior to drying.

[00207] According to another dry formulation embodiment, wet and dry ingredients are combined by mixing the wet ingredients with all or some of the dry ingredients in a mixer until a homogenous mixture is formed. The mixture is dried by evaporation or lyophilization, for example, to form a dry, powdery product that is then blended with any remaining dry ingredients in a tumbler until a homogeneous mixture is formed.

[00208] Methods of preparing an edible composition for an animal are disclosed.

[00209] In an embodiment, the method comprises contacting an edible composition or a component thereof with a fTAS2R receptor polypeptide disclosed herein for a time sufficient to reduce the amount of a bitter compound from the edible composition or component thereof. The time to reduce the amount of the bitter compound can be determined by one of skill in the art. The contacting can occur in a continuous, semi-continuous, or batch process. In an embodiment, the edible composition is for a feline

[00210] In an embodiment, the method comprises adding a compound to an edible composition to decrease the palatability of the edible composition to an animal, wherein the compound is an agonist or a positive modulator of a feline bitter taste receptor. In an embodiment, the palatability is decreased to an extent that a feline consumes 10 to 30% less of the edible compositionwith the added compound than the edible compositionwithout the added compound. In an embodiment, the decrease in palatability is measured as decrease in calories of edible composition consumed, weight of edible composition consumed, or volume of edible composition consumed.

[00211] A method of formulating an edible composition with enhanced palatability for an animal is disclosed.

[00212] In an embodiment, the method comprises determining the presence of a compound which is an agonist, antagonist, or modulator of a feline TAS2R receptor polypeptide in an edible composition; and enhancing palatability of the edible composition by if the compound is an agonist or a positive modulator, increasing the amount of an antagonist for the receptor in the edible composition or reducing the amount of the compound in the edible composition, or if the compound is an antagonist or a negative modulator, increasing the amount of the compound in the edible composition. The amount of the compound can be increased by applying a flavor composition comprising the compound to the edible composition such that the flavor composition is incorporated into or a leastleas partially coats

the edible composition.

[00213] Also disclosed are methods of administering a bitter compound to an animal (e.g. a feline) in need thereof. The skilled artisan will appreciate that in some cases a human or other animal may be in need of a bitter compound (e.g. a pharmaceutical, a nutrient, or the like) and that it can be challenging to administer the compound to the animal.

[00214] In an embodiment, the method comprises administering a feline edible composition to a feline, wherein the edible composition comprises a feline bitter compound and a compound that alters perceived bitterness of the edible composition, masks the bitter compound in the edible composition, or acts as an agonist, antagonist, or modulator of a feline TAS2R receptor in the feline to alter bitter taste perception by the feline. In an embodiment, the bitter compound comprises a therapeutic, a nutritional supplement or an oral care product. A nutritional supplement refers to a supplement intended to provide nutrients that may otherwise not be consumed in sufficient quantities and includes vitamins, minerals, fiber, probiotics, fatty acids, and amino acids. A therapeutic or pharmaceutical refers to a compound, element, or mixture that when administered to a subject, alone or in combination with another compound, element, or mixture, confers, directly or indirectly, a physiological effect on the subject. An oral care product refers to a product used to promote healthy teeth, gums, freshen breath or prevent or treat oral disease.

[00215] Methods of manufacturing feline edible compositions are also disclosed.

[00216] In an embodiment, the method comprises contacting a feline food composition or a component thereof with a TAS2R receptor polypeptide herein for a time sufficient to remove a bitter compound from the food product or component. In an embodiment, the TAS2R receptor is bound to a solid support that can be separated from the food composition. In an embodiment, the contacting is a continuous operation. In an embodiment, the food composition is contacted with a plurality of TAS2R receptor polypeptides.

[00217] In an embodiment, the method comprises determining the presence of one or more bitter compounds in an edible composition; determining a bitterness profile of the edible composition based on the one or more bitter compounds determined to be present; and adding a compound to or removing a compound from the edible composition to enhance the palatability of the edible composition, wherein the compound alters the bitterness profile of the edible composition, masks one or more of the bitter compounds present in the edible composition, or acts as an agonist, antagonist or modulator of a feline bitter taste receptor. In an embodiment, adding the compound to the edible composition comprises applying a coating solution to the edible composition comprising the compound such that a coating at

least partially surrounds the feline edible composition. In an embodiment, the edible composition is a basal food, a flavor composition, a treat, a therapeutic, or a nutritional supplement. The presence of a bitter compound in a edible composition can be determined by a method disclosed herein, or by any other method known in the art. A bitterness profile of an edible composition refers to an enumeration of bitter compounds determined to be present in the edible composition, and optionally further includes the amount of a given bitter compound in the edible composition. In an embodiment, the edible composition is for a feline.

[00218] Also disclosed are repellent compositions. In an embodiment, the repellent compositon can comprise a feline TAS2R receptor agonist or positive modulator in a sufficient amount to elicit rejection, for example at least 0.05% to about 30% by weight, and optionally aromatics or perfumes such as rosemary oil, mint oil, cinnamon oil, limonene, or eugenol, and one or more inert ingredients such as a liquid diluents, carriers, thickeners, surface-active agents, preservatives, aromatics, deodorizers, antibacterial agents, antifungal agents, antimicrobial agents, biocide agents, and one or more of several types of adjuvant including, but not limited to, wetting agents, spreading agents, sticking agents, foam retardants, buffers and acidifiers. Suitable liquid diluents include water, petroleum distillates, or other liquid carriers with or without surface active agents. Examples of carriers include bentonite, fullers earth, additional clays, talc, chalk, quartz, attapulgite, montmorillonite or diatomaceous earth, vermiculite, highly dispersed silicic acid, alumina and silicates, calcite, marble, pumice, sepiolite and dolomite, inorganic and organic meals, sawdust, coconut shells, corn cobs and tobacco stalks. In an embodiment, the repellent compositon can further comprise a propellant gas for dispensing as a spray, such as Figen 11/12 or propane/butane, e.g. in a ratio of 15:85. In an embodiment, the fTAS2R agonist is denatonium, aloin, or PTC.

[00219] Other embodiments of the present invention are described in the following non-limiting Examples.

EXAMPLES

Example 1. Determining feline bitter taste receptor (TAS2R) gene and polypeptide sequences

[00220] In this example, feline TAS2R genes were identified, by querying the NCBI *Felis catus* whole genome shotgun contigs database with human bitter receptor gene sequences. Human gene sequences used are identified by NCBI Gene IDs in Table 2.

Table 2. NCBI Gene IDs for all functional and pseudogene hTAS2Rs used to identify feline bitter genes.

Functional Genes	
Human Bitter Receptor gene	Gene ID
TAS2R1 (TAS2R1; TRB7)	50834
TAS2R3 (TAS2R3)	50831
TAS2R4 (TAS2R4)	50832
TAS2R5 (TAS2R5)	54429
TAS2R7 (TAS2R7; TRB4)	50837
TAS2R8 (TAS2R8; TRB5)	50836
TAS2R9 (TAS2R9; TRB6)	50835
TAS2R10 (TRB2; TAS2R10)	50839
TAS2R13 (TRB3; TAS2R13)	50838
TAS2R14 (TRB1; TAS2R14)	50840
TAS2R16 (TAS2R16)	50833
TAS2R19 (TAS2R19; TAS2R23; TAS2R48; MSTP058; TAS2R23; TAS2R48)	259294
TAS2R20 (TAS2R20; TAS2R49; TAS2R56; TAS2R49)	259295
TAS2R30 (TAS2R30; TAS2R47; TAS2R47)	259293
TAS2R31 (TAS2R31; TAS2R44; TAS2R53; TAS2R44)	259290
TAS2R38 (PTC; TAS2R38; TAS2R61)	5726
TAS2R39 (TAS2R39; TAS2R57)	259285
TAS2R40 (GPR60; TAS2R40; TAS2R58)	259286
TAS2R41 (TAS2R41; TAS2R59)	259287
TAS2R42 (TAS2R24; TAS2R55; hTAS2R55; TAS2R55)	353164
TAS2R43 (TAS2R43; TAS2R52)	259289
TAS2R45 (GPR59; TAS2R45; ZG24P)	259291
TAS2R46 (TAS2R46; TAS2R54)	259292
TAS2R50 (TAS2R50; TAS2R51; TAS2R51)	259296
TAS2R60 (TAS2R56; TAS2R60)	338398

Pseudogenes	
Human Bitter Receptor gene	Gene ID
TAS2R2P (PS9; TAS2R2; TAS2R02; TAS2R2)	338396
TAS2R12P (PS10; TAS2R12; TAS2R12; TAS2R26)	266656
TAS2R15P (PS8; TAS2R15)	266657
TAS2R18 (PS4; TAS2R18; TAS2R65; TAS2R65; TAS2R65P)	338414
TAS2R62P (PS1; TAS2R62; TAS2R62)	338399
TAS2R63P (PS6; TAS2R63)	338413
TAS2R64P (PS2; TAS2R64; TAS2R64P)	338412
TAS2R67P (PS5)	448991
TAS2R68P (PS7; TAS2R68P)	100653053

[00221] Individual contigs among the hits were downloaded for manual identification of start (ATG) and stop (TAA, TGA, or TAG) codons and to determine if the gene is likely full length. When sequences from both feline genome assemblies were obtained, they were compared.

[00222] Predicted functional genes were identified based on a set of rules selected to include a protein which is approximately 300 amino acids in length, the start site and stop site are in similar locations as the human protein when the blasted sequences are aligned, then the sequence was compared to the sequence of the orthologous canine bitter gene to verify that similarity was reasonable. Table 3 identifies canine bitter gene sequences used.

Table 3. NCBI Gene IDs for all functional and pseudogene canine TAS2Rs used

	Functional Genes	
	Canine Bitter Receptor gene	**Gene ID**
1	TAS2R1 (CAFA-TAS2R1)	100271742
2	Cafa-TAS2R2	100271741
3	TAS2R3 (CAFA-TAS2R3)	100271736
4	TAS2R4	100688996
5	TAS2R5 (CAFA-TAS2R5)	100271743
6	TAS2R7 (CAFA-TAS2R7)	100271739
7	TAS2R10 (CAFA-TAS2R10)	100271734
8	Cafa-TAS2R12	100271738
9	TAS2R38 (CAFA-TAS2R38)	100271737
10	TAS2R39 (CAFA-TAS2R39)	100271735
11	TAS2R40	608842
12	TAS2R41	482734
13	TAS2R42 (CAFA-TAS2R55)	100271731
14	Cafa-TAS2R43	100271744
15	TAS2R62-like	608741
16	Cafa-TAS2R67	100271740

	Pseudogenes	
	Canine Bitter Receptor gene	**Gene ID**
1	TAS2R8P	100682910
2	TAS2R9P	100686911
3	Cafa-TAS2R44P	GenBank: AB249699.1
4	TAS2R46-like	100682759
5	TAS2R60-like	100856773
6	TAS2R104-like	100682833

[00223] Table 4 below summarizes the full length feline genes identified. The % protein similarity between the feline gene and closest human homologue is presented in the table.

Table 4. Full length Feline Bitter Receptor Genes Identified

Predicted Feline Gene	Functional human homologue	Best % similarity to human sequence
TAS2R1	yes	60.5%
TAS2R2	NO	74.8%
TAS2R3	yes	74.4%
TAS2R4	yes	71.9%
TAS2R7	yes	74.4%
TAS2R9	yes	68.3%
TAS2R10	yes	67.8%
TAS2R12	NO	51.0%
TAS2R38	yes	67.6%
TAS2R42	NO	56.1%
TAS2R43	yes	59.0%
TAS2R44	yes	59.9%
TAS2R67	NO	47.6%

[00224] Cloning of each of the feline bitter genes to confirm the DNA sequence was performed after amplifying the desired gene by polymerase chain reaction (PCR) using the genomic DNA of a single cat. Potential primers to amplify each feline gene were designed using commercial software. Sets of primers were selected from among those designed based on predicted annealing temperature, fidelity, potential for dimerization and mispriming, and location of the desired sequence in order to amplify the feline gene sequence and determine the DNA sequence from isolated feline genomic DNA. Primer pairs used to amplify each gene are shown in Table 5.

Table 5. Primers for genomic amplification

Gene Name	F/R	SEQ ID NO	Sequence	Length
fTAS2R1	F	32	TCATGGTGGAGGTGAAGGATTG	22
fTAS2R1	R	33	AGGTATGGCAGGCATCGTCAGC	22
fTAS2R2	F	34	CAGGAATTGGCAGAAGGTCAGAT	23
fTAS2R2	R	35	GGAGAAGGAAATTGCCAGAAAGAG	24
fTAS2R3	F	36	AAATTGGGCAGAGACAAGAGACAGG	25
fTAS2R3	R	37	CGGCACCGGAACCACAAGAG	20
fTAS2R4	F	38	GGGGACAATTGGAAAAGGAAACG	23
fTAS2R4	R	39	CTCAAAGGCCCACGAAGTCAGAT	23
fTAS2R7	F	40	AGGATCATGAAAGGGAACGGGTCT	24
fTAS2R7	R	41	GACAAAGAGAAAGAGGCAAAATCG	24
fTAS2R9	F	42	CCGACAAAGAGGGCAGAAAAAGAC	24
fTAS2R9	R	43	GACCTCCTCCGGCTCAGAAGAAGT	24

Gene Name	F/R	SEQ ID NO	Sequence	Length
fTAS2R10	F	44	GATATACGTTGGGCGCTCCTACT	23
fTAS2R10	R	45	AGTGAAACCCTTACAGTGAATAG	23
fTAS2R12	F	46	CAAGCAGTGTGACAGCAGCAGGTA	24
fTAS2R12	R	47	GGAGAGGAAGGAAAGAAACGCACA	24
fTAS2R38	F	48	GAAGTCCTGGCTTGTAATGTA	21
fTAS2R38	R	49	CAAAACAAACTTGGGGAACTT	21
fTAS2R42	F	50	ACACTGGAATCGCAAAGAAACACG	24
fTAS2R42	R	51	GATCCTCAAAGACTCCTCAATAAG	24
fTAS2R43	F	52	GCACAACCAGCGACATCAGACATT	24
fTAS2R43	R	53	CCCAGGCGCCCCAAAAGA	18
fTAS2R44	F	54	GCACAACCAGCGACATCAGACATT	24
fTAS2R44	R	55	CCGGTGAGGGTAGATTATTTCCA	23
fTAS2R67	F	56	ACCCAGGCGCCCCAGTATCT	20
fTAS2R67	R	57	GCTTCCGGCATTTTTATTCC	20

[00225] The process of amplification and cloning of a representative gene, TAS2R38, is briefly described. The fTAS2R38 sequence was amplified via PCR using Easy A High Fidelity PCR Cloning Enzyme (Agilent, Santa Clara CA), custom primers, and feline genomic DNA as a template.

[00226] The resulting PCR product was ligated into the pGEM-T Easy Vector (Promega, Madison WI). DH5-α bacterial cells (Life Technologies; Carlsbad, CA) were transformed with the vector. Plasmid was purified from cultures of the transformed DH5-alpha cells using the Plasmid Miniprep Kit (Omega BioTec, Norcross, GA). Sequencing of the gene using the purified plasmid DNA was performed by the Core DNA Sequencing Facility at the University of Illinois, Champaign-Urbana. The sequencing data was analyzed with SeqMan Pro (DNAStar, Madison WI) to determine the quality of the data and to edit the data.

[00227] The gene sequence determined from the isolated feline genomic DNA sequencing was compared against the sequences obtained from the whole genome shotgun contigs and analyzed to identify specific nucleotide differences, predicted protein sequence, and protein structure. Sequences disclosed in the sequence listing for each of the feline bitter taste receptor gene cDNAs and polypeptides are identified by the SEQ ID NOs shown in Table 6.

Table 6. SEQ ID NOs of feline bitter taste receptor gene cDNA and polypeptide sequences

SEQ ID NO.	Feline TAS2R Sequence
1	R1 cDNA

2	R1 polypeptide
3	R2 cDNA
4	R2 polypeptide
5	R3 cDNA
6	R3 polypeptide
7	R4 cDNA
8	R4 polypeptide
9	R7 cDNA
10	R7 polypeptide
11	R9 cDNA
12	R9 polypeptide
13	R10 cDNA
14	R10 polypeptide
15	R12 cDNA
16	R12 polypeptide
17	R38 cDNA
18	R38 polypeptide
19	R42 cDNA
20	R42 polypeptide
21	R43 cDNA
22	R43 polypeptide
23	R44 cDNA
24	R44 polypeptide
25	R67 cDNA
26	R67 polypeptide

[00228] In general, the feline gene is named after its homologous human counterpart, as shown in Table 7. However for a feline gene similar to many human genes, such as fTAS2R43, the feline gene is named as its homologous canine counterpart.

Table 7. Corresponding genes in felines, canines and humans

Predicted Feline Gene	Predicted Canine Gene	Human Gene
TAS2R1	TAS2R1	TAS1R1
TAS2R2	CAFA-T2R2	TAS2R2P
TAS2R3	TAS2R3	TAS2R3
TAS2R4	TAS2R4	TAS2R4
TAS2R5P	TAS2R5	TAS2R5
TAS2R7	TAS2R7	TAS2R7
TAS2R8P	TAS2R8P	TAS2R8
TAS2R9	TAS2R9P	TAS2R9
TAS2R10	TAS2R10	TAS2R10
TAS2R12	TAS2R12	TAS2R12P
TAS2R16P	N/A	TAS2R16
TAS2R38	TAS2R38	TAS2R38
TAS2R39P	TAS2R39	TAS2R39
TAS2R40P	TAS2R40	TAS2R40
TAS2R41P	TAS2R41	TAS2R41
TAS2R42	TAS2R42	hTAS2R42, 18P, 67P
TAS2R43	TAS2R43	hTAS2R13, 14, 19, 20, 30, 31, 43, 45, 46, 50, 15P, 63P, 64P, 68P
TAS2R44	CAFA-T2R44P	hTAS2R13, 14, 19, 20, 30, 31, 43, 45, 46, 50, 15P, 63P, 64P, 68P
TAS2R67	CAFA-T2R67	hTAS2R42, 18P, 67P
TAS2R60P	Tas2R60P-like	TAS2R60
TAS2R62P	TAS2R62-like	TAS2R62P

[00229] A sequence alignment of the 3rd through the 7th transmembrane (TM) regions of several human and feline bitter receptors is shown in Fig. 1. The sequence alignment illustrates the substantial degree of homology of this region in bitter taste receptors of the two species.

[00230] A sequence alignment of human TAS2R38 polypeptide (SEQ ID NO:31) and feline TAS2R38 polypeptide (SEQ ID NO:18) determined from sequencing genomic DNA of five individual cats is shown in Fig. 2. Amino acids in hTAS2R38 that differ from those in fTAS2R38 are boxed in Fig. 2. The positions of the human polymorphisms known to affect taste perception of 6-n-propylthiouracil (PROP), A49P, V262A, I293V (where AVI is a non-taster and PAV is a taster) are shaded grey in Fig. 2. The residues known to be important for phenylthiocarbamide (PTC) binding to the human TAS2R38 receptor are denoted in Fig. 2 by

a thick black box (residues 99-100, 103, 255, and 259) These amino acids either directly bind PTC, contribute to the binding pocket, or are involved in receptor activation by associating with other amino acids.

[00231] TOPCONS was used to identify the seven transmembrane regions and the extracellular and cytoplasmic loops of each fTAS2R polypeptide. Results of this analysis are presented in Table 1.

Example 2. Expression systems for feline TAS2R

A. Generation of Expression Vectors for feline TAS2R

[00232] This example describes generation of an expression vector for a representative feline bitter receptor, TAS2R38. An analogous process is conducted for each of the TAS2R receptors.

[00233] The full length gene of feline TAS2R38 was amplified by polymerase chain reaction (PCR) using gene-specific primers that span the entire coding region.

[00234] The TAS2R38 cDNA was subcloned into an expression cassette based on the plasmid/expression vector pcDNA3.1D-V5His (Life Technologies, Carlsbad, Calif., US), which contains within its multiple cloning sites the nucleotide sequence coding for the FLAG epitope to allow surface detection of the receptor, then the first 45 amino acids of the rat somatostatin receptor subtype 3 (RSS tag) to facilitate cell surface targeting of the transgene, and the nucleotide sequence coding for the herpes simplex virus (HSV) glycoprotein D epitope (HSV epitope) for facilitating immunocytochemical detection (HSV Tag) on the carboxy terminus.

[00235] The nucleic acid sequences encoding the FLAG tag, RSS tag, TAS2R38, and the HSV tag were fused, in that order, in frame to create a construct to allow translation into the receptor protein. The resulting receptor cDNA in the expression vector encodes the joined amino acid sequences of TAS2R38 preceded by the RSS tag and followed by the HSV tag.

[00236] The expression vector including the construct is called pcDNA3.11D-FLAGV5His-TAS2R38 and allows for expression of the TAS2R38 protein (SEQ ID NO:18).

[00237] Generation of an expression vector for each of the other fTAS2Rs disclosed herein was performed by analogous steps.

B. Generation of Cell Lines transiently expressing fTAS2R

[00238] Cell lines that transiently express a desired TAS2R disclosed herein were generated by transfecting the appropriate expression vector, e.g., pcDNA3.1D-FLAGV5His-

TAS2R38, constructed as described above in Ex. 2A into cells of a eukaryotic cell line (Life Technologies, Cat# R700-07).

[00239] On day 0, 60,000 cells per well were plated on poly lysine coated, black 96 well plates with clear bottoms (Costar). The following day the cells were transfected with 150ng TAS2R38 expression vector, e.g., pcDNA3.1D-FLAGV5His (Invitrogen) along with 45ng of Ga16 chimera containing the last 44 amino acids of rat gustducin (Gα16gust44) with 0.5ul Lipofectamine 2000 (Invitrogen) per well. Cells were then incubated 22-44 hours at 37°C 5% CO_2.

[00240] The expression of fTAS2R38 was evaluated by testing for the presence of a functional response to a known hTAS2R38 ligand (e.g., PTC), determined via automated calcium imaging using a Fluo-4AM (Life Technologies Corporation) Calcium Assay. Fluo-4AM is a fluorescent indicator of intracellular calcium dynamics (change in concentration) and allows monitoring changes in the calcium concentration, particularly an increase in response to receptor activation occurring after agonist exposure.

[00241] Generation of cell lines transiently expressing the other fTAS2Rs disclosed herein was analogous.

[00242] Expression of the fTAS2Rs in the various cell lines generated was evaluated by flow cytometry. The extracellular FLAG tag was detected with a FLAG-specific antibody conjugated to fluorescein isothiocyanate (FITC). The percentage of cells expressing a given fTAS2R was determined by percent of cells positive for the FITC signal. The level of fTAS2R expression was determined by the geometric mean of the fluorescence intensity measured. The results for each of the expressed fTAS2R are shown in Table 8.

Table 8. Flow cytometry results for cell lines transiently expressing fTAS2Rs

fTAS2R	% of cells expressing fTAS2R	Relative fTAS2R Expression level (Geometric Mean of fluorescence intensity)
Untransfected cells	0	8,929
TAS2R1	38	231,625
TAS2R2	37	295,625
TAS2R3	24	201,000
TAS2R4	36	331,125
TAS2R7	27	144,375
TAS2R9	24	113,250

fTAS2R	% of cells expressing fTAS2R	Relative fTAS2R Expression level (Geometric Mean of fluorescence intensity)
TAS2R10	30	298,500
TAS2R12	32	258,625
TAS2R38	31	268,750
TAS2R42	25	133,375
TAS2R43	24	246,375
TAS2R44	9	125,750
TAS2R67	12	118,000

C. Screening of transiently transfected cell lines.

[00243] Testing for a functional response of fTAS2R38 to the known hTAS2R38 ligands, PTC and PROP, and of fTAS2R43 to the known hTAS2R43 ligands, aloin, denatonium and saccharine), was determined via automated calcium imaging using the Fluo-4AM (Life Technologies Corporation) Calcium Assay.

[00244] The fTAS2R38 was activated 81% over baseline by 100 μM PTC, but was not stimulated by 30 μM PROP. The fTAS2R43 was activated 45% over baseline by 300 μM aloin, and 17% over baseline by 1 mM denatonium, but was not stimulated by 6.7 mM saccharin. Furthermore, the responses to PTC, denatonium and aloin were inhibited by 1mM probenecid.

[00245] Testing for a functional response of each of the other fTAS2Rs disclosed herein can be performed by analogous methods using known ligands to a corresponding homolog of each fTAS2R.

D. Generation of Cell Lines stably expressing fTAS2R

[00246] Cell lines stably expressing fTAS2R are also obtained.

[00247] For these experiments, the fTAS2R38 cDNA is subcloned into an expression cassette based on the plasmid/expression vector pcDNA3.1Zeo (Life Technologies, Carlsbad, Calif., US), which contains within its multiple cloning sites the nucleotide sequence coding for the first 45 amino acids of the rat somatostatin receptor subtype 3 (RSS tag) to facilitate cell surface targeting of the transgene, and the nucleotide sequence coding for the herpes simplex virus (HSV) glycoprotein D epitope (HSV epitope) for facilitating immunocytochemical detection(HSV Tag).

[00248] The nucleic acid sequences encoding the RSS tag, HSV tag, and fTAS2R38 are

fused, in that order, in frame to create a construct to allow translation into the receptor protein. The resulting receptor cDNA in the expression vector encodes the joined amino acid sequences of fTAS2R38 preceded by the RSS tag and the HSV tag.

[00249] The expression vector including the construct is called pcDNA3.1Zeo-TAS2R38 and allows for expression of the fTAS2R38 protein (SEQ ID:18).

[00250] Generation of an expression vector for the other fTAS2Rs disclosed herein is analogous. The restriction enzymes used are adapted accordingly.

[00251] Cell lines that stably express a desired fTAS2R disclosed herein are generated by transfecting the appropriate expression vector, e.g., pcDNA3.1Zeo-TAS2R38, constructed as described above in Ex. 2A into a eukaryotic host cell line (Life Technologies Cat# R700-07) transformed with the Gα16 chimera containing the last 44 amino acids of rat gustducin (G[alpha]16-gustducin 44 cells) as described in WO2004/055048 (US7919236).

[00252] On day 0, the G[alpha]16-gustducin 44 cells are plated in a 6-well plate at a density of 900,000 cells per well and grown overnight in a selective growth media (DMEM with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin).

[00253] On day 1, the medium is exchanged with 2 ml of antibiotic-free and serum-free growth medium. 10 μl Lipofectamine 2000 (Life Technologies Corporation) is dissolved in 250 μl DMEM and incubated for 5 minutes at room temperature. In parallel, 4 μg pcDNA3.1Zeo-TAS2R38 DNA is dissolved in 250 μl DMEM. These two resulting solutions are mixed and incubated for 20 minutes at room temperature before they are added to the cells into the cell culture medium. After 4 hours, the medium is replaced with antibiotic-free, serum-containing growth medium. The cells are incubated in humidified atmosphere (37 C., 5% CO2).

[00254] After 24 hours, the cells are re-plated in selective growth medium (DMEM with 10% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 200 μg/ml G418 and 200 μg/ml zeocin) and are further incubated in a humidified atmosphere (37 C, 5% CO2).

[00255] After 2 to 4 weeks of culture (replacing medium as necessary), zeocin-resistant colonies are selected and expanded.

[00256] The expression of fTAS2R38 is evaluated by testing for the presence of a functional response to a knownhTAS2R38 ligand (e.g., PTC and PROP), determined via automated calcium imaging using the Fluo-4AM (Life Technologies Corporation) Calcium Assay. Fluo-4AM is a fluorescent indicator of intracellular calcium dynamics (change in

concentration) and allows monitoring changes in the calcium concentration, particularly an increase in response to receptor activation occurring after agonist exposure. One clone is selected resulting in the G[alpha]16-gustducin 44/TAS2R38 cell line. The G[alpha]16-gustducin 44/TAS2R38 cell line was stimulated 90% over baseline in the presence of 100 μM PTC but was not stimulated with 30 μm PROP.

[00257] Generation of cell lines stably expressing the other fTAS2Rs disclosed herein is analogous

Example 3. Cell-based Screening for ligands and effectors of feline TAS2Rs

[00258] Identification of agonists, antagonists and modulators of feline TAS2R38 receptor is performed by a cell-based screening assay in which the effect of a test compound on cells transfected with feline TAS2R38 and Gα16gust44 is compared against the effect of the test compound on untransfected cells.

[00259] Prior to the screening assay, the cells are loaded with the calcium sensitive dye Fluo-AM (Life Technologies) for one hour at 37 °C as described in Example 2B. The dye is washed out and the cells are assayed in Hank's Balanced Salt Solution (HBSS; Life Technologies) containing 20mM HEPES in a Flexstation II (Molecular Devices). A 10 fold dilution series 0.01mM – 1mM of test compounds is used to stimulate the cells. PTC, a known human TAS2R38 agonist, is among the test compounds

[00260] The stimuli are injected and monitored for 100-180 seconds. Data is analyzed and graphed as a percentage over the baseline signal, which is the reading prior to stimulation. Stimulation of the fTAS2R38 expressing cell line by a particular test compound is considered to occur when the signal is greater than both the signal from the buffer alone in the receptor expressing cell line and the signal from the un-transfected cell line sample injected with the test compound.

[00261] Cell based screening for agonists, antagonists, and modulators for the other fTAS2Rs disclosed herein is analogous.

Example 4. Flavor and Repellant Compositions

[00262] Exemplary dry flavor compositions for an animal comprising an agonist, an antagonist, or a modulator of a feline TAS2R receptor disclosed herein are made in general accordance with the following formulation.

Table 9. Dry Flavor Composition

Component	% by weight
identified agonist, antagonist or modulator of a feline TAS2R receptor	0.01%-5%
grain-based meal or flour, such as corn, wheat, barley or rice;	0%-50%
animal by-product meal, such as poultry or pork meal;	0%-50%
brewers or distiller's yeasts;	0%-50%
phosphate salts;	0%-50%
fresh animal protein, such as poultry or pork protein;	0%-50%
seafood-based protein;	0%-50%
sugars or starches;	0%-20%
dairy ingredients;	0%-10%
animal fat;	0%-5%
amino acid ingredients;	0%-5%
phosphoric acid and/or sodium hydroxide;	0%-5%
citric acid;	0%-5%
specialized natural flavor spikes	0%-5%
Final pH from 4.0 – 8.0	
Final moisture from 1.0 – 5.0%	

[00263] The identified agonist in the dry flavor composition is denatonium, aloin, or PTC or the identified antagonist is probenecid.

[00264] Exemplary liquid flavor compositions for an animal comprising an agonist, an antagonist, or a modulator of a feline TAS2R receptor are made in general accordance with the following formulation.

Table 10. Liquid Flavor Composition

Component	% by weight
identified agonist, antagonist or modulator of a feline TAS2R receptor	0.01%-5%
animal protein, such as poultry or pork protein;	0%-40%
animal by-product meal, such as poultry or pork meal;	0%-40%
seafood-based protein;	0%-40%
grain-based meal or flour, such as corn, wheat, barley or rice;	0%-30%
brewers or distiller's yeasts;	0%-30%
phosphate salts;	0%-10%
sugars or starches;	0%-10%
dairy ingredients;	0%-10%
phosphoric acid and/or sodium hydroxide;	0%-10%
animal fat;	0%-5%
amino acid ingredients	0%-5%

citric acid	0%-5%
specialized natural flavor spikes	0%-5%
Final pH from 2.7 – 3.1	
Final moisture from 60.0 – 80.0%	

[00265] The identified agonist in the liquid flavor composition is denatonium, aloin, or PTC or the identified antagonist is probenecid.

[00266] An exemplary repellent composition in the form of an aerosol for spraying onto an object to deter companion cats from chewing or eating the object is made by formulating 50% active ingredient solution, the active ingredient being a feline TAS2R agonist or positive modulator, with 50% of a propellant gas such as Frigen 11/12 (a halogenated hydrocarbon) or propane/butane (e.g., in a 15:85 ratio) in an aerosol can. The active ingredient solution consists of from about 0.5% to about 30 % by weight of a feline TAS2R agonist or positive modulator dissolved in a liquid diluent, e.g., water, optionally 0.5 - 1.5% of an aromatic or a perfume, and up to 29.5% isopropanol. The feline TAS2R agonist is denatonium, aloin, or PTC.

[00267] Embodiment 1. An isolated feline TAS2R (fTAS2R) receptor polypeptide comprising an extracellular domain of a feline TAS2R receptor; a transmembrane region of a feline TAS2R receptor, or an intracellular domain of a feline TAS2R receptor, wherein the fTAS2R receptor comprises a sequence selected from SEQ ID NO:18, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26, wherein the isolated feline TAS2R (fTAS2R) receptor polypeptide does not consist of the amino acid sequence of SEQ ID NOs: 2, 4, 6, or 10.

[00268] Embodiment 2. The polypeptide of embodiment 1 wherein: the extracellular domain of the feline TAS2R receptor polypeptide comprises: amino acids 1, 68-84; 146-179; or 249-257 of SEQ ID NO:2; amino acids 1-10, 73-88; 151-186; or 256-264 of SEQ ID NO:4; amino acids 1-8; 72-88; 150-186; or 256-265 of SEQ ID NO:6; amino acids 1-2; 69-87; 151-183; or 253-261 of SEQ ID NO:8; amino acids 1-8; 72-88; 150-187; or 257-265 of SEQ ID NO:10; amino acids 1-6; 72-88; 150-183; or 253-262 of SEQ ID NO:12; amino acids 1; 69-87; 150-181; or 251-260 of SEQ ID NO:14; amino acids 1-8; 69-88; 150-185; or 252-261 of SEQ ID NO:16; amino acids 1-17: 83-98; 161-198; or 268-277 of SEQ ID NO:18; amino acids 1; 69-88; 150-185; or 255-264 of SEQ ID NO:20; amino acids 1-2; 69-87; 149-

181; or 251-260 of SEQ ID NO:22; amino acids 1-2; 69-87; 149-181; or 251-259 of SEQ ID NO:24; or amino acids 1-8; 72-88; 150-185; or 254-263 of SEQ ID NO:26; the transmembrane region of the feline TAS2R receptor polypeptide comprises: amino acids 2-22, 47-67, 85-105, 125-145, 180-200, 228-248, or 258-278 of SEQ ID NO:2; amino acids 11-31, 52-72, 89-109, 130-150, 187-207, 235-255, or 265-285 of SEQ ID NO:4; amino acids 9-29, 51-71, 89-109, 129-149, 187-207, 235-255, or 266-286 of SEQ ID NO:6; amino acids 3-23, 48-68, 88-108, 130-150, 184-204, 232-252, or 262-282 of SEQ ID NO:8; amino acids 9-29, 51-71, 89-109, 129-149, 188-208, 236-256, or 266-286 of SEQ ID NO:10; amino acids 7-27, 51-71, 89-109, 129-149, 184-204, 232-252, or 263-283 of SEQ ID NO:12; amino acids 2-22, 48-68, 88-108, 129-149, 182-202, 230-250, or 261-281 of SEQ ID NO:14; amino acids 9-29, 48-68, 89-109, 129-149, 186-206, 231-251, or 262-282 of SEQ ID NO:16; amino acids 18-38, 62-82, 99-119, 140-160, 199-219, 247-267, or 278-298 of SEQ ID NO:18; amino acids 2-22, 48-68, 89-109, 129-149, 186-206, 234-254, or 265-285 of SEQ ID NO:20; amino acids 3-23, 48-68, 88-108, 128-148, 182-202, 230-250, or 261-281of SEQ ID NO:22; amino acids 3-23, 48-68, 88-108, 128-148, 182-202, 230-250, or 260-280 of SEQ ID NO:24; or amino acids 9-29, 51-71, 89-109, 129-149, 186-206, 233-253, or 264-284 of SEQ ID NO:26; and the intracellular domain comprises: amino acids 23-46; 106-124; 201-227; or 279-298 of SEQ ID NO:2; amino acids 32-51; 110-129; 208-234; or 286-304 of SEQ ID NO:4; amino acids 30-50; 110-128; 208-234; or 287-316 of SEQ ID NO:6; amino acids 24-47; 109-129; 205-231; or 283-306 of SEQ ID NO:8; amino acids 30-50; 110-128; 209-235; or 287-311 of SEQ ID NO:10; amino acids 28-50; 110-128; 205-231; or 284-337 of SEQ ID NO:12; amino acids 23-48; 109-128; 203-229; or 282-300 of SEQ ID NO:14; amino acids 30-47; 110-128; 207-230; or 283-309 of SEQ ID NO:16; amino acids 39-61; 120-139; 220-246; or 299-334 of SEQ ID NO:18; amino acids 23-47; 110-128; 207-233; or 286-322 of SEQ ID NO:20; amino acids 24-47; 109-127; 203-229; or 282-299 of SEQ ID NO:22; amino acids 24-47; 109-127; 203-229; or 281-308 of SEQ ID NO:24; or amino acids 30-50; 110-128; 207-232; or 285-312 of SEQ ID NO:26.

[00269] Embodiment 3. The polypeptide of embodiment 1 or 2 comprising a transmembrane region 2, a transmembrane region 3, a transmembrane region 4, a transmembrane region 5, a transmembrane region 6, and a transmembrane region 7, wherein each transmembrane region comprises at least 20 consecutive amino acids of the corresponding transmembrane region sequence independently selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24,

and SEQ ID NO:26; or a transmembrane region 3, a transmembrane region 6, and a transmembrane region 7, wherein each transmembrane region comprises at least 20 consecutive amino acids of the corresponding transmembrane region sequence independently selected from SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26; an extracellular domain 3 comprising at least 15 consecutive amino acids selected from amino acids 146-179 of SEQ ID NO:2; amino acids 151-186 of SEQ ID NO:4; amino acids 150-186 of SEQ ID NO:6; amino acids 151-183 of SEQ ID NO:8; amino acids 150-187 of SEQ ID NO:10; amino acids 150-183 of SEQ ID NO:12; amino acids 150-181 of SEQ ID NO:14; amino acids 150-185 of SEQ ID NO:16; amino acids 161-198 of SEQ ID NO:18; amino acids 150-185 of SEQ ID NO:20; amino acids 149-181 of SEQ ID NO:22; amino acids 149-181 of SEQ ID NO:24; and amino acids 150-185 of SEQ ID NO:26; and an extracellular domain 4 comprising at least 8 consecutive amino acids selected from amino acids 249-257of SEQ ID NO:2; amino acids 256-264 of SEQ ID NO:4; amino acids 256-265 of SEQ ID NO:6; amino acids 253-261 of SEQ ID NO:8; amino acids 257-265 of SEQ ID NO:10; amino acids 253-262 of SEQ ID NO:12; amino acids 251-260 of SEQ ID NO:14; amino acids 252-261 of SEQ ID NO:16; amino acids 268-277 of SEQ ID NO:18; amino acids 255-264 of SEQ ID NO:20; amino acids 251-260 of SEQ ID NO:22; amino acids 251-259 of SEQ ID NO:24; and amino acids 254-263 of SEQ ID NO:26.

[00270] Embodiment 4. The polypeptide of any one of embodiments 1-3, further comprising a heterologous polypeptide.

[00271] Embodiment 5. The polypeptide of embodiment 4, wherein the heterologous polypeptide is linked to the amino terminus or the carboxy terminus of the feline TAS2R receptor polypeptide.

[00272] Embodiment 6. The polypeptide of any one of embodiments 1-5 comprising the amino acid sequence of SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26.

[00273] Embodiment 7. The polypeptide of any one of embodiments 1-6 consisting of the amino acid sequence of SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26.

[00274] Embodiment 8. The polypeptide of any one of embodiments 1-7 that is non-naturally occurring.

[00275] Embodiment 9. The polypeptide of any one of embodiments 1-8 having fTAS2R

receptor activity or binding a ligand of an fTAS2R receptor.

[00276] Embodiment 10. The polypeptide of any one of embodiments 1-9, wherein the sequence is SEQ ID NO:18.

[00277] Embodiment 11. The polypeptide of any one of embodiments 1-10, wherein the fTAS2R is fTAS2R38 and amino acid 74 of SEQ ID NO:18 is N.

[00278] Embodiment 12. The polypeptide of any one of embodiments 1-11, wherein the extracellular domain comprises a sequence of at least 15 consecutive amino acids of extracellular domain 2 or 3 or of at least 8 consecutive amino acids of extracellular domain 4 of a fTAS2R receptor sequence; the transmembrane region comprises a sequence of at least 20 consecutive amino acids of a fTAS2R receptor sequence transmembrane region, and the intracellular domain comprise a sequence of at least 17 consecutive amino acids of a fTAS2R receptor sequence intracellular domain.

[00279] Embodiment 13. A composition comprising at least two polypeptides of any one of embodiments 1-12

[00280] Embodiment 14. An isolated polynucleotide encoding the polypeptide of any one of embodiments 1-12.

[00281] Embodiment 15. An isolated polynucleotide encoding a feline TAS2R (fTAS2R) receptor polypeptide, or fragment thereof comprising a nucleotide sequence selected from: the nucleotide sequence of SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25;a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; a nucleotide sequence that hybridizes to the complement of the polynucleotide having SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25 under high stringency conditions; and the complement of the foregoing nucleotide sequences.

[00282] Embodiment 16. A polynucleotide comprising at least 15 contiguous nucleotides of SEQ ID NO:17, wherein the contiguous nucleotides contain nucleotide 220 and an A is present at nucleotide 220; or the complement of the nucleotide sequence.

[00283] Embodiment 17. The polynucleotide of embodiment 16, comprising at least 20 contiguous nucleotides.

[00284] Embodiment 18. The polynucleotide of embodiment 16 or 17, comprising at least 25 contiguous nucleotides.

[00285] Embodiment 19. The polynucleotide of any one of embodiments 14-18, wherein the nucleotide sequence is codon-optimized for expression in a non-feline cell.

[00286] Embodiment 20. The polynucleotide of embodiment 19, wherein the non-feline cell is *Escherichia coli*E., a *Saccharomyces cerevisae* cellyeast, a *Drosophila melanogaster* cell, a *Caenorhabditis elegans* cell, or a mammalian cell.

[00287] Embodiment 21 The polynucleotide of embodiment 20, wherein the mammalian cell is a murine or human cell.

[00288] Embodiment 22. The polynucleotide of any one of embodiments 14-21 that is non-naturally occurring.

[00289] Embodiment 23. A composition comprising at least two polynucleotides of any one of embodiments 14-22.

[00290] Embodiment 24. A primer pair for amplifying at least a portion of a nucleic acid encoding a feline TAS2R polypeptide.

[00291] Embodiment 25. The composition of embodiment 24 comprising a primer pair selected from the primer pairs of Table 5.

[00292] Embodiment 26. A feline TAS2R receptor polypeptide encoded by the polynucleotide of any one of embodiments 14-22.

[00293] Embodiment 27. An expression vector comprising the polynucleotide of any one of embodiments 14-22.

[00294] Embodiment 28. A host cell comprising the expression vector of embodiment 27.

[00295] Embodiment 29. The host cell of embodiment 28 wherein the cell is a mammalian cell, a fish cell, a yeast cell, a bacterial cell, or an insect cell.

[00296] Embodiment 30. The host cell of embodiments 28 or 29 wherein the cell is a human, murine, or feline cell.

[00297] Embodiment 31. The host cell of embodiment 28 or 29 wherein the cell is a bacterial, insect, or yeast cell.

[00298] Embodiment 32. A cell culture comprising at least one cell of any one of embodiments 28-31.

[00299] Embodiment 33. An oligonucleotide comprising a nucleotide sequence of at least 15 and up to 100 contiguous nucleotides of SEQ ID NO: 7, SEQ ID NO:9, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25; or the complement of the nucleotide sequence.

[00300] Embodiment 34. The oligonucleotide of embodiment 33, comprising at least 18

and up to 50 contiguous nucleotides.

[00301] Embodiment 35. The oligonucleotide of embodiment 33 or 34, comprising at least 18 and up to 30 contiguous nucleotides.

[00302] Embodiment 36. An isolated antibody or a fragment thereof, that specifically binds an fTAS2R receptor epitope of the polypeptide of any one of embodiments 1-12 and 26.

[00303] Embodiment 37. A method for identifying a compound that interacts with a feline TAS2R receptor polypeptide comprising: contacting a polypeptide of any one of embodiments 1-12 and 26 with a test compound, and detecting interaction between the polypeptide and the test compound.

[00304] Embodiment 38. The method of embodiment 37, wherein detecting interaction between the polypeptide and the test compound comprises measuring an electrical property, measuring a change in an ion concentration, measuring a change in protein conformation, measuring binding of the test compound to the polypeptide, measuring a change in phosphorylation level, measuring a change in transcription level, measuring a change in second messenger level, measuring a change in neurotransmitter level, measuring a change in a spectroscopic characteristic, measuring a change in a hydrodynamic (e.g., shape) property, measuring a change in a chromatographic property, or measuring a change in solubility.

[00305] Embodiment 39. The method of embodiment 37 or 38, further comprising identifying the test compound as a compound that interacts with the receptor.

[00306] Embodiment 40. A method for identifying a compound which modulates a feline TAS2R receptor polypeptide which comprises: contacting the polypeptide of any one of embodiments 1 to 12 and 24 with a TAS2R receptor ligand in both the presence and absence of a test compound in separate assays, and determining whether the test compound modulates binding of the ligand to the receptor polypeptide or activation of the receptor polypeptide by the ligand.

[00307] Embodiment 41. The method of embodiment 40, wherein determining whether the test compound modulates binding of the ligand to the receptor or activation of the receptor by the ligand comprises measuring an electrical property, measuring an ion concentration, measuring a change in protein conformation, measuring a binding of the test compound to the polypeptide, measuring a change in phosphorylation level, measuring a change in transcription level, measuring a change in second messenger level, or measuring a change in neurotransmitter level.

[00308] Embodiment 42. The method of embodiment 40 or 41 further comprising

identifying the test compound as a modulator.

[00309] Embodiment 43. The method of any one of embodiments 37 to 42, wherein the polypeptide is bound to a solid support, expressed in a host cell, in a bilayer membrane, in a lipid monolayer, or in a vesicle.

[00310] Embodiment 44. A method of preparing an edible composition comprising contacting an edible composition or a component thereof with a polypeptide of any one of embodiments 1 to 12 and 26 for a time sufficient to reduce the amount of a bitter compound from the edible composition or component thereof.

[00311] Embodiment 45. The method of embodiment 44 wherein the polypeptide is bound to a solid support that can be separated from the edible composition.

[00312] Embodiment 46. The method of embodiment 44 or 45 wherein the contacting is a continuous operation, a semi-continuous operation, or a batch operation.

[00313] Embodiment 47. The method of any one of embodiments 44-46 wherein the edible composition is a feline food composition, and the composition or a component thereof is contacted with a plurality of different polypeptides.

[00314] Embodiment 48. A method of formulating an edible composition with enhanced palatability comprising determining the presence of a compound which is an agonist, antagonist, or modulator of a feline TAS2R receptor polypeptide of any one of embodiments 1 to 12 and 26 in an edible composition; and enhancing palatability of the edible composition by if the compound is an agonist or a positive modulator, increasing the amount of an antagonist for the receptor in the edible composition or reducing the amount of the compound in the edible composition, or if the compound is an antagonist or a negative modulator, increasing the amount of the compound in the edible composition.

[00315] Embodiment 49. The method of embodiment 48 wherein increasing the amount of the compound comprises applying a flavor composition comprising the compound to the edible composition such that the flavor composition is incorporated into or at least partially coats the edible composition.

[00316] Embodiment 50. The method of embodiment 48 or 49, wherein the edible composition comprises a food, a flavor composition, a treat, a pharmaceutical, an oral care material, a nutritional supplement, a chewable product, or a drinkable product.

[00317] Embodiment 51. A method of administering a bitter compound to an animal in need thereof comprising administering an edible composition to an animal, wherein the edible composition comprises a bitter compound and a compound that is an agonist, antagonist, or modulator of a feline TAS2R receptor polypeptide of any one of embodiments

1 to 12 and 26 that alters acceptance of the edible composition by the animal compared to acceptance of the edible composition without the compound.

[00318] Embodiment 52. The method of embodiment 51 wherein the bitter compound comprises a pharmaceutical, oral care material, a repellant, or a nutritional supplement.

[00319] Embodiment 53. A method of preparing an edible composition for controlling palatability of the edible composition to an animal comprising adding a compound to an edible composition to decrease the palatability of the edible composition to an animal, wherein the compound is an agonist of or a positive modulator of a feline TAS2R receptor polypeptide of any one of embodiments 1 to 12 and 26.

[00320] Embodiment 54. The method of embodiment 53 wherein the palatability is decreased to an extent that an animal to whom the edible composition is administered consumes 10 to 30% less of the edible composition with the compound than of the edible composition without the added compound.

[00321] Embodiment 55. The method of embodiment 53 or 54 wherein the decrease is measured in calories of edible composition consumed, weight of edible composition consumed, or volume of edible composition consumed.

[00322] Embodiment 56. A method for making a flavor composition for coating or incorporating into an edible composition to be administered to an animal comprising: mixing an agonist or an antagonist of a feline TAS2R receptor polypeptide of any one of embodiments 1 to 12 and 26, wherein the agonist is denatonium, aloin, or PTC and the antagonist is probenecid with a carrier to obtain a flavor composition; optionally, mixing into the flavor composition a palatability enhancer, a compound to help adhere the flavor composition to the edible composition, or a compound for providing color or aroma; wherein the flavor composition is a liquid, solid, powder, paste, gel, spreadable formulation, granule, or sprayable formulation.

[00323] Embodiment 57. A flavor composition for coating or incorporating into an edible composition to be administered to an animal comprising: an agonist or an antagonist of a feline TAS2R receptor polypeptide of any one of embodiments 1 to 12 and 26, wherein the agonist is denatonium, aloin, or PTC and the antagonist is probenecid; optionally, a palatability enhancer; optionally, a compound to help adhere the flavor composition to the edible composition; and optionally, a compound for providing color or aroma; wherein the flavor composition is a liquid, solid, powder, paste, gel, spreadable formulation, granule, or sprayable formulation.

[00324] Embodiment 58. The flavor composition of embodiment 57 or the method of any

one of embodiments 53-56, wherein the edible composition is a food, treat, nutritional supplement, pharmaceutical, oral care material, chewable product, repellant, or drinkable product.

[00325] Embodiment 59. The flavor composition of embodiment 57 or 58 or the method of any one of embodiments 53-56 wherein the edible composition is a dry food, a soft food, a semisoft food, a liquid, a tablet, capsule, caplet, granule, paste, colloidal mixture, dispersion, or gel.

[00326] Embodiment 60. The method of any one of embodiments 48 to 56 or the flavor composition of any one of embodiments 57 to 58, wherein the edible composition is for administration to a feline.

[00327] Embodiment 61: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R38. Embodiment 62: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R42. Embodiment 63: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R43. Embodiment 64. The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R44. Embodiment 65: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R67. Embodiment 66: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R12. Embodiment 67: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R10. Embodiment 68: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R9. Embodiment 69: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R7. Embodiment 70: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R4. Embodiment 71: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R3. Embodiment 72: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R2. Embodiment 73: The polypeptide of any one of embodiments 1-12 and 26, wherein the fTAS2R receptor comprises a domain of fTAS2R1.

[00328] As used herein, the terms "a" and "an" do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced item. The term "or" means "and/or". The terms "comprising", "having", "including", and "containing" are to be

construed as open-ended terms (i.e., meaning "including, but not limited to"). The modifier "about" used in connection with a quantity is inclusive of the stated value and has the meaning dictated by the context (e.g., includes the degree of error associated with measurement of the particular quantity).

[00329] Recitation of ranges of values are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. The endpoints of all ranges are included within the range and independently combinable.

[00330] Unless defined otherwise, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

[00331]

[00332] Embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of these embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIMS

1. An isolated feline TAS2R (fTAS2R) receptor polypeptide comprising a sequence selected from SEQ ID NO:18, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26.

2. The polypeptide of claim 1, further comprising a heterologous polypeptide.

3. The polypeptide of claim 2, wherein the heterologous polypeptide is linked to the amino terminus or the carboxy terminus of the feline TAS2R receptor polypeptide.

4. A composition comprising at least two polypeptides of any one of claims 1-3.

5. An isolated polynucleotide encoding the polypeptide of any one of claims 1-3.

6. An isolated polynucleotide comprising a nucleotide sequence selected from:

the nucleotide sequence of SEQ ID NO: 17, SEQ ID NO: 7, SEQ ID NO: 11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, or SEQ ID NO: 25;

a nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 18, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, or SEQ ID NO: 26; and

the complement of the foregoing nucleotide sequences.

7. An expression vector comprising the polynucleotide of claim 5 or 6.

8. A host cell comprising the expression vector of claim 7.

9. The host cell of claim 8 wherein the cell is a mammalian cell, a fish cell, a yeast cell, a bacterial cell, or an insect cell.

10. The host cell of claims 8 wherein the cell is a human, murine, or feline cell.

11. An isolated antibody or a fragment thereof, that specifically binds an fTAS2R receptor polypeptide of any one of claims 1-3.

12. A method for identifying a compound that interacts with a feline TAS2R receptor polypeptide comprising:

contacting a polypeptide of any one of claims 1-3 with a test compound, and

identifying the test compound as a compound that interacts with the receptor when interaction is detected between the polypeptide and the test compound.

13. The method of claim 12, wherein detecting interaction between the polypeptide and the test compound comprises

measuring an electrical property, measuring a change in an ion concentration, measuring a change in protein conformation, measuring binding of the test compound to the polypeptide, measuring a change in phosphorylation level, measuring a change in transcription level, measuring a change in second messenger level, measuring a change in neurotransmitter level, measuring a change in a spectroscopic characteristic, measuring a change in a hydrodynamic (e.g., shape) property, measuring a change in a chromatographic property, or measuring a change in solubility.

14. The method of claim 12, wherein the polypeptide is bound to a solid support, expressed in a host cell, in a bilayer membrane, in a lipid monolayer, or in a vesicle.

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15. A method for identifying a compound which modulates a feline TAS2R receptor polypeptide which comprises:

contacting the polypeptide of any one of claims 1-3 with a TAS2R receptor ligand in both the presence and absence of a test compound in separate assays, and

identifying the test compound as a modulator when the test compound is determined to modulate binding of the ligand to the receptor polypeptide or activation of the receptor polypeptide by the ligand.

16. The method of claim 15, wherein determining whether the test compound modulates binding of the ligand to the receptor or activation of the receptor by the ligand comprises

measuring an electrical property, measuring an ion concentration, measuring a change in protein conformation, measuring a binding of the test compound to the polypeptide, measuring a change in phosphorylation level, measuring a change in transcription level, measuring a change in second messenger level, or measuring a change in neurotransmitter level.

17. The method of claim 15, wherein the polypeptide is bound to a solid support, expressed in a host cell, in a bilayer membrane, in a lipid monolayer, or in a vesicle.

18. A method of modifying an edible composition comprising a bitter compound, the method comprising

contacting the edible composition or a component thereof with a polypeptide of any one of claims 1-3 for a time sufficient to reduce the amount of the bitter compound from the edible composition or component thereof,

thereby producing a modified edible composition comprising reduced amounts of the bitter compound.

19. The method of claim 18 wherein the polypeptide is bound to a solid support that can be separated from the edible composition.

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20. The method of claim 18 wherein the contacting is a continuous operation, a semi-continuous operation, or a batch operation.

21. The method of claim 18 wherein the edible composition is a feline food composition, and the composition or a component thereof is contacted with a plurality of different polypeptides.

1/2

FIG. 1

TM 3 … TM 4 (positions 110, 120, 130, 140, 150)

Sequence	Alignment	Position
human Tas2R16	WEFFNILTFWLNSLLTVFYCIKVSSFTHHIFLWLRWRILRLFPWILLGSL	134
human Tas2R4	FMFLDSSSVWFVTLLNILYCVKITNFQHSVFLLLKRNISPKIPRLLLACV	137
feline Tas2R4	WMFLESTSLWLVTLLNALYCVKITDFQHSVFLLLKRKLSPKIPRLLLACV	138
human Tas2R9	WTFANNSSLWFTSCLSIFYLLKIANISHPFFFWLKLKINKVMLAILLGSF	138
feline Tas2R9	WTLSNHSSVWFTACLSIFYLLKIANISHPVFLWLKLNVTRVVLGLFLASF	138
human Tas2R10	WVIGNQSSMWFATSLSIFYFLKIANFSNYIFLWLKSRTNMV-LPFMIVFL	136
feline Tas2R10	WIIINQSNIWFATSLSTFYFLKIANFSHHMFLWLKGRINVV-LPLLMGSL	137
feline Tas2R12	WTGSNYFCITCTTCLSVFYFFKIANFSNPLFLWIKWRIHKVLLTIVLAAV	138
human Tas2R38	WMIANQANLWLAACLSLLYCSKLIRFSHTFLICLASWVSRKISQMLLGII	148
feline Tas2R38	WMITNQVGLWLTTCLSLLYCSKIARFSHTLLHCVASWVSRKVPQMLLGAM	148

TM 4 (positions 160, 170, 180, 190, 200)

Sequence	Alignment	Position
human Tas2R16	MITCVTIIPSAIGNYIQIQLLTMEHLPRNSTVTDKLENFHQYQFQAHT--	182
human Tas2R4	LISAFTTCLYITLSQASP---FPELVTTRNNTSFNISEGILSLVVSLV--	182
feline Tas2R4	LISAFSTLLYVVLTQTSP---FPELLTGSNGTVCDINKSILSLVTSLV--	183
human Tas2R9	LISLIISVPKNDDMWYHL---FKVSHEENITWKFKVSKIPG--TFKQLTL	183
feline Tas2R9	LTSIIISVFLKEGSWGHV----EVNHEENITWEFRVSKAPS--AFKLIIL	182
human Tas2R10	LISSLLNFAYIAKILND----YKT-KNDTV-WDLNMYKSEY--FIKQILL	178
feline Tas2R10	FISWLFTFPQIVKILSD----SKVGNGNAT-WQLNMPKSEF--LTKQILV	180
feline Tas2R12	F-SFCLSLPFKDTVFTSL---IKNKVNAERNWTVSFTTRTYELFLSHMLL	184
human Tas2R38	LCSCICTVLCVWCFFSRPHFTVTTVLFMNNNTRLNWQIKDLNLFYSFLFC	198
feline Tas2R38	LFSCICTAICLGDFFSRSGFTFTTMLFVNN-TEFNLQIAKLSFYHSFIFC	197

TM 5 (positions 210, 220, 230, 240, 250)

Sequence	Alignment	Position
human Tas2R16	-VALVIFFILFLASTIFLMASL---TKQIQHHSTGHCNPSMKARFTALRS	228
human Tas2R4	-LSSSLQFIINVTSASLLIHSLRRHIQKMQKNATGFWNPQTEAHVGAMKL	231
feline Tas2R4	-LSSFLQFIMNVTSASLLIHSLRRHIQKMQKNATDFWNPQTEAHMGAMKL	232
human Tas2R9	NLGVMVPFILCLISFFLLLFSLVRHTKQIRLHATGFRDPSTEAHMRAIKA	233
feline Tas2R9	NLGALVPFALCLISFVLLLFSLFRHAKQMQLYATGSRDCSTEAHMRAIKA	232
human Tas2R10	NLGVIFFFTLSLITCIFLIISLWRHNRQMQSNVTGLRDSNTEAHVKAMKV	228
feline Tas2R10	NIGVLLLFTLFLITCFLLIISLWRHSRRMQLNVTGFQDPSTEAHMKAMKV	230
feline Tas2R12	NIMFIIPFAVSLASFVLLICSLWSHTRQMKGRGG---DPTTKVHVRAMKA	231
human Tas2R38	YLWSVPPFLLFLVSSGMLTVSLGRHMRTMKVYTRNSRDPSLEAHIKALKS	248
feline Tas2R38	TLASIPSLLFFLISSGVLIVSLGRHMRTMRAKTKDSHDPSLEAHIKALRS	247

TM 6 … TM 7 (positions 260, 270, 280, 290, 300)

Sequence	Alignment	Position
human Tas2R16	LAVLFIVFTSYFLTILITIIGTLF-DKRCWLWVWEAFVYAFILMHSTSLM	277
human Tas2R4	MVYFLILYIPYSVATLVQYLPFYAGMDMGTKSICLIFATLYSPGHSVLII	281
feline Tas2R4	MIYFLILYIPYSLATLLQYLPS-VRMDLGATSICMIISTFYPPGHSVLII	281
human Tas2R9	VIIFLLLLIVYYPVFLVMTSSALIPQGKLVLMIGDIVTVIFPSSHSFILI	283
feline Tas2R9	VTIFLLFFIMYYAVFLVVTSSFLIPQGRVVLMFGGIVTVIFPSSHSFILI	282
human Tas2R10	LISFIILFILYFIGMAIEISCFTVRENKLLLMFGMTTTAIYPWGHSFILI	278
feline Tas2R10	LISFIILFILHFIGLAIEIACFTMPEKKLLFIFGMTTTVLYPWGHSFILI	280
feline Tas2R12	MISFLLFFFMYYLSTIMMNLAYVILDSLVAKIFANTLVFLYPSGHTFLLI	281
human Tas2R38	LVSFFCFFVISSCVAFISVPLLILWRDKIGVMVCVGIMAACPSGHAAILI	298
feline Tas2R38	LVSFLCLYVVSFCAALVSVPLLMLWHNKIGVMICVGILAACPSIHAAILI	297

FIG. 2